Role of aquaporins in cell proliferation: What else beyond water permeability?

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ABSTRACT
In addition to the extensive data demonstrating the importance of mammalian AQPs for the movement of water and some small solutes across the cell membrane, there is now a growing body of evidence indicating the involvement of these proteins in numerous cellular processes seemingly unrelated, at least some of them in a direct way, to their canonical function of water permeation. Here, we have presented a broad range of evidence demonstrating that these proteins have a role in cell proliferation by various different mechanisms, namely, by allowing fast cell volume regulation during cell division; by affecting progression of cell cycle and helping maintain the balance between proliferation and apoptosis, and by crosstalk with other cell membrane proteins or transcription factors that, in turn, modulate progression of the cell cycle or regulate biosynthesis pathways of cell structural components. In the end, however, after discussing all these data that strongly support a role for AQPs in the cell proliferation process, it remains impossible to conclude that all these other functions attributed to AQPs occur completely independently of their water permeability, and there is a need for new experiments designed specifically to address this interesting issue.

KEYWORDS
aquaporins (AQPs); cell cycle; cell proliferation; gene expression; glycerol; hydrogen peroxide permeability; neurogenic niche

Introduction
In many human tumors, changes have been reported in the expression of aquaporins (AQPs), and by playing a role in different cellular mechanisms, such as migration, angiogenesis, adhesion and cell proliferation, these proteins may be involved in tumor progression. Since the beginning, the idea behind a role for AQPs in tumor process was basically associated with their contribution to changes in cell volume necessary for cells to proliferate and differentiate. Initial studies demonstrated high AQP1 expression in proliferating microvessels of many tumors. Later, a role for AQP1 in angiogenesis was confirmed when, following the subcutaneous implantation of melanoma-B16F10 cells, considerably reduced tumor growth was observed in AQP1-null than in wild-type mice, due to impaired microvessel formation and faster tumor necrosis. Similarly, a lower migratory capacity was detected in melanoma cells that naturally do not express AQP1 than in AQP1-expressing cells; and a mechanism that would lead to net cell displacement confirmed the contribution of ion pumps and AQPs for allowing net propulsive flows of water. Together these findings strengthen the idea that AQPs play an important role in angiogenesis and cell migration.

However, here we will focus our attention on the different ways and mechanisms that have been proposed so far to explain the participation of AQPs in the cell proliferation process itself. Reviewing the literature, we find that this association has been suggested mainly in relation to a role for these proteins in cell volume changes, or in the case of a subgroup of AQPs, called aquaglyceroporins, to their permeability to solutes such as glycerol and hydrogen peroxide. Additionally, various studies have demonstrated that AQP expression is modified over the course of the cell cycle and, more recently, we have shown that overexpression of AQP1 and AQP3 alters the levels of essential checkpoint proteins (cyclins) relevant for cell cycle progression, in addition to modifying the levels of transcription factors, and cytokines.

In this review, we have sought to gather the most relevant evidence associating AQPs with cell...
proliferation and we will discuss whether the canonical function of water permeation of these proteins would be always essential for this process or the role in proliferation might be independent of this function.

**AQPs and cell volume**

Proliferation and cell growth are closely associated with changes in cell volume. In fact, factors that affect cell volume also have an effect on mechanisms that control cell proliferation.

Recently, we have shown that cells with AQP1 and AQP3 overexpression have a constitutively larger cell volume and complexity. These results are in agreement with the well-accepted hypothesis that, during progression through the cell cycle, cell volume increases as a result of the accumulation of diverse substrates, protein synthesis, and DNA duplication. In addition, we proved that cells overexpressing AQP1 or AQP3 have enhanced hydrogen peroxide permeability and altered expression of proteins necessary for cell cycle progression. All this indicates that the relationship of both AQP1 and AQP3 with a more proliferative phenotype goes beyond their water permeability.

