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Expression of Cadherins in Astrocytic Tumors
(星細胞系腫瘍におけるカドヘリンの発現)

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I、Introduction

Cell adhesion molecules (CAMs) are proteins that play an important role in the interaction between cells, between cells and their surrounding matrix, and in the tissue building of multicellular organisms. There are four major classes of CAMs: the cadherin superfamily, the selectin superfamily, the immunoglobulin-gene superfamily, and the integrin family.

Cadherin CAMs are glycoproteins discovered by Takeichi et al²⁸⁾. They have been roughly classified into type E (epithelial-cell derived), type P (placenta-derived)¹⁶⁾, and type N (nerve-derived)¹⁰⁾. These types are referred to as the classical cadherins, but to date about 10 different types of cadherins have been discovered.

In recent years it has been learned that cadherins are the CAMs that possess the most popular, powerful, and stable adhesiveness in the other CAMs^{27,32)}, and a great deal of attention is being focused on their role in tumor metastasis and invasion. So, we chose cadherins in this study.

Although the expression of cadherin in meningioma³³⁾, ependymomas, choroid plexus papilloma⁸⁾, and gliomas was recently studied by Shinoura²⁵⁾, its relationship to the clinical symptoms, particularly to dissemination, has not been studied. The present study investigates the manner in which cadherins are expressed in glioblastomas and anaplastic astrocytomas, the difference between expression of cadherin in primary and recurrent glioblastomas and anaplastic astrocytomas, and the correlations of cadherins with clinical symptoms.

II. Materials and Methods

1). Patient Selection

Twenty-two astrocytic tumor tissue samples were retrieved from post operated patients. The samples consisted of surgical specimens taken from 22 astrocytic tumors at initial, second, and later operations between 1982 and 1994. All specimens were fixed in 20% formalin and processed in paraffin for routine light microscopy.

Tumors were classified and graded as 13 cases of glioblastoma (GBM: WHO Grade IV) and 9 anaplastic astrocytoma (AAS: WHO Grade III), according to the WHO scheme.

There were 8 male and 5 female GBM patients ranging in age from 27 to 71 (mean: 51.1); the mean observation period was 24.7 months. AAS patients consisted of 6 males and 3 females ranging in age from 31 to 64 (mean: 43.8); the mean observation period was 26.9 months.

The observation period was defined as the interval between the first diagnosis made by CT and MRI images, and June 1995 or the time of death. The presence of dissemination was determined on the basis of CSF smear findings, CT, and MRI findings.

2). Immunohistochemical studies

Sections were cut and stained for immunohistochemical studies using monoclonal antibodies to GFAP (glial fibrillary acidic protein, DAKO Corp.), E-cadherin (HECD-1, Takara) and N-cadherin (NCAD-2, Becton Dickinson)²⁰. Sections were cut at a thickness of 4 μ m, then deparaffinized and hydrated through a series of xylenes and alcohols. Prior to staining, the sections were pretreated using an antigen retrieval technique that consisted of heating the slides in a microwave oven at the highest power (500 wt) for 5 cycles of 2 minutes in 0.01 mM citrate buffer (pH 6.0). The primary antibodies were allowed to react with E-cadherin (1:500 dilution) and N-cadherin (1:1000 dilution) overnight at 4 °C. The LSAB (labeled streptavidin biotin) method (DAKO Corp.) was employed after dissolving 10 mM Ca^{2+} in primary antibody solutions, and color was developed with DAB (3,3'-diaminobenzidine4HCl). TBS (pH7.6, containing 1.0 mM Ca^{2+}) was used for each of the washing procedures. The sections were counterstained with hematoxylin. Normal goat serum was used as the negative control.

We used serial sections of normal brain tissue as the positive control for N-cadherin and assessed correlations between N-cadherin and GFAP.

The cadherin positive rates were determined by counting the cells in about 10 fields at x400 magnification. Findings were scored as follows: +++ (strongly positive) when 90% or more of the cells were positive; ++ when 50 to 90% were positive; + when 10 to 50% were positive; - when less than 10% were positive.

