The role of hypoxia-inducible factor-1α, aquaporin-4, and matrix metalloproteinase-9 in blood-brain barrier disruption and brain edema after traumatic brain injury

Laboratory investigation

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Object. The present study investigated the role of hypoxia-inducible factor-1α (HIF-1α), aquaporin-4 (AQP-4), and matrix metalloproteinase-9 (MMP-9) in blood-brain barrier (BBB) permeability alterations and brain edema formation in a rodent traumatic brain injury (TBI) model.

Methods. The brains of adult male Sprague-Dawley rats (400–425 g) were injured using the Marmarou closed-head force impact model. Anti–AQP-4 antibody, minocycline (an inhibitor of MMP-9), or 2-methoxyestradiol (2ME2, an inhibitor of HIF-1α), was administered intravenously 30 minutes after injury. The rats were killed 24 hours after injury and their brains were examined for protein expression, BBB permeability, and brain edema. Expression of HIF-1α, AQP-4, and MMP-9 as well as expression of the vascular basal lamina protein (laminin) and tight junction proteins (zona occludens-1 and occludin) was determined by Western blotting. Blood-brain barrier disruption was assessed by FITC-dextran extravasation, and brain edema was measured by the brain water content.

Results. Significant (p < 0.05) edema and BBB extravasations were observed following TBI induction. Compared with sham-operated controls, the injured animals were found to have significantly (p < 0.05) enhanced expression of HIF-1α, AQP-4, and MMP-9, in addition to reduced amounts (p < 0.05) of laminin and tight junction proteins. Edema was significantly (p < 0.01) decreased after inhibition of AQP-4, MMP-9, or HIF-1α. While BBB permeability was significantly (p < 0.01) ameliorated after inhibition of either HIF-1α or MMP-9, it was not affected following inhibition of AQP-4. Inhibition of MMP reversed the loss of laminin (p < 0.01). Finally, while inhibition of HIF-1α significantly (p < 0.05) suppressed the expression of AQP-4 and MMP-9, such inhibition significantly (p < 0.05) increased the expression of laminin and tight junction proteins.

Conclusions. The data support the notion that HIF-1α plays a role in brain edema formation and BBB disruption via a molecular pathway cascade involving AQP-4 and MMP-9. Pharmacological blockade of this pathway in patients with TBI may provide a novel therapeutic strategy. (DOI: 10.3171/2010.6.JNS10207)

Key Words • basal lamina • tight junction protein • blood-brain barrier permeability

Cerebral edema following TBI can cause brain swelling and increased intracranial pressure, in turn producing secondary neural damage, brain herniation, and death. In severe TBI, brain edema poses a critical clinical problem due to its association with increased morbidity and mortality.20,23 Despite its clinical importance, all present treatment options for edema, including intravenous infusion of high molecular weight compounds, simply provide symptomatic relief due to the fact that molecular mechanisms underlying this phenomenon remain poorly understood. The lack of effective drugs to attenuate the formation and progression of TBI-induced edema has led to increased interest in the role that antiedematous molecules may play in mitigating this phenomenon.

Recently, AQPs and MMPs have been shown to be integral components of the pathophysiology of brain edema...
The MMPs are intercellular proteases that cleave the extracellular matrix, including major components of the basal lamina and tight junctions between endothelial cells. This proteolytic cleavage results in disruption of the BBB, vessel permeability increases, and the BBB is no longer able to regulate the passage of molecules between the vasculature and parenchyma. Such BBB dysregulation leads to an increase of water in the extracellular space of the brain, defining edema. In many types of brain pathologies, including TBI, MMP-9 is markedly upregulated, and this is thought to cause BBB disruption. The role of MMPs in BBB disruption is further supported by the observation that treatment with MMP inhibitors decreases such disruption and improves brain edema. Similar to the case of AQP-4, however, the molecular cascade leading to the upregulation of MMP-9 after TBI is not clearly understood.

Hypoxia-inducible factor-1α (HIF-1α), an upstream transcription factor induced by hypoxia, regulates the subsequent expression of many kinds of proteins responding to the various pathophysiological conditions induced by hypoxia. While some studies have shown HIF-1α to be upregulated in TBI, its potential role in tissue injury or restoration after TBI remains controversial. Thus, whether HIF-1α contributes to the formation of brain edema by regulating AQP-4 and MMP-9 remains to be clarified.

