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MOLECULAR MECHANISMS OF BRAIN TUMOR EDEMA

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Abstract—Despite their diverse histological types, most brain tumours cause brain oedema, which is a significant cause of patient morbidity and mortality. Brain tumour oedema occurs when plasma-like fluid enters the brain extracellular space through impaired capillary endothelial tight junctions in tumours. Under-expression of the tight junction proteins occludin, claudin-1 and claudin-5 are key molecular abnormalities responsible for the increased permeability of tumour endothelial tight junctions. Recent evidence suggests that the membrane water channel protein aquaporin-4 (AQP4) also plays a role in brain tumour oedema. AQP4-deficient mice show remarkably altered brain water balance after various insults, including brain tumour implantation. AQP4 expression is strongly upregulated around malignant human brain tumours in association with reduced extracellular volume, which may restrict the flow of extracellular fluid from the tumour bed into the brain parenchyma. Elimination of excess fluid leaking into brain parenchyma requires passage across three AQP4-rich barriers: a) the glia limitans externa, b) the glia limitans interna/ependyma, and c) the blood–brain barrier. Modulation of the expression and/or function of endothelial tight junction proteins and aquaporins may provide novel therapeutic options for reducing brain tumour oedema.

Key words: aquaporin, blood–brain barrier, tight junction, vasogenic edema, water channel.

Brain oedema is defined as a net increase in water content of the brain parenchyma. Redistribution of water between the intracellular and extracellular compartments, or an increase in cerebral blood or cerebrospinal fluid volume does not therefore constitute brain oedema. All aggressive brain tumours, such as malignant gliomas and metastatic tumours, and many benign tumours, such as meningiomas, produce brain oedema regardless of their cell type of origin (Thapar and Rutka, 1995). Because the skull is non-compliant, brain oedema causes increased intracranial pressure (ICP), potentially leading to brain ischaemia, herniation and death. The lethal effect of oedema is illustrated by the 10-fold reduction in brain tumour operative mortality in the 1960s, which resulted from treating brain oedema with corticosteroids (Jelsma and Bucy, 1967).

Current understanding of brain oedema is based on Klatzo's (1994) classification into cytotoxic and vasogenic types. Cytotoxic oedema refers to cell swelling, as seen in early hypoxia, which primarily affects the astrocytes. Vasogenic oedema, of which brain tumour oedema is the archetypal example, is produced by fluid flow into the extracellular space (ECS) of the brain parenchyma through an incompetent blood–brain barrier (BBB). The amount of brain swelling is a dynamic balance between oedema fluid formation and absorption. Research into brain tumour oedema has concentrated on oedema fluid formation, and so relatively little is known about the mechanisms of oedema fluid elimination.

In humans, peripheral tumour metastases to brain (Ito et al., 1990) and primary brain tumours such as glioblastoma multiforme (GBM) (Groger et al., 1994) produce up to 90 ml of oedema fluid each day. The excess fluid is accommodated within the skull, which contains the brain, cerebrospinal fluid (CSF) and blood. In normal human adults the intracranial volume contains intracellular (1100–1300 ml) and interstitial (100–150 ml) spaces, and the CSF (75–100 ml) and blood (75–100 ml) spaces. Exchange of fluid amongst these compartments occurs in response to osmotic and hydrostatic forces at the interfaces between intracellular and extracellular compartments (cell membranes), blood and brain (BBB), CSF and brain (ventricular ependyma, glia limitans), and blood and CSF (choroid plexus and arachnoid granulations). In addition, up to 30 ml of water in brain is produced daily from glucose metabolism. Because the skull is rigid, an increase in brain parenchymal volume results in displacement of fluid from the low-pressure CSF (approximately 10 mm Hg in man) and venous (approximately 10 mm Hg) compartments, and later the high-pressure arterial (approximately 100 mm Hg mean) compartment (Monro-Kellie doctrine). The ability of the brain to compensate for a rise in ICP as total brain water increases is in large part due to the capacity of the CSF and venous compartments to be compressed.

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Abbreviations: AQP, aquaporin; BBB, blood–brain barrier; CSF, cerebrospinal fluid; ECS, extracellular space; GBM, glioblastoma multiforme; ICP, intracranial pressure; JAM, junctional adhesion molecule; SF/HGF, scatter factor/hepatocyte growth factor; VEGF, vascular endothelial growth factor; ZO, zonula occludens.

