Expression of Membrane-Type 1, 2, and 3 Matrix Metalloproteinases Messenger RNA in Ovarian Carcinoma Cells in Serous Effusions

Ben Davidson, MD,1 Iris Goldberg, PhD,5 Aasmund Berner, MD, PhD,1 Jahn M. Nesland, MD, PhD,1 Vered Givant-Horwitz, MSc,4 Magne Bryne, PhD,1,3 Bjørn Risberg, MD, PhD,1 Gunnar B. Kristensen, MD, PhD,2 Claes G. Tropé, MD, PhD,2 Juri Kopolovic, MD,5 and Reuven Reich, PhD4,6

Key Words: Membrane-type matrix metalloproteinases; Serous effusions; Ovarian carcinoma; Polymerase chain reaction; Messenger RNA in situ hybridization

Abstract

We studied the levels of matrix metalloproteinase (MMP)-2, membrane-type (MT)1-MMP, MT2-MMP, and MT3-MMP in 43 malignant pleural and peritoneal effusions using reverse transcription–polymerase chain reaction (RT-PCR) and cellular localization of MT1-MMP in 66 effusion specimens and 85 corresponding primary and metastatic tumors using messenger RNA (mRNA) in situ hybridization (ISH). In 43 effusions, MMP-2 mRNA was detected in 37, MT1-MMP in 25, and MT2-MMP in 32. Expression of MT1-MMP and MT2-MMP was found in 21 specimens; in 16 MT-MMP–positive specimens, mRNA for only 1 of 2 enzymes was expressed. MT3-MMP mRNA was not detected. High levels of MMP-2 mRNA were detected in 37, MT1-MMP in 25, and MT2-MMP in 32. Expression of MT1-MMP and MT2-MMP was found in 21 specimens; in 16 MT-MMP–positive specimens, mRNA for only 1 of 2 enzymes was expressed. MT3-MMP mRNA was not detected. High levels of MMP-2 mRNA were detected more often in effusions with high MT1-MMP and/or MT2-MMP mRNA expression. Using ISH, MT1-MMP mRNA was localized to cancer cells in 27 of 58 malignant effusions; focal signals were detected in mesothelial cells in 7 of 42. MT1-MMP was localized to tumor cells in 32 of 85 primary and metastatic solid lesions, and stromal cells expressed MT1-MMP in 3. Tumor cell MT1-MMP expression in effusion specimens did not differ from primary or metastatic lesions. MT-MMP expression in tumor cells in effusions showed no association with effusion site or tumor type using ISH and RT-PCR.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteolytic enzymes with a central role in extracellular matrix (ECM) remodeling in a variety of physiologic and pathologic conditions.1 In addition to their role in ECM degradation, MMPs seem to be involved in complex interactions with other cellular and extracellular proteins, thereby assuming an important role in normal cellular function, as well as in tumor invasion and metastasis.2 Membrane-type (MT) MMPs contain propeptide, catalytic, and hemopexin-like domains, as do other MMPs, but contain an additional transmembrane domain, as well as a cytoplasmic tail at the C-terminus.3,4 MT-MMPs are activated intracellularly by furin or related enzymes, as well as extracellularly by plasmin.3,4 Six MT-MMPs have been identified, with a different distribution in normal and neoplastic tissues.5-10 MT1-MMP, MT2-MMP, MT3-MMP, and MT5-MMP share about 50% structural homology, whereas MT4-MMP and MT6-MMP may represent a new subgroup of MT-MMPs, characterized by absence of the aforementioned cytoplasmic tail.10 MT-MMPs (with the exception of MT4-MMP) activate MMP-2 (gelatinase A), an enzyme that has a key role in local invasion and dissemination of a large variety of tumors.3,4,11-12 through a 2-step cleavage reaction, following the formation of a membrane complex with MMP-2 and tissue inhibitor of metalloproteinase-2.12 The efficiency in mediating this reaction seems to be highest for MT1-MMP, followed by MT3-MMP, and is lower for MT2-MMP.3 MT1-MMP also can activate procollagenase-3 (MMP-13), while recombinant forms of all 3 enzymes can cleave a large number of ECM proteins.3

