Original Paper

Distribution of Aquaporins 1 and 4 in the Central Nervous System

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ABSTRACT: The aquaporins (AQP), a protein family, were first discovered in the early 1990s. The primary role of aquaporins is to facilitate water transport across multiple cell types. In the spinal cord and brain responsible for most of the water diffusion are AQP4 and AQP1. In this paper, we describe the structure, localization and role of this water channel family, especially AQP4 and AQP1. AQP4 is involved in various pathologies such as: stroke, brain tumors, Neuromyelitis optica (NMO), Alzheimer's Disease (AD), traumatic brain injury, Parkinson's Disease, hydrocephalus, schizophrenia, epilepsy, major depressive disorder, autism. Brain edema is the most important acute complication of the hypoxic-ischemic and it has no pathogenic treatment. Imaging and histopathology studies have shown that inhibition of AQP4 reduces brain edema.

KEYWORDS: *Aquaporin 4, brain edema, ischemic stroke*

Introduction

The aquaporins (AQP) are channels that are selectively permeable to water, first identified in the early 1990s. Now, the aquaporin family is made up of 11 subtypes, from AQP0 to AQP10.

According to permeability characteristics the mammalian aquaporins have now been subdivided into three categories: (i) the aquaporins (AQP0-AQP6) are permeable to water; (ii) the aquaglyceroporins (AQP3, AQP7, AQP8) are permeable to water, urea and glycerol; and (iii) the neutral solute channels, including AQP9 and AQP10, are permeable to water, glycerol, pyrimidines, urea, monocarboxylates and purines [1,2].

These proteins have different functions and localizations in the human body. AQP1 was identified in kidneys, in descending thin limbs of Henle, in the basolateral membranes of proximal tubules and apical brush border; in tissues with an important role in secretion like choroid plexus (cerebrospinal fluid), cholangiocytes (bile), capillary endothelium in many organs and non-pigmented epithelium in the anterior chamber of the eye [3].

AQP0 (major intrinsic protein of lens fiber) is a part of the cells' skeleton [4].

AQP2 (vasopressin) was identified in kidneys, in collecting duct principal cells [3].

AQP3 is present in multiple organs like airways [5], eye [6], skin [7] and kidneys [8].

AQP4 is present in brain and retina, most abundant in astroglial cells but also in ependymal cells lining the ventricles [9,10].

AQP5 is distributed in salivary glands, submucosal glands of airways and lacrimal glands [5].

AQP6 is present in renal collecting ducts. AQP7 was identified in adipose tissue [11].

AQP9 is distributed in hepatocytes [12].

In this article we will focus on the description of aquaporins present in the brain, especially AQP1 and AQP4.

The main aquaporins present in the brain are AQP1, AQP4 and AQP9, but recent studies have identified AQP3, AQP5, and AQP8 in cultures of astrocytes and neurons; AQP5 was observed on astrocytes and AQP8 on oligodendrocytes, but their roles remain to be determined [13].

Materials and Method

We have utilized here brain tissue from 6 patients that died of non-CNS related causes.

The patients had been admitted and followed-up in the department of Neurology, Clinical Hospital of Neuropsychiatry, University of Medicine and Pharmacy of Craiova, Romania (Table 1).

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A written informed consent had been obtained from their caretakers regarding utilization of tissue material for research purposes.

Table 1. Patients from whom brain tissue was utilized in this study

Age	Gender	Pathology
56	F	Digestive tract tumor
64	F	Miocardial infarction
68	M	Lung tumor
69	F	Digestive tumor
85	M	Bronchopneumonia, tuberculosis
75	M	Digestive tumor

After macroscopic examination, tissue blocks were sampled from all major isocortical areas, routinely processed for paraffin embedding, then 4µm-tick sections were cut and flattened on poly-L-lysine coated glass slides. A hematoxylin and eosin staining confirmed no obvious histopathological changes in the cortex and the white matter, ie slight patchy gliosis, stasis and isolated perivascular and pericellular oedema.

Frontal, temporal, parietal and occipital tissue blocks were selected from each patient and further processed for immunohistochemistry. Briefly, the slides were dewaxed, re-hydrated in decreasing alcohol series until distilled water, and then antigen retrieval was performed by microwaving the slides in citrate buffer pH6 at 600W for 21 minutes. Endogenous peroxidase was blocked in 1% water peroxide for 30 minutes, the unspecific antigenic binding sites were blocked with goat serum for 30 minutes, and then the first two primary antibodies were added overnight at 4°C, diluted in PBS and 3% albumin (AQP1 1, mouse; and AQP4, rabbit) (Table 2).