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Brain tumour oedema: a defect in the blood–tumour barrier

The existence of the BBB was first demonstrated in 1885 by the German pathologist Ehrlich (1885) who reported that i.v. injection of albumin-bound dyes into rats stained all body tissues except for the brain. Subsequent electron microscopy studies showed that i.v. administered electron dense particles, such as horseradish peroxidase, are prevented from entering the brain parenchyma by capillary endothelial cells (Reese and Karnovsky, 1967). Cerebral capillary endothelial cells differ from those of peripheral capillaries in that they are not fenestrated and contain few endocytotic vesicles, thus limiting transcellular flux (Sage et al., 1998). Individual brain microvessel endothelial cells make contact by tight junctions, further limiting intercellular flux (Reese and Karnovsky, 1967; Rubin and Staddon, 1999). The permeability properties of the BBB reflect largely the tightness of these junctions (Bar-Sella et al., 1979; Rubin and Staddon, 1999). Astrocytes, whose processes form end-feet that surround brain microvessels (Janzer and Raff, 1987; Hayashi et al., 1997), and pericytes, which contact microvessel endothelial cells, probably secrete factors that confer BBB properties to adjacent endothelial cells. Transplanted astrocytes *in vivo* (Janzer and Raff, 1987; Hayashi et al., 1997) and astrocytes *in vitro* (Janzer and Raff, 1987; Hayashi et al., 1997) induce BBB properties in adjacent non-neural endothelial cells from different species. Interestingly, some of the BBB-inducing properties of astrocytes are mimicked by corticosteroids (Underwood et al., 1999; Antonetti et al., 2002; Romero et al., 2003), which are used clinically to reduce brain tumour oedema. The absence of brain pericytes in platelet-derived growth factor receptor β deficient mice is associated with endothelial cell proliferation and increased microvascular permeability (Hellstrom et al., 1999, 2001).

Increased vascular permeability in human brain tumours has been demonstrated by computed tomographic measurements of blood–tumour barrier permeability (Groothuis et al., 1991) and by immunohistochemical detection of plasma proteins in tumour parenchyma (Seitz and Wechsler, 1987). The leakiness of the blood–tumour barrier occurs primarily because of defects in inter-endothelial tight junctions, although there may be other contributing abnormalities in endothelial pinocytosis and endothelial fenestrations (Long, 1979; Nir et al., 1989; Shibata, 1989). Abnormalities in the appearance of tight junctions in human gliomas correlate with increasing malignancy (Shibata, 1989). Electron microscopy has also shown that i.v.-injected horseradish peroxidase in rodents with GBM is excluded locally from the brain parenchyma by morphologically normal tight junctions, but not by defective tight junctions (Nir et al., 1989). In human metastatic tumours the blood vessels fail to form tight junctions (Long, 1979) and exhibit the characteristics of their parent tissue such as expression of peripheral endothelial markers like aquaporin (AQP) 1 (Saadoun et al., 2002a). This is interesting because metastatic tumours recruit endothelial cells from the surrounding brain to form blood vessels. Cerebral endothelial cells that vascularise brain tumours probably dedifferentiate in response to signals produced by the surrounding tumour cells. Cultured endothelial cells from brain (Dropulic and Masters, 1987; Stolz and Jacobson, 1991) and other organs (Stolz and Jacobson, 1991) can be induced to lose features characteristic of their tissue of origin and adopt an immature phenotype by changing culture media and exposing to growth factors.

There is no general agreement about whether the microvessels of peritumoral brain are leaky. Long (1979) did not find morphological evidence of increased permeability in microvessels around metastatic adenocarcinomas, whereas Stewart et al. (1985) reported structural defects in endothelial tight junctions around human GBM. The latter findings may be related to the ill-defined margins in GBM and cell infiltration in surrounding brain.

The importance of the vascular bed in the initiation and progression of vasogenic oedema has been shown in animal models. Excision of the lesioned cortex immediately after focal freeze injury completely abolishes the expected vasogenic oedema and removal of the lesion a few hours after freeze injury prevents advancement of the oedema front (Aarabi and Long, 1979). There is good evidence that vasogenic oedema fluid arises from plasma. In cat models of tumour implantation, brain abscess and focal freeze injury, the sodium content of the oedema fluid was the same as plasma (129–135 mM; Bothe et al., 1984) and i.v.-injected 99mTc-labelled albumin was found in the oedema fluid (Gazendam et al., 1979).

