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Research Highlight

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Targeting AQP4 localization as a novel therapeutic target in CNS edema

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CNS edema is a pathological phenomenon after trauma, infection, tumor growth, or obstruction of blood supply, and it also can be fatal or lead to long-term disability, psychiatric disorders, substance abuse, or self-harm [1,2]. One exciting possibility would be to control excessive water accumulation in cells. However, all trials that inhibit water channel protein failed in clinic. A recent study by Kitchen *et al.* [3] reported that targeting the astrocytes' surface localization of water channel protein aquaporin-4 (AQP4) significantly relieves CNS edema. Astrocytes are the most abundant cell type of the brain and generally have a greater capacity than neurons to survive stresses [4]. Astrocyte cell function is critically affected by the lack of oxygen supply (hypoxia) to the brain, which is usually associated with CNS edema [5]. Their work holds new promise for our ability to use water-transfer strategies to treat CNS edema. Cytotoxic and vasogenic edema are primary interrelated etiological factors for the progress of CNS edema [6]. Vasogenic edema also depends on the extent of cytotoxic edema and the nature/severity of the underlying cause of the cytotoxic edema. So, understanding the pathogenesis of cytotoxic edema is important for the treatment of CNS edema. Aquaporins (AQPs) are historically known to be passive transporters of water. Lines of evidence in the last decade have highlighted the diverse function of AQPs beyond water homeostasis, including regulation of renal water balance, brain-fluid homeostasis, triglyceride cycling, and skin hydration [7]. Moreover, a subgroup of AQP water channels, termed 'aquaglyceroporins', also facilitates transmembrane diffusion of small, polar solutes not only water but also solutes [8,9]. AQP4 is the major subtype of AQPs expressed in astrocytes throughout the nervous system and facilitates astroglial cell migration via increasing plasma membrane water permeability, which in turn upregulates the transmembrane water fluxes during astroglial cell movement and is thus considered as an interesting therapeutic target in various neurological disorders. Astrocyte swellingmay also cause cytotoxic component disruptions of the blood–brain barrier, suggesting that astrocytes seem so sensitive to cytotoxic edema. AQP4 is a recognized contributor for the formation of cytotoxic brain edema, which is mainly a phenomenon of intracellular swelling of astrocytes. Knockdown of 'AQP4' or removal of the perivascular AQP4 pool by α-syntrophin or α-syntrophin deletion has been convincingly proven to counteract osmotically induced acute brain edema following ischemia and other brain injuries [10–12]. A previous study revealed that the NH2-cytosolic terminus of AQP4 interacts with metabotropic glutamate receptor 5 and assembles with the catalytic subunit of Na,K-ATPase to form a complex that has the potential function for the regulation of water permeability and potassium homeostasis in the astrocytes [13] (**Fig. 1**). In addition, AQP4 may trigger astrocytic Ca^{2+} responses, which is partly dependent on autocrine purinergic signaling (P2 purinergic receptor) activation in response to hypoosmotic stress [14] (**Fig. 1**). Additionally, subcellular relocalization of AQP4 in primary astrocytes is induced by calmodulin (CaM), calcium, and PKA in response to hypotonicity [15]. Further study proved that hypoxia-driven astrocyte swelling induces the increased abundance of AQP4 and initiates AQP4 cell-surface relocalization in a CaM- and PKA-dependent manner [3] (**Fig. 1**).

Previous studies showed an increase in AQP4 membrane localization in primary human astrocytes, which was not accompanied by a change in AQP4 protein expression level [16,17]. The increased AQP4 expression and the redistribution/surface localization can be two different concepts. Water transport via AQP4 can be regulated by translocation of AQP4 to the membrane in eukaryotes. Furthermore, AQP4 can be phosphorylated at multiple serine residues, which plays an incredible role in driving AQP4 subcellular relocalization. Casein kinase (CK) II-regulated Ser276 phosphorylation increases AQP4 lysosomal targeting and degradation under stress [18] (**Table 1**). Moreover, mutations at four putative CK2 phosphorylation sites (Ser276, Ser285, Thr289, and Ser316) also cause accumulation of AQP4 in the Golgi apparatus of astrocytes, demonstrating that CKII is necessary for AQP4 translocation [19] (**Table 1**). Glutamate, a crucial mediator of neuron astrocyte interactions, may increase astrocyte water permeability PKG-mediated AQP4 phosphorylation at serine 111 [20] (**Table 1**). Meanwhile, the vasopressin-induced reduction in water

