Aquaporin 4 and its Relationship with Brain Astrocytomas – Literature Review

Aquaporina 4 e sua Relação com Astrocitomas Cerebrais – Revisão da Literatura

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Abstract

Background Aquaporins (AQPs) are a family of membrane proteins that regulate the osmotic permeability of the plasma membrane. There are described in the literature a total of 13 types of Aquaporins in mammals, each with different places of expression. In addition to water, some AQPs allow the passage of glycerol and ammonia, being called Aquaglyceroproteins. In the central nervous system, AQPs 1 and 4 are expressed, being responsible for the water regulation in the blood-brain barrier. These two AQPs are believed to participate in the pathophysiological process that governs the behavior of various CNS diseases, such as trauma and primary tumors. More particularly, there are quite controversial data in the literature on the expression of AQP4 in tumors and its relationship with disease progression and treatment possibility.

Objective This paper aims to perform a literature review on the function and expression of AQP4 in the CNS and primary tumors of this system, to compile what is in the literature on the subject and raise new possible research hypotheses.

Methods The PUBMED platform was used for bibliographic survey using "Aquaporin 4," "expression" and "astrocytomas" as keywords. Articles older than 2008 and articles that did not address AQP4 expression in astrocytomas were excluded. In the selected articles, the following topics were investigated: AQP4 structure, brain and tumor localization, and relationship with peritumoral edema.

Results Regarding the structure and location of AQP4, the literature presents two isoforms of AQP4: M1 and M23. Both form clusters of AQP4 called "orthogonal arrays of proteins - OAPs." In the tumor tissue, the literature shows a decrease in the formation of OAPs and an increase in the expression of both AQP4 isoforms, besides losing their polarity, diffusing through the cytoplasmic membrane. As for the function of AQP4 in

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tumors, AQP4 assists in cell migration and invasion, in addition to participating in cell proliferation and apoptosis. Regarding the relationship with cerebral edema, there are controversial knowledge. Studies have shown that increased AQP4 aggravates cytotoxic edema of tumor cells and, by assisting in cell migration and angiogenesis, indirectly assist in the formation of vasogenic edema by breaking the blood-brain barrier. Other studies, however, point to the increase in AQP4 as a protective mechanism to combat vasogenic edema that occurs in tumor formation. Furthermore, the literature presents a therapeutic proposal in which, by inhibiting AQP4 expression, tumor migration and cerebral edema decrease in rats with glioblastoma.

Discussion As shown in the literature, there is a difference in histopathological structure between high and low grade gliomas. However, there are common changes between them. These common changes could then be used as a factor of severity or evolution of low-grade to high-grade tumors. Moreover, it is not yet possible to perceive the true relationship of AQP4 expression and increased VEGF evolution of peritumoral edema. Finally, it can be hypothesized that since the expression ratio between AQP4 isoforms in normal tissue is greater than in some tumors, the decrease in this ratio is due either to decreased M23 expression or increased of the isoform M1.

Conclusion Further studies are needed to understand the physiology and pathophysiology involving AQP4 in astrocytomas to create effective therapeutic proposals to combat this disease.

Resumo

Introdução As aquaporinas (AQPs) são uma família de proteínas de membrana que regulam a permeabilidade osmótica da membrana plasmática. Existem descritos na literatura um total de 13 tipos de aquaporinas em mamíferos, cada um com diferentes locais de expressão. Além da água, alguns AQPs permitem a passagem de glicerol e amônia, sendo chamados de aquagliceroproteínas. No sistema nervoso central, as AQPs 1 e 4 são expressas, sendo responsáveis pela regulação da água na barreira hematoencefálica. Acredita-se que esses dois AQPs participem do processo fisiopatológico que regula o comportamento de várias doenças do SNC, como trauma e tumores primários. Mais particularmente, há dados bastante controversos na literatura sobre a expressão de AQP4 em tumores e sua relação com a progressão da doença e possibilidade de tratamento.

Objetivo Este artigo tem como objetivo realizar uma revisão da literatura sobre a função e expressão da AQP4 no SNC e tumores primários deste sistema, a fim de compilar o que está na literatura sobre o assunto e levantar novas hipóteses de pesquisa possíveis.

Método A plataforma PUBMED foi utilizada para levantamento bibliográfico utilizando "Aquaporin 4," "expression" e "astrocitomas" como palavras-chave. Artigos com idade superior a 2008 e artigos que não abordaram a expressão de AQP4 em astrocitomas foram excluídos. Nos artigos selecionados, foram investigados os seguintes tópicos: estrutura da AQP4, localização do cérebro e do tumor e relação com o edema peritumoral.

Resultados Em relação à estrutura e localização da AQP4, a literatura apresenta duas isoformas da AQP4: M1 e M23. Ambos formam aglomerados de AQP4 chamados "arranjos ortogonais de proteínas - OAPs." No tecido tumoral, a literatura mostra uma diminuição na formação de OAPs e um aumento na expressão de ambas as isoformas AQP4, além de perder sua polaridade, difundindo através da membrana citoplasmática. Quanto à função da AQP4 nos tumores, a AQP4 auxilia na migração e invasão celular, além de participar da proliferação celular e apoptose. Em relação à relação com o edema cerebral, existem controvérsias. Estudos demonstram que o aumento da AQP4 agrava o edema citotóxico das células tumorais e, auxiliando na migração celular e na angiogênese, auxilia indiretamente na formação de edema vasogênico por quebra da barreira hematoencefálica. Outros estudos, no entanto, apontam para o aumento da

AQP4 como mecanismo protetor para combater o edema vasogênico que ocorre na formação de tumores. Além disso, a literatura apresenta uma proposta terapêutica em que, ao inibir a expressão da AQP4, a migração tumoral e o edema cerebral diminuem em ratos com glioblastoma.

Discussão Como mostrado na literatura, há uma diferença na estrutura histopatológica entre os gliomas de alto e baixo grau. No entanto, existem mudanças comuns entre eles. Estas alterações comuns poderiam então ser usadas como um fator de gravidade ou evolução de tumores de baixo grau a alto grau. Além disso, ainda não é possível perceber a verdadeira relação entre a expressão da AQP4 e o aumento da evolução do VEGF no edema peritumoral. Finalmente, pode-se supor que, como a razão de expressão entre as isoformas de AQP4 no tecido normal é maior do que em alguns tumores, a diminuição dessa razão é devida à diminuição da expressão de M23 ou ao aumento da isoforma M1.