Carcinoma of the ovary continues to be the major cause of mortality due to gynecologic cancer in the Western countries, with a 5-year survival rate of 35%.13 As manifested by their frequent diagnosis at an advanced stage, these tumors...
tend to spread widely within the peritoneal cavity, often resulting in the accumulation of ascitic fluid, and occasionally to distant organs. The presence of MMPs in ovarian neoplasia and their contribution to the invasive phenotype of these tumors has been demonstrated in in vitro studies, as well as in studies of clinical specimens (reviewed by Stack et al). The presence of MT1-MMP in ovarian carcinoma cells, as well as in peritumoral stromal cells was demonstrated in 2 studies, using immunohistochemistry and messenger RNA (mRNA) in situ hybridization. MMP-2 was localized to the same cell populations in both studies. However, the relative expression of MT1-MMP in effusion specimens in comparison with primary and metastatic tumors has not been studied, nor has the expression of MT2-MMP and MT3-MMP in ovarian carcinoma. Thus, the object of the present study was to study mRNA expression of MT1-MMP, MT2-MMP, and MT3-MMP in serous effusions of patients diagnosed with ovarian carcinoma. In addition, we attempted to locate the cellular source of MT1-MMP and to compare the expression of this enzyme in carcinoma cells in pleural and peritoneal effusions with that of the corresponding primary tumors and metastatic lesions.

Materials and Methods

Effusion Specimen Protocol

Fresh, nonfixed peritoneal and pleural effusions (volume range, 20-2,000 mL) were submitted to the Division of Cytopathology, Department of Pathology, the Norwegian Radium Hospital, Oslo, during the period January 1998 through March 1999. Effusion specimens, as well as relevant clinical data, were obtained from the Department of Gynecologic Oncology, Norwegian Radium Hospital. Specimens were centrifuged in an Eppendorf 5810 centrifuge (Eppendorf, Hamburg, Germany) for 10 minutes at 2,000 rpm. The resulting pellet was used for the preparation of 4 cytologic smears (2 alcohol-fixed, Papanicolaou-stained and 2 air-dried, rapid Romanowsky–stained smears) and a formalin-fixed, paraffin-embedded cell block. The remaining material was frozen in RPMI and dimethyl sulfoxide medium. Cell blocks were prepared using the Shandon Lipshaw Cytoblock kit (Shandon, Pittsburgh, PA).

Morphologic Examination

Cytologic smears from all samples were evaluated by a senior cytopathologist (A.B. or B.R.) and reported as positive, suggestive of carcinoma, or negative for malignant cells. Owing to the possibility of sampling a different population in cell blocks, sections from all cell blocks were evaluated in a double-blind manner and graded as described by 2 senior cytopathologists (A.B. and B.R.). The diagnostic criteria used were consistent with established guidelines. Cell block sections subsequently were stained using monoclonal antibodies directed against epithelial markers to confirm the morphologic diagnosis, as previously described.

Reverse Transcription–Polymerase Chain Reaction

We analyzed 43 malignant effusions for the presence of MMP-2, MT1-MMP, MT2-MMP, and MT3-MMP. Distribution of the studied specimens according to histologic type and location is given in Table 1. All specimens contained a distinct population of carcinoma cells. Specimens were defined as pure when the tumor cell population consisted of more than 80% of the total cell population (23 samples). Mixed effusions (20 samples) consisted of an inflammatory or mesothelial component exceeding 20% of the total cell population.

Polymerase chain reaction (PCR) analysis was performed on reversely transcribed mRNA using the primers listed in Table 2. Products were separated on 1% agarose gels. The HT-1080 fibrosarcoma cell line was used as the external control.

Table 1

<table>
<thead>
<tr>
<th>Histologic Type</th>
<th>Peritoneal</th>
<th>Pleural</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>24</td>
<td>12</td>
<td>36</td>
</tr>
<tr>
<td>Mixed carcinomas</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Germ cell*</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Primary peritoneal carcinoma</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>13</td>
<td>43</td>
</tr>
</tbody>
</table>

* Malignant endodermal sinus tumor.

