Keratins 7 and 20 as Diagnostic Markers of Carcinomas Metastatic to the Ovary

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The most common carcinomas metastatic to the ovary that mimic ovarian primaries are colonic adenocarcinomas and endometrial carcinomas. Conventional histochemical staining procedures, even in combination with additional immunohistochemical assays, are of limited value in distinguishing between these metastases and primary ovarian carcinomas. In this study we investigated whether the application of monoclonal antibodies against keratins 7, 8, and 20 could help in differentiating between these categories. The reactivity patterns of 40 carcinomas metastatic to the ovary were compared with those of their primary carcinomas on the one hand and with various primary ovarian carcinomas and mesotheliomas on the other. Colon cancer metastatic to the ovary was keratin 7 negative and keratin 20 positive in 94% of the cases; in contrast, all primary ovarian carcinomas were keratin 7 positive and keratin 20 negative, with the exception of two cases of mucinous cystadenocarcinoma. Ovarian metastases of gastric cancer usually contained keratins 7 and 20. Metastases of endometrial cancer to the ovary and primary ovarian carcinomas usually showed similar keratin expression. We propose that keratins 7 and 20 antibodies may be of help to distinguish between primary ovarian carcinomas and carcinoma metastases in the ovary.

When a carcinoma in the ovary is diagnosed, the possibility of a metastatic tumor should be considered, because up to 7% of all ovarian neoplasms are metastases, which may histologically mimic primary ovarian carcinomas.1,3,13 Approximately 40% of adenocarcinoma metastases in the ovary originate from the colon. Colorectal carcinoma metastases frequently form large cystic masses and are often unilateral, thus resembling primary endometrioid or mucinous ovarian adenocarcinomas.10 In addition, breast and endometrial carcinomas regularly metastasize to the ovary.

The contributions of immunohistochemistry to resolve the nature of an adenocarcinoma in the ovary have been limited. Carcinoembryonic antigen (CEA), for example, has been tested as a marker to distinguish between primary ovarian adenocarcinoma and colon cancer metastasis but has been found in both.4,5 As epithelial tissues differ in their pattern of keratin expression and this pattern is often maintained in carcinomas and in their metastases, we hypothesized that the distribution of specific keratin subtypes might be used to distinguish between primary and metastatic ovarian cancer in the case of a metastasis to determine the site of origin of the primary carcinoma.10-21

On the basis of keratin expression studies in malignancies of the gastrointestinal and urogenital tract, we selected a subgroup of keratins, ie, keratins 7, 8, and 20, for our study. Keratin 7 has been shown to be ubiquitously present in ovarian carcinomas but not in colonic carcinomas.13,15,16 Keratin 20 has been found to have a complementary distribution pattern, with ovarian carcinomas usually being negative but colon carcinomas being positive.17 In this study we compared the expression patterns of keratins 7, 20, and 8 (which served as positive control) in primary ovarian carcinomas with those of carcinomas of various sites (breast, stomach, colon, and endometrium) as well as mesotheliomas in an effort to determine whether or not primary and metastatic ovarian tumors could be distinguished by their pattern of keratin expression.

MATERIALS AND METHODS

The tissue material used in this study was obtained from the pathology departments of Erasmus University Hospital in Rotterdam, the Diagnostic Centre SSDZ in Delft, Groene Hart Hospital in Gouda, and Canisius-Wilhelmina Hospital in Nijmegen. Paraflin blocks of primary ovarian carcinomas, mesotheliomas, and various ovarian metastases as well as the corresponding primary tumors in most cases were collected (Table 1). Histopathological diagnosis was based on light microscopy, occasionally supplemented by special stains (periodic acid-Schiff and mucicarmine) or immunohistochemistry (mostly CEA and epithelial membrane antigen [EMA]).

Keratin Monoclonal Antibodies

Three monoclonal antibodies were used in this study: 1. OVAL 12/30 (IgG2) (BioGenex Laboratories, San Ramon, CA) is specific for keratin 7. It stains a subgroup of adenocarcinomas, eg, those of the lung, ovary, and breast as well as most transitional cell carcinomas.18 Adenocarcinomas from the gastrointestinal tract have been described not to react.17 The antibody reacts with formalin-fixed, paraflin-embedded tissue.

2. CAM 5.2 (Becton-Dickinson, San Jose, CA) recognizes keratin 8 only and stains malignant tumors derived from virtually all types of epithelia.

