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EXPRESSION OF THE AQUAPORIN-1 WATER CHANNEL IN HUMAN GLIAL TUMORS

OBJECTIVE: Malignant glial tumors are associated with cerebral edema. The aquaporins (AQPs) are a family of membrane proteins that provide a major pathway for water transport in mammals. In the central nervous system, AQP1 is selectively expressed in the choroid plexus and thought to participate in cerebrospinal fluid production. Prior studies have suggested that AQP1 may be up-regulated in glial tumors, potentially contributing to tumor-associated edema. The objective of this study was to investigate the expression of AQP1 in a large series of human glial tumors.

METHODS: Thirty-six human glial tumors were obtained from the University of California, San Francisco Neurosurgery Tissue Bank. AQP1 expression was evaluated by reverse transcriptase polymerase chain reaction, complementary deoxyribonucleic acid gene array, Western blot analysis, and immunohistochemical analyses.

RESULTS: AQP1, normally restricted to choroid epithelia, was highly expressed in glioblastomas. Complementary deoxyribonucleic acid array, Western blot analysis, and immunohistochemical analysis revealed intense up-regulation of AQP1 expression in all glioblastomas studied.

CONCLUSION: The abnormal up-regulation of AQP1 in glial tumors suggests a potential pathological role for this membrane water channel and raises the possibility that selective AQP1 inhibition might offer a new therapeutic target for treatment of tumor-associated edema.

KEY WORDS: Aquaporins, Brain tumor, Edema, Gene array, Reverse transcriptase-polymerase chain reaction

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A family of molecular water channels called aquaporins (AQPs) has been identified in mammals. AQPs are small integral membrane proteins (molecular weight, ~30,000) that provide a major pathway for water transport in the kidney, brain, secretory epithelia, and other organs (16). Several AQPs have been identified in regions of the central nervous system that are thought to participate in the production and absorption of brain fluid (2, 6, 17). AQP1, first identified in red blood cells and renal proximal tubular epithelium (3), is expressed in the ventricular-facing membrane of the choroid plexus epithelium. In the kidney, AQP1 functions primarily as a water pore to facilitate transmembrane transport of water driven by osmotic gradients. The specific localization of AQP1 in the choroid plexus suggests that it plays an important role in facilitating water transport across the choroid plexus epithelium apical membrane during secretion of cerebrospinal fluid (5, 8, 13). AQP4 is expressed in astrocytic end-feet at the blood-brain barrier and plays an important role in cerebral water balance and development of brain edema (6). AQP9 is also expressed in astrocytes and is up-regulated after ischemia (1).

Expression of AQPs has been documented in a small group of human brain tumors. Endo et al. (4) demonstrated AQP1 immunoreactivity on glioblastoma cell lines implanted in rodents. Differential gene expression analysis identified up-regulation of AQP1 in four primary human glioblastomas multiforme (7). Saadoun et al. (11) confirmed AQP1 up-regulation by immunohistochemistry in five high-grade astrocytomas. AQP4, which normally is expressed on the foot processes of astrocytes, also seems to be up-regulated in

glioblastomas (12). The purpose of this study was to characterize AQP1 expression in larger number of human glial tumors by reverse transcriptase-polymerase chain reaction (RT-PCR), Western blot, immunohistochemistry, and differential gene expression analysis.

PATIENTS AND METHODS

RT-PCR Analysis

Human brain tumors were immediately homogenized in Trizol reagent (Invitrogen Corp., Carlsbad, CA) for messenger ribonucleic acid (mRNA) isolation by use of the Oligotex mRNA mini kit (Qiagen, Studio City, CA). The complementary deoxyribonucleic acids (cDNAs) were generated from mRNA by SuperScriptII (Invitrogen Corp.) ribonuclease H reverse transcriptase primed with oligo$(dT)_{18}$ and random hexamers. After reverse transcription, PCR products were amplified with gene-specific primers designed to amplify a portion of the coding sequences of each of the nine known human AQPs: AQP1 through AQP9. The reactions contained 1 μl of the RT reaction as template cDNA and were performed for 30 cycles, in a 30-second, 94°C denaturation step; a 30-second, 60°C annealing step; and a 1-minute, 72°C extension step. PCR products were separated on 2% agarose gels and visualized with ethidium bromide staining.

