# Cell-Adhesion Molecule Expression and the Proliferation of Malignant Mesothelioma: A Post-Mortem Examination

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**Abstract.** *Aim: In order to determine if metastatic malignant mesothelioma cells are more aggressive than primary malignant mesothelioma cells, an analysis of the expression of the adhesion molecules E-cadherin and β-catenin, concomitant with an assessment of the proliferative activity at primary and metastatic sites, was conducted in post-mortem samples. Materials and Methods: E-cadherin or β-catenin expression was graded according to the percentage of positively-stained tumor cells. The proliferative activity was quantified by the Ki-67 labeling index. Results: Histologically, the majority of metastatic tumors matched the primary tumor. In the epithelioid component of primary tumors, E-cadherin and β-catenin expression ranged from 1+ to 4+. Conclusion: Malignant mesothelioma cells acquire a higher proliferative potential after metastasis, without any significant changes in their histology, although metastasis produces no definite trend on the expression of E-cadherin or β-catenin.*

Malignant mesothelioma arises from the serosal cells of the pleura, and less frequently, from the peritoneum. It is a highly malignant tumor with very poor prognosis (1, 2), and most patients die within 2 years of diagnosis. Malignant mesothelioma is histologically sub-classified as epithelioid (mostly composed of epithelial-shaped cells), sarcomatoid (mostly composed of spindle-shaped cells), or biphasic (composed of both types of cells) (3).

E-cadherin is a transmembrane protein that forms a calcium-dependent cell-cell adhesion complex together with β-catenin (4). The E-cadherin-β-catenin complex is essential for the formation of stable cell-cell adhesions, and reduced expression of these proteins is associated with the aggressiveness of various types of cancer cells, because the disruption of the stable cell-cell adhesion contributes to the release of cancer cells from the primary lesion (5-7).

It is currently unknown whether metastasized malignant mesothelioma cells are more aggressive than primary tumor cells. Therefore, in the present study we investigated the aggressiveness of primary and metastatic mesothelioma cells. To estimate the aggressiveness of the cancer, we examined the expression of adhesion molecules, E-cadherin and β-catenin, as well as the proliferative activity represented by the Ki-67 labeling index (8).

## Materials and Methods

*Patients and tissue specimens.* Permission for the usage of tumor tissues for histological studies was obtained from the family of each patient before autopsy. Primary and metastatic tumors obtained from 7 patients with pleural malignant mesothelioma who were autopsied at the Hyogo College of Medicine were used in this study. The patient group consisted of 4 men and 3 women, aged 59-68 (mean=63.9) years. Five patients were treated with chemotherapy alone, 1 received both chemotherapy and radiotherapy, and 1 was treated with chemotherapy and surgery. For all patients, the interval between initial diagnosis and death ranged from 7 to 60 months (mean=32.3 months).

The primary and metastatic tumors were fixed in 0.01 M phosphate-buffered 10% formalin (pH 7.4), and several paraffin-embedded blocks were produced from each tumor. Each block was serially-sectioned (5-μm thickness) for histological analysis by hematoxylin-eosin staining and for immunohistochemical analyses.

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*Key Words:* Malignant mesothelioma, E-cadherin, β-catenin, Ki-67, metastasis.

Table I. *A summary of the immunohistochemical staining of pleural malignant mesothelioma.*