Recently the molecular constituents of brain endothelial tight junctions have been elucidated (Huber et al., 2001; Fig. 1). Tight junctions consist of transmembrane and intracellular protein components (Rubin and Staddon, 1999; Wolburg and Lippoldt, 2002; Vorbrodt and Dobrogowska, 2003). The transmembrane proteins, which include the claudin family, occludin and junctional adhesion molecule (JAM), bind to their counterparts on adjacent cells, in effect 'gluing' two cells together. Although occludin is expressed ubiquitously in tight junctions, members of the claudin family are expressed in different tissues. Brain endothelial cells express occludin, JAM, claudin-1 and claudin-5 (Rubin and Staddon, 1999; Wolburg and Lippoldt, 2002; Vorbrodt and Dobrogowska, 2003). At the cytoplasmic surface of cells, the claudins and occludin bind zonula occludens 1 (ZO1), ZO2, ZO3 and other less well-characterised proteins, each of which anchor to the actin cytoskeleton. ZO1, ZO2 and ZO3 are members of the membrane-activated guanylate cyclase protein family (Stevenson et al., 1986; Haskins et al., 1998), suggesting their involvement in intracellular signalling. This complex organisation confers plasticity to the tight junction, allowing it to function as a gate that can be transiently opened by the cell in response to second messengers to permit passage of leucocytes or solutes (Vestweber, 2002). Phosphorylation of occludin and claudins may regulate tight junction permeability (Rubin and Staddon, 1999). Protein kinase C (Andreeva et al., 2001) and casein kinase 2 (Smales et al., 2003) have been shown to phosphorylate occludin on tyrosine residues.

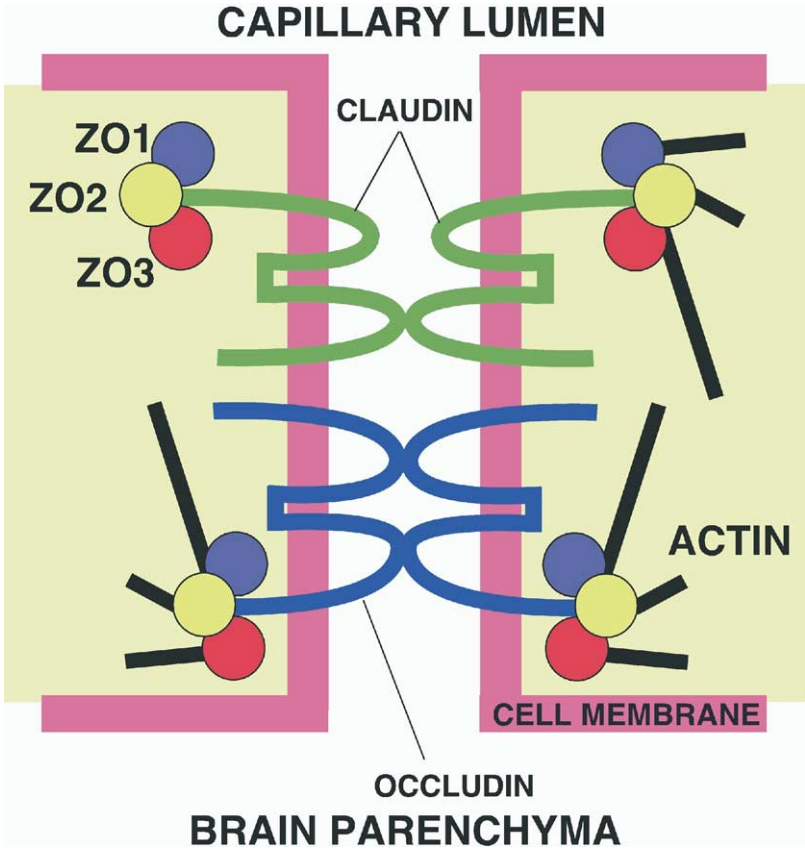

Fig. 1. Molecular constituents of endothelial tight junctions. Transmembrane proteins (occludin and claudins) bind their counterparts on adjacent endothelial cells, thus 'gluing' the cells together. Intracellularly, occludin and claudins are attached to ZO1, ZO2 and ZO3, which are in turn attached to the actin cytoskeleton.

Some defects in brain tumour endothelial vessel tight junctions have been characterised. Occludin, claudin-1 and claudin-5 are downregulated in human gliomas and are absent in metastatic tumour vessel tight junctions (Liebner et al., 2000; Papadopoulos et al., 2001b; Rascher et al., 2002). The occludin protein content of human astrocytomas is inversely related to the Daumas-Duport histological grade (Papadopoulos et al., 2001b). It has been suggested that in human high-grade gliomas, occludin is downregulated and phosphorylated (Papadopoulos et al., 2001a,b). Phosphorylation of occludin inhibits its interactions with ZO1, ZO2 and ZO3 (Kale et al., 2003) and increases tight junction permeability (Rubin and Staddon, 1999).