Immunohistochemical Analysis

Fresh brain tumor tissue was fixed overnight in 10% formalin (Fisher Scientific, Pittsburgh, PA). The next day, the tissue was dehydrated in ethyl alcohol and xylene, paraffin-mounted, and sectioned at 8-μm thickness. Sections were embedded on slides and stored at room temperature. For immunohistochemical analysis, slides were warmed to 60°C for 10 minutes. Tissue was rehydrated and rinsed in 1× phosphate-buffered saline (PBS) (pH 7.4). Endogenous peroxidase activity was quenched by immersion in 3% H_2O_2 in methanol for 10 minutes. A nonspecific blocking procedure with 5% normal goat serum (Vector Laboratories, Inc., Burlingame, CA) was performed before application of primary antibodies. Affinity-purified rabbit polyclonal antirat AQP1 antibody (Chemicon International, Temecula, CA) was used. This antibody is raised against a 19-amino acid synthetic peptide (251–269) from the cytosolic carboxy-terminal domain of AQP1. The slides were incubated overnight in anti-AQP1 antibody (1:1000) at 4°C in PBS containing 5% normal goat serum, pH 7.4. The next day, sections were incubated in a 1:500 biotinylated goat antirabbit immunoglobulin G secondary antibody (Vector Laboratories, Inc.) for 1 hour at room temperature in PBS containing 5% normal goat serum, pH 7.4, and then incubated in 1:100 Vectastain ABC reagent (Vector Laboratories, Inc.) for 1 hour at room temperature in PBS containing 5% normal goat serum, pH 7.4. Diaminobenzidine (Sigma Chemical Co., St. Louis, MO) was used to develop the reaction product. After multiple rinses in PBS and a final rinse in distilled water, sections were counterstained with hematoxylin (Biomeda, Foster City, CA), dehydrated, cleared, and coverslipped.

Western Blot Analysis

Western blot analysis was performed on fresh frozen brain tumor tissue or on control brain (obtained from resection for epilepsy). Samples were homogenized in buffer (250 mmol/L sucrose; 10 mmol/L Tris·HCl, pH 7.4; and 20 μg/ml phenylmethylsulfonyl fluoride) and centrifuged at 1000 rpm for 10 minutes at 4°C. The soluble fraction was dissolved in sodium dodecyl sulfate (Pierce, Rockford, IL) and centrifuged at 10000 rpm for 10 minutes at 4°C. Protein concentration was determined by Bradford assay (Bio-Rad, Hercules, CA). Ten micrograms of each sample were loaded onto 12% Tris-glycine gels (Invitrogen Novex, Carlsbad, CA) in Tris-glycine sodium dodecyl sulfate buffer (Invitrogen Novex), and run at 90 V for 2 hours at room temperature. The gel was transferred to a polyvinylidene fluoride membrane overnight at 4°C. The membrane was rinsed with Tris-buffered saline (TBS)-T buffer (0.02 mol/L Tris, 0.225 mol/L NaCl, 0.1% Triton, and distilled water), and then placed in blocking solution of TBS-T with 3% nonfat milk (Sigma Chemical Co.) for 30 minutes before application of primary antibodies at room temperature. Anti-AQP1 primary antibody (1:1000) was incubated overnight at 4°C in TBS-T buffer containing 1% bovine serum albumin (Sigma Chemical Co.), pH 7.6. After three rinses in TBS-T buffer, the membrane was incubated in horseradish peroxidase-linked donkey anti-rabbit immunoglobulin G (Amersham Pharmacia, Piscataway, NJ) diluted 1:5000 in TBS-T buffer containing 1% bovine serum albumin, pH 7.6, for 30 minutes at room temperature. After rinsing in TBS-T buffer, the membrane was incubated with ECL Plus chemiluminescent detection reagents (Amersham Pharmacia Biotech, Piscataway, NJ) for 5 minutes and exposed to film (Hyperfilm ELC; Amersham Pharmacia Biotech).