| Case no. | Histological type | Component | Mesothelial marker | Calretinin | D2-40 | TM | CEA | TTF-1 |
|---|---|---|---|---|---|---|---|---|
| 1 | Epithelioid | Epithelioid | (+) | (+) | (−) | (+) | (−) | (−) |
| 2 | Epithelioid | Epithelioid | (+) | (−) | (−) | (−) | (−) | (−) |
| 3 | Biphasic | Epithelioid | (+) | (+) | (−) | (+) | (−) | (−) |
| | | Sarcomatoid | (−) | (−) | (−) | (−) | (−) | (−) |
| 4 | Biphasic | Epithelioid | (+) | (+) | (+) | (−) | (−) | (−) |
| | | Sarcomatoid | (−) | (−) | (−) | (−) | (−) | (−) |
| 5 | Biphasic | Epithelioid | (+) | (+) | (−) | (+) | (−) | (−) |
| | | Sarcomatoid | (−) | (−) | (−) | (−) | (−) | (−) |
| 6 | Sarcomatoid | Sarcomatoid | (+) | (−) | (−) | (−) | (−) | (−) |
| 7 | Sarcomatoid | Sarcomatoid | (+) | (−) | (−) | (−) | (−) | (−) |

TM, Thrombomodulin; CEA, carcinoembryonic antigen; TTF-1, thyroid transcription factor 1.

*Immunohistochemistry.* Immunohistochemical staining was carried-out using an avidin-streptavidin immunoperoxidase method with an anti-human mesothelial cell mouse monoclonal antibody (HBME-1 clone, 1:50 dilution; Dako, Glostrup, Denmark), an anti-human calretinin rabbit polyclonal antibody (Nichirei, San Francisco, USA, diluted), anti-human D2-40 mouse monoclonal antibody (1:200 dilution; Dako), an anti-human thrombomodulin mouse monoclonal antibody (1009 clone, 1:500 dilution; Novocastra, Newcastle Tyne, UK), an anti-human carcinoembryonic antigen rabbit polyclonal antibody (1:1000 dilution; Dako), an anti-human thyroid transcription factor-1 mouse monoclonal antibody (1:1000 dilution; Novocastra), an anti-human E-cadherin mouse monoclonal antibody (36B5 clone, 1:50 dilution; Novocastra), an anti-human β-catenin mouse monoclonal antibody (17C2 clone, 1:80 dilution; Novocastra), and an anti-human Ki-67 mouse monoclonal antibody (1:100 dilution; Dako). Antigen retrieval was carried-out for the immunohistochemical staining of calretinin, D2-40, thrombomodulin, E-cadherin, β-catenin, and Ki-67. The antigen retrieval and the immunohistochemical staining were carried out using an automated Benchmark system (Ventana Medical System, Tucson, AZ, USA) according to the manufacturers' instructions.

*Immunohistochemical assessment.* Positive immunostaining was defined as ≥5% of the tumor cells being positively stained. The immunostaining of E-cadherin and β-catenin was graded according to the percentage of tumor cells stained positively as follows: negative, <5%; 1+, 5-24%; 2+, 25-49%; 3+, 50-74%; 4+, ≥75%. The Ki-67 labeling index (the percentage of the tumor that was Ki-67-positive) was determined by examining >500 tumor cells in the area with the highest labeling index using the Win Roof software program (Mitani Co., Tokyo, Japan).

## Results

*Tumor histology.* The histological types of the 7 primary malignant mesotheliomas were epithelioid (n=2), bi-phasic (n=3), and sarcomatoid (n=2) (Figure 1) (Table I). The tumor cells of primary tumors expressed at least 1 of the 4 mesothelioma markers (mesothelial marker, calretinin, D2-40, and thrombomodulin), but did not express the anti-human carcinoembryonic antigen or thyroid transcription factor-1, both of which are markers of lung adenocarcinoma (Table I).

Multiple metastases were found in all 7 patients (Tables II, III and IV). When the histological type of the primary tumor was purely epithelioid or sarcomatoid, the histological types of the metastatic tumor matched the primary tumor (Tables II, III and IV). In contrast, when the histological type of the primary tumor was biphasic, the histological type of the metastatic tumors was either the biphasic or epithelioid type (Tables II, III and IV).

*The pattern of cell-adhesion molecule expression in primary and metastatic tumors.* The sarcomatoid components of primary or metastatic tumors were negative for E-cadherin and β-catenin (Tables II and III). All epithelioid components in 5 primary tumors (2 epithelioid type tumors and 3 biphasic type tumors) showed positive staining for E-cadherin at grade 1+, 2+ or 3+, and positive staining for β-catenin at grade 1+, 3+ or 4+ (Figure 2) (Tables II and III).