Conclusão: Novos estudos são necessários para compreender a fisiologia e a fisiopatologia da AQP4 em astrocitomas, a fim de criar propostas terapêuticas efetivas para combater essa doença.

Background

There is currently a total of 13 types of Aquaporins in mammals, each with its expression in different body tissues such as red blood cells, lungs, pancreas, kidneys and nervous tissue. All AQPs form tetramers in membranes in which monomers, each of ~30kd molecular size, contain six transmembrane helical domains and two helical segments surrounding cytoplasmic and extra-cellular vestibules. The vestibules are connected by a narrow aqueous pore allowing single-file water transport in which water selectivity is coferred by electrostatic and steric factors.[1] They are proteins that regulate the flow of water into and out of the cell. However, some of the AQPs such as AQP3 and AQP8 also allow the passage of glycerol and ammonia, called aquaglyceroproteins.[2,3] In the central nervous system there is an increase in AQP 1 and AQP 4 in astrocytes submitted to neoplasms and trauma.[4] The structure, function and location of AQPs in the various organs are known. However, there is still to be discovered about the relationship and interaction that these proteins play in the various pathophysiological processes involving such organs. These findings would allow the genesis of new ways to approach pathologies such as neoplasia, especially of the CNS, in which AQP 4 has a great influence, not yet fully known.

Gliomas are the most common central nervous system tumors. Glioblastoma multiforme (GBM) is the most common and malignant brain tumor with high mortality and poor prognosis. The vast majority of patients develop symptoms within ~3 months and die within 8 to 18 months of diagnosis. Less than half live more than 6 months and only 3% have a 2-year survival. Malignant gliomas have a very aggressive and relapsing behavior, giving the patient a very poor prognosis. For this reason, new therapies are needed to change this scenario.[3]

Therefore, the aim of this review is to address the literature on the structure and location of this protein in the CNS, as well as its function and expression in the healthy CNS and brain gliomas. Finally, this study will address the relationship between this protein and peritumoral edema that is present in the literature and the new therapeutic approaches in research.

Methods

The PUBMED database platform was used for consultation using the keywords "Aquaporin 4," "Expression" and "Astrocytoma." The language was used as a selection criterion, preferably choosing articles in Portuguese, Spanish and English. This study included all articles found since 2008, including other systematic reviews. Articles that did not address Aquaporin 4 expression in astrocytomas anywhere in the paper were excluded from this review. In the articles surveyed, the following topics were investigated: AQP4 structure, brain and tumor localization, relationship with edema and relationship with VEGF.

Results

Structure and Location of AQP4 in Brain and Tumors

Aquaporins are a family of integral membrane proteins that regulate the osmotic permeability of the plasma membrane, allowing water to pass through the membrane while blocking entry of ions or charged solutes.[2] It contains 6 transmembrane α-helices, with 2 asparagine-proline and alanine (NPA) loops, each with its cytoplasmic B and extracellular E portion oriented 180 degrees apart.[2] There are a total of 13 types of aquaporins in mammals.[5] In the Brain tissue there are three types of aquaporins: AQP 1, AQP 4 and AQP 9.[6]

AQP4 is usually expressed at the end of astrocyte perivascular cytoplasmic protrusions (►**Fig. 1**), ependimoglia and glia limitans, in the black and gray substance where there is 10 to 15 times more molecular AQP4 than in the rest of brain tissue.[2,4,7–10] AQP4 is also found in the hippocampal