Gene Array Analysis

Total RNA was extracted from freshly resected and snap-frozen tumor samples stored at −80°C with Trizol reagent. Both primary (n = 26) and recurrent (n = 4) glioblastomas were analyzed in duplicate. In some cases, more than one independent sample from each tumor was processed to examine the variability of gene expression within the tumor. Three separate samples of brain tissue (cerebellum, left motor cortex, and right motor cortex) were used as controls. A sample of gliotic tissue without tumor also was included as a control. mRNA was isolated from the total RNA samples in accordance with the manufacturer's protocol for the Fastrack kit (Invitrogen Corp.). Double-stranded cDNA was synthesized with a Life Technologies Superscript cDNA Synthesis System (Invitrogen Corp.) by use of oligo$(dT)_{24}$ primers with a T7 RNA polymerase promoter site added to its 3' end (Genset, La Jolla, CA). The isolated cDNA was used for in vitro transcription with the T7 Megascript system (Ambion, Austin, TX) in the presence of biotin-11-cytidine 5'-triphosphate and biotin-

16-uridine 5'-triphosphate (Enzo Diagnostics, New York, NY). Next, 25 to 50 μg of the cRNA product in buffer (40 mmol/L Tris-acetate, pH 8.1/100 mmol/L potassium acetate, 30 mmol/L magnesium acetate) was fragmented at 94°C for 35 minutes. It was then used as a hybridization mix with 0.1 mg/ml herring sperm DNA (Sigma Chemical Co.), plus four control bacterial and phage cRNA (1.5 pmol/L BioB, 5 pmol/L BioC, 25 pmol/L BioD, and 100 pmol/L Cre) samples to serve as internal controls for hybridization efficiency as directed by the manufacturer (Affymetrix, Santa Clara, CA). Aliquots of the hybridization cRNA mixtures (10 μg cRNA in 200 μl hybridization mix) were hybridized to each Genechip array (Affymetrix) and washed and scanned with the GeneArray scanner G2500A (Hewlett Packard) according to procedures developed by the manufacturer (Affymetrix). Scanned output files were visually inspected for hybridization artifacts and then analyzed with Genechip 3.1 software. Arrays were scaled to an average intensity of 100 and analyzed independently. The expression level of the AQP1 transcript was calculated by dividing the level of AQP1 expression in the tumor sample by the average level of AQP1 expression from normal brain (three independent samples) to provide the amount of fold increase in gene expression.

Magnetic Resonance Imaging Edema Index

Tumor volume was estimated on preoperative T1-weighted magnetic resonance imaging scans (1 mm slice thickness) with gadolinium enhancement. Peritumoral brain edema was evaluated on T2-weighted fast spin echo scans. Tumor and peritumoral brain edema volumes were approximated similar to the method described by Tamiya et al. (14). Maximum perpendicular diameters of tumor and peritumoral brain edema were measured on axial scans, and the maximum extent in the coronal plane was measured by integrating the number of axial 1-mm slices. Volumes were approximated by use of the formula for an ellipsoid ($V = \frac{4}{3}\pi abc$), where a, b, and c are radii (half diameter of maximum extent). Edema index was calculated as $V_{edema} + V_{tumor}/V_{tumor}$ and as such was always at least 1 (yields a value of 1 when no edema is present).

RESULTS

RT-PCR Analysis

RT-PCR was performed to identify transcripts encoding human AQPs in brain tumor specimens (*Fig. 1*). Reverse-transcribed cDNA prepared from human glioma specimens was PCR-amplified with specific primers for human AQP1 to AQP9. As expected, the glial AQP water channel AQP4 was expressed in all glial tumor samples (data not shown). AQP1, which is normally restricted to choroid plexus epithelia, also was highly expressed in glioblastomas. *Figure 1* shows a representative RT-PCR analysis of AQP1 expression in 4 of the 36 tumors analyzed.