The purpose of this study was to determine, in a rodent model of TBI with prominent edema, whether a molecular cascade involving HIF-1α, AQP-4, and MMP-9 is causally related to edema formation. The study further seeks to provide novel data, which may support potential therapeutic targets within this cascade, aimed at ameliorating cerebral edema.

**Methods**

**Rat Closed-Head Trauma Model**

All animal experimental procedures were approved by the Institutional Animal Investigation Committee of Wayne State University and were in accordance with National Institutes of Health guidelines for care and use of laboratory animals. A total of 90 adult (400–425 g) male Sprague-Dawley rats (Charles River) were used. Rats were divided into a Sham-Operated Group (controls) and 4 TBI groups (18 animals in each group). The TBI groups included 1) untreated animals (the Untreated TBI Group), 2) animals treated with anti–AQP-4 antibody (the AQP-4-Antibody–Treated TBI Group), 3) animals treated with the MMP-9 inhibitor minocycline (the Minocycline-Treated TBI Group), and 4) animals treated with the HIF-1α inhibitor 2-methoxyestradiol (the 2ME2-Treated TBI Group). To produce TBI, a modified Marmarou force impact model was used. Unlike other TBI models that directly impact the cerebral cortex (fluid percussion and cortical impact), the Marmarou model is a closed-head TBI model that induces significant cerebral edema. It is more representative of actual TBI, which rarely involves penetration of the brain. Briefly, the anesthetized rats were placed prone on a foam-covered platform. A 450-g weight was first aligned with the surface of a steel helmet, which was directly attached to the skull between bregma and lambda, and was then dropped directly onto the helmet from a height of 2 m. Placement of the helmet ensures that the skull is not fractured by the impact, and it guarantees that the brain is not directly contused. Control (sham-operated) animals were anesthetized and had the helmet attached to their skulls, but were not subjected to the weight drop.

**Inhibition of AQP-4, MMP-9, and HIF-1α**

In 3 of the TBI groups anti–AQP-4 IgG (1 μg/kg, Sigma Aldrich Co.), minocycline (1 mg/kg, Sigma Aldrich), or 2ME2 (2.5 mg/kg, Sigma Aldrich) dissolved in 0.25 ml of isotonic saline was administered intravenously for 30 minutes after injury. Minocycline, a lipophilic tetracycline derivative, is well known to inhibit MMP-9 activity. 2-Methoxyestradiol (2ME2) is a naturally occurring metabolite of estradiol, which is known to posttranscriptionally downregulate the expression of HIF-1α. Rats from the Untreated TBI Group were administered 0.25 ml of isotonic saline intravenously. All rats in the TBI groups were killed 24 hours postinjury, and their brains were used for analysis of edema (6 in each group), BBB disruption (6 in each group), and protein expression (6 in each group).

**Assessment of BBB Permeability and Brain Edema**

Dextran coupled with FITC (FITC-dextran) was chosen to determine the extent of BBB disruption and permeability alterations at 24 hours after TBI. Breakdown of the BBB allows water and blood-borne substances to pass into the brain parenchyma more readily. Fluorescein isothiocyanate–dextran (average molecular weight 40,000 D) is commonly used to measure vascular permeability because it normally remains in the capillary lumen with no leakage outside of the vessels. Rats were injected via the tail artery with 0.25 ml of 5% FITC-dextran per 100 g body weight 10 minutes before they were killed.
Coronal sections (20 μm) were cut from each brain using a freezing microtome. Images for analysis of FITC-dextran leakage from cerebral vessels were obtained at ×400 original magnification using an image analysis system (QCapture Pro 5.1, QImaging). For spectrophotometric analysis, tissues were thoroughly flushed with 0.9% saline to eliminate vascular stasis due to FITC-dextran. Each sample was then weighed and placed in 6 ml of a 50% trichloroacetic acid solution. The samples were homogenized and centrifuged for 45 minutes at 4000 rpm to form a pellet. The aqueous upper phase was analyzed using an AD 340 plate reader (Beckman Coulter, Inc.). A standard curve for control was generated using an FITC-dextran serial dilution. The magnitude of BBB disruption was then quantified by measuring the extent of tracer leakage.