3. IT-ks 20.8 (DAKO A/S, Glostrup, Denmark) specifically reacts with keratin 20.6 Immunoreactivity has been reported in adenocarcinomas of the colon, stomach, bile duct, and pancreas as well as in transitional cell carcinomas, and Merkel cell carcinomas. Focal staining also has been noted in...
TABLE 1. Summary of Keratin Immunohistochemical Staining Patterns in Primary Malignancies and Metastases to the Ovary

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Keratin 7</th>
<th>Keratin 8</th>
<th>Keratin 20</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No Staining</td>
<td>5%-50%</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>Metastatic colonic carcinoma†</td>
<td>17</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Primary colonic carcinoma†</td>
<td>15</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Metastatic gastric carcinoma</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Primary gastric carcinoma</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Metastatic breast carcinoma</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Primary breast carcinoma</td>
<td>6</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Metastatic endometrial carcinoma</td>
<td>10</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Primary endometrial carcinoma</td>
<td>10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Serous cystadenocarcinoma</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Mucinous cystadenocarcinoma</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Poorly differentiated carcinoma</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

* Number of cases.
† Including the adenocarcinoma of the appendix.
‡ Percentage of positive tumor cells.

mucinous ovarian tumors. This antibody reacts with formalin-fixed, paraffin-embedded tissue samples.

Immunohistochemistry

Immunohistochemical staining of formalin-fixed, paraffin-embedded tissue was performed with the streptavidin-biotin-peroxidase-complex method. Consecutive 4-µm paraffin sections were rehydrated, endogenous peroxidase activity was blocked (by incubation in 1% H2O2), and sections were pretreated with 0.1% pronase (Sigma, St. Louis, MO; P5147, type XIV) in Tris-HCl buffer (5 minutes at 37°C). After washing in phosphate-buffered saline (PBS), the slides were incubated for 20 minutes in normal goat serum (diluted 1:5 in PBS) with the primary antibody (diluted 1:10 in PBS/bovine serum albumin [BSA] for IT-Ks 20.8, h100 for OV-TL 12/30, and 1:80 for CAM 5.2) for 60 minutes at room temperature. Sections stained with monoclonal antibody IT-Ks 20.8 were incubated overnight at 4°C. As a detection system, we used Multilink I (BioGenex) followed by streptavidine-peroxidase conjugate (BioGenex). As a chromogen, we used a diaminobenzadine solution containing 0.65% imidazol. The staining was intensified by incubation in 0.5% CuSO4 and in 0.9% NaCl for 5 minutes. Finally, the sections were weakly counterstained with Mayer’s hematoxylin, dehydrated, and mounted. As a negative control, sections were incubated with PBS instead of the primary antibody.

Microscopic Analysis

Immunostaining was semiquantitatively evaluated by estimating the number of stained cells in the following categories: no staining and only a few scattered positive cells (less than 5%) was considered to be negative (−), staining in 5% to 25% of cells (+), staining in 25% to 50% (++), staining in 50% to 75% (+++) and staining in 75% to 100% (++++). All negative controls were negative. Equivocal reactions were considered negative. All slides were scored by two of the authors (C.W., F.S.).

RESULTS

The reactivity patterns of the major groups of epithelial malignancies with the antibodies used are summarized in Table 1 and diagrammatically represented in Fig 1. Keratin 8 staining was found in all lesions, although the staining intensity and the number of stained cells varied somewhat.

Primary Ovarian Carcinomas and Mesotheliomas

Keratin 7 immunoreactivity was found in all primary ovarian adenocarcinomas (Fig 2A) and mesotheliomas rather extensively for the most part; however, two tumors were negative. Keratin 20 was not detected in the mesotheliomas (Fig 2B). Serous cystadenocarcinomas were negative (Fig 2C).

Mucinous cystadenocarcinomas showed variable staining, ranging from negative (three cases) to more than 50% of the tumor cells in one of the remaining

FIGURE 1. Diagram of immunohistochemical results with the primary ovarian carcinomas, mesotheliomas, and metastases to the ovary. Shaded areas indicate the percentage of patients with a moderate to strong immunoreactivity in more than 25% of the tumor cells.
two cases. In the moderately differentiated adenocarcinomas, keratin 20 staining was not detectable.

**Colonic Adenocarcinomas**

Keratin 7 staining was found in four of 16 colon carcinoma metastases, focally in one case and extensively in three cases. Twelve cases were entirely negative. In most cases staining in the primary tumor (Fig 3A) and in the metastases were comparable, although staining in a metastasis occasionally occurred with a negative primary and vice versa.

