Diffuse Mesothelin Expression Correlates with Prolonged Patient Survival in Ovarian Serous Carcinoma

M. Jim Yen,1 Chih-Yi Hsu,2 Tsui-Lien Mao,1 T-C. Wu,1 Richard Roden,1 Tian-Li Wang,1 and Ie-Ming Shih1

Abstract

Purpose: Mesothelin is an emerging marker for cancer diagnosis and target-based therapy, yet relatively little is known about the clinical significance of mesothelin expression in tumors. In this study, we correlate mesothelin immunoreactivity to clinicopathologic features in ovarian serous carcinoma.

Experimental Design: Mesothelin expression levels were compared among 81 publicly available serial analysis of gene expression (SAGE) libraries of various carcinoma and normal tissue types. Immunohistochemistry using a well-characterized mesothelin monoclonal antibody (5B2) was done to evaluate mesothelin expression in 167 high-grade and 31 low-grade ovarian serous carcinomas. Immunohistochemistry staining scores were correlated with patient survival, tumor site, tumor grade, in vitro drug resistance, and differentiation status of tumor cells.

Results: SAGE analysis showed that mesothelin was overexpressed in 50% of ovarian and pancreatic carcinomas but rarely in other cancer types, including liver, colon, kidney, prostate, and breast. Mesothelin immunoreactivity (55% of tumor cells) was present in 55% of ovarian serous carcinomas with no difference in expression between high-grade and low-grade serous tumors ($P = 0.82$). Based on Kaplan-Meier analysis, we found that a diffuse mesothelin staining (>50% of tumor cells) in primary high-grade ovarian carcinomas correlated significantly with prolonged survival in patients who had advanced-stage disease and had received optimal debulking surgery followed by chemotherapy ($P = 0.023$). Mesothelin expression did not correlate significantly with patient age, tumor site, in vitro drug resistance, or tumor differentiation status ($P > 0.10$).

Conclusion: Our results provided new evidence that mesothelin expression is associated with prolonged survival in patients with high-grade ovarian serous carcinoma.

Mesothelin is a glycoprotein that has been reported as a tumor-associated marker in several types of human cancer, including nonmucinous ovarian carcinomas and adenocarcinomas arising from the pancreaticobiliary tract, endometrium, and lung (1). Mesothelin was highly expressed in these tumors but not in a wide variety of normal tissues. The mesothelin gene was first cloned by Chang and Pastan (2), and it encodes a precursor protein that is processed to yield the 40-kDa mesothelin protein and a 31-kDa soluble fragment named megakaryocyte-potentiating factor. Mesothelin is attached to the cell membrane by a glycosylphosphatidylinositol linkage, and it has recently been shown to bind to CA125 (or MUC16), which is expressed in many ovarian serous carcinomas. The interaction between mesothelin and CA125 can mediate heterotypic cell adhesion as anti-mesothelin antibody blocks binding of CA125-expressing OVCAR3 cells to another cell line expressing mesothelin (3). Although the biological functions of mesothelin remain largely unknown as mesothelin knockout mice did not display detectable phenotypes (4), there is evidence that mesothelin may have potential as a new cancer diagnostic marker and a novel molecular target for gene therapy (5). For example, mesothelin alone or in combination with other gene products can provide a valuable marker for the differential diagnoses of cancer types (1). It has also been shown that a soluble form of the mesothelin (megakaryocyte-potentiating factor) family can provide useful new marker(s) for the diagnosis of ovarian carcinoma as well as monitoring its response to therapy (6). Mesothelin has been used to mediate the targeting of adenoviral vectors and an antibody-based gene therapy in mesothelin-expressing tumors (7–9).

Ovarian carcinoma is the leading cause of cancer mortality in patients who suffer from gynecologic neoplasms. Among the different types of ovarian cancer, serous carcinoma is the most
common and lethal type. Although mesothelin has been documented as a tumor-associated marker in ovarian serous carcinoma, a larger-scale analysis focusing on the correlation of mesothelin expression and clinicopathologic variables has never been done. Such a correlation study is important as it can provide a prognostic marker for predicting clinical outcome in cancer patients and shed new light on the function of mesothelin in ovarian cancer. The purpose of this study is to use immunohistochemistry to assess the clinical significance of mesothelin expression in a large series of serous carcinomas that are well annotated with clinicopathologic information.