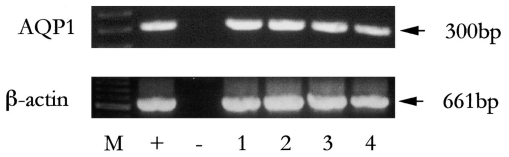

FIGURE 1. *Representative RT-PCR analysis of human glioma specimens. RT-PCR analysis with AQP1-specific primers demonstrated the expression of AQP1 (300-base pair PCR product) in glioma samples (*Lanes 1–4*). Human β-actin (661-base pair PCR product) also was amplified as an internal control. Kidney mRNA was positive for both primer pairs (+). As a negative control, a glioma sample was analyzed without reverse transcriptase (−).* M, *marker.*

Immunohistochemical Analysis

Immunohistochemical analysis was performed on all tumors; representative data from two Grade IV glioblastoma multiforme tumors are shown (*Fig. 2*). *Figure 2A* shows typical Grade IV histological characteristics with anaplastic glial cells and foci of microvascular proliferation. The corresponding AQP1 immunoreactivity from the same tumor is shown in *Figure 2B*. Strong AQP1 immunoreactivity is observed on the membranes of the neoplastic astrocytes. It is interesting that AQP1 immunoreactivity seemed to be localized to astrocyte membranes and was not evident in areas of microvascular proliferation. *Figure 2C* shows a different Grade IV tumor with neoplastic astrocytes and multiple mitoses. Again, the corre-

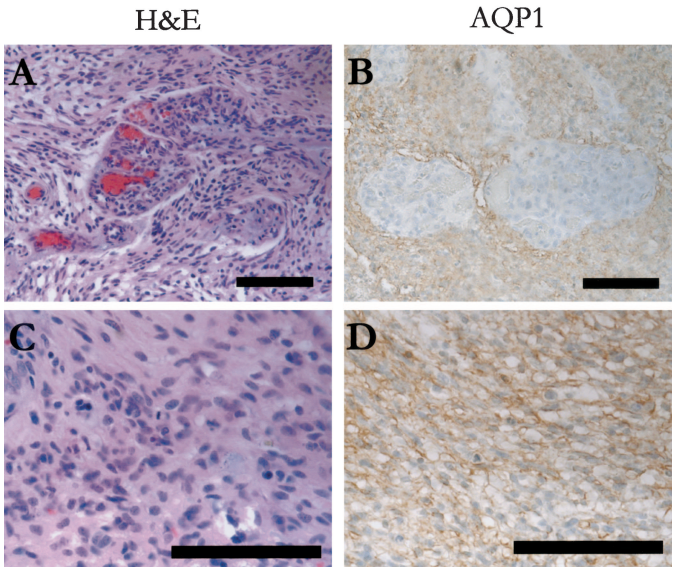

FIGURE 2. *Photomicrographs showing representative immunohistochemical analyses of AQP1 protein expression in human glioma specimens.* A, *glioblastoma multiforme showing multiple foci of microvascular proliferation (hematoxylin and eosin).* B, *immunoperoxidase AQP1 stain showing multiple negative foci of microvascular proliferation but strongly positive immunoreactivity on the membranes of the neoplastic astrocytes.* C, *glioblastoma multiforme composed of neoplastic astrocytes with multiple mitoses (hematoxylin and eosin).* D, *immunoperoxidase AQP1 stain showing strong membrane immunoreactivity.* Scale bars, *50 μm.*

sponding AQP1 tumor immunoreactivity is localized primarily to glial membranes (*Fig. 2D*). AQP1 immunoreactivity in control brain was localized to the choroid plexus as described previously (8) (data not shown).

Western Blot Analysis

Western blot analysis confirmed robust AQP1 protein expression in gliomas (*Fig. 3*). In glial tumors of various grades (Grade II, n = 3; Grade III, n = 3; Grade IV, n = 4), there was a dramatic up-regulation of AQP1 protein expression (*Fig. 3*). In addition, it seemed qualitatively that higher-grade astrocytomas (Grades III and IV) expressed more AQP1 protein than low-grade astrocytomas (Grade II) (*Fig. 3*). In nontumorous brain (obtained from epilepsy resection), there was a faint band at 28 kDa, presumably reflecting low basal levels of expression of AQP1 or possible inclusion of a small amount of choroid plexus with the medial temporal lobe surgical specimen (*N*, *Fig. 3*). Protein from cerebral cortex demonstrated no AQP1 band (−, *Fig. 3*). Protein from mouse and rat cortex also failed to demonstrate any AQP1 expression (data not shown).