Table 2

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Primer</th>
<th>Primer Sequence</th>
<th>Product Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2</td>
<td>Sense</td>
<td>5’CACCTACACCAAGAACATGCC 327</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5’AAACACGGCTTCTCCTCCTG</td>
<td></td>
</tr>
<tr>
<td>MT1-MMP</td>
<td>Sense</td>
<td>5’CCATGCTGACTCCGATGTA 532</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5’TCCATCAGATCGTGGTAAAT</td>
<td></td>
</tr>
<tr>
<td>MT2-MMP</td>
<td>Sense</td>
<td>5’CGCCGAGCCGCGCCATGTC 172</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5’CTTCCGACATAGCCCCGAAAC</td>
<td></td>
</tr>
<tr>
<td>MT3-MMP</td>
<td>Sense</td>
<td>5’TTCGACTAAGCCCAAGATGTC 173</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5’TCCATGCTGACCTCCGAGG</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>Sense</td>
<td>5’TTCCGACCCATCCACAAAA 314</td>
<td></td>
</tr>
<tr>
<td>(control)</td>
<td>Antisense</td>
<td>5’GCAATACAGGCCACCCCTCAT</td>
<td></td>
</tr>
</tbody>
</table>

bp, base pairs; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MMP, matrix metalloproteinase; MT, membrane-type.
control in all reactions. Band size was evaluated by using the MMP/glyceraldehyde-3-phosphate dehydrogenase ratio.

**mRNA In Situ Hybridization**

**Effusion Specimens**

The studied material consisted of 58 malignant effusion specimens, obtained preoperatively, intraoperatively, or at disease recurrence, from 50 patients diagnosed with ovarian carcinoma and 3 patients diagnosed with primary peritoneal carcinoma. These consisted of 44 peritoneal and 14 pleural effusions. Eight reactive effusions, obtained from patients with various malignant neoplasms or a clinical suspicion of malignant neoplasm, also were studied (total, 66 effusion specimens). The distribution of the studied malignant effusions according to histologic type and site is given in **Table 3**.

**Tumor Specimens**

Eighty-five archival surgical specimens, consisting of primary tumors (n = 36) and metastatic lesions (n = 49) from

The aforementioned patients, also were studied. Formalin-fixed, paraffin-embedded tissue blocks were obtained from archival material in the Department of Pathology, the Norwegian Radium Hospital, or from other hospitals in Norway. All tissue specimens underwent microscopic confirmation of diagnosis, tumor type, and histologic grade, according to established criteria.20 Tumor distribution according to histologic type and biopsy site is shown in **Table 4**.

Tissue sections (4-µm-thick) of formalin-fixed, paraffin-embedded specimens were mounted on silane-coated slides. Sectioning was performed in ribonuclease-free water. Slides were dewaxed and rehydrated using xylenes (twice, 10 minutes each time) and isopropyl alcohol (5 minutes). Hybridization using the MT1-MMP probe was performed as previously described.21 Hybridization results were interpreted as absent, weak, or intense. Strong, dark blue staining was interpreted as intense. Labeling of fewer than 20% of the cells was defined as focal, while labeling of 20% or more was interpreted as diffuse. A minimum of 300 cells, when present, were evaluated.

**Statistical Analysis**

Statistical analysis of MT1-MMP in situ hybridization results consisted of the following comparative analyses: (1) in situ hybridization results in malignant epithelial vs reactive mesothelial cells in effusion specimens; (2) in situ hybridization results in tumor vs stromal cells in tissue specimens; (3) in situ hybridization results in tumor cells in effusion specimens vs their respective primary tumors and solid metastases; (4) in situ hybridization results in tumor cells originating from peritoneal effusions vs tumor cells in pleural effusions; and (5) in situ hybridization results in serous carcinoma cells and cells originating from other tumor types in effusion specimens.

Analyses were undertaken applying the SPSS-PC package (version 8.0, SPSS, Chicago, IL). Probability of less than .05 was considered statistically significant. Comparative analyses of malignant cytologic specimens, primary tumors, and metastatic lesions were performed using the Wilcoxon

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**Table 3**

Distribution of 58 Malignant Serous Effusions Studied Using In Situ Hybridization According to Histologic Type and Location

<table>
<thead>
<tr>
<th>Histologic Type</th>
<th>Peritoneal</th>
<th>Pleural</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>32</td>
<td>11</td>
<td>43</td>
</tr>
<tr>
<td>Primary peritoneal carcinoma</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Mucinous</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clear cell</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mixed carcinomas</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Germ cell/sex cord–stromal*</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Borderline†</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>44</strong></td>
<td><strong>14</strong></td>
<td><strong>58</strong></td>
</tr>
</tbody>
</table>

* Includes 1 malignant endodermal sinus tumor and 1 malignant granulosa cell tumor.
† Unequivocally malignant cells of serous type were detected in the pleural effusion specimen, inconsistent with the primary tumor diagnosis of borderline mucinous carcinoma. The nature of the cells in the borderline tumor in question was confirmed using a battery of epithelial markers (Ber-EP4, B72.3, E-cadherin, and the 5 carbohydrate antigens studied by Davidson et al37).