Next day the slides were incubated with anti-rabbit Alexa Fluor596 (Thermo Fisher Scientific, Waltham, United States, 1:150) and anti-mouse horse radish peroxidase (HRP) (Vector Laboratories, Peterborough, UK) for 1 h.

Table 2. Antibodies utilized in this study

Name	Clone	Epitope	Dilution	Source
Aquaporin 4	Rabbit, polyclonal	Amino acids 244-323, of human AQP4	1:1000	Santa Cruz Biotechnology, Inc, Texas, U.S.A.
Aquaporin 1	Mouse, IgG1	Complete sequence of human AQP1	1:300	Thermo Fisher Scientific
GFAP	Mouse	Glial fibrillary acidic protein	1:200	Dako, Glostrup, Denmark

After thorough washing, the HRP was visualized by precipitating tyramide Alexa 488, 1:200 for 5 minutes (Thermo Fisher). Then the anti-GFAP (mouse) primary antibody was next added overnight, and the next day the signal was visualized with anti-mouse Alexa Fluor 555. All slides were coverslipped with a DAPI-based mounting medium (Vector Laboratories). Fluorescent images were captured utilizing a Nikon Eclipse 90i microscope (Nikon CEE GmbH, Wien, Austria) equipped with a 16-megapixel Nikon DS-Ri2 CMOS camera, and the Nikon NIS-Elements image analysis software.

Fluorescence images were obtained by sequential scanning of each channel with custom made narrow-band filters for DAPI, Alexa 488, Alexa 555 and Alexa 594 spectra (Chroma Technology Corp., Bellows Falls, USA). For colocalization analysis, all images were stored in Nikon's proprietary format and subjected to a 5 iterations blind deconvolution algorithm in NIS Elements software package.

Results

We have first evaluated the relative disposition of AQP1 and AQP4 in the cortex and white matter of control individuals, with no clear CNS-associated pathology. Most of the AQP4 was expressed, as already described [14], in the pia mater, petechial in the cortical astrocytes, and denser in the white matter astrocytes. When we evaluated AQP1 in the cortex, there only were a few astrocyte-like cells stained, and apparently with little/no colocalisation with AQP4 (Fig.1A). Most of the AQP1 positive astrocytes were located in the middle-deep cortical layers, and not in the superficial layers. On higher resolution deconvoluted images, there was almost no colocalisation between the two aquaporins. In the white matter, however, both signals were extremely intense but with AQP1 located mostly as a rim between the gray matter and the white matter, with a reduced expression in the white matter itself (Fig.1B). There were, however, areas of clear-cut colocalisation here, compared to the gray matter.