Normal large intestine and colorectal cancers were used as the positive control for immunohistochemical staining of E-cadherin. Since N-cadherin is widely distributed in the central nervous system, retina, lens, renal tubule, myocardium, vascular endothelial cells, the testes, and so on ¹⁰⁾, normal brain tissue and renal tubules were employed as the positive controls for N-cadherin.

III、Results

E-cadherin was stained in the cell membranes and intracellular granules of the normal large intestine control, but no expression was detected in the normal brain except in the arachnoid membrane (Fig. 1a, b), nor was expression detected in either the GBM or the AAS cases.

N-cadherin, on the other hand, was stained in the neurons of the normal brain tissue control. Expression of N-cadherin was seen even in small neurons (Fig. 2), but none was detected in normal astrocytes in comparisons with GFAP immunostain (Fig. 3a, b). N-cadherin was observed in both GBM and AAS tumor cells.

The pattern of N-cadherin expression in the GBM cases was in cell membrane and cytoplasm near the cell membrane. Furthermore, strong expression in the normal neurons incorporated into the tumors was observed in all 25 specimens (Fig. 4a), and N-cadherin was positive in the endothelium of intratumoral vessels in 26 of the 28 specimens (Fig. 4b). There was wide variation in the expression of N-cadherin in small tumor cells (Table 1), with no expression at all in some tumors and strong expression in others. Expression was generally weak in giant cells, with the positive rate exceeding 50% in only one of 15 specimens. In 15 out of 16 specimens, strong expression was observed in cells that might be gemistocytes with abundant cytoplasm, and strong expression was found in all cells forming gliovascular rosettes (Fig. 4c). Hardly any expression was detected in central cells surrounded by perinecrotic pseudopalisades, and the expression was seen only in cells in the periphery (Fig. 4d).

N-cadherin expression in the AAS cases was nearly the same as in GBM cases. In 14 out of 15 specimens, strong expression was observed in neurons. Expression of N-cadherin was also seen in intratumoral vessels in 18 out of 19 specimens. Strong expression was observed in giant cells in 1 out of 7 specimens, while strong expression was seen in gemistocytes in 6 out of 10 specimens. Each specimens showed widely variable expressions in small tumor cells as well as in GBM, but the expression generally tended to be stronger than in GBM (Table 2).

The clinical data of the GBM cases are summarized in Table 1. Lower N-cadherin expression in small tumor cells sampled at the second operation was observed in 11 (84.6%) of the 13 cases. Dissemination or metastasis was observed during the course of the disease in 9 (81.8%) of these patients, with contralateral cerebral and dural metastasis observed in 2 (cases 3 and 11) and in 1 (case 5) respectively. In addition, skin metastasis was observed in case 7. N-

cadherin expression was reduced at the time of recurrence. Photomicrographs of immunostaining in the primary and a recurrent tumor in case 8, which show a typical dissemination, are shown in Fig. 5, a and b.

The clinical background of the cases with AAS is shown in Table 2. Progressive reduction of N-cadherin expression was observed in 3 (33.3%) of the 9 patients from the time of the second operation. Dissemination was observed only in case 14.

IV. Discussion

Cadherins are a Ca^{2+} -dependent type of cell adhesion molecules discovered by Takeichi et al²⁸⁾. They are proteins that are capable of passing through cell membranes. It is said that after having passed through the cell membrane, cadherins bind to the intracellular lining proteins catenin α , β , and γ , and bind to the cytoskeletal protein actin through vinculin, and that they possess powerful adhesiveness³¹⁾. In the absence of Ca^{2+} , cadherins are very unstable; they possess properties by which cells are easily separated by protease²⁷⁾.

Since Yoshida et al³⁶⁾ reported the cadherin monoclonal antibody EC CD-1 in 1984, research on cadherins has developed rapidly. Cadherins play an important role in the tissue building of multicellular organisms and in organogenesis. E-cadherin is said to be distributed widely in epithelial tissue, but its initial function in ontogenesis is compaction in the process that completes the morula formation. It has been proved by Vestweber³⁴⁾ that antibodies to E-cadherin inhibit this compaction.