Two principal reasons why tumours have defective endothelial tight junctions include reduced numbers of normal astrocytes in tumour tissue, and excessive secretion of angiogenic factors. Because aggressive tumours are deficient in normal astrocytes, they lack the astrocyte derived factors required for the formation of a normal BBB (Janzer and Raff, 1987). Brain tumour cells secrete a mixture of angiogenic factors, such as vascular endothelial growth factor (VEGF, originally called vascular permeability factor; Bates et al., 1999) and scatter factor/hepatocyte growth factor (SF/HGF; Lamszus et al., 1999; Arrieta et al., 2002). High VEGF immunoreactivity has been reported in human anaplastic astrocytoma and GBM (Lafuente et al., 1999; Ludwig et al., 2000b), oedematous meningioma (Goldman et al., 1997) and metastatic tumours to the brain (Ludwig et al., 2000a). In cultured brain or retinal endothelial cells, VEGF (Antonetti et al., 1999; Wang et al., 2001; Behzadian et al., 2003) and SF/HGF (Jiang et al., 1999) cause downregulation and phosphorylation of the tight junction component proteins occludin and ZO1, with a corresponding increase in monolayer permeability. In contrast, corticosteroids cause de-phosphorylation of occludin and ZO1, and decreased endothelial monolayer permeability (Underwood et al., 1999; Antonetti et al., 2002; Romero et al., 2003). Many malignant tumours outgrow their blood supply and become hypoxic, which is probably a potent stimulus for secretion of angiogenic factors by brain tumour cells. The VEGF promoter has hypoxia responsive elements (Shibata et al., 2000), and hypoxia has been shown to stimulate human GBM cells to secrete VEGF (Plate et al., 1994).

About 60% of meningiomas cause brain oedema, even though they do not originate from brain cells, but arise from the coverings of the brain (extra-axial tumours). Meningiomas grow in extra-cerebral spaces, have extra-axial blood supply, and are separated from the brain by the pia mater. Electron microscopy of human peri-meningioma tissue showed increased ECS volume, suggesting that the

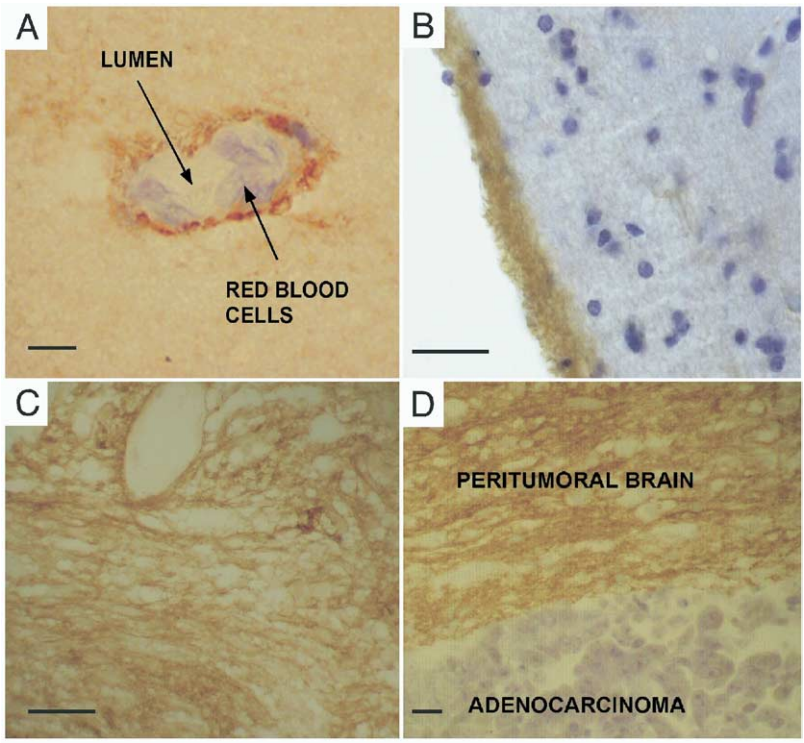

Fig. 2. AQP4 expression (brown) in human brain and brain tumours. In normal brain, there is immunostaining of (A) pericapillary astrocyte foot processes, and (B) the glia limitans. Note massive upregulation of AQP4 immunoreactivity and loss of polarized expression in (C) glioblastoma, and (D) the gliotic region around metastatic carcinoma. Haematoxylin counterstain. Scale bars=10 μm (A), 35 μm (B), 30 μm (C), 10 μm (D).