**Table 4**

Distribution of 36 Primary Tumors and 49 Metastatic Lesions Studied Using In Situ Hybridization According to Histologic Type and Site

<table>
<thead>
<tr>
<th>Histologic Type</th>
<th>Ovary</th>
<th>Omentum</th>
<th>Intestine</th>
<th>Peritoneum</th>
<th>Lymph Node</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>30</td>
<td>21</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td><strong>70</strong></td>
</tr>
<tr>
<td>Mucinous</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td><strong>2</strong></td>
</tr>
<tr>
<td>Combined</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td><strong>4</strong></td>
</tr>
<tr>
<td>Germ cell</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td><strong>1</strong></td>
</tr>
<tr>
<td>Borderline*</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td><strong>1</strong></td>
</tr>
<tr>
<td>Primary peritoneal carcinoma*</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td><strong>7</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>36</strong></td>
<td><strong>24</strong></td>
<td><strong>8</strong></td>
<td><strong>5</strong></td>
<td><strong>4</strong></td>
<td><strong>8</strong></td>
<td><strong>85</strong></td>
</tr>
</tbody>
</table>

* A total of 7 biopsy specimens for 3 patients. Ovarian biopsy specimens are from the surface of the ovary, as mandated by the diagnostic criteria for this tumor.
signed rank test. In cases for which more than one metastatic lesion was available, the lesion showing the most intense staining was included in the statistical evaluation.

**Results**

**Reverse Transcription–Polymerase Chain Reaction**

MMP-2 mRNA was detected in 37 of 43 effusions. MT1-MMP was detected in 25 of 43, while MT2-MMP was detected in 32 of 42 specimens using reverse transcription (RT) PCR [Image 1]. In 1 specimen, a failed reaction for MT2-MMP was seen in 2 experiments. MT2-MMP mRNA expression was detected more often in effusions consisting predominantly of carcinoma cells (20/22 [91%]) than in those with a mixed cellular population (12/20 [60%]), while MMP-2 and MT1-MMP were detected equally in both effusion types [Table 5]. Furthermore, strong expression of MT2-MMP was seen in 16 (73%) of 22 pure effusions, but only in 5 (25%) of 20 mixed effusions. Dual expression of MT1-MMP and MT2-MMP was found in 21 specimens, while in 16 MT-MMP–positive specimens, mRNA for only 1 of the 2 enzymes was expressed. Six specimens were negative for both MT1-MMP and MT2-MMP mRNA. Strong expression of both MT1-MMP and MT2-MMP mRNA was detected in 8 specimens, 6 of which were classified as pure. MT3-MMP mRNA was not detected in any of the studied effusions. High levels of MMP-2 mRNA were detected more often in effusions with high MT1-MMP and/or MT2-MMP mRNA expression. Specifically, 14 (74%) of 19 specimens with negative or absent MMP-2 mRNA expression were negative for MT1-MMP. In addition, 12 cases showed strong expression of both MMP-2 and MT2-MMP.

Expression of MMP-2 was detected in 13 (100%) of 13 pleural effusions and 24 (80%) of 30 peritoneal effusions. Expression of MT1-MMP mRNA (positive in 8/13 pleural effusions and 17/30 peritoneal effusions) and MT2-MMP mRNA (positive in 10/13 pleural effusions and 22/29 peritoneal effusions) was comparable in effusions from both sites. The mRNA for none of the 3 enzymes showed preferential expression in a given tumor type (serous vs nonserous).

