[J Tokyo Wom Med Univ
81 (Extra) E9～E13 (2011)]

Short Report

Expression of Aquaporine 1 and 4 in Meningiomas: An Immunohistochemical Study

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(Accepted February 25, 2011)

A family of water channel protein called aquaporines (AQP) are small membrane-spanning proteins that are expressed at plasma membranes. In the central nervous system, eight AQPs (1, 3, 4, 5, 6, 8, 9, and 11) were expressed. AQP4 was expressed in astrocytes and AQP1 in cholloid plexus, predominantly. Recently some AQPs played a role in cell migration and proliferation. To elucidate the relationship between histological grades of meningioma and the expressions of AQP1 and 4, we investigated the expression of AQP1 and 4 in meningiomas. In grade 1, faint positive reactions for AQP1 in some endothelial cells of neovasculature in small areas were found, but not for AQP4. In grade 2, immunoreactions for AQP1 and 4 were detected in endothelial cells of neovasculature. In addition, positive reactions for AQP1 were recognized in some tumor cells and stromal cells. Our results suggested the evidence of the role of AQPs in the differentiation and biological behaviors of meningiomas.

Key Words: aquaporine, meningioma, immunohistochemistry

Introduction

Brain edema, associated with various cerebral injuries, is a critical and crucial event as the accumulation of excessive fluids can cause fatal cerebral herniation. Water molecular channels called aquaporines (AQP) are small membrane-spanning proteins expressed at plasma membranes[1)2)]. There are thirteen AQPs which are divided into 3 groups. Recently AQSs have been demonstrated to play a role in cell migration[3)] and proliferation[4)]. In the central nervous system, eight AQPs (1, 3, 4, 5, 6, 8, 9, and 11) are expressed. AQP4 is expressed in astrocytes and AQP1 in cholloid plexus, predominantly.

Meningiomas are tumors arising from arachnoid cells of dura mater which are frequently associated with peritumoral edema. Although we examined the expressions of AQP4 in grade 1 meningiomas, negative findings were obtained in tumor cells[5)]. To our knowledge, only a few studies have been reported in the past on AQP expression in meningiomas[6)]. In this study, we investigated the expression of AQP1 and 4 in meningiomas.

Materials and Methods

Specimens from 16 resected meningiomas at our hospital and consultation cases from other hospitals were studied (Table). Written informed consent was obtained from all subjects including those from consultation cases. Tumors were reclassified according to the 2007 WHO classification by one neuropathologist (T.S.). No information on corticosteroid treatment, including dexamethasone or cerebral edema surrounding the meningiomas was obtained in this study (Table).

Immunostainings

Tissue specimens were fixed in 20% formaldehyde, dehydrated, and embedded in paraffin. Each specimen was processed for conventional histology to verify the nature of the tissue.

Deparaffinized sections (5 μm thickness) were treated with methanol containing 0.3% H_2O_2 for 30 minutes and rinsed in phosphate-buffered saline (pH 7.4). Sections were then incubated with antihuman AQP1, antihuman AQP4 polyclonal antibody (H80, diluted 1:50; Santa Cruz Biotechnology, Santa Cruz, CA, USA), antihuman VEGF polyclonal anti-

Table Summary of cases and results

	Age	Gender		Grade	MIB-1LI
1	48	F	Transitional	1	>0.5
2	57	F	Angiomatous	1	>0.5
3	45	F	Meningotheliomatous	1	>0.5
4	47	M	Transitional	1	>0.5
5	56	F	Meningotheliomatous	1	>0.5
6	64	M	Meningotheliomatous	1	>0.5
7	35	M	Meningotheliomatous	1	>0.5
8	49	F	Meningotheliomatous	1	>0.5
9	55	F	Meningotheliomatous	1	>0.5
10	63	F	Meningotheliomatous	1	>0.5
11	63	F	Meningotheliomatous	1	>0.5
12	61	F	Atypical meningioma	2	5.0%
13	58	F	Atypical meningioma	2	3.5%
14	46	M	Clear cell meningioma	2	1.5%
15	47	F	Atypical meningioma	2	2.5%
16	43	M	Clear cell meningioma	2	4.3%