Materials and Methods

Mesothelin expression analysis using the serial analysis of gene expression database. Mesothelin expression levels were compared among 81 publicly available serial analysis of gene expression (SAGE) libraries (http://cgap.nci.nih.gov/SAGE; refs. 10, 11) for carcinomas and normal tissues of ovary, pancreas, liver, colon, kidney, prostate, and breast. The selection of libraries used for analysis was based on their availability in the SAGE database. Mesothelin tag counts for each library were retrieved by filtering for tag sequences that matched uniquely to mesothelin according to the April 15, 2005 SAGEMap available on the public National Center for Biotechnology Information FTP site (ftp://ftp.ncbi.nlm.nih.gov/pub/sage/map/Hs/NlaIII/SAGEmap_tag_ud-rel.zip). Using a minimum tag count setting of > 1, these mesothelin tags were tallied and normalized per 100,000 total tags for each SAGE library.

Tissue samples. Formalin-fixed, paraffin-embedded tissue samples of 198 ovarian serous tumors, including 167 serous high-grade carcinomas and 31 low-grade serous carcinomas, were retrieved from the archival file of the Department of Pathology at the Johns Hopkins Hospital. The acquisition of paraffin tissues was approved by the Johns Hopkins Institutional Review Board. The paraffin tissues were organized into tissue microarrays, which were made by removing three 1.5-mm-diameter cores of tumor from each block. The selection of areas for core was made by two pathologists (C.-Y.H. and I-M.S.) based on review of the corresponding H&E slides. To evaluate whether expression of mesothelin was a prognostic marker in patients with ovarian serous carcinoma, we analyzed 105 advanced-stage, high-grade serous carcinomas, of which 92 were International Federation of Gynecology and Obstetrics stage III and 13 were International Federation of Gynecology and Obstetrics stage IV. High-grade serous carcinomas have been conventionally classified into “moderately” and “poorly” differentiated carcinomas based on whether they are predominantly papillary or solid, respectively.

Immunohistochemistry. Expression of mesothelin was assessed by immunohistochemistry using a mouse monoclonal antibody (SB2) that specifically reacts with the mesothelin (Promega, Madison, WI). This antibody has been well characterized and been used in immunohistochemistry to assess its diagnostic potential (1, 12–14).

Immunohistochemistry was done on paraffin sections at a dilution of 1:500 followed by the EnVision+System using the peroxidase method (Dako, Carpenteria, CA). The percentage of positive cells was estimated by randomly counting ~500 tumor cells from three different high-power fields (>40) within one specimen. For the negative control, an isotype (IgG1)-matched antibody, MN-4, was used in parallel (13). A positive reaction was defined as discrete localization of the brown chromagen on the cell surface. Because mesothelin immunoreactivity, if present, was always strong, we used the percentage score in this study. Cases with ~5% of tumor cells positively reacting were scored as negligible. We used the following scoring system for mesothelin immunoreactivity: 0, <5%; 1+, 5% to 50%; 2+, 51% to 75%; 3+, 76% to 95%; 4+, >95%. Scoring was done by two independent investigators who concurred on the results.

A third pathologist scored the cases with discrepant scores, and the tumor was assigned based on the consensus of two.

Reverse transcriptase-PCR. To validate the immunohistochemistry results, we did reverse transcriptase-PCR to semiquantitatively measure the levels of mesothelin transcript copy number in eight representative cases with either diffuse or undetectable mesothelin immunoreactivity. mRNA was isolated from frozen tumor tissues, and cDNA was prepared as previously described (16). The sequences of forward and reverse primers were 5′-TACGTCAGAGAATGTGAGCA-3′ and 5′-TGAAGCAGTGAGGAGGCGGTG-3′ that amplified 238 bp of mesothelin coding sequence. PCR was done as detailed in a previous report (16), and the PCR product was visualized by staining with ethidium bromide on 1.5% agarose gels.