Cell volume regulation (CVR) mechanisms comprise: a) regulatory volume decrease (RVD), mediated by potassium chloride and taurine efflux and usually in response to hypotonicity-induced cell swelling, and b) regulatory volume increase (RVI), mediated by sodium influx and usually in response to hypertonicity-induced cell shrinkage. AQPs are crucial for the regulation of cell volume, playing a role in mediating osmotic water flow, and recent research has provided further evidence for a general mechanism for cell volume regulation by which AQPs may function as a component of a sensing complex required to activate RVD/RVI.

In addition, AQPs can regulate the subcellular localization of other membrane proteins such as transient receptor potential vanilloid 4 (TRPV4), a volume sensitive calcium channel that participates in osmoregulation. Jo et al. showed that TRPV4–AQP4 interactions constitute a molecular system that fine-tunes astroglial volume regulation by integrating osmosensing, calcium signaling, and water transport, and when over-activated, they can trigger pathological swelling. In lung epithelial cells, salivary glands, and probably sweat glands, the presence of AQP5 is fundamental to cell volume regulation. The dynamic regulation of AQP5 abundance by changes in extracellular osmolality is suggested by TRPV4 activation mediating hypotonic reduction, and EGFR activation mediating hypertonic induction. Moreover, in renal cortical collecting ducts expressing AQP2, hypotonic stress caused translocation of TRPV4 to the plasma membrane, indicating a functional interaction between the two proteins and suggesting that AQP2 forms part of a sensory and signaling pathway that results in TRPV4 translocation, possibly via sensing of extracellular osmolality. Hypotonicity-induced translocation of AQP1 has also been described in HEK293 cells.

Another example that illustrates the role of AQPs in cell volume regulation can be seen in sperm, where AQP3 is actively involved in the signaling pathway that activates RVD to protect sperm from excess cell swelling, thereby optimizing postcopulatory sperm behavior. In line with this, the RVD of sperm of AQP3−/− mice was inhibited compared to wild-type mice and these knockout mice had lower fertility.

It is becoming increasingly clear that AQPs also have a role in cell volume regulation beyond a passive mechanism driving water, and this role may be related to AQP-mediated signal transduction, although the mechanisms that may be involved in this are not yet well understood.

**AQP permeability to small solutes**

Among the physiological functions of AQPs, we should highlight osmotic volume regulation and the transport of biochemical precursors or metabolic waste products. All AQPs transport water, and a subset, the aquaglyceroporins, also transport glycerol and urea. In addition, several metabolically important small neutral solutes and ions have been identified as AQP substrates, namely hydrogen peroxide, carbon dioxide, nitric oxide, ammonia, metalloids such as arsenite and antimonite, selenite, boric acid, and silicic acid. AQP6 with low intrinsic water permeability may function primarily as an anion transporter and AQP9 has been shown to be permeable to a wide range of non-charged solutes, like mannitol, sorbitol, purines and pyrimidines.

At present, the most plausible cellular and/or molecular mechanisms associating AQPs with
proliferation are related to their ability to permeate water, glycerol and/or hydrogen peroxide. Next, we will consider individually each of these solutes.

**Glycerol**

Glycerol is an important intermediate of metabolism\(^57\) that is largely metabolized to glycerol-3-phosphate, a key intermediate for the production of ATP. As mentioned above, a subset of AQPs, the aquaglyceroporins, AQP3 AQP7, AQP9 and AQP10, as well as AQP11, transport glycerol. Biochemical studies showed impaired cellular glycerol metabolism and biosynthesis in AQP3-deficient epidermal cells, with reduced ATP content, decreased biosynthetic incorporation of glycerol into lipids and impaired MAP kinase signaling.\(^15\) These studies suggest that AQP3-facilitated glycerol transport is a key determinant of cell proliferation in some cell types.