Gene Array Analysis

To further study the expression of AQP1 in high-grade astrocytomas, we studied data from an extensive cDNA microarray analysis that included approximately 7000 human genes and expressed sequence tags (as described in Patients and Methods). We compared the expression levels of AQP1 in 26 primary and 4 recurrent glioblastomas with normal brain (*Table 1*). For the primary tumors there were 13 men and 13 women with a mean age of 63 years (range, 28–82 yr). The average expression of AQP1 in these tumors was increased 4.1 ± 0.8-fold higher than that in normal brain. The mean age of patients with recurrent tumors was 37 years (range, 13–69 yr), and the patient group comprised two men and two women. Similar to the primary tumors, the expression of AQP1 in recurrent tumors was increased an average of 3.9 ± 1.3-fold. In all samples, the increased expression of AQP1 was confirmed by immunohistochemical and Western blot analysis (*Figs. 2* and *3*). A control sample of gliotic tissue demonstrated no increase in AQP1 expression.

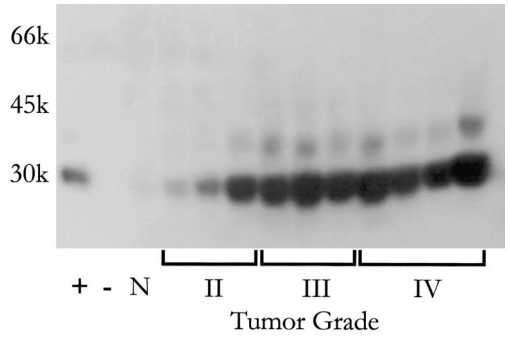

FIGURE 3. *Western blot analysis of ACP1 protein in human glioma specimens. The dense band at approximately 28 kDa is visible in lanes corresponding to glial tumors. +, positive control (kidney); −, negative control (cortex);* N, *human temporal lobe (patient with epilepsy);* Grade II, *astrocytoma;* Grade III, *anaplastic astrocytoma;* Grade IV, *glioblastoma multiforme.*

TABLE 1. Gene array analysis of aquaporin-1 expression in human glioblastomas[a]

Tumor type and patient sex/age (yr)	Fold increase in AQP1 expression[b]
Primary	
M/28	4.4
F/38	3.0
F/67	3.8
F/69	3.9
M/78	5.7
M/66	4.6
M/50	3.8
M/82	4.4
F/61	4.6
F/71	3.2
M/73	3.8
F/61	4.1
F/73	2.9
M/77	4.9
F/55	2.5
M/62	4.1
M/62	3.6
F/73	4.0
F/73	5.4
M/56	4.2
M/79	5.7
F/58	3.9
F/34	3.6
M/67	4.1
F/71	4.9
M/62	4.5
Secondary	
M/13	5.5
F/69	3.4
M/51	4.6
F/54	2.4
No tumor (peritumoral gliosis)	
M/51	1

[a] AQP1, aquaporin-1.
[b] Mean increase ± standard error of the mean: primary tumors, 4.1 ± 0.8 (n = 26); secondary tumors, 3.9 ± 1.3 (n = 4); no tumor, 1.

Magnetic Resonance Imaging Edema Index

Of the tumors studied by Western blot analysis (four Grade IV, three Grade III, and three Grade II), we were able to obtain preoperative magnetic resonance images to calculate edema

index in 70% (7 of 10). Edema index was estimated as described in Patients and Methods. Edema index was higher for high-grade (Grades III and IV) lesions (edema index, 7.4 ± 2.9; n = 4) than for low-grade (Grade II) tumors (edema index, 1.7 ± 0.5; n = 3).

