ABSTRACT

Cadherins are a family of intercellular glycoproteins responsible for calcium-dependent cell adhesion and are currently divided into four types: epithelial (E), neuronal (N), placental (P), and vascular (V). Since cadherins are known to be indispensable for not only morphogenesis in the embryo but also maintenance of tumor cell nest, we examined the expression of E-cadherin in 31 meningiomas (11 syncytial, 12 transitional, 8 fibroblastic) and 3 arachnoid villi by immunoblot and immunohistochemical analyses. In the immuno blot analysis, E-cadherin was detected at the main band of M, 124,000 in all of the arachnoid villi, as well as syncytial and transitional types of meningiomas, but not in the fibroblastic type. The immunohistochemical examination showed that E-cadherin was expressed at the cell borders of syncytial and transitional types, but the expression was absent in the fibroblastic type. Immunoelectron microscopy showed that E-cadherin was localized at the intermediate junctions in arachnoid villi, while it was detected diffusely at the cell surface in meningiomas. It is suggested from these data that the expression of E-cadherin might be closely related to the differentiation and organogenesis of meningioma cells.

INTRODUCTION

Ca²⁺-dependent cell adhesion molecules designated as cadherin are known to play an essential role in embryonic development and morphogenesis (1–7), and are currently divided into four types: E² (6–8), P (6, 7), N (9, 10), and V (11, 12). Each type of cadherin has different tissue distributions and binding specificities (2, 5, 13) and a unique pattern of expression that coincides with the cell movement and rearrangement in animal embryogenesis (3, 6).

Shimoyama et al. demonstrated that E-cadherin, known as uvomorulin (14), L-CAM (15), Arc-1 (16), and cell-CAM120/80 (17) also are expressed in almost all epithelial tissues and cancer cells and suggested that it might be indispensable for the maintenance of not only normal tissue but also cancer cell nest (18, 19). Recently, it was suggested from many experimental data that the loss of E-cadherin might be related to invasion and metastasis of tumors. For example, the expression of E-cadherin was demonstrated to be unstable in culture cells of the highly metastatic ovarian tumor (20). Because loss of E-cadherin can generate dedifferentiation and invasiveness of human carcinoma cells, Frixen et al. (21) suggested that E-cadherin might act as an invasion suppressor. The epithelial cells transformed with sarcoma virus in Madin-Darby canine kidney also showed loss of uvomorulin, which might be a critical step in the promotion of epithelial cells to malignant or invasive phenotypes (22). Furthermore, it was suggested that the reversible down-regulation of this molecule plays a basic role in tumor invasion (23, 24).

RESULTS

Immunoblot Analysis

HECD-1 preferentially reacted with M, 124,000 proteins compatible with E-cadherin in arachnoid villi (Fig. 1, lane 2) as well as syncytial (Fig. 2, lanes 2–4) and transitional (Fig. 2, lanes 5–8) meningiomas, but there was no reaction in fibroblastic meningiomas (Fig. 2, lanes 9–11). Positive controls of colon epithelium (Figs. 1 and 2, lane 1) showed the same...
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Fig. 1. Western blot analysis of the colon epithelium (positive control, lane 1) and arachnoid villi (lane 2). The samples were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 7.5% polyacrylamide gel and were transferred onto a nylon membrane. The latter was stained with monoclonal antibody HECD-1 using peroxidase-conjugated anti-mouse IgG. Both lines expressed M, 124,000 proteins which are compatible with E-cadherin. Left ordinate, mobilities of molecular markers in thousands.

Immunohistochemistry.

Expression Pattern of E-Cadherin in Arachnoid Villi. Because arachnoid cells are known to show many characteristic features in common with meningioma cells (25–27), it is widely accepted that meningiomas originate from arachnoid villi. The latter basically consist of 4 portions: the cap cell cluster, the central core, the arachnoid cell layer, and the fibrous capsule (31, 32). E-cadherin was identified in the cap cell cluster (Fig. 3A, asterisk), the central core (Fig. 3B), and the arachnoid cell layer (Fig. 3, A and C, arrows) but was not detected in the fibrous capsule (Fig. 3C, arrowhead).

Expression Pattern of E-Cadherin in Meningiomas. The syncytial type of meningiomas was composed of polygonal cells which were closely packed in a sheet-like arrangement. E-cadherin was localized at the cell-cell border of syncytial tumor cells but not at the interstitial tissues of the fibrous septum (Fig. 4).

The transitional type is a mixed tumor consisting of syncytial and spindle cells and is characterized by whorls. The expression pattern was quite different between syncytial and spindle cells: E-cadherin was intensely detected in the syncytial cells (Fig. 5A) but weakly in the spindle cells (Fig. 5B).

The fibroblastic type contained interlacing bundles of spindle cells intermingled with conspicuous fibrils. In this type, E-cadherin was not detected in all of the cells studied (Fig. 6). The expression patterns of E-cadherin in arachnoid villi and meningiomas are summarized in Table 1.