Cadherins also play an important role in the development of the central nervous system, and strong expression of E-cadherin is observed during the initial period in the ectodermal development. A short time after expression of N-cadherin is first detected in the invagination of the neural crest, the neural tube is formed in this area³⁴⁾. E-cadherin disappears later^{22, 29)}, but it persists in the dorsal root ganglia, etc., and expression continues until after birth²¹⁾. N-cadherin, on the other hand, is said to be expressed not only in the central nervous system, but also in a variety of organs, including the retina, lens, myocardium, renal tubule, vascular endothelial cells, and the testes¹¹⁾. However, it is expressed either during neural tube formation or in the initial stage of a variety of cells differentiating into the nervous system. It is believed that N-cadherins play an important role also in the network formation of the central nervous system, such as the morphogenesis of the nervous system, neurite elongation, and nerve fiber migration¹⁴⁾.

The role of cadherins in cancer has been attracting a great deal of interest in recent years. There have been reports that cancers must change their morphology freely in an early stage in order to acquire the ability to invade and metastasize. Cell adhesion molecules play an important role in their morphogenesis and in the adhesiveness between cells, and attenuation and disappearance of E-cadherin expression have actually been reported to have often been observed in precancerous lesions¹²⁾ and in cancer tissue^{19, 23, 24, 26)}. In addition, a reduction of E-cadherin expression was also reported in highly

invaded areas and lesions with distant metastasis^{15, 30}. These facts were confirmed in experiments in which human cancer cell lines were used⁷.

In comparison with GFAP, immunostaining of N-cadherin in the serial sections of normal brain tissue was expressed in normal nerve cells, but not in normal astrocytes. These data were same with Shinoura's report²⁵. In the vicinity of the invaded tumor cells, a strong expression of N-cadherin in neurons was observed, which indicates the process of degeneration with disappearance of the nucleoli. N-cadherin seems therefore to be an antigen, possibly serving as an excellent marker of neurons and neuronal neoplasms. On the other hand, expression of N-cadherin was observed in tumor cells such as AAS and GBM, although no expression was detected in normal astrocytes in the adult human brain. In the present study, N-cadherins are essentially located on the cell surface and cytoplasm near the cell membrane in the tumor cells of GBM and AAS. These phenomena are considered that the N-cadherin regulation of cell adhesion is said not only in extracellular component but also in intracellular component. And these facts are thought that the antibody of N-cadherin are reacted to the intracellular components¹³.

The expression of N-cadherin in this study is recognized in the gliovascular rosettes of almost all cases of both GBM and AAS. Moreover, it was strongly positive in the tumor cells, whether small or gemistocytic. Whereas GFAP mediates in the adhesion between tumor cells and blood vessel walls, N-cadherin mediates presumably in the adhesion between individual tumor cells and in the adhesion between tumor cells and the pericyte of blood vessels. Thus it is formed gliovascular rosettes. This may be concluded from the fact that the reaction of almost all intratumoral blood vessels is strongly positive. Moreover, the expression pattern depends on the properties of the tumor cells and is strongly positive.

Examination of respective tumor cell properties in GBM patients showed a strong expression of N-cadherin in small immature cells in 9 (29.0%) out of 31 specimens. This was greater than the 1 (6.7%) out of 15 large-cell specimens, and the expression was different in each of the first, second and later surgical specimens in the small tumor cells. The activity and proliferating capacity of giant cells themselves are said not to be high^{1, 5}, so it may be better to use small cells as an index of tumor cell activity. Actually, N-cadherin is not expressed in normal tissue and is detected only when neoplastic transformation occurs. However, when tumor cells become clinically or pathologically malignant through repeated recurrences, N-cadherin expression decreases or disappears. In fact, 7 patients with GBM dissemination showed progress reduction of N-

cadherin since the second operation, and contralateral cerebral or dural metastasis was discovered in 3 of them. Only in case 7, skin metastasis was also observed in the 7 GBM patients with dissemination. If N-cadherin is a cell adhesive factor and acts to reinforce intercellular adhesion, a decrease of N-cadherin would result in a reduction of the adhesion between cells, and the tumor cells would be separated to invade the surrounding brain tissue and disseminate into the CSF. If the reduction in the expression of N-cadherin was greater during the second operation than during the first, this could serve as a parameter for foreseeing the risk of dissemination occurrence.