**mRNA In Situ Hybridization**

MT1-MMP levels in cancer cells were evaluated in 58 malignant effusion specimens. Substantial populations of reactive mesothelial cells were found in 34 (59%) of 58 specimens, as well as in all 8 reactive effusions (total = 42). MT1-MMP mRNA was localized to cancer cells in 27 (47%) of 58 malignant effusions [Image 2A]. Diffuse signals (more than 20% of the cells) were detected in tumor cells in 7 of...
the 27 specimens, while focal signals were seen in the remaining 20. Diffuse and focal signals were detected in mesothelial cells, respectively, in 2 and 5 (total, 17%) of 42 effusions [Table 6]. The difference in expression between the two cell populations reached statistical significance ($P = 0.009$). As for RT-PCR, MT1-MMP expression in tumor cells did not relate to effusion site or tumor type.

In situ hybridization results for MT1-MMP in effusions showed good association with RT-PCR results [Table 7]. MT1-MMP was localized to tumor cells in 32 (38%) of 85 primary ($n = 15$) and metastatic ($n = 17$) solid lesions [Image 2B]. Stromal cells expressed MT1-MMP in 3 (4%) of 85 specimens (Table 6). Carcinoma cells in 6 tumors showed an intense signal, but the signal was not observed in stromal cells. The differences in MT1-MMP expression between tumor and stromal cells were statistically significant in primary ($P = .001$) and metastatic ($P = .002$) lesions. Comparison of tumor cell expression in effusions with surgical specimens of both primary and metastatic lesions did not reveal significant differences ($P > .05$).

**Discussion**

The role of MT-MMPs in the activation of MMPs has been shown in vitro and in vivo, best documented for MT1-MMP–mediated activation of MMP-2.$^{22}$ MT1-MMP was localized to tumor and/or stromal cells in various malignant tumors, including carcinomas of the ovary,$^{16,17}$ pancreas,$^{23}$ bladder,$^{24}$ head and neck,$^{25}$ liver,$^{26}$ uterine cervix,$^{21,27}$ stomach,$^{28}$ colon,$^{25,29}$ and lung.$^{29}$ However, fewer studies investigated the expression of other MT-MMPs in epithelial tumors.$^{30-32}$ These studies evaluated thyroid,$^{30}$ breast,$^{31}$ and urinary bladder$^{32}$ carcinomas. While MT1-MMP and MT2-MMP were detected in carcinoma cells of all the aforementioned tumor types, MT3-MMP was undetectable in thyroid and breast carcinomas and was faintly detectable in benign bladder mucosa and bladder carcinomas.$^{30-32}$

The present study analyzed mRNA expression of MMP-2, MT1-MMP, MT2-MMP, and MT3-MMP in serous effusions of patients with ovarian carcinoma using RT-PCR, with subsequent evaluation of MT1-MMP expression in effusions and primary and metastatic lesions using mRNA in situ hybridization.

Our results agree with the aforementioned reports, as MT3-MMP mRNA was not detected in any of our samples.
Unlike the findings for thyroid, breast, and bladder carcinomas, the expression of MT2-MMP mRNA seems to be more frequent than that of MT1-MMP in ovarian carcinoma cells in effusions (32/42 vs 25/43 malignant effusions). This observation was further underscored when the analysis was limited to specimens with a predominant carcinoma cell population (20/22 vs 14/23). One may thus hypothesize that MT2-MMP, rather than MT1-MMP, is the central MT-MMP in ovarian carcinoma cells in effusions. However, although MT1-MMP apparently is detected less often, its association with MMP-2 mRNA expression and in situ hybridization results support a role for MT1-MMP, in addition to MT2-MMP, in these cells.

MMP-2 was detected in the majority of specimens in the present study. This finding is in agreement with the report of Fishman and coworkers, pointing to the central role of MMP-2 in ovarian carcinoma, as well as with recent findings (Davidson, Reich, Berner, et al, unpublished data, 2000) in a study using immunohistochemistry and mRNA in situ hybridization that demonstrated up-regulation of MMP-2 in ovarian carcinoma cells on protein and mRNA levels compared with primary ovarian carcinomas, with a concomitant decrease in MMP-9 protein levels. MMP-2 was colocalized to carcinoma and mesothelial cells. However, expression in the tumor cell population predominated (Davidson, Reich, Berner, et al, unpublished data, 2000). In the present study, 37 of 43 specimens, both pure and mixed effusions, expressed MMP-2. Production of this enzyme by both cell populations thus may enhance ECM degradation of peritoneal tissues, possibly facilitating cancer cell invasion of abdominal structures, a characteristic of tumor spread in ovarian carcinoma. The association of MT1-MMP and MT2-MMP mRNA with high levels of MMP-2 mRNA suggests that both enzymes may have a role in the activation of MMP-2 in serous effusions.