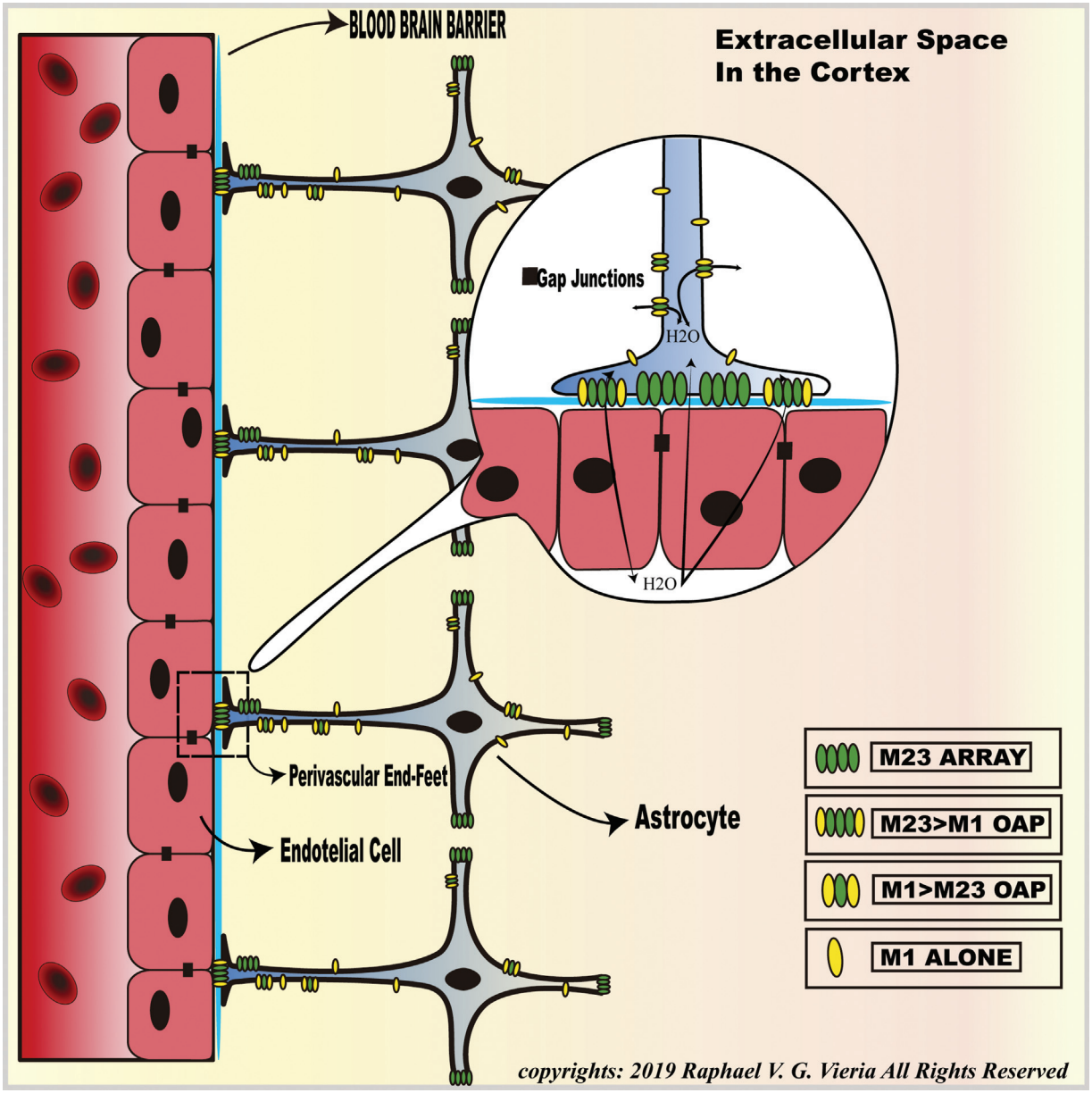

Fig. 1 Extracellular structure and astrocytic interaction with the normal brain blood-brain barrier.

dentate gyrus, medial habenular nucleus, cerebellum, neocortex, and supra-optic and suprachiasmatic nuclei of the hypothalamus.[2] The polarized expression of AQP4 coincides with the location of potassium channels, however, in gliomas, the location is lost and the water channels disperse throughout the cell surface.[11] The protein comes in two different isoforms, M1 and M23, with 22 fewer amino acids.[4] Both isoforms have the same water permeability, but different aggregation properties.[12] With the mixture of the two isoforms and the agrin and dehydroglycan proteins, the so-called Orthogonal Arrays of Particles (OAPs) of different sizes are formed, which are present mainly at the extremities of the astrocyte protrusions[4,13] (►**Fig. 2**). OAPs rich with the presence of the M23 isoform promote the formation of large OAPs with little mobility, standing stationary at the end of the astrocyte perivascular process.[12] M1isoform rich OAPs are small and diffuse freely into the cell membrane, mainly for astrocyte processes, to aid in cell migration[12] (►**Fig. 2**). The M1 isoform can exist alone in the cell, also moving freely through the membrane.[4,12] The isoform M23, when it does not agglomerate with the isoform M1, does so among themselves, forming larger and less mobile clusters.[4,12] Agrine is an extracellular matrix proteoglycan and destroglycan is a component of the dystrophin-destroglycan complex. The agrina connects to the dystrophin-dehydroglycan complex, which in turn connects to OAPs. There complexes also contain the inwardly rectifying potassium channel Kir4.1, a protein involved in spatial buffering of K^+ ions released, because of synaptic activity, into the extracellular space.[14] Noel et al. Showed that this chain of molecules undergoes glioma alterations.[4] The hypothesis that Agrina and destroglycan are responsible for the polarization of AQPs in the vascular extremities of astrocytes has been tested *in vitro*[15] and *in vivo*[4,16]. *In vitro* it has been shown that rats

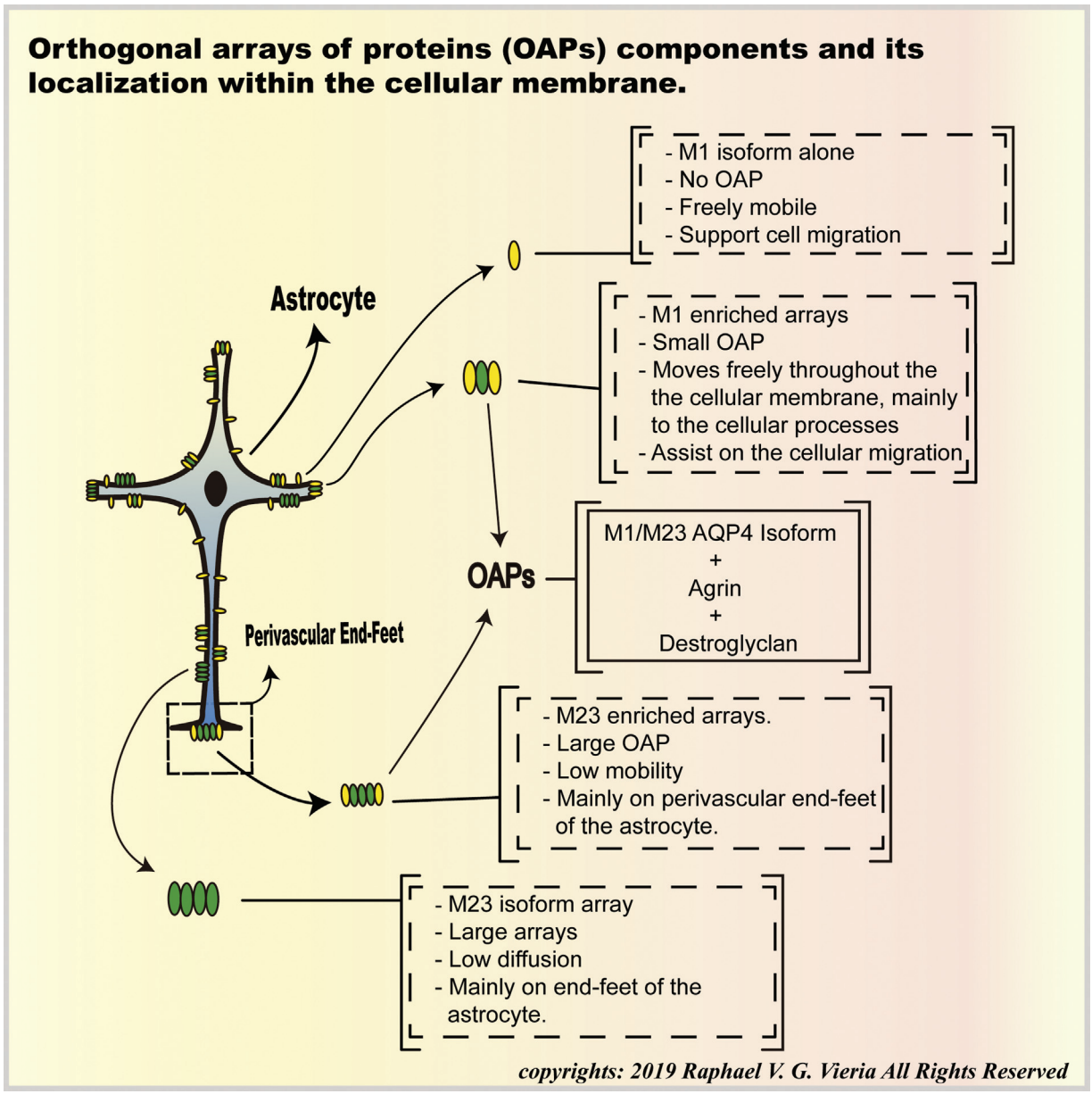

Fig. 2 OAP components and their location in the astrocyte membrane.