Edema was directly measured by assessing water content in the brains at 24 hours post-TBI. Briefly, each brain was harvested and immediately weighed after extraction to determine its wet weight before being dried in an 80°C oven for 72 hours, after which it was weighed again to obtain the dry weight. The formula (wet weight – dry weight)/wet weight × 100 was used to calculate the water content expressed as a percentage of the wet weight.

Expression of AQP-4, MMP-9, HIF-1α, Laminin, ZO-1, and Occludin

Western immunoblot analysis was used to assess expression of AQP-4, MMP-9, and HIF-1α, as well as expression of vascular wall proteins, including the basal lamina (laminin), and of the endothelial tight junction, including zona occludens-1 (ZO-1) and occludin. Tissue samples containing the entirety of both cerebral hemispheres, including gray and white matter, were processed in lysis buffer with protease inhibitors on ice. Equal volumes (10 μl) of tissue extracts normalized by protein concentration were separated by electrophoresis through 10% polyacrylamide gel (Bio-Rad Laboratories, Inc.) and were then transferred to a nitrocellulose membrane (Bio-Rad Laboratories, Inc.). Seven different primary antibodies were used: polyclonal rabbit anti–AQP-4 (1:500, Santa Cruz Biotechnology, Inc.), polyclonal rabbit anti–MMP-9 (1:2,000, Santa Cruz Biotechnology, Inc.), polyclonal rabbit anti–HIF-1α (1:2,000, Santa Cruz Biotechnology, Inc.), monoclonal mouse anti–laminin (1:500, R&D Systems, Inc.), polyclonal rabbit anti–ZO-1 (1:1,000, Invitrogen) and polyclonal rabbit anti–occludin (1:1,000, Invitrogen). Equal loading of protein was confirmed and adjusted by intracellular protein β-actin (goat polyclonal anti–β-actin antibody, 1:1,000, Santa Cruz). These antibodies were incubated with the membrane at room temperature for 1 hour. After 3 wash cycles, the membrane was then incubated with secondary antibody conjugated to horseradish peroxidase (Sigma Aldrich) for 30 minutes. Finally, the targeted antigens were visualized by using standard chemical luminescence methods (Amersham ECL, GE Healthcare BioSciences Corp.). To quantify the relative levels of target protein expression, blot images were analyzed using an image analysis program (ImageJ 1.42, National Institutes of Health). Finally, the expression intensity of the proteins from different groups was statistically compared.

Statistical Analysis

All the data were expressed as means ± SEs. Statistical analysis was performed with SPSS for Windows, version 13.0 (SPSS, Inc.). Differences between 2 groups were analyzed using a 2-tailed unpaired Student t-test. The differences among multiple groups were assessed using a 1-way analysis of variance. Post hoc comparison between groups was further undertaken with the least significant difference method. Probability values < 0.05 were considered significant.

Results

Brain Edema, BBB Disruption, and Protein Expression After TBI

Brain edema was assessed as a percentage change in water content of brain tissues in each group. Animals with untreated TBI showed significantly (p < 0.05) increased brain edema compared with sham-operated controls (Fig. 1).

Leakage of FITC-dextran from cerebral microvessels was observed under fluorescence microscopy and quantified with spectrophotometry. As an intact BBB prevents extravasation of FITC-dextran from vessels, no leakage was observed surrounding vessels in sham-operated control rats. Obvious leakage from vessels into the surrounding parenchyma was observed after TBI (Fig. 2A). This observation is further corroborated by spectrophotometric analyses. Thus, a significant (p < 0.05) increase in the amounts of FITC-dextran was detected in the brain parenchyma of rats following TBI (Fig. 2B). Taken together the data confirm that both brain edema and BBB disruption are prominent in this TBI model.

Western immunoblot analyses of protein expression in brain tissues from rats subjected to TBI and not treated (Untreated TBI Group) compared with those from sham-operated controls revealed increased expression of AQP-4 (p < 0.05), MMP-9 (p < 0.01), and HIF-1α (p < 0.01) at 24 hours after TBI (Fig. 3A–C). In contrast, a significant post-TBI decrease in the expression of laminin (p < 0.01), ZO-1 (p < 0.01), and occludin (p < 0.05) was detected (Fig. 3D–F).

Brain Edema, BBB Disruption, and Protein Expression in TBI with Inhibition of AQP-4, MMP-9, and HIF-1α

Brain edema was significantly reduced in the rats treated with anti–AQP-4 antibody (p < 0.01), minocycline (p < 0.01), or 2ME2 (p < 0.01) after brain injury as compared with the Untreated TBI Group (Fig. 4). These results suggest that TBI-induced brain edema was alleviated by AQP-4, MMP-9, and HIF-1α.

