CLINICAL ARTICLE

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Differential Expression of the Tight Junction Protein, Occludin, in Brain Tumors

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Objective : Cerebral edema develops in the brain tumors by loosening of the endothelial tight junction. Tight junction(TJ) proteins, such as occludin and claudin bind adjacent cells tightly. Authors examine the expression rate of occludin in human brain tumors to evaluate the effect of altered expression of occludin on cerebral edema.

Methods : Seventy surgical specimens stored at -70°C were used. It included 14 astrocytic tumors, 27 meningiomas, 12 scwannomas, 7 pituitary adenomas, 6 hemangioblastomas, and 4 craniopharyngiomas. After protein extraction, expression of occludin was investigated by Western blot analysis. The tumors were classified according to World Health Organization(WHO) classification.

Results : The expression rates of occludin in brain tumors were : glioma (8/14=57.1%), meningioma (16/27=59.3%), schwannoma (10/12=83.3%), pituitary adenoma (6/7=85.7%), hemangioblastoma (6/6=100%), and craniopharyngioma (3/4=75.0%). The expression rate in glioma and meningioma was lower than other brain tumors. In gliomas, high grade tumor (1/4=25.0%) exhibited lower expression rate of occludin than low grade one (7/10=70.0%).

Conclusion : These results suggest that the expression of occludin is different among the various kinds of brain tumors. In gliomas, its expression is correlated with the histological grade. It may indicate that occludin plays a role in the development of edema in the brain tumors.

KEY WORDS : Brain neoplasm · Occludin · Tight junction · Brain edema.

Introduction

Cerebral edema, which increase intracranial pressure, leading to brain ischemia and herniation that contribute to morbidity and mortality[28]. Malignant brain tumors cause brain edema because their microvessel leak fluid from the lumen into the brain[16]. The microvessels of metastatic brain tumors also exhibit the characteristics of those in the parent tissue and fail to form tight junctions[20]. This hypothesis was confirmed by demonstrating increased permeability in human brain tumor, plasma protein immunoreactivity in human glioblastoma, and that edema fluid is an ultrafiltrate of plasma[9,10,27]. Up to date, tight junctions(TJs) proteins have been known such as, occludin, claudin, and junctional adhesion molecule that bind across adjacent cell membranes 'gluing' them together[7,8,12,22,23]. Occludin, the best-characterized transmembrane protein, is an essential functional component of tight junctions. It was widely expressed by essentially all epithelial and endothelial tissue[8,12]. In non-neoplastic human brain microvessels, occludin was expressed in 60% by immunoblotting[25]. 55-kDa occludin may be functionally important in human brain microvessels because loss of its expression strongly correlates with opening of the blood-brain barrier(BBB). However, the expression of TJ protein, occludin has mostly been investigated in gliomas[24]. This study was performed to determine the expression rate of occludin in human brain tumors and to verify the correlation of occludin expression to histological malignancy of gliomas.

Materials and Methods

Patients

Seventy patients who underwent surgery as brain tumors

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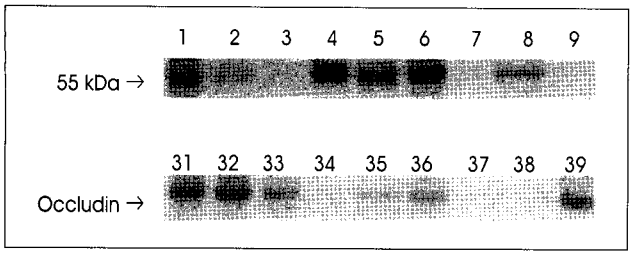

Fig. 1. Western blot analysis for detection of occludin expression immunoblotting by 1:1000 occludin monoclonal antibody. Note the presence of 55 kDa bands of occludin in lanes 1-2, 4-6, 8, 31-33, 35-36, and 39.

between January 2001 and December 2002 provided tissue specimens. All brain tissues were collected during craniotomies for their resection or biopsy and immediately stored in the deep freezer at -70°C prior to experimental study. Brain tissues were also examined and classified by a neuropathologist. Gliomas were classified as low (grades I-II) or high (grades III-IV) grade.