Limited data are available about the relative contribution of carcinoma cells and peritumoral stromal cells or mesothelial cells to MT1-MMP production in ovarian carcinoma. MT1-MMP mRNA has been localized to stromal cells and tumor cells in a study that included 19 invasive carcinomas, most often to stromal cells (18/19 vs 8/19 cases). In an additional study of various carcinomas, including 4 of ovarian origin, MT1-MMP protein expression was detected in carcinoma and stromal cells in all cases. MT1-MMP has been localized immunohistochemically to carcinoma cells in short-term cultures of ascitic specimens. However, mesothelial cell expression has not been studied previously. This issue became more intriguing in view of the relatively high expression of MT1-MMP in malignant effusions with substantial reactive (inflammatory or mesothelial) populations. We detected a consistently predominant MT1-MMP mRNA expression in carcinoma cells and a negligible one in stromal cells. This finding was valid for primary and metastatic tumors and was reproduced in effusion specimens when carcinoma cells were compared with reactive mesothelial cells. Inflammatory cells in mixed effusions were similarly negative. Concomitantly, carcinoma cells in primary tumors and metastatic lesions and effusions showed a comparable profile. The finding of MT1-MMP in carcinoma cells is in agreement with the findings of Fishman et al using short-term cultures of ascites specimens. The discrepancy between our results and those of Afzal and coworkers can possibly result from the use of different sensitivity of the detection systems. In addition, different tumors were studied in the 2 studies. Eight serous and 11 nonserous tumors were included in the study by Afzal and coworkers, while 47 of 58 effusions and 70 of 85 solid lesions of the serous type were included in our study.

The detection of metastatic ovarian carcinoma cells in pleural effusion specimens defines a stage IV disease irrespective of the presence or absence of solid metastases in distant organs and is associated with an unfavorable outcome. In contrast, the dissemination of ovarian carcinoma cells within the peritoneal cavity is postulated to be initiated by direct shedding from the surface of the ovary and may occur in localized tumors, ie, FIGO (International Federation of Gynecology and Obstetrics) stage Ic. This division into 2 apparently nonoverlapping pathogenetic mechanisms is not supported by the reports associating the presence of large-volume ascites and positive peritoneal cytology with poor outcome in ovarian carcinoma, unless one assumes that cells with markedly variable metastatic properties are found in peritoneal effusions. Although MMP-2 mRNA expression was detected more often in pleural effusions, the resemblance to the MT-MMP expression profile, using RT-PCR and in situ hybridization, provides further evidence that ovarian carcinoma cells in peritoneal and pleural fluids do not differ significantly from each other in their protease phenotype. These results are also in agreement with recent reports, in which both carbohydrate antigen expression and E-cadherin complex expression were similar for metastatic carcinoma cells in both compartments.
Considered together, it seems that further studies are necessary to determine the genotypic and phenotypic alterations, if any, that are involved in the transition from stage III to stage IV disease in ovarian carcinomas.

Expression of MMP-2, MT1-MMP, MT2-MMP, and MT3-MMP mRNA was studied in serous effusions from patients diagnosed with ovarian carcinoma, with further characterization of MT1-MMP expression in corresponding primary and metastatic lesions. The data presented suggest a positive correlation between MMP-2, MT1-MMP, and MT2-MMP mRNA expression and, possibly, a role in ovarian carcinoma pathogenesis, mainly through tumor cell production of these enzymes.

From the Departments of 1 Pathology and 2 Gynecologic Oncology, the Norwegian Radium Hospital, Oslo; 3 Oral Biology, University of Oslo, Oslo, Norway; 4 Pharmacology, Faculty of Medicine, and 5 David R. Bloom Center for Pharmacy, Hebrew University, Jerusalem, Israel; and 6 Pathology, Sheba Medical Center, Tel-Hashomer, Sackler School of Medicine, Tel-Aviv University, Israel.

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Address reprint requests to Dr Davidson: Dept of Pathology, the Norwegian Radium Hospital, Montebello N-0310, Oslo, Norway.

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References