Immunoelectron Microscopy

In arachnoid villi, E-cadherin was localized at the cell boundaries and concentrated in the junctional area (Fig. 7). In contrast, in meningiomas, it was detected diffusely at the cell surface (Fig. 8A). The expression of E-cadherin was more intense at the intermediate junctions which were associated with microfilament bundles (Fig. 8B, arrows) than at the other cell surface (Fig. 8, B and C, arrowheads). The reaction products were localized at the extracellular space (Fig. 8C, arrowheads). No specific staining was demonstrated within the cytoplasm or the other cell junctions except for intermediate junctions (Fig. 8C, arrows).

DISCUSSION

Selective cell adhesion is considered to be a fundamental process of morphogenesis. Recently, a number of cell adhesion molecules have been identified and demonstrated to be indispensable for organogenesis in embryonic development. By the biochemical and functional properties, these molecules have been classified into three families: cadherin, immunoglobulin, and integrin.

Fig. 2. Western blot analysis of the colon epithelium (lane 1) and meningiomas (lanes 2–11). Cell lysates of the syncytial (lanes 2–4), transitional (lanes 5–8), and fibroblastic (lanes 9–11) meningiomas were loaded on 7.5% polyacrylamide gel, transferred onto a nylon membrane, and detected by HECD-1. The weaker bands lower than M, 100,000 appeared to be degradation products of E-cadherin.
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Fig. 3. Immunohistochemical localization of E-cadherin in an arachnoid villus. The latter was reacted with HECD-1 using biotinylated anti-mouse IgG and avidin-biotin-peroxidase complex and was counterstained with hematoxylin. Arachnoid cell layer (arrows in A and C), cap cell cluster (asterisk in A), and arachnoid cells of the central core (B) were positive, whereas fibrous capsule (arrowhead in C) was negative. A and C, × 150; B, × 300. Bar, 50 μm.

Fig. 4. Immunohistochemical localization of E-cadherin in the syncytial meningioma. E-cadherin was detected at the cell border but not in the fibrous septum. × 400. Bar, 50 μm.

Fig. 5. Immunohistochemical localization of E-cadherin in the transitional meningioma. E-cadherin was detected in the syncytial area and whorls (A), but the expression was weak in the fibroblastic area (B). × 200. Bar, 50 μm.
Cadherins are intercellular glycoproteins of 723–747 amino acids responsible for Ca²⁺-dependent cell adhesion and are subdivided into various types such as E-, P-, N-, and V-cadherin with different adhesive specificities and tissue distributions (1–13). These types of cadherins are similar to each other in amino acid sequence and consist of the cytoplasmic domain (COOH-terminal region), the transmembrane domain (hydrophobic region), and the extracellular domain (NH₂-terminal region). Nose et al. (33) demonstrated that the epitope for antibodies capable of blocking cadherin action is located in the 113 amino acids of the NH₂-terminal region, and this region is essential for binding specificity of cadherin. In this study, the reaction products recognized with HECD-1 were localized at the extracellular space (Fig. 8C, arrowheads).

It has been demonstrated by immunoblot and immunohistochemical analyses that E-cadherin is expressed in almost all epithelial tissues (6, 18). The immunoelectron microscopic analysis showed that E-cadherin was preferentially localized at the intermediate junctions which were associated with actin bundles (3, 13, 34). The blocking antibody of E-cadherin induced a disruption of epithelial cell adhesion (3). Accordingly, E-cadherin is thought to be indispensable for the formation and maintenance of adult epithelial tissues.

In arachnoid villi, E-cadherin was detected, except for the fibrous capsule, by immunohistochemistry and was localized in a spotty pattern at the cell-cell borders by immunoelectron microscopy. Furthermore, the expression was preferentially intense around the intermediate junctions. Because arachnoid cells are known to be pluripotential cells exhibiting both epithelial and mesenchymal properties (27), it is reasonable that arachnoid cells express E-cadherin. Because arachnoid villi provide a main route for the cerebrospinal fluid absorption, arachnoid cells are always affected by the pulsation of the cerebrospinal fluid (35, 36). We suggest from the present data that E-cadherin might play an important role in the flexible adhesion of arachnoid cells even in the presence of the cerebrospinal fluid.

Shimoyama et al. (18, 19) suggested that E-cadherin also plays an important role in the formation and maintenance of tumor cell nests. In meningiomas, the expression pattern of E-cadherin was quite different among 3 histological subtypes; it was intensely expressed in the syncytial type, but heteroge-

### Table 1

<table>
<thead>
<tr>
<th>Expression of E-cadherin</th>
<th>Arachnoid villi Portions</th>
<th>Meningiomas Histological types</th>
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<tr>
<td>Present</td>
<td>Cap cell cluster</td>
<td>Syncytial</td>
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<td></td>
<td>Arachnoid cell layer</td>
<td>Transitional</td>
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<td>Core arachnoid cell</td>
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<td>Absent</td>
<td>Fibrous capsule</td>
<td>Fibroblastic</td>
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Fig. 6. Immunohistochemical localization of E-cadherin in the fibroblastic meningioma. There were no cells positive for HECD-1. × 200. Bar, 50 μm.