lacking agrin undergo alteration of membrane distribution of AQP4.[3] In rats with no dehydroglycan, there was loss of OAPs at the vascular end of the astrocytes, which correlated with down-regulation of AQP4 in the region.[3,4]

In same study, Noel et al showed that astrocytes at the extremities of glioblastomas present the classic star shape with the presence of AQP4 at the vascular end of astrocytic protrusions, the same pattern found in non-tumor astrocytes.[4] In the primary glioblastoma cell membrane, the density of OAPs is small even if there is contact with the basal lamina (►**Fig. 3**). In the relapsing Glioblastoma cell membrane, the occurrence of regular OAPs is also small, and of non-polarized distribution, but with density similar to the parenchyma and membrane area outside the astrocyte protrusion end. This shows the loss of cell polarization of astrocytes during glioma transformation[8] in both primary and recurrent tumors, and in the latter, the density of OAPs is closer to the normal pattern.[4] In addition, tumor regions with greater presence of small blood vessels showed greater immunoreactivity to AQP4 than regions with larger blood vessels[4] (►**Fig. 4**). It is possible to observe the redistribution of AQP4 already in the tumor infiltration zone around the tumor, where the neurovascular functional structure is normal.

Reinforcing the above, Ndoum et al., In 2013,[17] through MRI analysis and immunohistochemistry, showed that low-grade gliomas preserve their astrocyte processes, vascular structure and blood-brain barrier, and there is no significant angiogenesis in the lesion. (►**Fig. 5**). The author explained this fact by the tumor cell's ability to invade healthy tissue and receive nutrients through the phenotypically normal astrocyte structure. The opposite occurs in high grade gliomas. Astrocyte structure and basement membrane are lost around the tumor vasculature. There is diffuse reactivity of

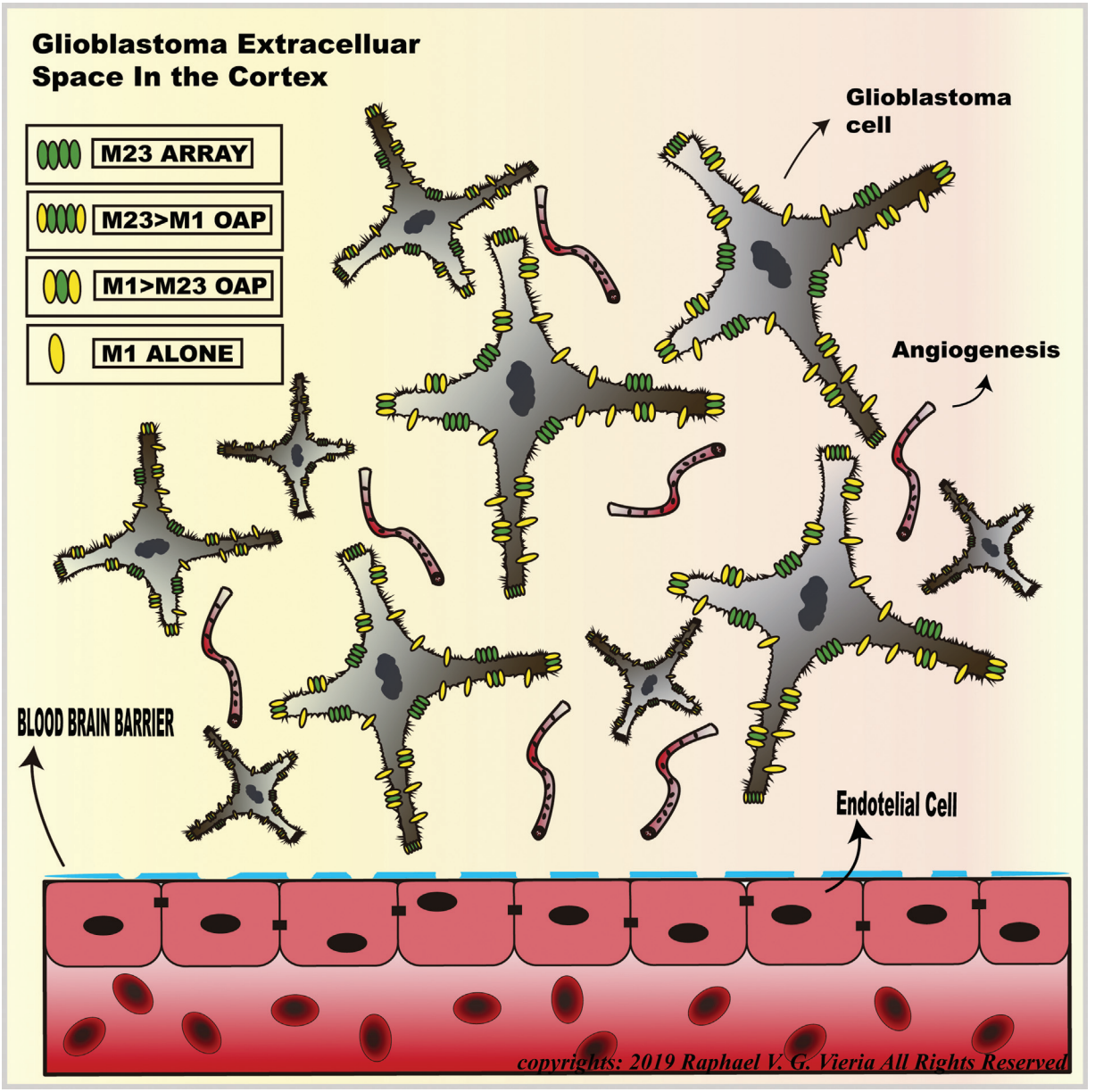

Fig. 3 Astrocyte extracellular structure in Glioblastoma and cellular localization of OAPs - AQP 4.

