

Gene Section

Review

AQP4 (aquaporin 4)

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Identity

Other names: HMIWC2, MIWC

HGNC (Hugo): AQP4

Location: 18q11.2

DNA/RNA

Description

Sequence length: 323 AA.
Total number of exons: 5.

Protein

Description

This gene encodes a member of the aquaporin family of intrinsic membrane proteins that function as water-selective channels in the plasma membranes of many cells. The encoded protein is the predominant aquaporin found in brain. Two alternatively spliced transcript variants encoding distinct isoforms have been found for this gene.

Subunit structure: homotetramer. Part of a complex containing MLC1, TRPV4, HEPACAM and ATP1B1.

Domain: contains two tandem repeats each containing three membrane-spanning domains and a pore-forming loop with the signature motif Asn-Pro-Ala (NPA).

Post-translational modification: phosphorylation by PKC at Ser-180 reduces conductance by 50%.

Phosphorylation by PKG at Ser-111 in response to glutamates increases conductance by 40%.

Structure: AQP4, a small 30-kDa monomer, is a hydrophobic transmembrane protein with cytosolic amino and carboxy terminal ends (Verkman, 2005). The molecule spans the cell membrane 6 times, forming 5 interhelical loops designated as A, C, and E on the extracellular surface and B and D on the intracellular surface. A consistent 3-amino acid hydrophobic motif, asparagine-proline-alanine (NPA), is present in both the B and E loops.

Each monomer folds into a structure that forms an independent water channel, characterized by wide external openings and a narrow central constriction where the NPA motifs interact. AQP4 monomers assemble into tetramers, with each monomer being individually functional.

Water movement through the channel is governed by an osmotic gradient across the membrane, with flow limited by size restriction and electrostatic repulsion.

Variants: AQP4 occurs in mainly two splices variants, the M1 and M23 isoform (Jung et al., 1994). M23 forms higher order assemblies within the plasma membrane, termed orthogonal arrays of particles (OAPs), whereas M1 exists as individuals tetramers. Phosphorylation of AQP4 can also regulate array formation.



Expression

In the brain, AQP-4 is expressed at the glia limitans everywhere, ependymal lining, cerebellum, hippocampal dentate gyrus, and in the supraoptic and paraventricular nuclei of the hypothalamus. Low AQP-4 expression has also been found in the neocortex, hippocampal areas, nucleus of the stria terminalis, and the medial habenular nucleus (Venero et al., 1999). AQP-4 is expressed in a polarized way by astrocytic foot processes at the borders between major water compartments and the brain parenchyma (Nielsen et al., 1997; Rash et al., 1998). The perivascular expression of AQP4 coincides with the K⁺ channel protein Kir 4.1 at blood-brain barrier (BBB) level (Nagelhus et al., 1999).

Localisation

Subcellular localization: membrane; multi-pass membrane protein.

Function

AQP4 is implicated in the pathogenesis of normal pressure hydrocephalus, pseudotumor cerebri and cerebral edema (Badaut et al., 2002). AQP4-null mice have a much better outcome after water intoxication, meningitis and brain ischemia (Manley et al., 2004). AQP4-null mice have a significantly greater increase in brain water content and intracranial pressure than the wild-type mice, suggesting that brain water elimination is defective after AQP4 deletion (Papadopoulos et al., 2004a; Papadopoulos and Verkman, 2007).

AQP4, by controlling the bidirectional water flux is responsible for the formation of cellular brain edema, but counteracts vasogenic edema (Saadoun et al., 2002). In vasogenic edema, AQP4 is thought to have a protective role, through brain water clearance, whereas in cytotoxic edema it is the main contributor to astrocytic cell swelling (Manley et al., 2004; Papadopoulos et al., 2004a; Papadopoulos et al., 2004b). Water intoxicated AQP4-null mice show a significant reduction in astrocytic foot process swelling and a decrease in brain water content (Manley et al., 2000).

Nicchia et al. (2005) have shown that AQP-4 knockdown in rat and human cells was associated with a depolymerization of actin with a change of morphology characterized by a remarkable F-actin cytoskeleton rearrangement in AQP-4 knock-down mouse astrocytes.

Moreover, AQP-4 can interact with α -syntrophin, a member of the dystrophin-dystroglycan complex, indicating an involvement of AQP-4 protein in altering the cell cytoskeleton (Warth et al., 2004). Accordingly, Nico et al. have demonstrated that in the brain of mdx mouse, an animal model of the Duchenne muscular dystrophy, glial cells showed a significant reduction in both protein and mRNA content of the dystrophin-associated proteins (DAPs), including AQP-4, Kir 4.1, syntrophin and α - β -dystroglycan, coupled with a

decrease in dystrophin isoform (Dp71) (Nico et al., 2010). Moreover, alterations of the vascular basement membrane and reduction of the expression of its components laminin and agrin and translocation of α - β -dystroglycan receptors in the glial cytoplasmic endfeet have been demonstrated (Nico et al., 2010).

Homology

The AQP4 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, fruit fly, mosquito, *M. oryzae*, *A. thaliana*, and rice.

Implicated in

Melanoma

Note

Melanoma cells implanted into the striatum of wild type and AQP4-null mice produce peritumoral edema and comparable sized-tumors in both groups after a week. However, the AQP4-null mice have a higher intracerebral pressure and water content (Manley et al., 2004).