In vitro drug resistance assay. Drug sensitivity was assessed by an agar-based cell culture system with radioactive thymidine incorporation as the end point (Oncotech, Tustin, CA). There were 61 cases for which the results of this assay were available to analyze. The protocol of the assay has been detailed at http://www.oncotech.com/innovation/PublicationDetails.asp?id=28. Briefly, fresh tumor specimens immediately removed at the time of surgery were placed into the RPMI (Life Technologies, Gaithersburg, MD) and shipped to Oncotech for analysis. The tumor tissue was minced and enzymatically digested to disaggregate the tumor cell, which were then plated in soft agar. Tumor cells were then exposed to antineoplastic agents at a concentration of a maximum tolerated dose for 5 days in vitro. Tritiated thymidine was introduced during the last 2 days of culture as a measure of cell proliferation, and the drug-treated cells were compared with the untreated control. Assay results were divided into three categories: extreme, intermediate, and low drug resistance using the criteria described on the company’s web site. Extreme resistance was interpreted as drug resistance, whereas intermediate and low drug resistance was interpreted as drug sensitive in this study as in previous reports (17, 18).

Statistical analysis and clinical correlation. The preexisting clinical information including clinical stage, treatment history, surgical findings during debulking, recurrence status, and survivorship was collected from clinical note, operation note, and discharge summary that were deposited in a centralized database. All the patients’ identifiers were decoded before data analysis. We defined optimal debulking as the residual tumor nodules with sizes <1 cm after surgery. Survival curves were established using the Kaplan-Meier method, and their differences were analyzed using the log-rank test. Overall survival time was defined as the number of months between diagnosis and death or between diagnosis and most recent follow-up. All patients with advanced high-grade serous carcinomas were treated with combined chemotherapy, including paclitaxel and cisplatin, after optimal debulking surgery. Spearman correlation was used to determine the association between mesothelin expression and differentiation status (moderate or poor). Statistical association between mesothelin expression and patient age, tumor grade, tumor site (primary or recurrent), and in vitro drug resistance status was determined using Fisher’s exact test.

Results

To comprehensively analyze mesothelin expression levels in common types of carcinomas, we did a sequence tag analysis based on the SAGE libraries that were available in the public database (http://cgap.nci.nih.gov/SAGE). SAGE provides an unbiased genome-wide analysis of all transcripts and has been used extensively in characterizing gene expression (19, 20). As shown in Fig. 1, half of the ovarian and pancreatic carcinoma libraries showed up-regulation of mesothelin (increased mesothelin-matched tag sequences) compared with their normal counterparts. Specifically, overexpression of mesothelin (normalized tag count > 5) was found in 5 of 10 ovarian carcinoma libraries and in three of six pancreatic carcinoma libraries.
By contrast, none of the libraries representing ovarian surface epithelial cells and pancreatic ductal epithelium had mesothelin-matched tag counts of >5. A wide variety of normal tissues also did not express mesothelin-matched tags, indicating a high specificity of mesothelin expression in carcinoma tissues.

Because ovarian carcinoma is one of the two tumor types that most frequently overexpress mesothelin based on SAGE analysis, in this study, we focused on analyzing the relationship between mesothelin expression and clinicopathologic features of ovarian serous carcinomas. Among ovarian serous carcinoma tissues, mesothelin immunoreactivity was heterogeneous with immunoreactive cells ranging from 0% to 99% (Fig. 2). Immunoreactivity of mesothelin was almost exclusively detected on the cell surface. In contrast, mesothelin immunoreactivity was absent from all six normal ovaries containing ovarian surface epithelium. Using an arbitrary cutoff of 5%, mesothelin expression (>5% of tumor cells) was found in 55% of ovarian serous carcinomas (Table 1). To validate immunohistochemistry results, we did reverse transcriptase-PCR in eight representative cases, in which the frozen samples were available and showed that PCR product of mesothelin was robust in representative tumors with diffuse mesothelin immunoreactivity (3+ and 4+) but was barely detectable in tumors with score of 0.