According to various reports, the frequency of dissemination is thought to range from 6.8% to 23%^{2,3,6)} and a high rate of dissemination (59.5%) is found at autopsies even though the patient exhibited no clinical manifestation⁴⁾. Although Shinoura²⁵⁾ reported that, based on his studies using cell lines, invasion of GBM has no close relation to N-cadherin, in our present study the decreased expression of N-cadherin after the second operation, especially in the GBM patients (84.6%), and the detection of dissemination in 81.8% of these patients, suggests a possible relationship between N-cadherin and tumor cell invasion or dissemination.

Various factors may be involved in the occurrence of dissemination, such as: 1) positional relationship between the tumor and the cerebral ventricles or subarachnoid space⁴⁾, 2) age³⁵⁾, 3) duration of illness³⁵⁾, 4) properties of the tumor cells^{9,35,37)}, 5) opening the cerebral ventricles during surgery; and so on. There were 12 cases among the GBM patients in this study in which the contrast-enhanced area was in contact with the cerebral ventricular wall, and dissemination was found in 9 of them. Dissemination was also observed in 1 of 4 patients with decreased N-cadherin expression after the opening of a cerebral ventricle. In the other 3 patients whose ventricles were opened, neither dissemination nor decrease in N-cadherin expression was observed at the time. Nevertheless, there was one exceptional case: in case 11 no evidence of dissemination was observed, although the patient exhibited a decrease in N-cadherin and an enhanced area adjacent to a cerebral ventricle.

Even though in 8 of the AAS cases a contrast-enhanced area was in contact with a cerebral ventricular wall, dissemination was observed only in case 14, who showed reduced expression of N-cadherin in the specimen after the second operation. N-cadherin expression was reduced in 2 of the other 7 cases, but was unchanged or had increased in 5. This suggests that dissemination tends not to occur even when it fulfills the condition of tumor cell contact with a cerebral ventricle because AAS is more benign than GBM and N-cadherin

expression is strong and cell adhesion is present.

Thus, while it cannot be denied that factors 1)-3) above are causative dissemination factors, the principal factor is the expression level of N-cadherin. Friede et al⁹⁾, have explained that from the viewpoint of tumor cell properties, the intercellular junctions of small tumor cells become weak because of their lack of cell processus, and that therefore tumor cells are released.

Whereas Okumura et al^{17,18)}, have demonstrated tumor cell invasion in vitro experiments on cultured cells, our research appears to represent a method of evaluation that views invasion and dissemination from a new perspective.

It may also be related to the development of a new category of drugs that prevent dissemination by exploiting N-cadherin. We believe the present study may shed new light and new research on the adhesion of tumor cells of GBM and AAS.

V、Conclusions

- 1) No expression of E-cadherin was detected outside the arachnoid membrane.
- 2) N-Cadherin seems useful in identifying neurons.
- 3) Decreased expression of N-cadherin at the time of recurrence was observed in cases of glioblastoma that became disseminated, suggesting decreased N-cadherin contributes to seeding of tumor cells.

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VII. Legends of Figure

Fig. 1a: Immunohistochemical staining for E-cadherin in a normal colon. Cell membrane and intracellular granular bodies express E-cadherin. (X200)

b: Immunohistochemical staining for E-cadherin in a normal brain. E-cadherin expression is not seen in normal brain tissue except for the arachnoid membrane. (X100)

Fig. 2 Immunohistochemical staining for N-cadherin in normal brain tissue. N-cadherin expression is seen in neurons and small neurons. (X100)

Fig. 3 These microscopic figures are serial sections of normal brain tissues .

a: GFAP staining is expressed in normal astrocytes. (X200)

b: But in the same astrocytes N-cadherin staining is not expressed. (X200)

Fig. 4 N-cadherin expression in various structures of astrocytic tumors.

a: Degenerative neurons of case 1 are strongly expressed by N-cadherin. (X200)

b: Intratumoral vessels of case 6, such as endothelial proliferations, are expressed by N-cadherin. (X100)