associated oedema is of the vasogenic type (Gilbert et al., 1983). The presence of brain oedema may be related to the secretion of VEGF by meningioma cells (Yoshioka et al., 1999; Paek et al., 2002; Pistolesi et al., 2002). It is thought that VEGF enters the peritumoral space and causes the formation of a cerebral/pial blood supply to the tumour (Yoshioka et al., 1999; Paek et al., 2002; Pistolesi et al., 2002). Human peri-meningioma endothelial cells show morphological changes, such as nuclear enlargement and many pinocytotic vesiles, consistent with de-differentiation due to exposure to VEGF (Vaz et al., 1998). The cerebral/pial-derived microvessels lack tight junctions allowing brain oedema to develop in the same way as it does in aggressive gliomas and metastases.

Flow of oedema fluid in brain ECS

The hydrostatic pressure gradient (systemic arterial pressure minus ICP) is the primary force driving vasogenic oedema fluid entry into the ECS. After focal cortical freeze injury in cats, elevation of the mean arterial pressure dramatically accelerated the spread of oedema, and oedema spread was slow with reduced blood pressure (Klatzo, 1994). Having crossed the defective blood–tumour barrier, oedema fluid enters the ECS of the tumour bed. Compared with normal brain tissue, aggressive human brain tumours such as GBM and medulloblastomas have an apparently expanded ECS volume (Bruehlmeier et al., 2003; Vargova et al., 2003), probably resulting from necrosis, abnormal tumour cell shape and defective ECS matrix. Increased ECS volume may provide a low resistance pathway facilitating the flow of oedema fluid throughout the tumour bed.

Prior to entering the ECS of the brain parenchyma, the oedema fluid must cross a rim of reactive glia surrounding the tumour (Zhang and Olsson, 1997). The role of reactive gliosis in brain tumour biology is poorly understood, but may limit the excess fluid entering the brain parenchyma. The gliotic tissue presents a significant barrier to the flow of extracellular fluid (Roitbak and Sykova, 1999), probably due to the high density of hypertrophied astrocytic processes and enhanced formation of extracellular matrix. After forebrain stab injury in mice, gliosis has been shown to reduce BBB permeability, leucocyte infiltration, and neuronal death (Bush et al., 1999).

Once inside the ECS of the brain parenchyma, further movement of oedema fluid is resisted by the cells and their processes. Dilation of the ECS by the hydrostatic pressure of the oedema fluid creates a pathway for its movement. Vasogenic oedema spreads by bulk flow (Reulen et al., 1977) preferentially through the white matter, which presents a more orderly arrangement of extracellular channels and thus offers less resistance to oedema fluid flow than the densely tangled cellular structures of the grey matter (Thapar and Rutka, 1995).

Elimination of oedema fluid: role of AQP water channels?

Several groups have proposed the involvement of AQPs in the pathophysiology of brain oedema (Venero et al., 2001;