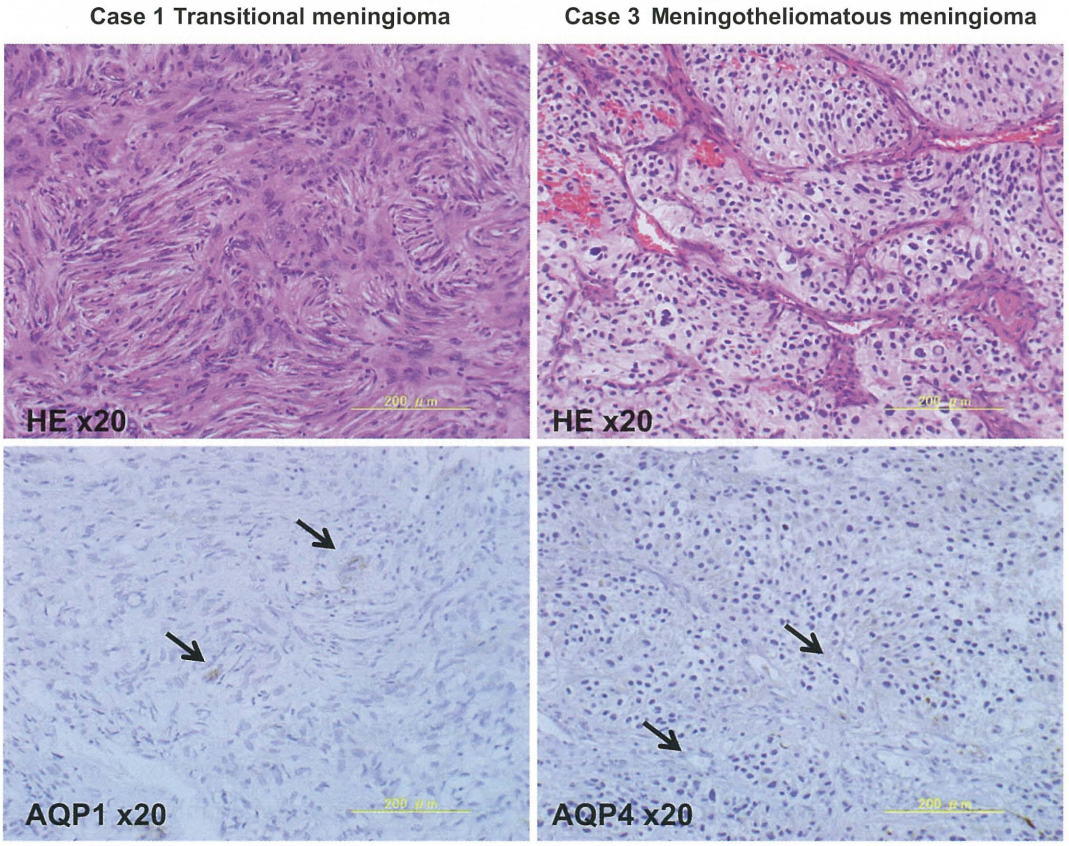

Fig. 1 Histological and immunohistochemical findings of grade 1 meningioma
Upper left: Histological findings of case 1 (HE staining ×20).
Upper right: Histological findings of case 3 (HE staining ×20).
Lower left: Immunohistochemical staining for AQP4. No positive reactions for AQP4 in tumor cells of endothelial cells (×20).
Lower right: Immunohistochemical staining for AQP1. Tumor cell showed negative for AQP1. Arrow indicated faint and positive reactions for AQP1 in endothelial cells (×20).