AQP4 by the tumor and loss of uniformity in AQP4 reactivity where the blood brain barrier is still intact[10] (►**Fig. 3**).

AQP4 Function in Brain and Gliomas

The main function of AQP 4 are to regulate the exchange of intracellular and extracellular water molecules, provide a transportation route for the rapid movement of water, participate in water regulation *in vivo*, and maintain the water balance *in vivo*.[18]

AQP4 is known to participate in the formation of cerebral edema following trauma or other brain diseases.[18] The distribution of AQP4 in the astrocyte cytoplasm suggests that this protein has the function of controlling the flow of water into and out of the brain parenchyma.[2,4,10,19,20] As a bidirectional water channel, AQP 4 facilitates brain water accumulation in cytotoxic edema and clearance of excess brain water in vasogenic and interstitial edema.[1] Moreover, astrocytes are known to express AQP4 in OAPs primarily in cell protrusions. Some of these protrusions involve the basal lamina of the cerebral vessels, fundamentally participating in the maintenance of the blood-brain barrier.[3] The deletion of AQP4 can reduce water permeability through the cell plasma membrane in the brain.[6] In addition to controlling the flow of water into and out of the cell, it has been postulated that AQP 4 actively participates in the cell migration process.[3,9,19] By expelling water, the cell can easily change its morphology. As in gliomas, the expression of aquaporin 4 is increased, the tumor cell has a great capacity for migration and tissue invasion. This hypothesis was tested by Zhao et al.[9] using LN229 glioblastoma cells, in which there is low expression of AQP4, showing low capacity for migration and tissue invasion *in vitro*. LN229 cell chemotaxis, compared with the control group, was lower. In addition to chemotaxis, chemokinesis, which is

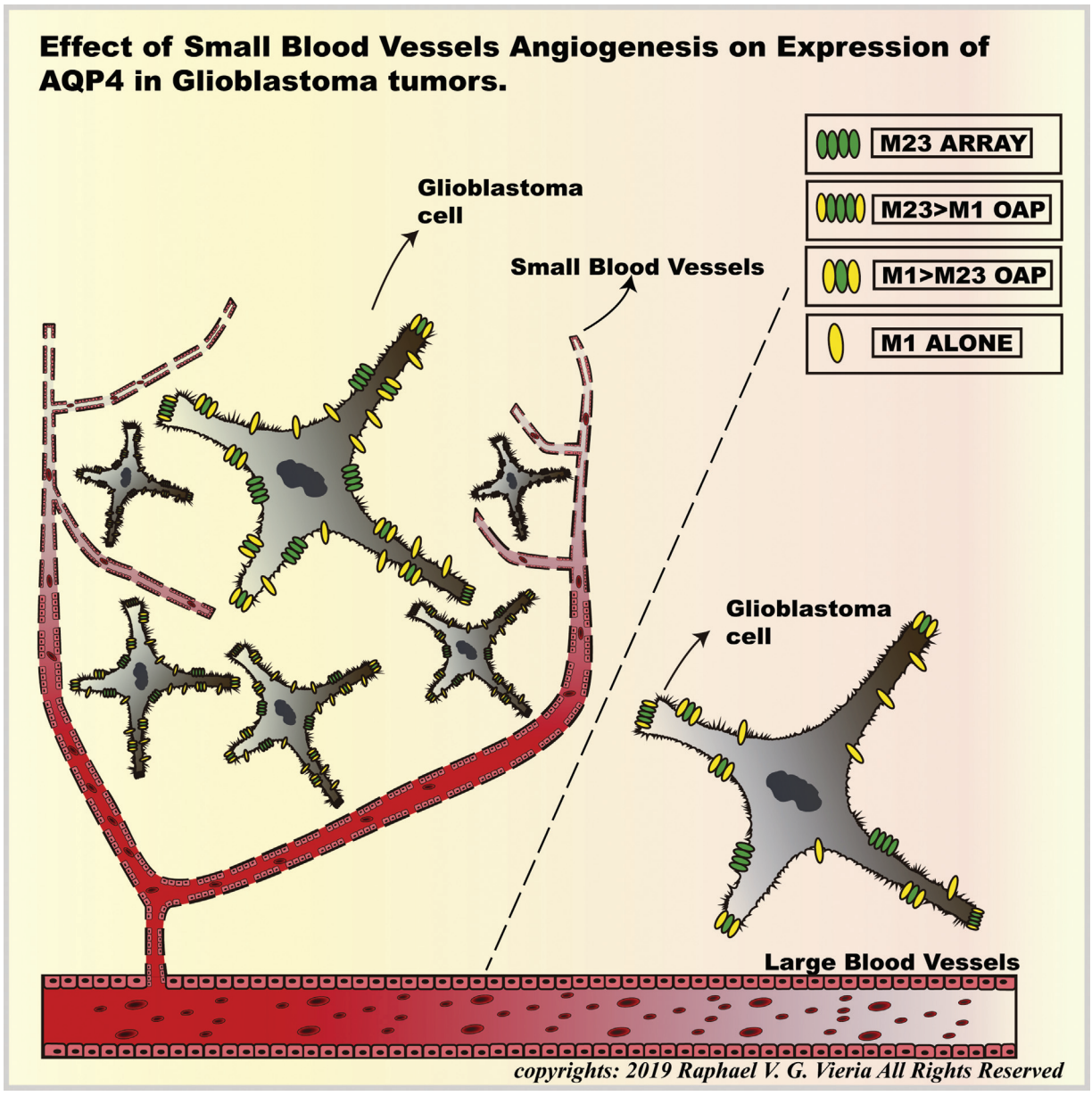

Fig. 4 Effect of neoangiogenesis on AQP4 expression in small OAPs in Glioblastoma.