DISCUSSION

By use of RT-PCR, immunohistochemistry, and Western blotting, we demonstrated dramatic up-regulation of the membrane water channel AQP1 in human glial tumors. The DNA microarray analysis confirmed these results and demonstrated approximately a fourfold increase in AQP1 expression in glioblastomas as compared with normal brain.

This study confirms and extends prior analyses of AQP expression in glial tumors. Endo et al. (4) demonstrated AQP1 immunoreactivity in glioblastoma cell lines implanted in rodents, and Markert et al. (7) identified up-regulation of AQP1 in four primary human glioblastomas multiforme. A prior immunohistochemical study confirmed AQP1 up-regulation in astrocytic tumors (five low-grade and five high-grade astrocytomas) (11). In that study, there seemed to be a graded increase in AQP1 from low- to high-grade tumors. Our Western blot analysis of Grade II, III, and IV tumors also suggests qualitatively that low-grade glial tumors may up-regulate AQP1 to a lesser extent than high-grade tumors but still dramatically more than control brain. However, a larger number of astrocytomas, in particular the low-grade lesions, will need to be analyzed to determine whether there are significant quantitative differences among different grades of tumors.

The immunohistochemical data suggest that the increase in AQP1 protein is localized primarily if not exclusively to membranes of neoplastic astrocytes. On the basis of cellular morphology, no AQP1 expression was observed in cerebral endothelium. The de novo expression of AQP1 in glial tumors could have significant functional significance for these cells. The exact mechanism for the up-regulation of AQP1 in cells that do not normally express this protein is unknown. Possible mechanisms include dedifferentiation or loss of cell type-specific transcriptional regulation. In addition, concomitant corticosteroid use may affect endogenous or tumor-related AQP1 expression. However, this factor is difficult to assess because the majority of these patients are treated with high-dose corticosteroids. Corticosteroid regulation of AQP1 should be studied in appropriate animal models to address this issue.

We demonstrated recently that AQP4, the glial water channel normally expressed in astrocytes, provides the principal route for water transport in astrocytes (16). When AQP4 was deleted from astrocytes, water permeability decreased by sevenfold. Thus, expression of AQP1 in addition to AQP4 in glial tumor cells could significantly increase water permeability in these cells. However, further experiments with primary glial tumor cells, using our new calcein-quenching method for measurement of cell membrane water permeability, will be required.

The demonstrated role of AQPs in transmembrane water transport and their marked up-regulation in neoplastic glial membranes suggests a potential role in formation of tumor-associated edema. A common property of malignant glial tumors is marked edema in surrounding brain tissue (peritumoral edema), but the mechanism of this is uncertain (10). It is possible that AQP1 up-regulation allows excess water to flow into cells and out into surrounding brain interstitium, perhaps in combination with blood-brain barrier breakdown observed in glioma microvessels. We demonstrated recently that AQP1 can increase water permeability fivefold in choroid plexus epithelial cells (9). Thus, it may be speculated that abnormally high AQP1 expression in glial tumors may "re-set" the water balance in local areas of brain around the tumor or prevent clearance of existing extracellular fluid. The clinical relevance of these observations remains to be established. Tumor-associated edema is associated with significant clinical morbidity such as focal neurological dysfunction, mass effect, and seizures. Inhibition of AQP expression or function may offer a new therapeutic option for tumor-associated cerebral edema. The only known inhibitor of AQP1 is mercury chloride. Studies are under way to identify nontoxic, potent, and selective AQP1 inhibitors with high-throughput screening (15). Future studies are required to determine whether inhibition of AQP1 up-regulation by genetic modification or direct inhibition of AQP1 protein via selective inhibitors will abrogate tumor-associated edema and improve clinical outcome.