The staining grade of E-cadherin and β-catenin at the metastatic sites varied even for the same patient. Furthermore, there was no relationship between the primary and metastatic tumor staining grades; the grade of E-cadherin or β-catenin staining at each metastatic site was variable (increased, decreased, or unchanged) compared to that of the primary tumor, and did not appear to have a specific pattern of expression.

## Discussion

The above mentioned results show that expression levels of E-cadherin or β-catenin were relatively low even in the epithelioid components of malignant mesothelioma. The E-cadherin-β-catenin complex is essential for the formation of stable cell-cell adhesions, and reduced expression is associated with invasion and metastasis of cancer cells (5-7).

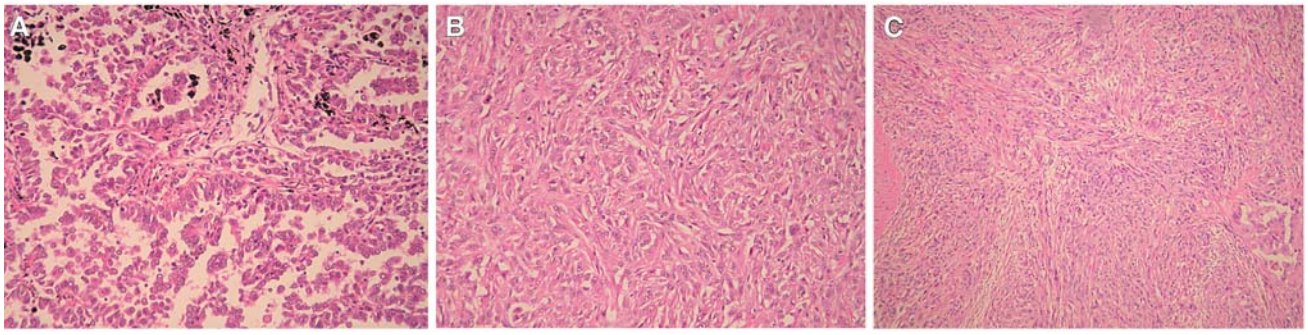


Figure 1. *The histological findings of pleural malignant mesothelioma of the (A) epithelioid type, (B) sarcomatoid type, and (C) biphasic type. HE (Hematoxylin&eosin) stain was used for staining the cells (original magnification, ×20).*

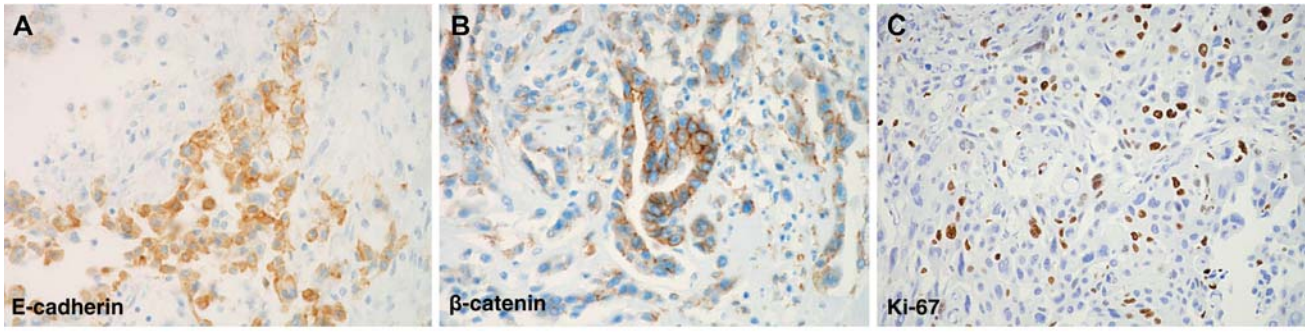


Figure 2. *Representative immunohistochemical staining of E-cadherin, β-catenin, and Ki-67 using HE stain (original magnification, ×20). Samples were obtained from malignant mesothelioma (epithelioid type).*