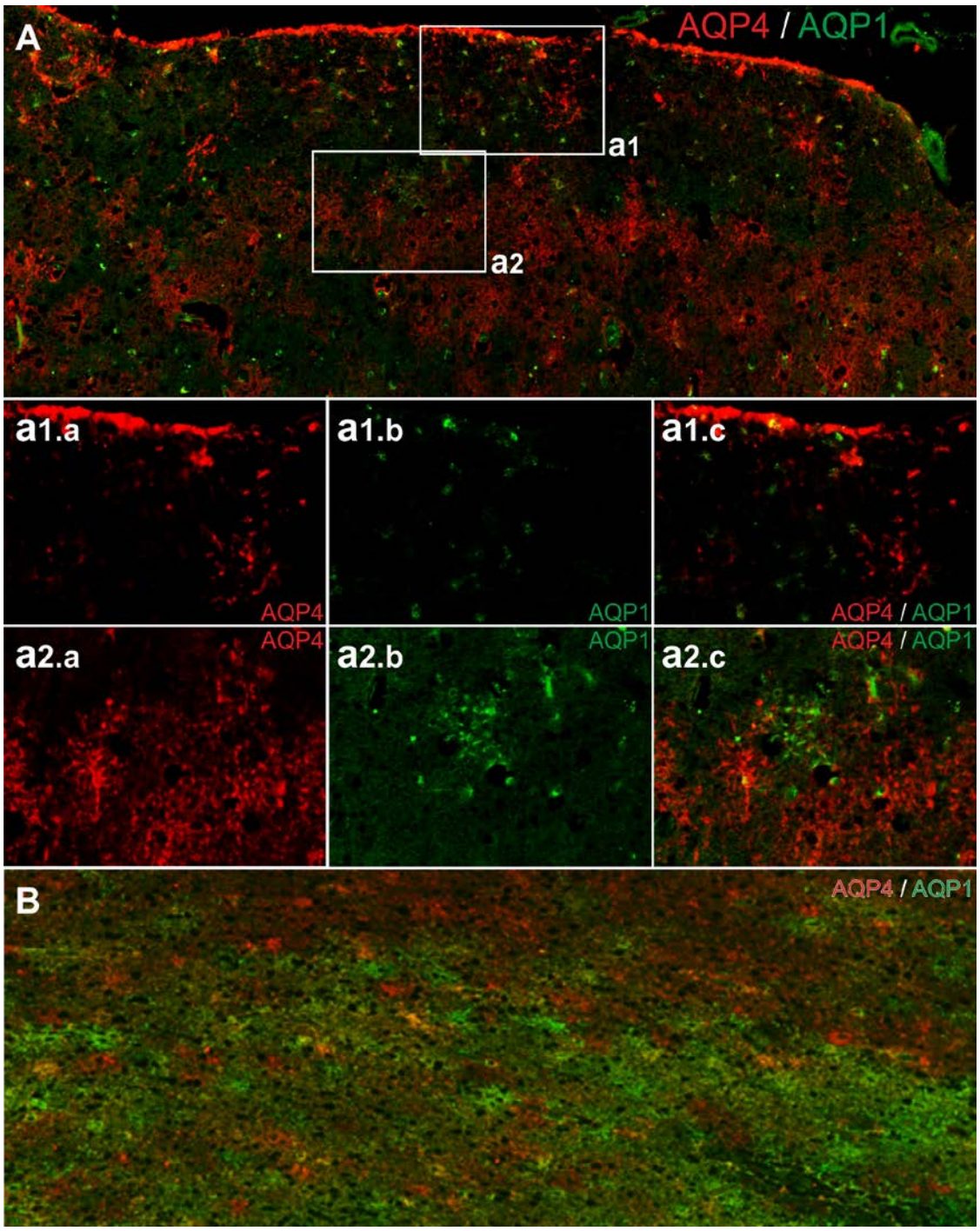

Fig.1. Histopathological localization of AQP1 and AQP4 in the cerebral cortex (A,a1,a2), and in the white matter in the normal aging brain. A, B, 5× scan; a1, a2, 40×

We next intended to see the most frequent AQP1 cellular expression, and thus we have evaluated its expression concomitantly with GFAP (Fig.2A-C).

In all cases, there was a complete proximity of the two signals, as is the case of AQP4. Since both are membrane-bound proteins, aquaporin signal is surrounding the GFAP cytoskeleton, and this was the expression pattern throughout the analyzed images. On high resolution images, we could identify astrocytes with isolated AQP1 or AQP4 expression, as well as astrocytes that expressed both markers (Fig.2D-I).

It was interesting that even in very close proximity these cells had such different phenotypes. In some instances AQP1 and AQP4 positive astrocytes seem to intersect their elongations, but without any colocalisation of the signal, a proof that they are indeed completely separate entities (Fig.2G-I).

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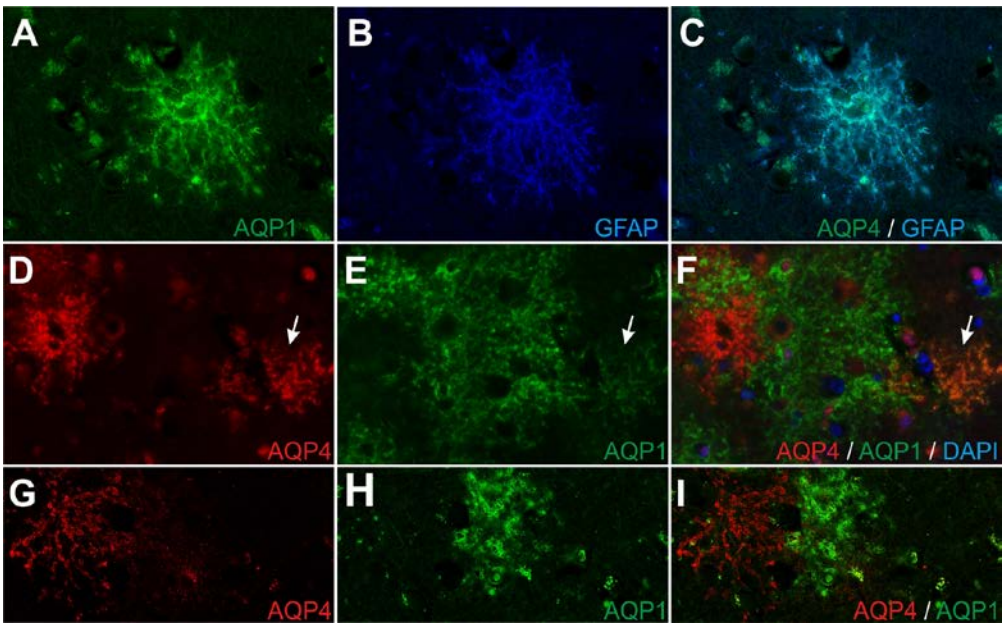