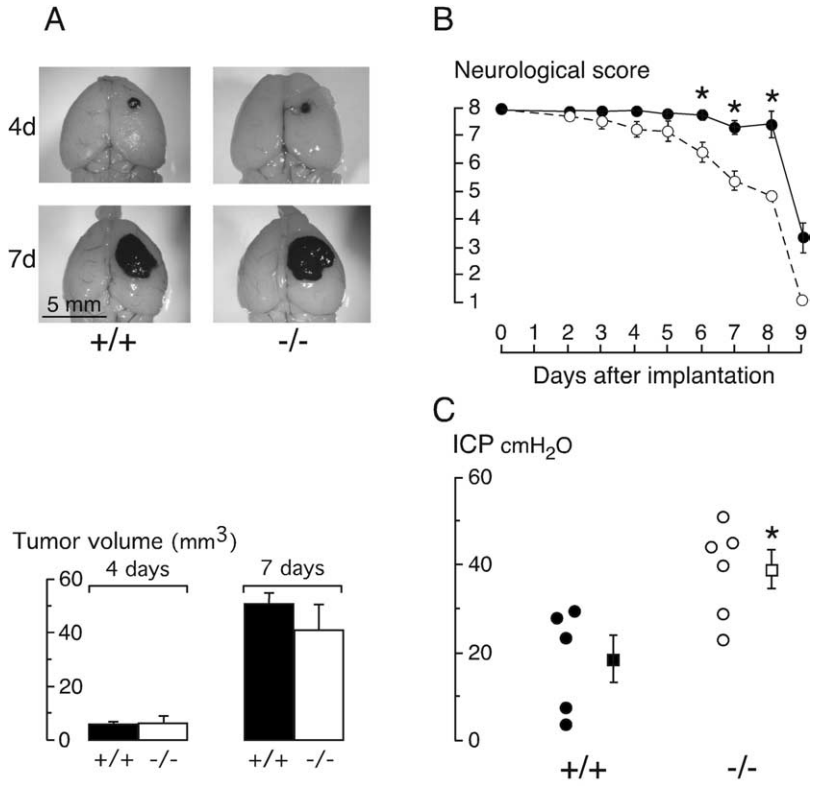

Fig. 3. Brain swelling in mice with implanted melanoma brain tumours. (A) The tumours grow at comparable rates in wild-type (+/+, ●) and AQP4-null (−/−, ○) mice. However, the AQP4-null mice have (B) worse (lower) neurological score, and (C) higher ICP, measured 1 week post-implantation. * *P*

Papadopoulos et al., 2002; Agre and Kozono, 2003; Amiry-Moghaddam and Ottersen, 2003). The AQPs are a family of water channel proteins, which comprises at least 12 members in mammals (Verkman, 2002; Agre and Kozono, 2003). The AQPs provide a major pathway for osmotically-driven water movement across plasma membranes in some cell types. AQP4 is the predominant water channel in normal brain; it is strongly expressed in astrocyte plasma membranes, and has been shown to increase the water permeability of cell membranes isolated from mouse brain (Ma et al., 1997) and cultured cortical astrocytes (Solenov et al., 2004). As shown in Fig. 2, the sites of AQP4 expression include the pericapillary astrocyte foot processes, the external glial limiting membrane, the ependyma, and the subependymal glia limitans interna (Nielsen et al., 1997; Rash et al., 1998). The localisation of AQP4 at the CSF–brain and blood–brain interfaces suggests its involvement in fluid transport into and out of the brain. Although AQP1 and AQP9 have also been found in the brain, their expression is limited. In normal brain, AQP1 is found on the ventricular-facing surface of the choroid plexus, where it facilitates the secretion of CSF (Oshio et al., 2003b). AQP9 has been detected in the glia limitans and tanycytes, but its precise expression pattern remains unclear (Elkjaer et al., 2000; Badaut et al., 2001). The possible role of AQP9 in the brain is discussed further in the review by Badaut and Regli (2004) in this issue of *Neuroscience*.

Immunohistochemical studies of human brain tumours (Saadoun et al., 2002b, 2003; Badaut et al., 2003) report strong upregulation of AQP4 in astrocytes in the vicinity of oedematous brain tumours. Fig. 2A shows AQP4 expression around a normal human brain microvessel and Fig. 2B shows expression in the glia limitans. There is marked increase in AQP4 expression in astrocytes in GBM (Fig. 2C) and reactive astrocytes around carcinoma metastasis to brain (Fig. 2D). Unlike normal brain, brain tumour-associated AQP4 expression is not polarized to astrocyte foot processes, but is seen throughout the entire astrocyte cell membrane. Correlation between increased AQP4 expression and brain tumour oedema provides indirect evidence for the importance of AQP4 in the pathophysiology of brain tumour oedema. Due to the lack of AQP4 inhibitors, it is not yet possible to verify a role of AQP4 directly in vasogenic brain oedema. However, AQP4-null mice have been valuable in defining the role of AQP4 in fluid transport and balance in brain [see review by Manley et al. (2004) on AQP4 knockout studies].

Despite the strong expression of AQP4 in the brain, AQP4-null mice are surprisingly normal in terms of gross behaviour, brain morphology, blood vessel anatomy, ICP, bulk intracranial compliance, and expression of other AQPs (Ma et al., 1997; Manley et al., 2000; Papadopoulos et al., 2004). Experiments using AQP4-null mice suggest the involvement of AQP4 in both cytotoxic and vasogenic