independent of concentration gradient, was also lower in the group with lower AQP4 expression.[19] Lack of AQP4 seems to change cell morphology. The cell body of LN229 cells became thin and elongated. Reduction of AQP4 decreases the cell's ability to adhere to the substrate due to reduced actin-F protein polymerization. Moreover, these cells showed higher cell-cell adhesion capacity and lower invasion capacity according to the Matrigel Boyden chamber test *(in vitro)*. *In vivo*, the same pattern is repeated. Mice injected with glioblastoma cells without AQP4 showed less cellular invasion than the control group.[19]

Increasing AQP4 expression to some extent may facilitate the reabsorption of water accumulated in the extracellular space, whereas a large increase in AQP4 expression may cause water accumulation in glial cells leading to cell death.[9]

Studies by Ding et al., 2013, showed that AQP4 directly participates in the processes of glioblastoma cell proliferation and apoptosis. By reducing the amount of AQP4 using RNA inhibitor, human glioblastoma cells LN229 and U87 suffered apoptosis and had their replication cycle shortened.[10]

AQP4 Expression in Gliomas

Mou et al.,[21] showed that AQP 4 expression is higher in the peritumoral region than in the tumor itself and in normal brain tissue, besides increasing according to the histological grade of the tumor. In addition, a positive correlation was observed between AQP4 expression with VEGF and HIF-1α. This result differs from that found in older literature, which showed higher protein expression in the center of the tumor.[3] Tan et al.[22] compared MRI images and AQP4 mRNA expression between high-grade and low-grade gliomas. The author found higher mRNA values in high-grade tumors than in low-grade solid tumors, which is consistent with most of the literature.

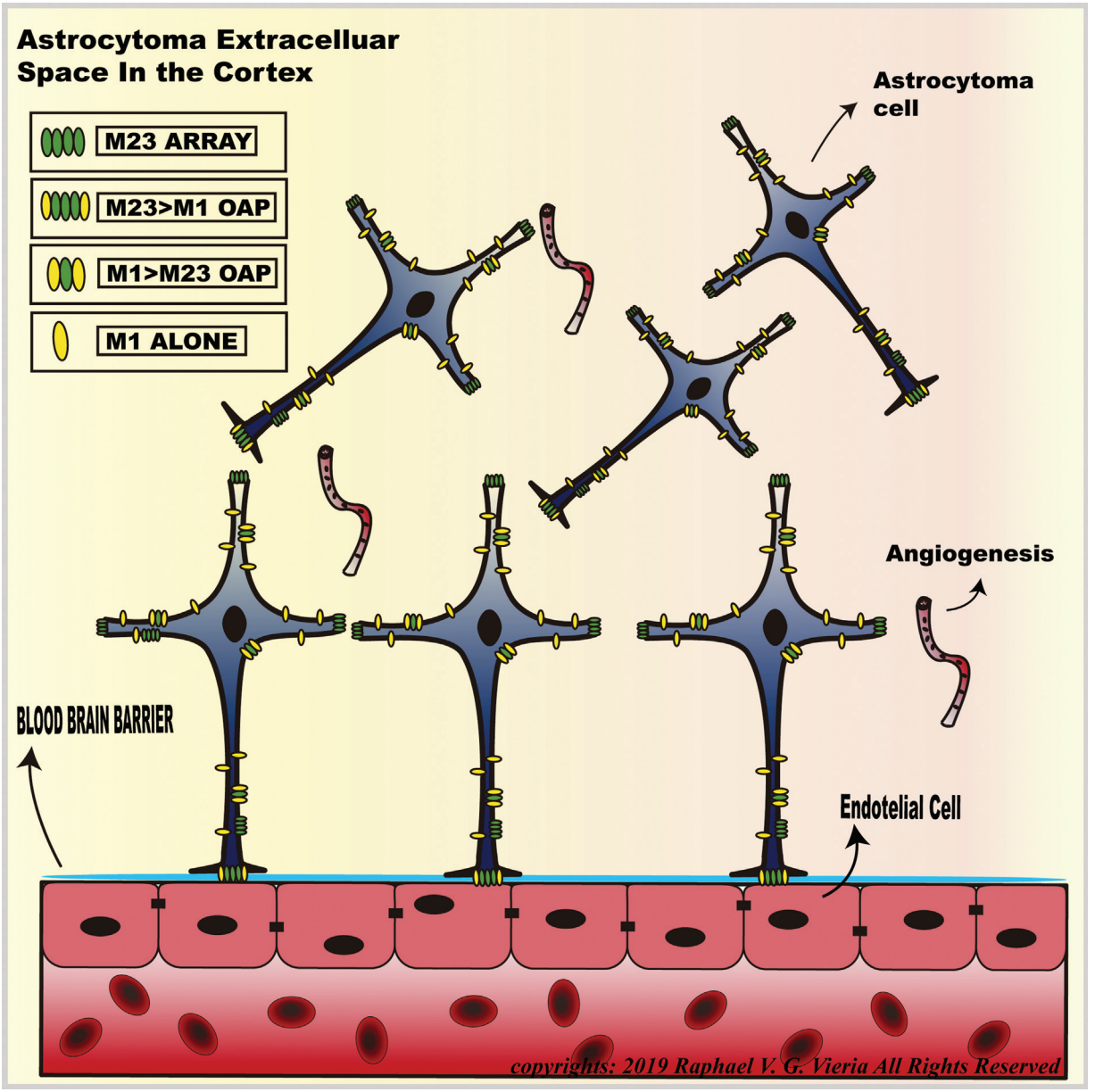

Fig. 5 Astrocyte extracellular structure in astrocytomas and cellular localization of OAPs - AQP 4.