Fig.2. AQP1 is also expressed intensely in astrocytes (A-C), and AQP1 positive astrocytes do co-express occasionally AQP4 (arrow), or are only AQP1-positive (D-F). Close astrocytes that do not overlap their AQP1/AQP4 signals, 40×

Lastly, we have followed blood vessels, and tried to asses if AQP1 would colocalises with luminal components of the blood brain barrier. After evaluating triple immunostainings, for AQP1, AQP4 and GFAP, most of the perivascular AQP1 signal showed a clear-cut colocalisation with GFAP and AQP4 (Fig.3A-D). AQP1 was completely colocalised with GFAP in what it regards the signal area, while AQP4 showed a much wider signal, with more astrocytes being stained in the parenchyma around the blood vessels. We could thus conclude that most AQP1 in the normal human brain is expressed by astrocytes, not only around blood vessels, but to a lesser extent compared to the expression area of AQP4.

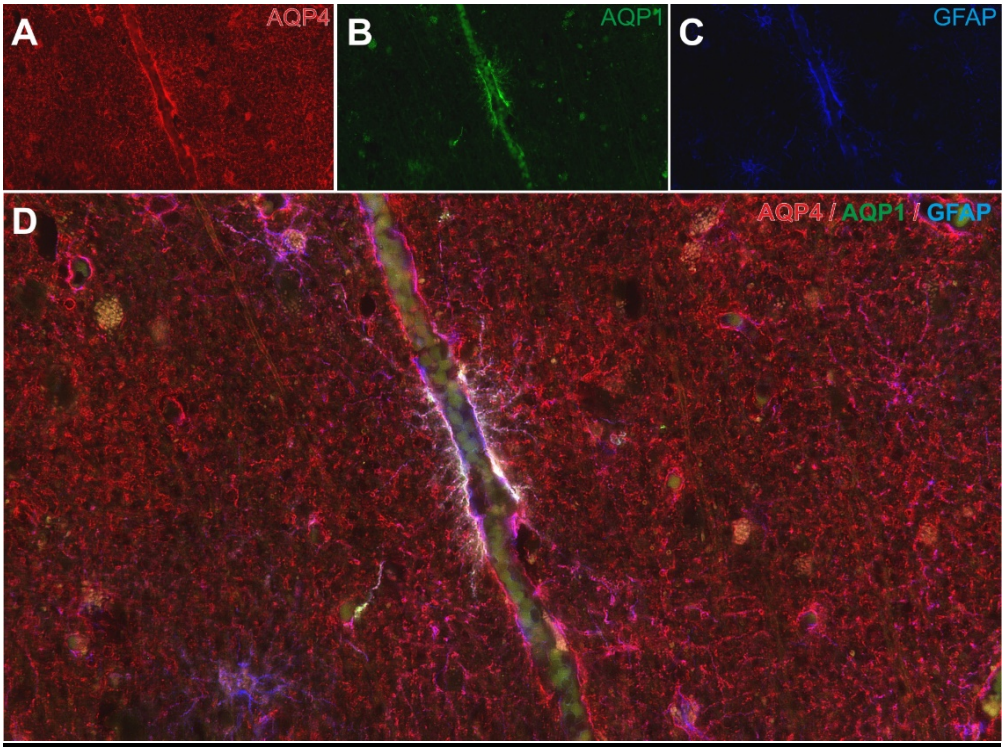

Fig.3. AQP1 is not so intensely expressed around blood vessels. 20×

Discussion

AQP structure

This ancient family of proteins, aquaporins, are present in all forms of life, showing their importance in keeping normal physiology of all organisms. They are present in a larger number in multicellular organisms compared to unicellular organisms, where there are only a few [15,16].