Table II. *The E-cadherin expression of primary and metastatic sites of malignant mesothelioma.*

| Case no. | Histological type | Component | Primary site | Metastatic site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pleura | Diaphragm | Liver | Lung | Heart | Peritoneal | Mesenteric LN | Pulmonary hilar LN | Parabronchial LN |
| 1 | Bi-phasic | Epithelioid | (2+)* | (1+)* | (1+)* | (−)* | | | | | |
| | | Sarcomatoid | (−)* | (−)* | (−)* | (−)* | | | | | |
| 2 | Bi-phasic | Epithelioid | (1+) | | (1+)* | (−)* | (−)* | | | (3+) | |
| | | Sarcomatoid | (−)* | | (−)* | NC | (−)* | | | (−)* | |
| 3 | Bi-phasic | Epithelioid | (1+)* | | (2+)* | (3+) | | (2+)* | (3+) | | |
| | | Sarcomatoid | (−)* | | (−)* | NC | | (−)* | (−)* | | |
| 4 | Sarcomatoid | Sarcomatoid | (−)* | | (−)* | | | | | | |
| 5 | Sarcomatoid | Sarcomatoid | (−)* | | | (−) | (−) | | | | (−)* |
| 6 | Epithelioid | Epithelioid | (3+) | (2+)* | (1+)* | (1+)* | | | | | (1+)* |
| 7 | Epithelioid | Epithelioid | (1+)* | | (1+)* | | | | (1+)* | | (−)* |

E-cadherin expression was graded according to the percentage of tumor cells stained positively as follows: Negative, <5%; 1+, 5-24%; 2+, 25-49%; 3+, 50-74%; 4+, ≥75%. NC, No component; LN, lymph node. *reduction of E-cadherin immunohistochemical expression.

Therefore, reduced expression of E-cadherin and β-catenin in malignant mesothelioma may partly contribute to the poor prognosis of the patients. However, there was no recognizable pattern of change in E-cadherin and β-catenin staining in the epithelioid components at metastatic sites compared to the primary tumors, suggesting that metastasis does not necessarily accelerate the disruption of cell-cell adhesions, at least for these 2 adhesion molecules.

Table III. *β-Catenin expression of primary and metastatic sites of malignant mesotheliomas.*

| Case no. | Histological type | Component site | Primary | Metastatic site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pleura | Diaphragm | Liver | Lung | Heart | Peritoneal | Mesenteric LN | Pulmonary hilar LN | Parabronchial LN |
| 1 | Bi-phasic | Epithelioid | (3+) | (4+) | (2+)* | (1+)* | | | | | |
| | | Sarcomatoid | (−)* | (−)* | (−)* | (−)* | | | | | |
| 2 | Bi-phasic | Epithelioid | (4+) | | (2+)* | (2+)* | (−)* | | | (1+)* | |
| | | Sarcomatoid | (−)* | | (−)* | NC | (−)* | | | (−)* | |
| 3 | Bi-phasic | Epithelioid | (3+) | | (3+) | (−)* | | (3+)* | (3+) | | |
| | | Sarcomatoid | (−)* | | (−)* | NC | | (−)* | (−)* | | |
| 4 | Sarcomatoid | Sarcomatoid | (−)* | | (−)* | | | | | | |
| 5 | Sarcomatoid | Sarcomatoid | (−)* | | | (−)* | (−)* | | | | (−)* |
| 6 | Epithelioid | Epithelioid | (3+) | (1+)* | (3+) | (1+)* | | | | | (1+)* |
| 7 | Epithelioid | Epithelioid | (1+)* | | (2+)* | | | | (3+) | | (3+) |

β-Catenin expression was graded according to the percentage of tumor cells stained positively as follows: negative, <5%; 1+, 5-24%; 2+, 25-49%; 3+, 50-74%; 4+, ≥75%. NC, No component; LN, lymph node. *reduction of b-catenin immunohistochemical expression.