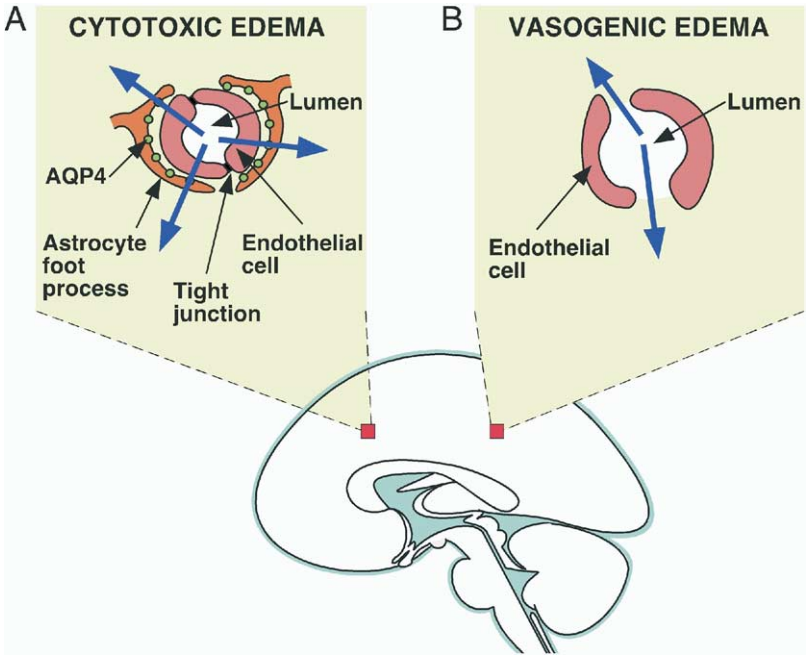

Fig. 4. Proposed mechanism of AQP4 involvement in oedema fluid formation. (A) In cytotoxic oedema, the entry of excess fluid into the brain parenchyma is AQP4 dependent, because oedema fluid flows from the vascular compartment, through intact BBB and AQP4-rich astrocyte foot processes, and accumulates primarily in astrocytes. (B) In vasogenic oedema, water accumulation is AQP4-independent because the BBB is leaky, permitting the entry of plasma fluid directly into the brain ECS circumventing astrocyte foot processes.

brain oedema. AQP4 deletion protected mice from brain swelling in two models of primarily cytotoxic oedema: water intoxication and permanent focal cerebral ischaemia (Manley et al., 2000). Similar findings were reported in mice lacking α-syntrophin (Amiry-Moghaddam et al., 2003) or dystrophin (Vajda et al., 2002), which secondarily manifest altered distribution of brain AQP4 expression. In contrast, AQP4 deletion caused increased brain swelling in three models of vasogenic brain oedema: intraparenchymal fluid infusion, focal freeze injury and brain tumour (Papadopoulos et al., 2004). Intracerebral fluid infusion mimics the resolution phase of brain swelling by producing a controlled amount of brain oedema without the potentially confounding variations in BBB permeability. Cold injury is a highly reproducible model of 'vasogenic' brain oedema resulting from increased BBB permeability and fluid extravasation. In the tumour model, melanoma cells were stereotactically implanted into the brain. As illustrated in Fig. 3A, 7 days post-implantation, the tumours were equal in size in AQP4-null and wild-type mice (Papadopoulos et al., 2004). However, the AQP4-null mice developed worse neurological score (Fig. 3B) and higher ICP (Fig. 3C) than wild-type controls. These experiments provided evidence that AQP4 deletion limits brain swelling in cytotoxic oedema, but aggravates brain swelling in vasogenic oedema.

These opposing actions of AQP4 in cytotoxic and vasogenic oedema are probably related to the bidirectional water transport through the AQP4 channel (Meinild et al., 1998; Amiry-Moghaddam et al., 2003). We hypothesise that influx of oedema fluid into the brain parenchyma in cytotoxic oedema involves water movement through the intact BBB and astrocyte foot processes. Fluid entry into the brain in vasogenic oedema involves movement across the leaky tumour microvascular endothelium, which is not surrounded by astrocyte foot processes. Therefore, the formation of cytotoxic oedema is AQP4-dependent, but the formation of tumour-associated oedema is AQP4-independent (Fig. 4). However, elimination of excess brain water in both cytotoxic and vasogenic brain oedema is likely to be AQP4-dependent because excess fluid flows through AQP4-containing barriers. Fluid flows through the glia limitans into the subarachnoid space, through the ependyma into the ventricles, and through the BBB and astrocyte foot processes into the blood (Reulen et al., 1977; Marmarou et al., 1994; Fig. 5).