To determine the aquaporins structure, great efforts have been made, given its unique and specific permeability characteristics. AQP monomers are~30kDa and usually contain shorter helical segments that do not spread over the entire membrane and six membrane-spanning helical segments. At the level of membranes aquaporins form stable tetramers and each monomer contains an independent water pore [17].

The studies have provided high-resolution structural data show that the helical domains entering the membrane surround extracellular and cytoplasmic vestibules that are bound by a narrow aqueous pore [18].

The responsibility of electrostatic and steric factors for the water selectivity of AQPs and passage of water molecules through this narrow aqueous pore are suggested by molecular dynamics simulations and structural data [19,20].

In the aquaglyceroporins the pore is less constricted than in the water-selective AQPs (diameter of 3.4 Å versus 2.8 Å, respectively) [21].

AQP4 is found as two main isoforms that are produced by alternative splicing: a shorter M23 isoform, which is generated by translation initiation at Met23 and the relatively long M1 isoform, which is generated by initiation of translation at Met1 [22].

The Role of Aquaporins in Nervous System

The major roles performed by aquaporins in nervous system are neuroexcitation, astrocyte migration and facilitating water movement into and out of the central nervous system (CNS) [23].

AQP 1 is present at the levels of the choroid plexuses and plays a part in CSF secretion and AQP 4 is present in ependymal cells and subependymal astrocytes, especially on the perivascular end-feets and plays a role in CSF absorption [9,14,22,24].

At the level of the choroid plexus the cerebrospinal fluid is secreted at the level of the arachnoid granulations and is absorbed primarily into the venous sinuses. CSF can also be absorbed through other mechanisms such as transependymal flow into the brain [3].

In some studies it was demonstrated that the rate of CSF secretion was reduced up to 25% in AQP1 deficient mice compared with wild-type mice. These data suggest that some of the CSF secretion is influenced by AQP 1 but some is also not dependent on AQP 1 [25].

The migration of astrocytes is encountered in various brain injuries to form a glial scar and delimit the injured tissue. AQP4 is present in increased amount in high-grade glioblastoma and in reactive astrocytes. After the finding that AQP4 might facilitate cell migration, new studies on mouse animal models have found impaired astrocyte migration at the level of traumatic brain injury, in brains injected with fluorescently labeled astrocytes from AQP4 null mice, compared to labeled astrocytes from wild-type mice [26].

Another consequence of AQP4 deletion in mice is influencing neural signal transduction. In the supporting cells around electrically excitable cells AQP4 is positive, including Müller vs. bipolar cells in retina and astrocytes vs. neurons in spinal cord and brain. In the mice with absence of AQP4 it was found an impairment of visual, olfactory and auditory signal transduction as analyzed by behavioral methods and/or evoked potential [27,28,29].

Various studies in AQP4 null mice have suggested many other functions of AQP4.

In neuroinflammation it has been described a probable role of AQP4. In this case AQP4 deletion in mice was correlated with decreased severity of the symptoms of experimental autoimmune encephalomyelitis in contrast to the wild-type mice [30].

Recent studies focused their attention on the role of AQP4 in clearance of toxic protein aggregates from the nervous system by a ‘glymphatic’ mechanism [31].

AQP4 in Different Pathological Conditions

Stroke

Stroke represents one of the most important cause of disability and death in the world and ischemic stroke is more common than hemorrhages.

Ischemic stroke occurs due to sudden interruption of blood flow to an area of the brain

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and the consequence of this disruption is neurological dysfunction. Ischemic stroke represents 80% of all stroke patients and is most commonly caused by embolic or thrombotic occlusion of a cerebral artery [32].

Brain edema represents the most important acute complication of the hypoxic-ischemic and at this time there is no pathogenic treatment for this element.

Parenteral administration of hypertonic solutions is the only non-surgical treatment accepted for cerebral edema, which, by moving water from the brain parenchyma to the blood, reduces intracranial pressure [33].

Cerebral edema may be grouped in two phases: cytotoxic or initial phase edema, which occurs because under conditions of ischemia cells cannot maintain the work of their transmembrane adenosine triphosphate (ATP)-dependent Na+/K+pumps in ischemic stroke; and the so-called vasogenic edema in which the hemato-encephalic barrier is altered and the permeability is modified.