A widely-accepted idea to explain the role of AQP3 in tumor cell proliferation relates to the fact that expression of this protein provides the cell with higher glycerol permeability and ATP content, which are required for a greater demand for biosynthesis\(^58\) and AQP3 inhibition is predicted to reduce both tumor-cell migration and proliferation. We demonstrated previously that the inhibition of cell proliferation was proportional to the reduction in glycerol permeability produced by Auphen.\(^18\) Since the transport of glycerol by AQP3 is bidirectional,\(^59\) proliferation could be related to the entry or exit of glycerol in cells; and unlike other authors,\(^60-62\) we did not rule out the effect of Auphen on cell proliferation being caused by glycerol accumulation inside the cell due to blocking of the exit of glycerol.\(^18\)

AQP7 is the major pathway for glycerol efflux from adipocytes after lipolysis,\(^63,64\) its absence resulting in increases in adipocyte cell volume and adipose tissue mass.\(^65,66\) In the renal proximal tubule, AQP7 is a reabsorption pathway that may be important for preventing glycerol from being excreted into urine.\(^67\) In pancreatic \(\beta\) cells, AQP7 acts as an outward glycerol transport channel and its absence is associated with a significant reduction in cell proliferation. Accordingly, the reduction in \(\beta\)-cell mass observed in Aqp7\(^-/-\) mice can be explained at least in part by a reduction in cell proliferation through protein kinase C and the c-myc cascade, with a reduction in the transcript levels of the esponding two genes.\(^68\)

AQP9 is the primary route of hepatocyte glycerol uptake for gluconeogenesis.\(^69\) In mouse memory T cells, it can act as a metabolic switch, enabling long-term survival of the cells by enabling triglyceride synthesis to build up an energetic reserve, allowing survival under nutrient-poor conditions.\(^70\) In skin, studies on AQP9-deficient mice suggest that this AQP also plays a central role in glycerol metabolism,\(^71\) but its function in this organ has yet to be studied extensively. Conversely, other research showed that higher intracellular glycerol content was associated with a lower proliferation rate.\(^72\)

It was recently proposed that AQP10 may be an alternative pathway for glycerol efflux in human adipocytes\(^73\) and AQP11, located intracellularly mainly in the endoplasmic reticulum and periphery of lipid storage droplets, an intracellular gateway for glycerol from the lipid stores in human adipocytes.\(^74\) To date, the role of glycerol transport by AQP10 and AQP11 in cell proliferation has not been investigated.

**\(H_2O_2\)**

An increase in levels of reactive oxygen species (ROS), particularly hydrogen peroxide (\(H_2O_2\)), can activate signaling pathways to stimulate cell proliferation,\(^75,76\) differentiation.\(^77,78\) migration,\(^79\) apoptosis,\(^80,81\) adaption to hypoxia, immune function, and other processes.\(^82\) Therefore, hydrogen peroxide is an important signaling compound and it has recently been identified as a substrate for several members of the aquaporin superfamily in various organisms, suggesting additional physiological roles in redox signaling and in cellular mechanisms for minimizing oxidative stress. Recently, Almasalmeh et al.\(^83\) suggested that all water-permeable AQPs are \(H_2O_2\) channels, yet \(H_2O_2\) permeability varies with the isoform.

The fact is that while some AQPs, AQP8, AQP3, AQP1 and AQP11, have been shown to be permeable to \(H_2O_2\), this needs to be confirmed in other isoforms. We\(^13\) and others\(^17,83-85\) have shown that both AQP1 and AQP3 mediate uptake of \(H_2O_2\) in cells, postulating that transport of \(H_2O_2\) into mammalian systems by AQPs might interfere with intracellular signaling, amplifying cascades that depend on ROS, or increase the phosphorylation status of a cell (AKT/protein kinase B) and thus favor proliferation cascades.
The release of H$_2$O$_2$ from mitochondria via AQP8 could be important during reoxygenation after hypoxia, when oxygen supply leads to excess generation of H$_2$O$_2$ in the local environment (e.g., in heart and muscle). Furthermore, cell glucose uptake and proliferation were found to be elated with intracellular H$_2$O$_2$ levels and AQP8 expression, indicating that AQP8 is able to modulate H$_2$O$_2$ transport through the plasma membrane affecting redox signaling linked to cell proliferation in leukemia. It is plausible that AQP11 was controlling intracellular ROS accumulation by acting as an endoplasmic reticulum H$_2$O$_2$ channel.

**Cell cycle and AQPs**

Elucidation of the molecular mechanisms that control the progression of the cell cycle have been crucial to improving our understanding of cell division. To comprehend the cellular mechanisms underlying the association of AQPs with proliferation and tumor progression, we now need to consider the role these proteins have over the course of cell cycle progression. We summarize here findings in the most important studies that have reported a direct connection between AQPs and the cell cycle.