Next, we examined whether mesothelin expression correlated with clinicopathologic features. First, we determined if mesothelin expression was related to different types of ovarian serous carcinomas, which comprises mainly high-grade and low-grade serous carcinomas. Both types of carcinomas are characterized by distinctive clinical, morphologic, and molecular genetic features (21, 22). The frequency of mesothelin expression (>5% of tumor cells) in high-grade serous carcinomas (55%; 95% confidence interval, 47-63%) was similar to low-grade serous carcinomas (58%; 95% confidence interval, 41-75%) without statistical significance (P = 0.82). Second, we evaluated whether expression of mesothelin was related to a patient's overall survival in high-grade serous carcinoma. Low-grade serous carcinomas were less common, and the case number was not sufficient for statistical analysis of cumulative survival rates in this study. In high-grade serous carcinomas, we focused on

Fig. 1. Mesothelin expression profiles based on SAGE analysis. Tumor (T) and normal (N) tissues or cell lines from ovary, pancreas, liver, colon, kidney, prostate, and breast were compared. Tag counts per 100,000 total tags are normalized (y-axis). Ovarian and pancreatic carcinomas are the two tumor types that overexpress mesothelin. Mesothelin is not expressed in any normal tissue types.
advanced-stage diseases (92 International Federation of Gynecology and Obstetrics stage III and 13 International Federation of Gynecology and Obstetrics stage IV cases), because the vast majority of patients with high-grade serous carcinomas were diagnosed at these two stages. Among 167 high-grade cases, there were 105 patients who received optimal debulking surgery followed by paclitaxel/cisplatin–based combined chemotherapy, and their long-term follow-up information was available. Therefore, these patients were analyzed for correlation between mesothelin expression and overall survival. We separated high-grade cases into two groups: group A with diffuse immunoreactivity (immunostaining score: 2-4) and group B with negligible or focal immunoreactivity (immunostaining score: 0-1). Using this cutoff, we were able to show that patients in group A (n = 29) had a significantly better overall survival than patients in group B (n = 76; Fig. 3). The median survival was 60 months among group A patients and 34 months among group B patients. There was a statistically significant difference in overall survival between both groups (P = 0.023, log-rank test). The statistical significance in overall survival even improved if only stage III patients were analyzed (P = 0.011). Survival correlation was not done in stage IV patients, because there was a limited case number (n = 13) for statistical analysis. Patients in both groups had a similar age distribution (63.2 ± 12.3 versus 62.7 ± 10.9 years) and received optimal tumor debulking surgery followed by paclitaxel/cisplatin–based chemotherapy. We have also attempted to use different cutoffs to separate patients into groups (immunostaining score: 0 versus 1-4) but were unable to show any statistical significance (P = 0.28, log-rank test).

To further explore possible mechanisms for how mesothelin expression is related to favorable overall survival in patients with high-grade serous carcinomas, we correlated mesothelin expression with other clinical and histopathologic features. Expression of mesothelin did not correlate with the status of in vitro drug resistance for paclitaxel (P = 0.106) or cisplatin (P = 1), which are commonly used in treating ovarian cancer patients. We found that mesothelin was more frequently expressed in primary high-grade serous carcinomas compared with recurrent counterparts, but the difference did not reach a statistical significance (P = 0.178). High-grade serous carcinomas have been conventionally classified into moderately and poorly differentiated carcinomas based on whether they are predominantly papillary or solid, respectively. By classifying high-grade carcinomas into moderately and poorly differentiated carcinomas, mesothelin expression did not correlate (P = 0.35) with either differentiation status category.

**Discussion**

Mesothelin is an emerging marker for diagnosis and target-based therapy in cancer. Ovarian cancer is one of the most common types of carcinoma that overexpresses mesothelin. In this study, we first compared the available SAGE data for mesothelin expression and then analyzed mesothelin immunoreactivity in a large number of ovarian serous carcinomas and correlated mesothelin expression to several clinicopathologic features. Our SAGE analysis confirmed previous reports (1, 2, 23–25) that mesothelin was expressed in the majority of ovarian carcinomas but not in a wide variety of normal adult tissues, suggesting that mesothelin is an ideal marker for cancer diagnosis and target-based therapy.