Table IV. *The Ki-67 labeling indices of primary and metastatic sites of malignant mesotheliomas.*

| Case no. | Histological type | Component | Primary site | Metastatic site | | | | | | | | Average ratio of Ki-67 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pleura | Diaphragm | Liver | Lung | Heart | Peritoneal | Mesenteric LN | Pulmonary hilar LN | Parabronchial LN | |
| 1 | Bi-phasic | Epithelioid | 13.2 (1) | 18.6 (1.4) | 14.7 (1.1) | 16.5 (1.3) | | | | | | 1.3 |
| | | Sarcomatoid | 7.7 (1) | 14.3 (1.9) | 16.6 (2.2) | 11.1 (1.4) | | | | | | 1.8 |
| 2 | Bi-phasic | Epithelioid | 17.4 (1) | | 43.2 (2.5) | 32.3 (1.9) | 26.8 (1.5) | | | | | 1.9 |
| | | Sarcomatoid | 12.4 (1) | | 10.3 (0.8) | NC | 12.3 (1) | | | | | 1.1 |
| 3 | Bi-phasic | Epithelioid | 15.2 (1) | | 52.3 (3.4) | 32.1 (2.1) | | | 29.4 (1.9) | 34.2 (2.3) | | 2.4 |
| | | Sarcomatoid | 10 (1) | | 18.1 (1.8) | | NC | | 16.4 (1.6) | 11.2 (1.1) | | 1.5 |
| 4 | Sarcomatoid | Sarcomatoid | 6.4 (1) | | 9.1 (1.4) | | | | | | | 1.4 |
| 5 | Sarcomatoid | Sarcomatoid | 4.6 (1) | | | | 10.5 (2.3) | 8.2 (1.8) | | | 18.5 (4.0) | 2.7 |
| 6 | Epithelioid | Epithelioid | 16.7 (1) | 21.8 (1.3) | 15.3 (0.9) | 23.4 (1.4) | | | | | 19.9 (1.2) | 1.2 |
| 7 | Epithelioid | Epithelioid | 5.3 (1) | | 9.5 (1.8) | | | | 15.2 (2.9) | | 20.9 (3.9) | 2.9 |

NC, No component; LN, lymph node; Ki-67 LI, Ki-67 labeling index. The numbers in parentheses indicate the ratio of the Ki-67 labeling index of the metastatic site to that of the primary site.

*Differential cellular proliferation between primary and metastatic tumors*. The Ki-67 labeling indices of epithelioid components in metastatic tumors were increased compared to those in primary tumors (cases 1-5) and the ratio of the average Ki-67 labeling index between metastatic and primary tumors in the 5 epithelioid tumors ranged 1.2-2.9 (mean±standard error: 1.9±0.8). In the case of one patient (case 4) with a sarcomatoid tumor, the ratio of the average Ki-67 labeling index between metastatic and primary tumors ranged between 0.8-1.4. However, the ratio in the remaining 4 sarcomatoid tumor cases (cases 3, 5, 6, and 7) was >1 and ranged between 1.1-2.7 (mean±standard error: 1.7±0.8).

At the majority of metastatic sites of malignant mesothelioma, the Ki-67 labeling indices for the same histological component were higher than those in the primary tumor. These results indicate that the metastasized tumor cells had acquired greater proliferative potential without any change in the histological type.

## Conclusion

In metastatic malignant mesothelioma, an increase in proliferation indicates that metastasis accelerates the spread of the tumor cells. However, it seems unlike that the increased aggressiveness of metastatic malignant

mesothelioma was related to the disruption of cell-cell adhesions, at least in the case of E-cadherin and β-catenin.

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