Neither light microscopic observation (Fig. 5A) nor spectrophotometric quantitative analysis (Fig. 5B) demonstrated any significant decrease of FITC-dextran extravasation.
sation in tissue from animals treated with anti–AQP-4 antibody as compared with the Untreated TBI Group (p = 0.3). In contrast, significantly decreased leakage was detected in brains from rats treated with either minocycline (p < 0.01) or 2ME2 (p < 0.01). These results suggest that BBB disruption following TBI can be ameliorated by inhibition of either MMP-9 or HIF-1α. Conversely, inhibition of AQP-4 does not appear to affect TBI-induced FITC-dextran leakage.

Significant (p < 0.05) reduction in the expression of AQP-4 was detected in brain tissues from brain-injured animals treated with minocycline or 2ME2, as compared with those from untreated injured rats (Fig. 6A). Similarly, compared with untreated injured rats, a significant (p < 0.01) reduction in the expression of MMP-9 was detected in brains from rats treated with minocycline or 2ME2 after TBI (Fig. 6B). A significant (p < 0.05) reduction in the expression of HIF-1α was detected in tissues from injured rats treated with 2ME2 compared with those from the untreated TBI group (Fig. 6C). Reduced expression of HIF-1α was also observed in brain tissues from the Minocycline-Treated TBI Group compared with those from the Untreated TBI Group, although this trend did not achieve a significant (p = 0.06) level. Furthermore, compared with the Untreated TBI Group, a significantly (p < 0.01) higher expression of laminin was detected in animals treated with either minocycline or 2ME2 after TBI (Fig. 6D). Similarly, a significantly (p < 0.01) higher expression of ZO-1 was detected in injured animals treated with 2ME2 compared with untreated injured rats. However, no significant (p = 0.3) differences were found between the Minocycline-Treated TBI and Untreated TBI groups (Fig. 6E). Finally, while a significantly (p < 0.05) higher expression of occludin was detected in the 2ME2-

Fig. 1. Bar graph demonstrating brain edema measured as a percentage change in water content. At 24 hours after TBI, the mean brain water content was significantly higher in the Untreated TBI Group than in the Sham-Operated Group (controls). Values are means ± SEs. *p < 0.05.

Discussion

The major finding of this study is that concurrently elevated expression of HIF-1α, AQP-4, and MMP-9 in traumatically injured brain tissues temporally coincided with brain edema formation and BBB disruption. In the present TBI model, edema, as determined by brain water content, was reduced by selective inhibition of HIF-1α, AQP-4, or MMP-9. In addition, BBB disruption, as determined by FITC-dextran extravasation, was ameliorated by inhibition of either MMP-9 or HIF-1α. Inhibition of MMP-9 was also shown to reverse the loss of laminin.
Furthermore, while inhibition of HIF-1α caused increased expression of both BBB basal lamina and tight junction proteins, this inhibition produced the reverse effect in the expressions of AQP-4 and MMP-9. Taken together, the data suggest a functional interaction linking AQP-4, MMP-9, and HIF-1α, which is possibly dysregulated by TBI. The data further suggest that HIF-1α regulates the expression of AQP-4 and MMP-9, which in turn are crucial in edema formation and BBB disruption post-TBI (Fig. 7).

**HIF-1α, MMP-9, and AQP-4 in BBB Function**

Normally, cerebral blood vessels differ from extracerebral vessels in their participation in forming the blood-brain barrier (BBB), a structural, biochemical and physiological construct that has recently been expanded to include not only interendothelial tight junctions, but also the foot processes of perivascular astrocytes, pericytes, and their intervening basal lamina. The BBB limits the passage of many substances, including high-molecular weight plasma proteins, from the vasculature into the brain parenchyma. On the endothelial side, tight junctions are cholesterol-enriched regions along the plasma membrane containing specialized transmembrane proteins and supported by accessory cytoplasmic proteins. Occludin, one of the transmembrane proteins, and ZO-1, an accessory cytoplasmic protein, are thought to play a critical role in BBB integrity. Decreased expression of these proteins is associated with increased BBB permeability to high-molecular weight proteins, similar to dextran.