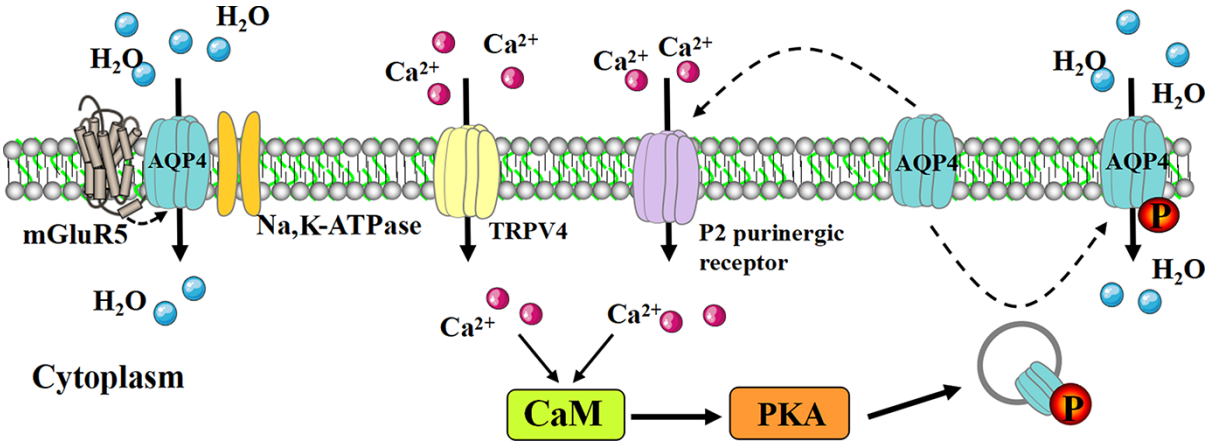

Figure 1. AQP4 promotes water permeability in astrocytes AQP4 is elicited by metabotropic glutamate receptor 5 (mGluR5) and interacts with the catalytic subunit of Na,K-ATPase to form a complex that has the potential function to regulate water permeability. Furthermore, AQP4 may trigger astrocytic Ca^{2+} responses via activation of autocrine purinergic signaling (P2 purinergic receptor). Both P2 purinergic receptor and TRPV(transient receptor potential vanilloid 4) can facilitate the influx of calcium ions in astrocytes, which activates CaM. CaM can also promote the activation of PKA. Additionally, subcellular relocalization of AQP4 in primary astrocytes is driven by CaM and PKA. Adapted from Kitchen *et al.* [3] with permission from Cell Press.

Table 1. The kinases for AQP4 phosphorylation

Kinase	Residue	Effect	References
CKII	Ser276, Ser285, Thr289, and Ser316	Regulate the degradation and translocation of AQP4	[18,19]
PKG	Ser111	Increase AQP4-induced astrocyte water permeability	[20]
PKC	Ser180	Reduce AQP4-triggered water permeability	[21,22]
PKA	Ser276	Promote AQP4-induced astrocyte water permeability	[3]
CaMKII	-	Restrain AQP4 water permeability function	[23]

permeability is implicated in PKC-dependent serine 180 phosphorylation of AQP4 [21] (Table 1). In addition, PKC activator also decreases brain edema following cerebral ischemia by downregulation of AQP4 expression [22] (Table 1). So, PKC itself can reverse the brain edema, while PKA plays a key role in mediating AQP4 translocation. PKA-phosphorylated AQP4 at Ser-276 is required for tonicity-mediated AQP4 relocalization. Recently, research further confirms that PKA phosphorylates AQP4 at Ser276 by activating CaM-dependent cyclic AMP to relieve CNS edema in response to hypotonic treatment [3]. Many lines of evidence revealed that targeting CaM dependence of AQP4 subcellular relocalization is a viable option for CNS edema therapies. Inhibition of calmodulin-dependent protein kinase II (CaMKII) and mutation of a putative phosphorylation site for CaMKII restrain lead-triggered increase of water permeability in AQP4-expressing astrocytes [23] (Table 1). Nevertheless, the precise site of AQP4 phosphorylated by CaMKII is still unclear. Overall, understanding the phosphorylation sites of AQP4 provides an additional treatment strategy for cerebral edema.