In brain tumours, AQP4 may also be involved in limiting the entry of oedema fluid from the tumour bed into the brain parenchyma. Indirect evidence suggests a correlation between the size of the ECS and the level of AQP4 expression, with AQP4-expressing gliotic tissue (Saadoun et al., 2002b) presenting a significant barrier to extracellular fluid flow (Roitbak and Sykova, 1999). In the first few weeks after birth, rat brain ECS volume decreases (Lehmenkuhler et al., 1993) in parallel to increased AQP4 expression (Wen et al., 1999). There is strong AQP4 expression in the glia limitans (Nielsen et al., 1997; Saadoun et al., 2003), which consists of tightly packed astrocyte foot processes (Brightman, 2002). AQP4-null mice have higher ECS volume compared with wild-type controls (D. K. Binder, unpublished observations). The reported increased AQP4 expression in peritumoral reactive astrocytes (Saadoun et al., 2002b) may therefore reduce ECS volume in the peritumoral gliotic rim. This would slow the

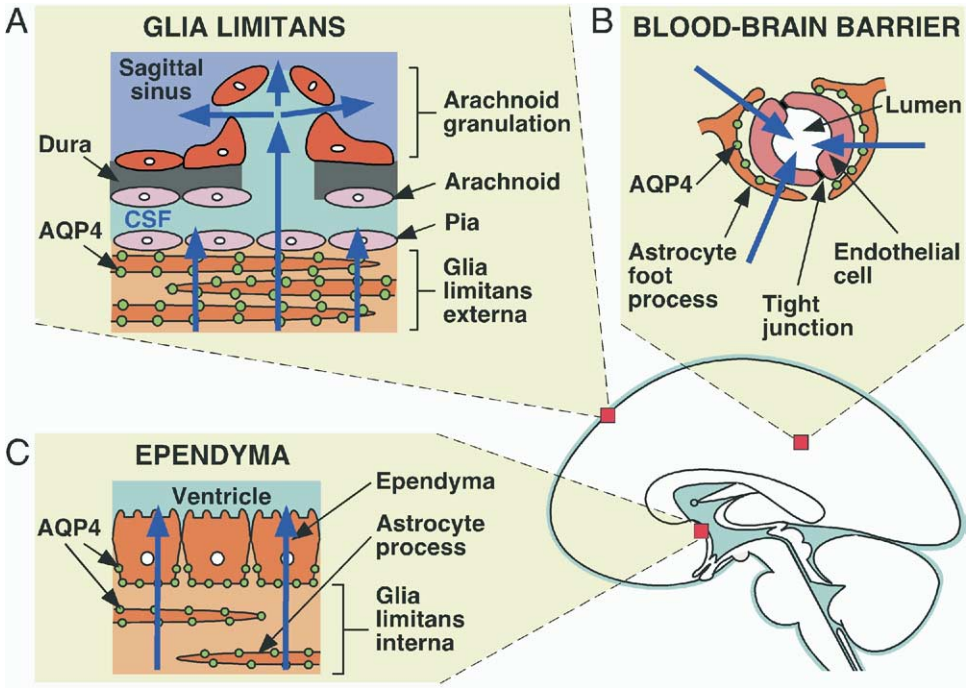

Fig. 5. Proposed role of AQP4 in elimination of cytotoxic and vasogenic oedema fluid. In both cytotoxic and vasogenic types of brain oedema, excess fluid is eliminated through (A) the glia limitans externa into the subarachnoid space, (B) the BBB into the bloodstream, and (C) the glia limitans interna and ependyma into the ventricles.

flow of oedema fluid from the tumour bed into the brain ECS.

It has been reported recently that AQP1 is expressed in tumour cells and peritumoral astrocytes in human high-grade gliomas (Saadoun et al., 2002a; Oshio et al., 2003a). This observation is intriguing because AQP1 is not normally found in astrocytes; its expression is restricted to the choroid plexus, where it provides the major water pathway for secretion of CSF (Oshio et al., 2003b). It is not known whether tumour-associated AQP1 is functional or whether it plays a role in the pathophysiology of brain tumour oedema. Experiments comparing brain tumour oedema in AQP1 knockout mice versus wild-type controls are likely to clarify the role of AQP1 in brain tumour oedema.

CONCLUSIONS

Brain tumour oedema results from imbalance between water moving into and out of the brain. Two recent discoveries have advanced our understanding of both processes. First, molecular abnormalities of tumour endothelial tight junctions increase blood–tumour barrier permeability, thus accelerating the rate of water entry into the brain. Second, the elimination of excess water from the brain is controlled by the water channel AQP4. We therefore suggest that endothelial tight junction proteins and AQP4 represent targets for the design of novel drugs to treat brain tumour oedema. Such compounds might slow oedema fluid formation (by upregulating occludin, claudin-1 and claudin-5 in endothelial cells) or increase oedema fluid elimination (by upregulating AQP4 in astrocytes).

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