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COMMENTS

Water channels or aquaporins (AQPs) have received attention recently, and data are emerging that detail the distribution and localization of these proteins in various tissues. Thus far, 10 mammalian AQP homologs have been identified, and results of several studies have underscored the importance of these proteins to basic physiology in a variety of organ systems (1, 2). The distribution of these channels can be summarized as follows: AQP0, lens major intrinsic protein/lens fiber cells; AQP1, nonpigmented epithelial cells of the anterior chamber of the eye, bronchial vascular lining, choroid plexus, and the proximal tubules of the kidney; AQP2, renal collecting ducts; AQP4, brain glia and retina; AQP5, corneal epithelial cells of the eye and apical membranes of lacrimal and salivary glands; and AQP6, renal collecting ducts. With gene deletion in animal models, there is mounting evidence of the role of AQP in the pathogenesis of a variety of diseases, including nephrogenic diabetes insipidus (AQP1 and AQP2), congenital cataracts (AQP0), cerebral edema associated with traumatic brain injury, cerebral ischemia and brain tumors (AQP4), and Sjögren's syndrome (AQP5).

By use of reverse transcriptase polymerase chain reaction, complementary deoxyribonucleic acid gene array, Western blot, and immunohistochemical analyses, Oshio et al. demonstrated a massive upregulation of AQP1 in 36 human glial tumors. Although the authors failed to demonstrate a quantitative relationship between AQP1 upregulation and glioma grade, the data suggest a quantitative relationship. This article complements the findings reported previously that also demonstrated a massive upregulation of AQP1 (5) as well as AQP4 (3, 6) in human astrocytoma and metastatic adenocarcinoma. However, two important questions arise. First, although it is easier to understand the upregulation of AQP4 in brain tumors (most abundantly expressed in the normal brain), reasons for upregulation of AQP1 (expressed in the choroid plexus in normal brain) are intriguing and more complex. Is this abnormal upregulation of AQP1 a reflection of the fact that it is a more primitive channel phylogenetically? Little is known regarding the distribution and evolution of AQP1 and other AQP in brain development, and it is plausible that there is upregulation of this channel in brain tumors representing primitive cell lines. Second, what is the effect of corticosteroids on AQP expression in the brain in general and tumors in particular? This question is difficult to address because the majority of patients with gliomas are treated with corticosteroids. However, it should be noted that the AQP1 promoter region in other malignancies (erythroleukemia) has been shown to possess steroid-influencing domains (4). Experimental studies with mixed neuronal and glial cell cultures and in animal models of brain tumor may provide unique insights into the evolution of tumor-associated cerebral edema. Furthermore, specific pharmacological inhibitors, lacking at present, will provide new insights into the role of this family of proteins in health and disease and unravel important therapeutic targets in the future.

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The authors have demonstrated with convincing evidence that AQP is increased in glial tumors. The role of AQP in water regulation and transport raises interesting speculation that this upregulation may relate to tumor-induced edema. This remains entirely speculative, as the majority of edema seems to arise from a defective blood-brain barrier. The issue regarding whether corticosteroid use can influence AQP levels or activity cannot be answered by this study, but this merits further investigation.

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The authors combined gene array, reverse transcriptase polymerase chain reaction, Western blotting, and immunohistochemistry to compare AQP1 expression with tumor grade in a series of human glial tumors. They also make a comparison between magnetic resonance imaging indices of edema and AQP1 expression.

They demonstrate that whereas AQP1 is normally restricted to choroid epithelia, it is highly expressed in glioblastoma, suggesting a potential role for this membrane water channel in tumor-induced edema. The corollary is that selective inhibition of AQP1 might be of therapeutic benefit for patients in resolving symptoms. Such promise awaits further studies.

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AQP1 is a cellular membrane protein associated with water transport pathways. Because of the previously reported expression of AQP1 in a small group of gliomas, the authors hypothesized a role in tumor-associated brain edema and analyzed its expression in a larger number of gliomas. They found AQP1 to be up-regulated in high-grade gliomas and to a lesser extent in low-grade gliomas, although the data were insufficient to correlate with tumor grade to statistical significance. As a preliminary investigation, this article confirms the association of AQP1 with gliomas; however, any clinical correlation with edema and histological findings or any potential therapeutic application will require further studies.

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Positively charged polystyrene nanoparticles dot a negatively charged silica microsphere (photograph courtesy of James F. Gilchrist, Angela T. Chan, and Jennifer A. Lewis of the Frederick Seitz Materials Research Laboratory at the University of Illinois).