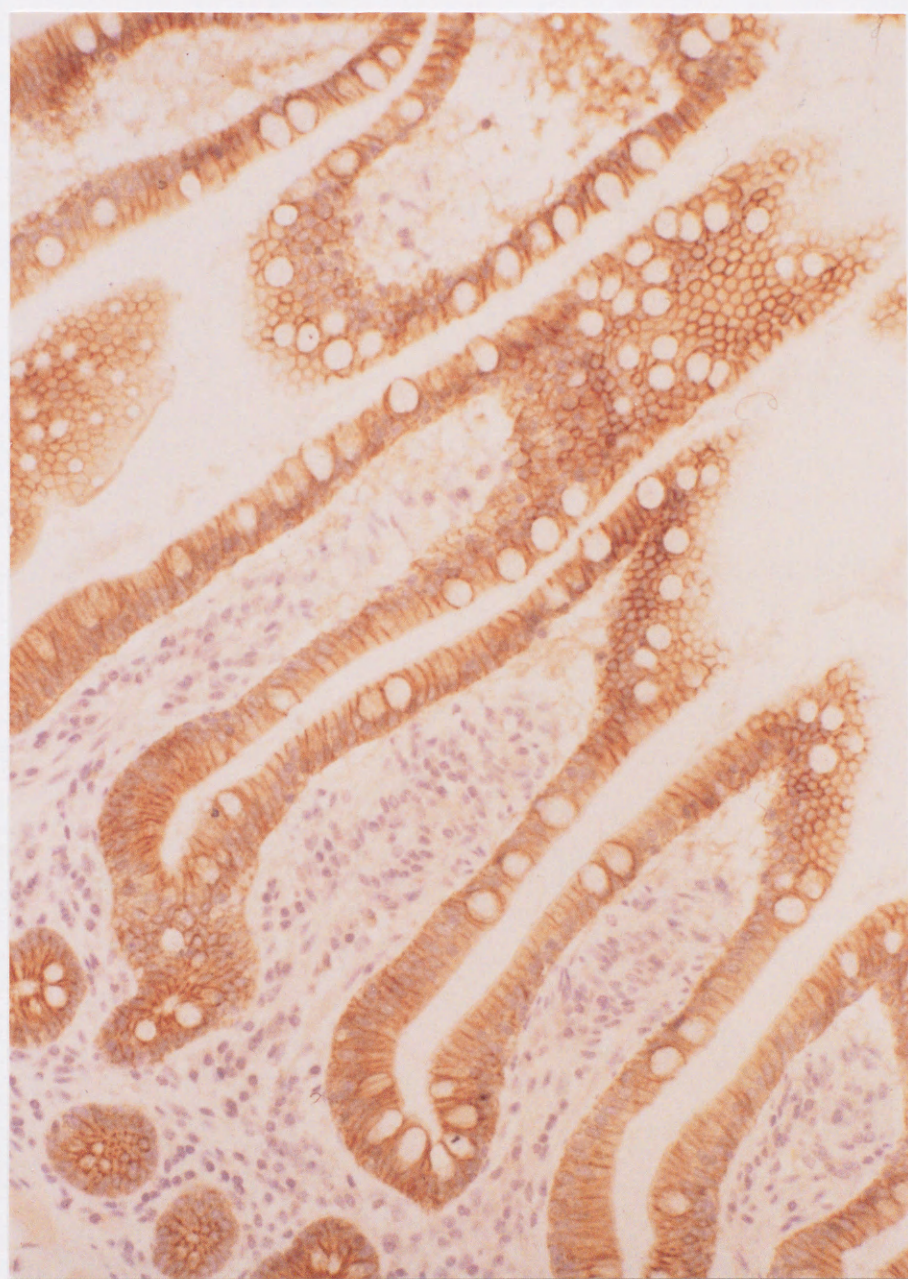
c: Tumor cells that consist of gliovascular rosettes of case 3 are extremely strongly expressed by N-cadherin. (X100)

d: Tumor cells that consisted of perinecrotic pseudopalisading of case 9 are not expressed. (X40)

Fig. 5 Difference of 1st operation tissue and 2nd operation tissue of case 8 by N-cadherin.

a: First operation materials of case 8 are strongly expressed by N-cadherin. (X100)

b: But 2nd-operation materials of case 8 are extremely reduced compared to 1st-operation materials. (X100)



↑

Fig 1. a