On the luminal side of the vessel wall, the perivascular basal lamina both surrounds and anchors endothelial

**Fig. 3.** Bar graphs showing the expression levels of AQP-4, MMP-9, HIF-1α, laminin, ZO-1, and occludin in brain tissue. In comparison with specimens from sham-operated controls, significantly higher levels of AQP-4, MMP-9, and HIF-1α and significantly lower levels of laminin, ZO-1, and occludin were detected in specimens from mice in the Untreated TBI Group. Equal protein loading was confirmed by intracellular β-actin. Representative immunoblots are presented. Values are means ± SEs. *p < 0.05; **p < 0.01.
cells to astrocytes, and it provides further structural and functional properties to the BBB. Laminin, a major structural protein of the basal lamina, is also known to be associated with pathological states of the BBB. Traumatic brain injury is known to upregulate various kinds of proteases including MMPs, which may destroy the integrity of the basal lamina and tight junctions. Laminin, occludin, and ZO-1 are all known substrates of various types of proteolytic proteins, including MMP-9. Once the structural integrity of the BBB is compromised by proteolysis, altered osmotic gradients and water channels cause accumulation of fluids in the extracellular and cellular compartments of the brain, which defines cerebral edema.

In the present study, the TBI-induced increased expression of HIF-1α, AQP-4, and MMP-9 was temporally associated with FITC-dextran leakage from microvessels within the brain parenchyma, in addition to decreased expression of regulatory proteins of BBB integrity and subsequent permeability. This outcome was reversed by inhibiting either HIF-1α or MMP-9. In contrast, inhibition of AQP-4 did not affect BBB leakage. Previous studies have demonstrated concurrent increased expression of HIF-1α and MMP-9 in different brain pathologies, including TBI, implicating these molecules in a pathophysiological cascade leading to secondary cell injury. A multitude of genes involved in inflammation, apoptosis...
Fig. 6. Bar graphs and representative immunoblots showing the level of expression of AQP-4, MMP-9, HIF-1α, laminin, ZO-1, and occludin in brain tissue from rats treated with monocycline or 2ME2 after TBI compared with that in tissue from untreated injured rats. Protein equal loading was confirmed by intracellular β-actin. Values are means ± SEs. *p < 0.05; **p < 0.01.

Fig. 7. Schematic diagram showing the mechanisms that may underlie traumatic brain edema formation. Traumatic brain damage induces HIF-1α expression, which, in turn, upregulates AQP-4 and MMP-9. MMP-9 may also upregulate AQP-4 and possibly HIF-1α. MMP-9 induces degradation of basal lamina (laminin) while HIF-1α induces degradation of basal lamina and tight junction proteins (ZO-1, occludin), resulting in BBB disruption, leading to brain edema. AQP-4 increases water permeability of BBB through water transport. These changes accelerate water movement from blood vessel to brain parenchyma, which causes brain edema.
and proteolysis are regulated by HIF-1α, and its increased expression has been reported to be correlated with BBB disruption. The present study further demonstrates that inhibition of HIF-1α mediates upregulation of laminin, ZO-1, and occludin, in addition to downregulation of MMP-9 expression. These results suggest that HIF-1α may be an upstream protein in causing BBB disruption through its regulatory expression of catalytic enzymes such as MMP-9. In the present study, while laminin expression was significantly increased following inhibition of either MMP-9 or HIF-1α, expression of ZO-1 and occludin was not. Tight junction proteins such as ZO-1 and occludin have been known to be impaired by other molecules in addition to MMP-9. For example, vascular endothelial growth factor (VEGF), an important target gene of HIF-1α, is also known to induce microvascular leakage by altering delocalization and expression of tight junction proteins.

A causal relationship between AQP-4 and BBB integrity has been previously suggested. As such, it has been reported that AQP-4 is essential for the maintenance of BBB. In contrast, some very recent studies have indicated that AQP-4 expression does not alter BBB integrity or brain morphological characteristics. In AQP-4–deficient mice, there is no BBB alteration with regard to morphological characteristics or permeability, suggesting no causal relationship between AQP-4 and BBB function. To date no study has demonstrated a causal relationship between AQP-4 alteration and impaired BBB function. The present data support the notion that AQP-4 induces cerebral edema, not by directly contributing to BBB breakdown, but via transcellular water channels.