Fig. 7. Immunoelectron microscopy of E-cadherin in the cap cell cluster of an arachnoid villus. The latter was reacted with the HECD-1 and avidin-biotin-peroxidase complex. The reaction products were localized at the cell surface where the cells come into contact (arrows). N, nucleus. × 11,000. No stain. Bar, 1 μm.
Fig. 8. Immunoelectron microscopy of E-cadherin in the syncytial meningioma. In A, E-cadherin was diffusely detected at almost all cell boundaries. At higher magnification, E-cadherin was intensely expressed at the intermediate junctions associated with the intracytoplasmic microfilament bundles (arrows in B), but at the other portions E-cadherins were not linked to each other and not associated with microfilament bundles (arrowheads in B and C). There were no reaction products at desmosomes (arrows in C) and within the cytoplasm. N, nucleus. A, × 9,000; B, × 15,000; C, × 18,000. Lead stain. Bar, 1 μm.
neously expressed in the transitional type, and was not expressed in the fibroblastic type.

Although it is widely accepted that meningiomas might derive from a single type of cell called an arachnoid cell, it is obscure why meningiomas exhibit various histological subtypes. Yamashima et al. (32) and Kida et al. (31) demonstrated ultrastructurally that the syncytial meningioma is similar to the cap cell cluster in arachnoid villi, while the fibroblastic meningioma is similar to the fibrous capsule. Accordingly, they suggested that the histological varieties of meningiomas conceivably derive from different cytogenesis within arachnoid villi (31, 32). The expression pattern of E-cadherin was closely correlated with the histological features (Table 1). We suggest from these data that E-cadherin is closely related to the differentiation of meningioma cells, especially in the syncytial type and the transitional type.

It is possible that the fibroblastic meningioma might express some unknown cell adhesion molecules other than E-cadherin. Nitta et al. (37) recently demonstrated that various types of collagens or laminin were expressed in the fibroblastic meningiomas, and their distribution was closely related to the basement membrane. Accordingly, we suggest that the integrin superfAMILY cell adhesion receptors (38, 39), such as fibronectin receptor complex, may be mainly implicated in the cell adhesion of the fibroblastic meningiomas.

In this study, we have demonstrated by immunoelectron microscopy that the expression pattern of E-cadherin is quite different between meningioma and arachnoid cells. E-cadherin was concentrated predominantly at the intermediate junctions in arachnoid villi, while in meningiomas, it was diffusely expressed at the cell surface, including the intermediate junctions.

Why did the distribution of E-cadherin change in meningiomas? In the previous study, the expression of E-cadherin was demonstrated to be not always associated with junctional structural changes in many embryonic cells (3, 7). The similar distribution change from normal to tumor cells was demonstrated in the fibroblastic receptor, which was implicated in the cell adhesion of fibronectin in the motile embryonic cells and tumor cells (41, 42). This receptor is present diffusely at the cell surface in both the motile embryonic cells and Rous sarcoma virus-induced tumors. In contrast, in the stationary cells, the fibronectin receptors were concentrated close to the focal contact sites and were linked to the cytoskeleton and fibronectin fibers. Accordingly, we suggest that the concentration of E-cadherin at the cell surface in arachnoid villi might provide stable maintenance of the structure of arachnoid villi; in contrast, the diffuse distribution of E-cadherin in meningiomas might contribute to the labile cell to cell adhesion and the establishment of new contacts.

Shimoyama and Hirohashi (43) demonstrated that the undifferentiated gastric adenocarcinomas without tight intercellular adhesion uniformly expressed E-cadherin at the surface of the cancer cells. They suggested that the abnormalities of some intrinsic molecules which anchor cadherin to the cytoskeleton might render cadherin ineffective (43). In meningiomas, it is unknown whether E-cadherin localized at the cell surface except that the intermediate junctions show normal cell binding function. By immunoelectron microscopy, the intercellular E-cadherins were not linked to each other in places and were not associated with the intracellular microfilaments. Because of these observations, the expression pattern of E-cadherin and the intrinsic molecules should be further examined to precisely clarify the role of cadherin.

In summary, E-cadherin might play an important role not only in the cell adhesion of human arachnoid villi but also in the morphogenesis and histogenesis of meningiomas.

REFERENCES

Immunohistochemical Localization of Cell Adhesion Molecule Epithelial Cadherin in Human Arachnoid Villi and Meningiomas

Yasuo Tohma, Tetsumori Yamashima and Junkoh Yamashita