Isoardo et al. compared AQP-4 expression in Glioblastoma multiforme between patients with and without seizures.[23] In his study, the average tumor size among patients with and without seizures was no different, as well as cortical involvement and MRI bleeding. Regarding the expression of AQP-4 by immunohistochemistry, a greater increase in expression was detected in patients with seizures than in those without seizures. The presence of AQP-4 was detected in the GBM cell membrane. In patients with epileptic seizures the distribution of AQP-4 was diffuse or perivascular, while in patients without seizures the immunohistochemistry was undetectable or showed a diffuse pattern of expression.[23] However, by PCR there was no difference in expression between the 2 groups and both showed increased expression of AQP-4.[23] Michael DeLay et al,[24] in 2012, showed that anti-VEGF antibody resistant glioblastomas show higher AQP4 expression. Through electron microscopy, Noel et al., In 2012, confirmed the greater amount of AQP4 in glioblastoma than in control tissue.[4] In the same study, Western blotting results showed that both M1 and M23 isoforms increased expression in the primary tumor. However, in relapsing glioblastoma tissues, AQP4 expression is lower, comparable to control tissue, and with M23 isoform more expressed than M1. In addition, tumor regions with greater presence of small blood vessels showed greater immunoreactivity to AQP4 than regions with larger blood vessels[4] (►**Fig. 3**). While AQP4 expression is increased in glioblastomas, the concentration of OAPs is reduced. According to Noel et al., 2012, this could be explained by the increased expression of the M1 isoform, which does not lead to the formation of dense OAPs (►**Fig. 3**). However, the expression ratio between M1 and M23 forms did not change in glioblastomas.[4] Similarly, Becker et al., 2016, found similar results when performing real-time PCR and electron microscopy to

compare different expression of AQP4 isoforms in different glioma grades. The author found a fall relationship in the presence of OAPs from low to high grade gliomas.[15] However, there was no correlation between tumor malignancy and M1 isoform expression, since this expression was uniform. In fact, there was an increase in M23 isoform expression on average 1.5 higher than M1 isoform, being higher in grade IV gliomas. However, the M23 / M1 ratio in a healthy brain is at least 3.[15] The author concludes, therefore, that the formation of OAPs depends on other mechanisms besides the change of the M23 / M1 isoforms relation.[15]

Contrary to what is shown in the previously mentioned studies, Zhao et al., 2012, observed a pattern of decreased AQP4 expression from grade II to IV gliomas. Their conclusion was that AQP4 expression does not depend entirely on tumor malignancy, but rather on tumor type. However, it has been shown that glioma cells with higher migration capacity show higher AQP4 expression.[9] Moreover, the study also showed that in low-grade gliomas, the concentration of AQP4 in astrocyte perivascular processes is higher than in high-grade gliomas.[9]

To relate radiological characteristics in MRI of gliomas and expression of AQP4, Tan et al., In 2016, compared diffusion imaging of kurtosis (mean kurtosis, radial kurtosis and axial kurtosis) and tension diffusion (mean diffusion) in High and low grade gliomas. Their study showed that there is greater expression of AQP4 in the solid part of high grade gliomas compared with low grade. Moreover, it showed that there is a directly proportional relationship between the mean, radial and axial kurtosis with the expression of AQP4, while the mean diffusion showed an inversely proportional relationship with the expression of AQP4.[25] The study also showed that the mean kurtosis value in the peritumoral edema area is higher in high grade gliomas than in low grade gliomas. This result may have been due to a larger tumor infiltration of the high grade gliomas,[25] which may mean a tumor staging method through imaging.

Wang et al[26] proposed to research the expression of AQP 1 and 4 in the main pediatric brain tumors. Regarding aquaporin 4 in astrocytomas, the author found that high-grade gliomas did not express AQP 4, while there was high protein expression in pilocytic astrocytomas, but with great variability. Houng et al[27] also demonstrated low AQP 4 expression in pilocytic astrocytoma samples, but high expression in low-grade diffuse astrocytoma samples, mainly around the microvasculature and with an intact blood-brain barrier.

AQP-4 Relationship with Edema

Brain edema is typically present in human brain cancers and affects both the course and outcome of pathology, therefore can be considered a prognostic factor.[28] The appearance and effects of edema in clinical progression of brain cancers has been known since long ago in clinical practice. Schoenegger et al. showed shorter survival time after surgery in patients with major peritumoral edema ($\geq$ 1 cm) in MRI.[28]

Cerebral edema is associated with various neurological disorders such as ischemia, trauma and tumor; all leading to increased intracranial pressure and its comorbidities such as herniation and death.[2] Under normal conditions, water moves in and out of the central nervous system, obeying osmotic pressure.[2] There are 3 mechanisms of formation of cerebral edema: cytotoxic, vasogenic and interstitial. Cytotoxic edema results from the disturbance of cellular metabolism, increasing the movement of liquid into the intracellular space. Vasogenic edema occurs due to alteration of the BBB, allowing greater passage of water and macromolecules, accumulating fluid in the extracellular space. Interstitial edema occurs due to an obstruction of the ventricular channels, causing hydrocephalus, in which the accumulated fluid leaks through the periventricular walls.[2] Filippidis et al., 2016, reviews the relationship of aquaporins with cerebral edema. The author points out several studies on the subject such as the reduction of intracranial pressure due to cytotoxic edema in rats without AQP4 expression. Fazzina et al., 2010, demonstrated that treatment with protein kinase C irreversible activator reduces cerebral edema by decreasing AQP4 expression.[29] Besides, AQP 4 deletion in mice reduces cytotoxic brain edema.[30] Yang et al., in 2008, showed that AQP4-overexpressing mice had an accelerated progression of cytotoxic brain swelling on acute water intoxication produced by intraperitoneal water injection. This lad to higher intracranial pressures (ICP). In contrast, ICP was lower in AQP4 knockout mice.[30]

Both benign and malignant tumors produce cerebral edema, which may be due to BBB defect and increased tumor angiogenesis (Papadopoulos et al., 2003). Saaduon et al., 2002, demonstrated that rats without AQP4 with edematous brain tumor showed higher intracranial pressure values and more neurological complications when compared with rats with AQP4, showing a possible protective effect of AQP4.[1] This information contrasts with the study of the same author, 2005, in which AQP4 promotes tumor metastasis by facilitating cell migration and angiogenesis,[1] thus promoting the formation of vasogenic edema.

Isoardo et al. compared the edema rate among patients with GBM who had epileptic seizures and who had no seizures. There was no difference in edema between the two groups and there was no difference between patients with positive and negative immunohistochemical results. The author explains these results by choosing patients with lesions of similar dimensions since the aim of the study was to determine if AQP-4 is related to the onset of epileptic seizures regardless of edema.[23]

The work of Yang et al., 2012, observed the relationship between VEGF and AQP4 in edema formation. By associating AQP4 expression with glioma cells expressing different amounts of VEGF, the author found that there is no difference in AQP4 expression by different amounts of VEGF.[31] However, there is increased expression of AQP4 in tumor tissue with increased VEGF, vascular permeability and water content. The author concludes that VEGF does not directly affect AQP4 expression, but that AQP4 redistribution in glioblastoma cells is a reaction to VEGF-induced vasogenic edema to facilitate resorption of excess fluid.[31]

Mou et al., 2010[21] found that in edema associated with gliomas, AQP4 was also regulated by local osmotic pressure and hypoxia. The degree of peritumoral edema could only be

directly related to AQP4 expression in the peritumoral region of the samples.