At this stage, AQP4 behaves like a bidirectional water channel. Recent studies have shown in vivo and in vitro the ability of 2-(nicotinamide)-1,3,4-thiadiazole (TGN-020) to inhibit AQP4 [34].

TGN-020's ability to reduce brain edema was demonstrated in imaging studies, nuclear magnetic resonance imaging (MRI) and histopathology in an animal model with cerebral ischemia pretreated with single dose of TGN-020 [24, 35].

Neuromyelitis optica (NMO)

NMO is a disease of the CNS that affects the optic nerves and spinal cord through inflammation and demyelination, leading to paralysis, blindness and death [36,37].

NMO was considered a form of multiple sclerosis (MS) until 2004, when serum antibodies against a perivascular brain antigen were positive in patients with NMO and remained negative in patients with MS [38]; it was subsequently demonstrated that these antibodies were raised against AQP4 [39].

AQP4-specific antibodies are helping to diagnose NMO and to differentiate NMO from MS. Anti AQP4-specific antibodies in NMO are IgG-like and produce astrocyte destruction by complement-dependent cytotoxicity; on the extracellular loops of AQP4 these bind three-dimensional conformational epitopes [40-42].

If injected intracerebrally in mice, AQP4-specific IgGs link to AQP4, and produce the histological changes of human NMO: leukocyte infiltration, astrocyte injure, neuronal cell death, deposition of activated complement proteins in perivascular area, myelin loss and [43].

The inflammatory cells present in NMO are primarily eosinophils, neutrophils, macrophages and few lymphocytes; whereas MS lesions are rich in macrophages and lymphocytes but low in granulocytes.

Current therapies for NMO include anti-B cell therapy plasma exchange and general immunosuppression [37].

Alzheimer Diseases (AD)

With the clarification of the glymphatic (glial lymphatic) system in 2012 as a system of brain clearing of toxins [31], some studies have associated a breakdown of the glymphatic pathway in AD [44].

It has been proposed that recycling of CSF flow is achieved by active fluid transport through the brain parenchyma from para-arterial to para-venous spaces and AQP4 realizes the clearance of interstitial solutes like tau and beta-amyloid, in order to prevent their accumulation.

In patients with AD the accumulation of beta-amyloid begins long before clinical signs of the disease and is a hallmark of AD [45].

Some studies have shown that the glymphatic system is most useful during sleep and dysfunctional sleep correlates with amyloid accumulation [31,46].

In the mid-to late-stage of AD the glymphatic function is low owed to the loss of polarity of AQP4 at the level of astrocytic extensions [47].

The role of AQP4 in regulating glutamate transporter-1 (GLT-1) has also been demonstrated. In AQP4 deficient mice the glutamate clearance is low and the astrocytes have reduced GLT-1 levels and these affects the memory and synaptic plasticity.

This demonstrates that AQP4 can be a new and exciting target in many diseases [48].

Brain tumours

The most common primary brain tumours is represented by astrocytomas, which is classified in four grades depending on malignancy. Glioblastoma or glioblastoma multiforme represents grade IV of astrocytomas.

Even with extensive surgical debulking, chemotherapy and radiotherapy, patients with glioblastoma generally live one year after diagnosis [49].

After the first description of Saadoun et al [50] of strong AQP4 positivity in astrocytomas,

with the highest levels in glioblastoma, many studies have reported similar results [51,52].

Sometimes glioblastomas also express AQP9 and AQP1 [53].

The correlation between tumor edema, as revealed by MRI, and the level of AQP positivity in tumor cells has proposed the role of AQP in tumor edema, although a more important idea is that AQPs promote tumor cell migration [54,55].

AQP4 is involved in various other pathologies such as: Parkinson's Disease, Traumatic brain injury, Hydrocephalus, Schizophrenia, Major depressive disorder, Epilepsy, Autism [56].

Conclusions

Besides describing the main characteristics of AQP1 and AQP4 in human normal brain and in its pathology, we have also showed that AQP1 is expressed in the normal human brain mostly in the astrocytes. Its expression is lower than that of AQP4, and their colocalisation degree also varies depending on the gray and white matter.

Acknowledgment

Rosu Gabriela-Camelia and Pirici Ionica equally contributed to the manuscript.

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