**HIF-1α, AQP-4, and MMP-9 in Brain Edema Formation**

In this study, TBI-induced brain edema was reduced by inhibiting HIF-1α, AQP-4, or MMP-9. These results suggest that increased HIF-1α, AQP-4, or MMP-9 expression in brain tissue can generate and exacerbate edema. Edema induced by TBI is generally classified into cytotoxic or vasogenic types. Cytotoxic edema, primarily involving astrocytes, refers to intracellular accumulation of water due to the inability of these cells to regulate their volume. In contrast, vasogenic edema refers to extracellular accumulation of water due to BBB disruption. Cytotoxic edema is a common feature of cerebral ischemia. There is also significant experimental and clinical evidence that TBI-induced edema is predominantly cytotoxic with some extent of vasogenic edema. In addition, recent studies have shown that cytotoxic edema develops earlier and persists longer than vasogenic edema after TBI. In the present study, while inhibition of AQP-4 caused improvement of brain edema, it did not significantly ameliorate the defective vessel leakage. This finding suggests that AQP-4–associated brain edema may be controlled by the upregulation of the water channel. AQP-4 may contribute to the formation of cytotoxic brain edema by regulating water balance across brain compartments. Thus, during pathological conditions in which an alteration of water homeostasis occurs, cellular swelling occurs due to influx of fluid from the vascular compartment independently of BBB function, leading to cytotoxic edema.

An unexpected result of the present study was the downregulation of AQP-4 by minocycline treatment. Moreover, the expression of HIF-1α was also decreased with minocycline, although this trend was not statistically significant. There has been no previous report that minocycline inhibits AQP-4 or HIF-1α. Since minocycline is a well-known inhibitor of MMPs, it might have indirectly decreased expression of AQP-4 and HIF-1α by affecting molecular pathways involving MMPs. The present study further demonstrated that inhibition of MMP-9 with minocycline could also reduce brain edema. Therefore, minocycline could have dual neuroprotective effects whereby it can ameliorate BBB disruption and reduce vasogenic brain edema by regulating MMP-9, in addition to reducing edema through decreased expression of AQP-4.

Some studies have demonstrated that the increased HIF-1α expression in TBI enhances the expression of various downstream proteins implicated in brain edema, BBB disruption, and apoptosis. Reduction in global cerebral blood flow and vessel permeability alteration were found to be potential factors in secondary ischemia in traumatically injured brain tissues, which in turn upregulate the expression of HIF-1α. In addition, a causal relationship between HIF-1α and MMP-9 was described here. Like the results in the present study, we previously also showed a temporal association of increased expression of HIF-1α and AQP-4 following TBI. Furthermore, inhibition of HIF-1α reduced the expression of AQP-4. These results suggest that HIF-1α and AQP-4 are part of a single molecular cascade.

In the present study, inhibition of HIF-1α reduced not only brain edema, but also the expressions of AQP-4 and MMP-9, suggesting that HIF-1α is a key protein in the formation of brain edema, possibly through regulation of AQP-4 and MMP-9. Whether enhanced HIF-1α is beneficial or harmful after brain damage remains debatable; in addition to inducing MMP-9 upregulation as shown here, HIF-1α also induces angiogenesis, glycolysis, and erythropoiesis, leading to neuroprotection. Recent studies have demonstrated that HIF-1α and its target genes may contribute to cell death, tissue destruction, and the formation of brain edema primarily in the acute phase after ischemic brain damage, suggesting that inhibition of HIF-1α is likely to be beneficial during the acute phase. Therefore, inhibition of HIF-1α immediately following TBI may be effective in improving neurological outcomes by protecting the brain from secondary injury following edema and BBB disruption. To determine the long-term therapeutic effects of inhibiting these proteins, as a means of neuroprotection and enhanced recovery processes in TBI, behavioral and neuropathological outcomes will be further assessed in our future studies.

**Conclusions**

The present data demonstrate a pathway involving HIF-1α, AQP-4, and MMP-9 in brain edema formation and BBB disruption. Elucidation of the pathway could
provides insights into effective pharmacological target therapies. To our knowledge, this is the first study to show a relationship between these proteins and the potential pathophysiological cascade leading to edema formation.

In TBI, increased expression of HIF-1α may play a central role in brain edema and BBB disruption by regulating both AQP-4 and MMP-9.

**Disclosure**

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Blood-brain barrier disruption and brain edema in TBI

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