Astrocytoma

Note

AQP4 expression has also been demonstrated to be up-regulated in edematous astrocytomas and metastatic tumors (Saadoun et al., 2002). An increased AQP4 expression has been demonstrated in glioblastoma multiforme (GBM) together with loss of polarized expression around the vessels and an AQP4 redistribution in glioma cells (Warth et al., 2004; Warth et al., 2005; Warth et al., 2007). Warth et al. (2007) investigated grade I-IV glioma by immunohistochemistry and the prognostic significance for patients' survival. In gliomas, a remarkable de novo AQP4 redistribution was observed in comparison with normal central nervous system tissue.

Moreover, the highest membranous staining levels were seen in pilocytic astrocytomas WHO grade I and grade IV glioblastomas, both significantly higher than in WHO grade II. AQP4 up-regulation was associated with brain edema formation and no association between survival and WHO grade-dependent AQP4 expression was seen. Moreover, in glioma cells co-localization of AQP4 with K⁺ channel protein Kir 4.1 is abolished and a mislocation of both Kir channels and AQP4 has been reported (Warth et al., 2007), suggesting that this molecular rearrangement occurs as a reaction to BBB damages, facilitating edema fluid flow. Mou et al. (2010) investigated changes of AQP4 protein expression in normal brain and in brain glioma tumor and peritumoral edematous tissues and analyzed the relationship of AQP-4 protein with edema index, VEGF and hypoxia inducible factor 1 alpha (HIF-1 α) protein. They demonstrated that expression of AQP-4 was higher in the tumor and highest in the peritumor tissue. Moreover, AQP-4 protein in tumor tissue of gliomas of different grades was not statistically

different. In normal brain tissues, AQP-4 was mainly expressed in the foot processes of astrocytes, but rare in the parenchyma. Finally, the degree of peritumoral edema positively correlated with the expression level of AQP-4 protein and this latter correlated with VEGF and HIF-1 α expression. Nico et al. (2009) evaluated AQP4 expression and content in GBM and correlated with VEGF-VEGFR-2 expression. They demonstrated that in the relapse after chemotherapy and radiotherapy, AQP4 reduced in parallel with VEGF-VEGFR-2 expression as compared with primary tumors, and in the peripheral areas of relapsed tumors AQP4 mimicked normal findings of perivascular rearrangements. These data indicate that in GBM chemotherapy and radiotherapy induce a down-regulation in AQP4 expression restoring its perivascular rearrangement and suggest its potential role in the resolution of brain edema. Moreover, the normally polarized rearrangement of AQP4 in peripheral areas in tumor specimens obtained after combined chemotherapy and radiotherapy could be expression of a process of normalization of tumor blood vessels. Tumor implantation experiments into AQP4-null mice have demonstrated that these mice have an increased intracranial pressure than wild-type controls (Papadopoulos et al., 2004a; Papadopoulos et al., 2004b). McCoy et al. (2010) using D54MG glioma cells stably transfected with either AQP1 or AQP4 demonstrated that protein kinase C (PKC) activity regulates water permeability through phosphorylation of AQP4. Activation of PKC with either phorbol 12-myristate 13-acetate or thrombin enhanced AQP4 phosphorylation, reduced water permeability and significantly decreased tumor cell invasion. Conversely, inhibition of PKC activity with chlerythrine reduced AQP4 phosphorylation, enhanced water permeability and tumor cell invasion.

Meningioma

Note

Ng et al. (2009) demonstrated that overexpression of AQP4 in meningiomas was associated with significant peritumoral edema.

Therapeutic perspectives

Note

Inhibition of AQPs expression and/or AQP-mediated water influx by acetazolamide, cyclophosphamide, topiramate, thiopental, phenobarbital and propofol, affects cancer cell proliferation, migration, metastasis and angiogenic potential (Monzani et al., 2007).

Inhibition of AQP-4 expression (by small interference RNA technology) or their function (with a blocking antibody or a small inhibitory molecule) may result in increased intracellular acidosis and cytotoxicity and reduced invasive potential of glioma cells. Ding et al. (2011), using small interference RNA and a pharmaceutical inhibitor to knock down the expression

of AQP-4, demonstrated a specific and massive impairment of glioblastoma cell migration and invasion in vitro and in vivo.

Moreover, they showed that down-regulation of matrix metalloproteinase-2 (MMP-2) expression coincides with decreased cell invasive ability. Accordingly, Badaut et al. (2011) using RNA interference have demonstrated that brain water motility decreases after astrocyte AQP-4 inhibition.

Corticosteroids are largely used in combination with chemotherapy and contribute to significantly reduce peritumoral brain edema by decreasing the permeability of tumor vessels and/or enhance the clearance of extracellular water (Sinha et al., 2004). Animal experiments showed a decrease of cerebral AQP-4 protein expression upon dexamethasone treatment (Ron et al., 2005), suggesting that AQP-4 may be considered one of the major molecular targets of the well-functioning steroid treatment in brain edema formation. Moreover, corticosteroids reduced AQP-4 mRNA level in experimental brain tumor model and after intracerebral hemorrhage in rats (Heiss et al., 1996; Gu et al., 2007).

The evidence that AQP-4 facilitates the migration of reactive astrocytes towards an injury site and the infiltration of malignant astrocytes in glioblastoma (Verkman et al., 2008) suggests that AQP-4 inhibitors may reduce reactive gliosis and infiltration of astrocytes.

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