Most (14) of the 16 metastatic colon carcinomas showed extensive keratin 20 immunostaining (Fig 3B), which also was noted in the corresponding primaries. In two other metastases focal staining was found, ranging from a few scattered cells to approximately 50% of cells. Two primaries were negative for keratin 20. The adenocarcinoma of the appendix and its metastasis were negative for keratin 7 and positive for keratin 20 (Fig 3C).

**Gastric Adenocarcinomas**

All ovarian metastases showed variable numbers of keratin 7-positive cells, whereas in two primary gastric carcinomas keratin 7 was not detectable. Three metastatic gastric carcinomas were positive for keratin 20 and one was negative. Invariably, the primary carcinomas showed fewer keratin 20-positive cells.

**Infiltrating Duct/Carcinomas of the Breast**

All carcinoma metastases were keratin 7-positive, as were five of the six primary tumors. One primary was negative. In contrast, keratin 20 was negative in eight cases and in the other case scattered cells were positive. All primaries were negative.

**Endometrial Adenocarcinomas**

Extensive keratin 7 staining occurred in nine of the 10 metastatic endometrial adenocarcinomas; one metastasis was negative. Often the primary was positive but less so than the metastasis. In the negative metastasis the primary showed more than 5% positive cells. Keratin 20 was not observed in any of the primary tumors of the metastatic tumors.

**DISCUSSION**

In adenocarcinomas metastatic to the ovary the primary site of the tumor is frequently unknown. The choice of the treatment modality may depend on the origin of the primary. Histological criteria do not differentiate adequately between various primary tumors. Immunohistochemical staining of primary specific markers may be of help in this respect. Carcinoembryonic antigen staining has been shown to be useful for gastrointestinal tract primary tumors metastatic to the ovary. However, the specificity of this marker is not sufficiently high, as CEA also is detected in a considerable number of noncolonic carcinomas. Another approach is the use of ovarian tissue specific antibodies, such as OV-632 and OC-125, but these reagents also lack sufficient specificity. Other researchers have used an application of panels of immunoreagents, including keratin antibodies.

Keratins, a complex family of proteins composed of 20 members in humans, have a specific distribution pattern in normal epithelia, which is often retained in neoplasms derived from them. Based on this knowledge, we investigated whether or not staining for keratins 7 and 20 might help to identify the primary localization of carcinomas metastatic to the ovary. We chose keratin 7 because this antibody also stains adenocarcinomas of the ovary and keratin 20 because of its specific staining of adenocarcinomas derived from the gastrointestinal tract.

We could confirm that carcinomas usually display the same expression pattern in the primary lesions and their metastases, but occasionally discrepancies were noted. It is conceivable that technical factors, such as tissue processing, are responsible for these discrepancies. It is also possible that in some tumors the expression pattern is altered during tumor progression.

The main finding of our study is that the keratin 7-negative, keratin 20-positive colon carcinoma metastases are readily distinguishable from the keratin 7-posi-
tive, keratin 20-negative primary ovarian carcinomas. This observation applied to all ovarian carcinomas, except the mucinous type in which two of five carcinomas displayed some immunoreactivity. Colon carcinomas were not invariably keratin 7 positive, as was noted previously. However, keratin 20, is invariably expressed in all colon cancer metastases and in a large majority of cases is absent in primary ovarian carcinomas. Keratin 20 expression was occasionally observed in mucinous adenocarcinomas. We hypothesize that this uneven staining in ovarian mucinous tumors could be related to foci of intestinal metaplasia of the Müllerian epithelium lining the cysts of the tumor. In colon carcinomas the keratin 7 expression was occasionally observed, in contrast to the primary ovarian carcinomas in which keratin 7 expression was extensive. The difference between primary ovarian carcinomas and metastatic adenocarcinomas of the stomach was less distinct, as both keratins 7 and 20 were expressed.

All metastatic breast carcinomas were positive for keratin 7 and negative for keratin 20, which is clearly different from metastatic colonic and gastric carcinomas but not from primary ovarian carcinomas. In almost all endometrial adenocarcinoma metastases to the ovary, keratin 7 was positive and keratin 20 was negative, as is the case in primary ovarian carcinomas. This may be related to the origin of both tumors from Müllerian epithelium. In mesotheliomas the same pattern of keratin expression was observed. The lack of expression of keratin 20 in mesotheliomas distinguishes them from metastases of gastric and colon cancer.

In conclusion, we have found the differences in the expression of keratins 7 and 20 to be helpful in the distinction between primary ovarian carcinomas and metastatic adenocarcinomas of the gastrointestinal tract to the ovary. In particular, keratin 7-negative, keratin 20-positive colon cancer metastases are readily distinguishable from keratin 7-positive, keratin 20-negative ovarian carcinomas.

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REFERENCES