The findings in the study by Nduom et al. In 2013 have clinical implications in that the breakdown of the BBB and astrocyte structure may lead to ultrafiltrate leakage through endothelial vessels and cause peritumoral vasogenic edema.[17]

Henker et al., In 2016, related 10 different types of polymorphisms with the preoperative volumetric characteristics of multiform glioblastomas. Using MRI and PCR, the study shows a strong relationship between 4–31G and 131G Aquaporin polymorphism and the ratio of tumor volume to peritumoral edema. The presence of this polymorphism determines lower measured peritumoral edema compared with tumor volume.[32] In situations where there is no polymorphism, the ratio of peritumoral edema to volume showed that the edema was on average twice as large as the tumor volume, and the necrosis area was one quarter of the total tumor volume.[32] AQP4–131G> A is the promoter area of the AQP4 gene. Thus, a change in this area may lead to alteration of AQP4 expression and consequent decrease of water accumulation and reduction of peritumoral edema.[32]

Discussion

Frequently, cell lines or primary cells cultures from glioblastoma are used to measure the cell volume regulation, but many glioma cell lines do not express the water channel proteins. Besides, the majority of freshly isolated glioma cells do not express any of these water channels in vitro in primary cell cultures. However, in glioma tissue, from which the cells were isolate, AQP 4 were detected.[4,13]

Could the occurrence of AQP4 redistribution in the astrocyte plasma membrane already in the tumor infiltration zone suggest an early factor to predict transformation to Glioblastoma? The tumor infiltration zone still has normal neurovascular structure and can be compared with normal tissue.[8] Moreover, the results shown by Nduom et al. 2013, could support the hypothesis that the finding of AQP4 redistribution by astrocytes could be considered as a malignancy factor for the tumor, since low grade tumors preserve their macrostructure more similar to normal tissue. High-grade tumors, on the other hand, do not preserve the blood-brain barrier, as well as diffuse expression of AQP4 throughout the cell membrane, even near vascularization.[17]

Increasing AQP4 expression to some extent may facilitate the reabsorption of water accumulated in the extracellular space, whereas a large increase in AQP4 expression may cause water accumulation in glial cells leading to cell death.[9] The expression level of AQP4 correlates with the level of cerebral edema. One of the factors that contribute to the maintenance of the blood-brain barrier is the concentration of AQP4 in perivascular astrocyte processes.[9,20] Electron microscopy has shown that gap junction opens in the high-grade astrocytoma microsvasculature. This, added to the loss of polarization of AQP4 in astrocytes can lead to increased edema.[9] Conversely, as a result of the breakdown of the blood-brain barrier, the redistribution of AQP4 into glioblastoma cells may act as a countermeasure to vasogenic edema. This reinforces the hypothesis that AQP may be involved in the dynamics of edema formation or resolution. The fact is that there is more about brain edema formation than just AQP4. It has been shown that Na^+-K^+-Cl^- cotransportes 1, matrix-metalloproteinase 9, thrombin, substance P and chemokine receptors are also involved in the process.[33]

Increasing the amount of AQP4 expression in higher VEGF tumor tissue[31] could explain the greater amount of AQP4 found in tumor cells near small vessels compared with large vessels,[4] since VEGF is the main modulator of angiogenesis. The author himself concluded that there is no statistical relationship between VEGF value and AQP4 expression, but these two factors could be physiopathologically involved.

The process of cellular invasion of malignant gliomas is complex and multifactorial. In addition to increased cellular mobility, other mechanisms such as reduced substrate and neighbor cell adhesion and extracellular matrix degradation are also involved. The role of aquaporin 4 in these mechanisms is not yet fully understood. The same could also be seen in the systematic review by Lan et al.[3]

Although the biomolecular behavior of brain gliomas has not been clarified, treatment methods are a target of research. Nico et al., 2009, found that the combination of chemotherapy and radiotherapy reduces AQP4 expression[4,8] and restores cell polarization.[8] It has also been shown that LN229 glioblastoma cells show lower AQP4 expression. As a result, these cells showed lower capacity for migration and tissue invasion.[8] Thus, the use of AQP4 inhibitory therapies for the treatment of cerebral gliomas would be possible. Ding et al., 2013 showed this possibility by using AQP4 inhibitory RNA in human glioblastoma cells causing apoptosis of these cells.[10] Another proposed idea is the use of AQP4-specific antibody linked with toxin to selectively damage AQP 4-expressing glioblastoma cells.[6] The use of curcumin to attenuate brain edema is also proposed, as one of its effects is the reduction of expression of AQP 4 and 1.[34]

In addition to molecular therapies, noninvasive evaluation methods for gliomas are being studied. The study of Tan et al.[22] correlates mean diffusion coefficient values obtained by serial MRI images between high and low grade gliomas and AQP4 mRNA expression. The study shows higher AQP4 expression in high grade tumors and a directly proportional relationship between AQP4 expression and mean diffusion values in solid parts of the tumor. However, there was no relationship between the diffusion coefficients for the peritumoral edema region. The author believes that the redistribution of AQP4 in the cell surface of high grade gliomas is responsible for the high values of mean diffusion coefficient, showing greater water flow in the solid region of these tumors. Such imaging study could serve as a new form of assessment of staging and expression of AQP in astrocytomas.

No studies were found in the literature associating the expression value of AQP4 with patient follow-up or survival after surgery. Such studies could show some relationship from which there was a predictive value of patient survival after possible surgery. Moreover, most of the laboratory studies had the limitation of a small sample space, impairing the impact or the statistical weight of the results.

Conclusion

With this review it is possible to realize that there is not only one mechanism that influences the relationship between AQP4 expression and the pathophysiology of cerebral gliomas. Some of these mechanisms have already been identified and hypotheses of therapeutic intervention are being tested. Given the morbidity and mortality of the disease, more studies are needed in the area, not only of therapeutic proposals, but also proposals to promote patient comfort or avoid submitting unnecessary procedures.

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