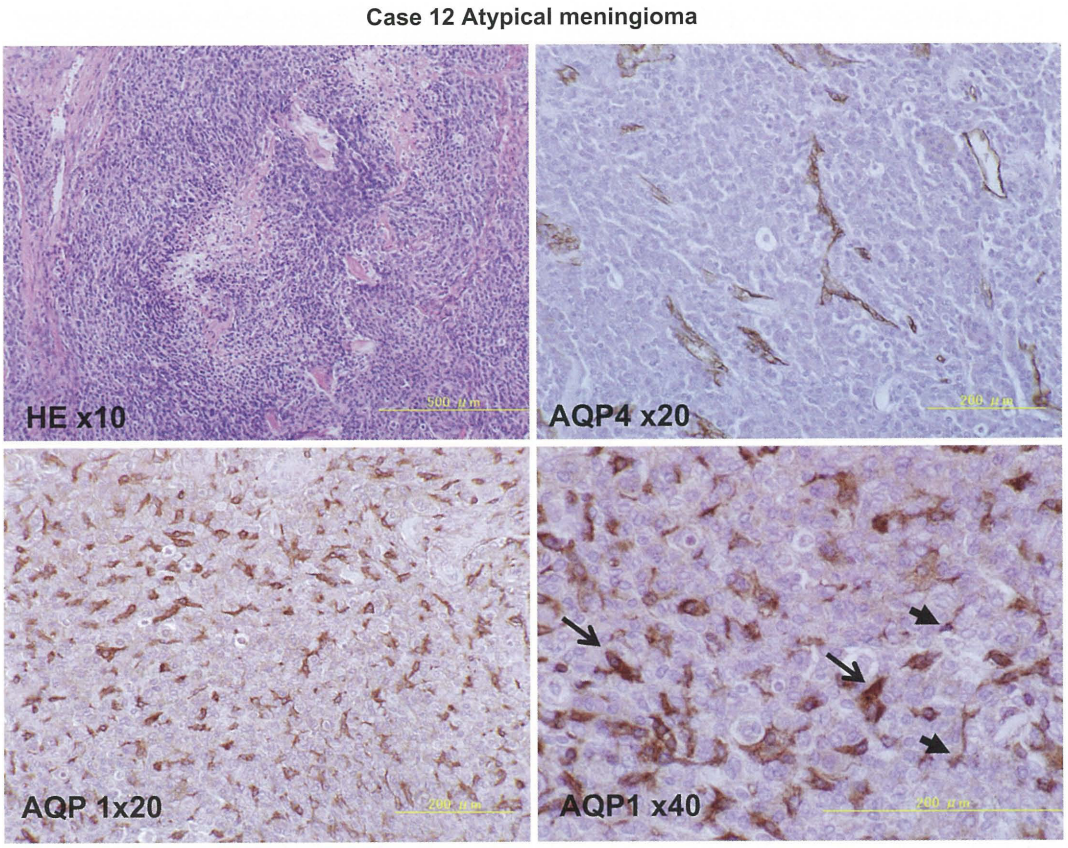

Fig. 2 Histological and immunohistochemical findings of grade 2 meningioma
Upper left: Histological findings of case 12 (HE staining ×10). High cellularity and coagulation necrosis.
Upper right: Immunohistochemical staining for AQP4. Positive reaction in endothelial cells.
Lower left and right: Immunohistochemical staining for AQP1. Positive reaction in endothelial cells (arrowheads), and some tumor cells (arrows).

body (C-1, diluted 1:1000; Santa Cruz Biotechnology, CA, USA) and antihuman MIB-1LI monoclonal antibody (diluted 1:100; DAKO, Kyoto, Japan). Before the reaction with anti-MIB-1 antibody, sections were pretreated in 10 mM Tris-Cl; 1 mM ethylene diamine tetra acetic acid (EDTA) buffer (pH 9.0) for 40 minutes in a microwave. Reaction products were visualized using a Vectastain avidin-biotin-peroxidase complex (ABC) kit (Vector Laboratories, Burlingame, CA, USA).