The most significant finding in this study is that we showed that diffuse mesothelin expression conferred a favorable clinical outcome in patients with high-grade serous carcinoma. A lack of correlation of mesothelin expression with several other clinicopathologic features, including tumor site (primary versus recurrent), tumor grade, drug resistance status, and differentiation

### Table 1. Expression of mesothelin in ovarian serous tumors

<table>
<thead>
<tr>
<th>Score</th>
<th>HGCA</th>
<th>LGCA</th>
<th>OSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>75</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>1+</td>
<td>52</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2+</td>
<td>27</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>3+</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4+</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>167</td>
<td>31</td>
<td>6</td>
</tr>
</tbody>
</table>

Abbreviations: HGCA, high-grade serous carcinoma; LGCA, low-grade serous carcinoma; OSE, ovarian surface epithelium from normal ovaries.
status of tumor cells indicates that mesothelin seems not to be associated with these variables. The mechanisms underlying a positive correlation of mesothelin expression and a prolonged overall survival in ovarian cancer patients remain elusive but immune response to mesothelin expressing tumor cells may be one of the mechanisms. This view is supported by the following evidence. A humoral response to mesothelin has been found in patients who suffered from ovarian cancer and mesothelioma (26). Elevated levels of mesothelin-specific antibodies were detected in the sera of 41.7% of patients with ovarian cancer, and the immunogenicity of mesothelin is associated with high mesothelin expression in tumor cells. Besides the humoral response, a cell-mediated immune response may also play an important role. It has been reported that vaccination with a granulocyte macrophage colony-stimulating factor–transduced allogeneic pancreatic tumor vaccine induced human mesothelin-specific CD8+ T cells in pancreatic cancer patients (27). Among those patients who received the vaccine, 3 of 14 patients had prolonged survival. It is also noted that among seven pancreatic tumor genes identified by SAGE as overexpressed in pancreatic cancer, only mesothelin was recognized by T cells from the three patients but not by T cell from other patients. Therefore, the perfect correlation between the generation of vaccine-dependent T-cell responses to mesothelin and long-term survival (albeit in a limited number of patients) makes it an extremely promising candidate for mesothelin-specific immunotherapy (5).

The above finding indicates that immune response to mesothelin-expressing ovarian carcinoma cells may result in a reduction of tumor load and contribute to a prolonged patient’s overall survival. It is interesting to note that a longer survival only occurred in tumors with diffuse mesothelin expression (> 50%) but not in those with focal expression. This finding implies that a tumor containing greater numbers of mesothelin-positive tumor cells was more likely to elicit a response from the immune system, whereas a tumor with focal mesothelin-positive cells would continue to progress unchecked as the majority of tumor cells fail to express the tumor antigen. Although the above view represents our most favored hypothesis, other mechanisms may also exist. For example, the cell-cell adhesion mediated by mesothelin and mucin 16 (CA125 molecules; ref. 3) may lead to cohesive tumor growth in ovarian carcinoma, which expresses mesothelin and prevents cancer cells from dissemination or metastasis in the peritoneal cavity.

In conclusion, this report represented the first large-scale analysis of mesothelin immunoreactivity in ovarian serous carcinomas and provided new evidence that diffuse mesothelin expression is associated with prolonged patient survival. Multi-institutional studies will be required to confirm whether mesothelin is an independent prognostic marker for ovarian cancer patients. It is also possible that future gene therapy directed towards enhancing mesothelin expression in cancer cells can offer improved clinical outcome to ovarian cancer patients.

References
Diffuse Mesothelin Expression Correlates with Prolonged Patient Survival in Ovarian Serous Carcinoma

M. Jim Yen, Chih-Yi Hsu, Tsui-Lien Mao, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/12/3/827

Cited articles
This article cites 26 articles, 15 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/12/3/827.full#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/12/3/827.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/12/3/827.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.