Western blot analysis

Brain tumor tissues that had been stored in the deep freezer were rinsed three times with phosphate-buffered saline (PBS), and lysed using a homogenization buffer containing 0.1M sodium phosphate buffer, pH6.1, 1mM EDTA, 1mM DTT, 0.1mM PMSF, and 1mM benzamidine1%. The homogenates were centrifuged for 15min at 12000rpm at 4°C. Their supernatants were diluted by the mixed solution of Laemmli sample buffer (SDS reducing buffer : 62.5mM Tris-HCl, pH6.8, 20% Glycerol, 2% SDS, 5% β,-Mercaptoethanol) 950μℓ and β-Mercaptoethanol 50μℓ at the ratio of 1:2, and then, boiled for 5min. After protein quantification performed by using the DC Protein Assay (Bio-Rad, Hercules, CA, USA), 15μg of proteins were loaded on each lane and subjected to SDS-PAGE on 10% acrylamide gel. Protein marker was used by the prestained SDS-PAGE standard (Bio-Rad Labs Ltd., Hemel Hemstead, Hertfordshire, UK). After electrophoresis, gel was equlibrated with towbin buffer (25mM Tris, 192mM glycine, 20% methanol) for 60min and proteins were then transferred overnight onto polyvinylidene difluoride(PVDF) membranes (Bio-Rad, Hercules, CA, USA). Membranes were saturated for 1h using 5% dry milk in TBS-T (10mM Tris-buffer pH 7.5, 100mM NaCl, 0.1% Tween 20) and incubated for overnight with 1:1000 occludin monoclonal antibody (Zymed, San Francisco, CA, USA) at 4°C. The membranes were then incubated for 1h with a 1:2000 mouse anti-mouse IgG antibody conjugated to alkaline phosphatase (Zymed, San Francisco, CA, USA) as secondary antibody. The detection followed with the ECL detection system (Amersham, Arlington Height, IL, USA). Blots were washed, and bands were visualized by ECL plus (Amersham, Arlington Heights, IL, USA) and autoradiography (Fig. 1).

Statistical analysis

The data were analyzed using SPSS 10.0 for windows (SPSS inc, Chicago. IL, USA). Statistical difference of occludin expression was analyzed by Kruskal-Wallis test and Fisher's exact test was employed to compare proportions in gliomas. Differences with a p-value of 0.05 or less regards as statistically significant.

Results

Patient characteristics

Seventy patients were enrolled in the study and their mean age was 43.6years (range, 2~79years). Twenty-six patients were male and 44patients were female. The study group was included 14 glioma patients who were consisted of 10 (14.3%) patients with low grade tumor and 4 patients (5.7%) with high grade tumors, 27 (38.6%) meningioma patients, 12 (17.1%) schwannoma patients, 7 (10.0%) pituitary adenoma patients, 6 (8.6%) hemangioblastoma patients, and 4 (6.0%) craniopharyngioma patients (Table 1).

Expression of occludin

As illustrated in figure 1 for representative examples, occludin was detected in specimen from most tumor types.

Table 1. Classification of 70patients with brain tumor and occludin expression according to pathological types

Pathological type	No. of patients(%)	Occludin expression(%)
Glioma	14 (20.0)	8 (57.1)
Low grade	10 (14.3)	7 (70.0)
High grade	4 (5.7)	1 (25.0)
Meningioma	27 (38.6)	16 (59.3)
Schwannoma	12 (17.1)	10 (83.3)
Pituitary adenoma	7 (10.0)	6 (85.7)
Hemangioblastoma	6 (8.6)	6 (100)
Craniopharyngioma	4 (6.0)	3 (75.0)
Total	70	49 (70.0)

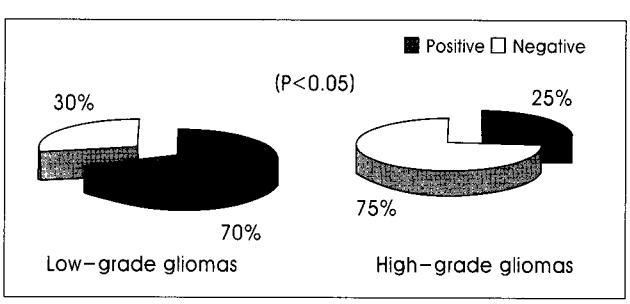

Fig. 2. Comparison of occludin expression between the low grade and high grade gliomas. Occludin expression is significantly elevated in the low grade gliomas.

Positive expression of occludin was shown in 49 (70.0%) of 70patients (Table 1). Expression rate of occludin was found in hemangioblastomas (100%), pituitary adenomas (85.7%), and schwannomas (83.3%). Occludin expression was relatively low in gliomas (57.1%) and meningiomas (59.3%). Its difference of expression according to pathological type was statistically significant (p=0.031 by Kruskal-Wallis test). In patients with glioma, the frequency of occludin expression in tumor samples was significantly correlated to the histological grade of glioma (p=0.004 by Fisher's exact test) (Fig. 2).

Discussion

The present study has shown that 55-kDa occludin was expressed in various pathology of brain tumors and its expression was significantly different each other. Brain tumors, such as gliomas and meningiomas that have accompanied the significant brain edema expressed the low level of occludin, however, brain tumors accompanying less brain edema like hemangioblastomas and pituitary adenomas demonstrated the high expression of occludin. Occludin expression was also increased in low-grade gliomas comparable to high-grade gliomas.