Immunoreactivity was visualized by using 0.025% 3.3'-diaminobenzidine tetrachloride/0/001% H_2O_2 (Dojin, Kumamoto, Japan). These slides were washed with phosphate-buffered saline (pH 7.4). The sections were counterstained with hematoxylin for a few seconds, dehydrated through graded alcohol, and coverslipped.

Results

The results of immunohistochemistry for MIB-1LI, AQP4 and VEGF of grade 1 meningioma were reported in a previous study[5]. High MIB-1LI were noted in grade 2 meningiomas compared with grade 1 meningiomas, immunoreaction for AQP1 were recognized only in endothelial cells in neovasculature of grade 1 (Fig. 1). In grade 2 meningiomas, immunoreactions for AQP1 were detected in endothelial cells in neovasculature, and some tumor cells (Fig. 2). No significant differences in immunoreactivity for AQP4 were noted in tumor cells between grade 1 and 2 meningiomas. No positive reactions were identified for AQP4 in tumor cells but were identified in endothelial cells of grade 2 meningiomas.

Positive reactions for VEGF-A were recognized in endothelial cells in grade 1 and grade 2 men-

ingiomas, in endothelial cells of grade 2 meningiomas, positive correlations were noted between VEGF-A and AQP1.

Discussion

Recent researches have elucidated that the physiological role of AQPs expands from water channels to other functions associated with tumor biology. Firstly, AQPs were found in high-grade malignant tumors and the expression of AQPs was correlated with metastatic potentials of malignant tumors, promoting tumor cell migrations.

Peritumoral edema is frequently associated with meningiomas (50-92%)[7]. However, only a few studies have been reported on the expression of AQPs in meningiomas. Ng et al reported that increased expression of AQP4 was associated with peritumoral edema in meningiomas[6], but all cases studied were grade 1 meningiomas and did not include grade 2 or 3 meningiomas. Our previous report showed no expression of AQP4 in tumor cells of grade 1 meningiomas[5].

Marton et al reported on the overexpression of AQP1 in atypical meningiomas in infancy[8]. Longatti et al indicated that dural invasion of meningiomas facilitated expressions of AQP1, suggesting some physiological role in cystic formation of cholloid plexus tumors[9]. In this study, we could obtain positive reactions of AQP1 in grade 2 meningiomas but not in grade 1 meningiomas. The biological roles of AQP1 for arachnoid cell differentiation were uncertain, but our results suggest that AQPs have some role in the differentiation of meningiomas.

In this study no information about cerebral edema could be obtained. Further studies are necessary to clarify the relations between cerebral edema and the expressions of AQPs of meningiomas.

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髄膜腫における aquaporine 1 および 4 の発現—免疫組織化学的検索

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Aquaporines は現在 13 種類が知られ，family を形成する蛋白で，各臓器に特異的に存在し，水チャンネルを調整する蛋白として知られている．近年，腫瘍の増殖，分化，遊走能にも影響を与えることが知られている．髄膜腫は腫瘍周囲の脳実質に著明な浮腫を起こすことで知られているが，一部の異型髄膜腫で AQP の発現の増強が報告されている．今回我々は 16 例の髄膜腫（Grade 1：11 例，Grade 2：5 例）における AQP1 および 4 の発現を免疫組織化学的に検討した．Grade 1 の髄膜腫では一部の新生血管内皮細胞に僅かな陽性所見を認めるのみであった．Grade 2 の髄膜腫では AQP1 および 4 の新生血管内皮における発現の増強が認められた．AQP1 は一部の腫瘍細胞および間質細胞に陽性所見が認められた．Grade 2 の髄膜腫では Grade 1 の髄膜腫に比して MIB-1LI も高く，髄膜腫の生物学的悪性度に AQP1 が何らかの影響を及ぼしていることが示唆された．