AQPs have been validated as an important drug target, but there is no single drug that has yet been successfully approved [24,25]. If AQP4 can be a target for the treatment of CNS edema, understanding the inhibitors or agonist of AQP4 phosphorylation may provide the clues for drug research of CNS edema. H-89, a strong suppressor for PKG and PKA, has a function of inhibiting AQP4-induced water edema [20] (Table 2). Phorbol myristate acetate (PAM), an activator of PKC, can effectively decrease the brain edema induced by AQP4 [22] (Table 2). KN-93, an inhibitor of CaMKII, diminishes the DHPG(dehydroxy-phenylglycol)-induced water permeability in GFP(green fluorescent protein)-AQP4 cells [22] (Table 2). In addition, CaMKII inhibitor KN-62 can reduce the lead-induced upregulation of water permeability in astrocytes transfected with 'AQP4' [23] (Table 2). In addition, some small molecule inhibitors also have the function of blocking the activity of AQP4. Acetazolamide (AZA), an inhibitor of sulfonamide carbonic anhydrase (CA), reduces the water permeability via AQP4 [26] (Table 2). TGN-020, a small molecule inhibitor of AQP4, acts as an inhibitor for AQP4-mediated brain edema associated with brain ischemia [27] (Table 2). Moreover, 14 antiepileptic drugs (AEDs) have been tested, and 7 of them are found to be able to suppress AQP4 function [28] (Table 2). Additionally, recent researches confirmed that trifluoperazine (TFP), an antipsychotic licensed by the US Food and Drug Administration and the UK National Institute for Health and Care Excellence and also a CaM antagonist, shows incredible effect on the prevention of AQP4 localization and CNS edema inhibition in rats [3] (Table 2).

In summary, among the 13 AQP membrane proteins, AQP4 is the primary water channel that regulates water permeability in CNS. The precise mechanism of CNS edema is complex. Excitingly, the latest research proved that translocation of AQP4 to the astrocyte cell surface can promote CNS edema induced by CaM and PKA under the condition of hypoxia. In addition, phosphorylation AQP4 has a close connection with AQP4 subcellular localization. Therefore, inhibiting phosphorylation of AQP4 may be a new method for the treatment of the CNS edema. Meanwhile, research also confirmed that the approved drug TFP has a function to block CNS edema, suggesting that TFP might be used to treat CNS edema. Furthermore, the inhibitors or activators of AQP4 phosphorylation also can provide important clues for the research of CNS edema therapeutic drugs.

Table 2. The small molecule inhibitors of AQP4

Small molecule	Category	Effect on AQP4	References
H-89	Suppressor for PKG and PKA	Inhibition of AQP4-induced water edema	[20]
PAM	Activator of PKC	Abolishment of the brain edema induced by AQP4	[22]
KN-93	Inhibitor of CaMKII	Diminished the DHPG-induced water permeability in GFP-AQP4(1) cells	[22]
KN-62	Inhibitor of CaMKII	Abrogation of AQP4-triggered water permeability	[23]
AZA	Inhibitor of sulfonamide CA	Reduced the water permeability via AQP4	[26]
TGN-020	Inhibitor of AQP4	Inhibitor for AQP4-mediated brain edema associated with brain ischemia	[27]
AEDs	AEDs	Suppressed AQP4 function	[28]
TFP	Antipsychotic	Prevention of the localization of AQP4 and further inhibition of CNS edema in rat	[3]

At present, most studies on the treatment of AQP4 in brain edema remain *in vivo* or *in vitro*. In future clinical trials, the role of AQP4 in CNS edema can also be verified by using the humanized three-dimensional self-organized models, organoids, and organ-on-a-chip platforms [29,30].

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Conflict of Interest

The authors declare that they have no conflict of interest.

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