Ultrastructural studies of human glioma microvessels have revealed 'open' interendothelial cell TJs[2]. TJs not only separate distinct physiological compartment, but they also confer selectivity to the transepithelial flux of molecules and ions through the intercellular spaces between the epithelial cells, the so-called paracellular pathway[11]. Occludin and claudin are transmembrane proteins of the TJ that probably participate in sealing the paracellular space[7]. Occludin was the first transmembrane protein of TJs that identified[8]. The expression level of occludin thus directly affects the strength of the junctional seal in a manner that appears to depend on the genetic background of the analyzed cells. A possible explanation of this is that occludin is a regulatory component of the TJ seal[26]. A recent study reported that occludin also participated in the regulation of signaling processes controlling epithelial transformation[18]. Claudins were more recently discovered and a family of TJ proteins of 22-24kDa[7]. Claudin-1 and -5 together with occludin are the most important constituents of BBB TJs[19]. The expression of TJ protein claudin-1 was lost in the majority of tumor microvessels, whereas claudin-5 and occludin were significantly downregulated only in hyperplastic vessels[19]. However, there is no evidence for a direct interaction between occludin and claudins[1]. Computed tomography(CT) enhancement after administration of contrast is a function of vascularity and the permeability of the BBB. Delayed CT enhancement, thus reflects BBB disruption rather than increased vascularity, which is also a feature of malignant brain tumors[10,21]. Expression of occludin also inversely correlated with the presence of contrast enhancement on CT scans[25]. The microvessels of metastatic brain tumors also exhibit the characteristic of those in the parent tissue and fail to form TJs[20]. In most microvessels of high grade brain astrocytoma, the BBB is also molecularly altered as revealed by changes in the expression of junctional proteins. This suggests a correlation between the molecular and the morphological alterations of TJ[19].

Vascular endothelial growth factor(VEGF) is a peptide produced by astrocytes and adenocarcinoma cells, which has been shown to increase microvessel endothelial permeability in vivo and in vitro[3,4]. Scatter factor/hepatocyte growth factor(SF/HGF) is a cytokine that increases endothelial permeability, is expressed by astrocytomas in vivo and is upregulated during the transition from low grade to malignant astrocytomas[17]. VEGF and SF/HGF may thus be important in the pathogenesis of brain tumor edema by inducing a reduction in the expression of endothelial TJ proteins including occludin[14,15]. Otherwise, dexamethasone is the mainstay of brain tumor edema treatment[13]. However, it is unlikely that dexamethasone has any effect on 55-kDa occludin expression in non-neoplastic human brain microvessels[25]. Treatment of primary human astrocytes with the cytokine interleukin-1β (IL-1β) caused down regulation of occludin but no change in expression of zonula occludens(ZO) proteins ZO-1 and-2. This suggested that in pathological conditions of the human central nervous system, elevated IL-1β expression fundamentally altered astrocyte-to-astrocyte connectivity[6].

Occludin bands have been variously identified as splice variants and phosphorylated forms[24]. The hyperphosphorylated form of occludin appears to play an important role in the functional assembly of TJs[29]. The 55 kDa occludin was present in non-neoplastic brain and there was a significant inverse correlation between occludin expression and increasing malignancy[25]. Our results in gliomas were also coincided with this description. Electron microscopy revealed TJ opening in high grade astrocytoma microvessels. Expression of the TJ protein occludin was reduced in these microvessels and this reduction was inversely correlated with the degree of cerbral edema. Normal astrocytes secreted factors that have induced barrier properties in endothelial cells, whereas high grade astrocytomas secreted VEGF, which has stimulated angiogenesis, down regulated occludin and increased endothelial cell permeability.

Otherwise, expression level and role of occludin have not studied except gliomas and metastatic brain tumors. In our study, there was a tendency that brain tumors accompanying

brain edema were correlated with less expression of occludin. However, the pathophysiology of brain edema is multifactorial, but that there may be common process operating regardless of the etiology[5].

The limitation of this study was that tumor pathology was heterogenous and the number was too small to reach statistical significance in each pathological groups. And also, volume of peritumoral edema was not evaluated with occludin expression. Further studies to understand TJ protein expression in brain tumor should be performed to facilitate the development of new anti-edema medication.

Conclusion

The results suggest that differential expression of occludin to the pathological types of primary brain tumors and its expression in glioma is higher in low grade tumor than in high grade one. A more understanding of TJ protein expression in brain tumors may allow the development of novel drugs that selectively control the BBB.

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