Almost two decades ago, Delporte et al. demonstrated that the expression of AQP1 may fluctuate during the cell cycle, the levels of AQP1 mRNA and protein being higher when cells are in the G0/G1 phase and lower when the cells enter the S and G2/M phases. Later, it was shown that AQP2 expression itself speeds up the proliferation and cell cycle progression of renal collecting duct cells by decreasing the transit time through S and G2/M phases of the cell cycle, probably by favoring an increase in cell volume.

A range of experimental approaches using drugs or specific culture conditions that allow manipulation of cell cycle progression have also contributed to our understanding of the role of AQPs in this process. For instance, we reported that cells with endogenous or exogenous, stable or transient, expression of AQP3 treated with Auphen, a potent inhibitor of the glycerol permeability of AQP3, were arrested in the S-G2/M phases of the cell cycle, suggesting the possibility that the inhibition of AQP3 permeability somehow detains the progression of the cell cycle, thereby slowing cell proliferation. In agreement with our results others have found that cell cycle of neurospheres derived from adult neuron stem cells of the subventricular zone (SVZ) in AQP4 knockout mice get arrested at the G2-M stage, functionally implicating AQP4 in the activation and differentiation of the mice SVZ neurogenic niche.

However, Yoneda et al. (2001) described alterations in AQP4 expression during astrocyte differentiation of a pluripotent embryonic carcinoma cell (P19) and during the cell cycle of astrocytoma cells. Their study revealed that in glioma cell lines, the levels of AQP4 mRNA were elevated in the G0/G1 phase when cells were arrested by transient expression of p21. Notably, quiescent astrocytes arrested in the G0/G1 phase, by serum starvation, showed a high expression of AQP4 and this expression was reverted when the cells moved to the S phase after serum supplementation, thus suggesting that AQP4 is specifically expressed in the G0/G1 phase but not in other phases of the cell cycle. A role for AQP5 expression was indicated in the proliferation and G1-S phase transition of esophageal squamous cell carcinoma, where AQP5 might affect the expression of genes involved in cell cycle progression, such as cyclin D1. Similarly, in osteosarcoma cells, U2OS or MG63 cells, knockdown of AQP1 inhibited cell proliferation and significantly increased cells population retained in G1 phase.

Looking for a direct connection between the expression levels of AQPs and the cell cycle pattern, that might explain the higher proliferation rates observed when using a cell line with stable overexpression of either AQP1 or AQP3, we performed a long series of experiments that led us to propose an important role of AQPs in proliferation. Analysis of the asynchronous cell cycle pattern in cells overexpressing AQP1 and AQP3 (Fig. 1) revealed a higher percentage of cells in the most proliferative phases, S and G2/M, with a consequent reduction in the percentage of cells in the G0/G1 phases, compared to the distribution in control cells that did not express AQPs. Closely analyzing cell cycle patterns of cells with AQP overexpression, one can see that they appear normal and certainly clearly different from those obtained with cells arrested in a given phase of their cell cycle, for instance, in the S or G2/M phases. Considering these modifications of the cell cycle together with the cell count and BrdU incorporation data, both showing higher numbers of cells, we concluded that overexpression of AQPs promotes the progression of cells.
through the cell cycle increasing their cell proliferation rate. According to these, cells that overexpressed AQP1 and AQP3 also had higher levels of cyclins D1 and E1,\textsuperscript{13,24} (Fig. 2), two cell cycle regulatory proteins crucial for phase transition during cell cycle progression,\textsuperscript{89-91} while levels of cyclin B were unchanged, indicating that cells were not arrested in S and G2/M phases; on the contrary, they potentially progress faster through the cycle.

A higher expression of cyclins might well explain changes in the cell cycle, and this helps to understand the greater proliferative capacity of cells that overexpress AQPs. We also performed cell cycle analysis in the AQP1-overexpressing cells after incubation with the synchronization drug, sodium butyrate, which synchronizes cells in G1 by inhibiting cyclin E activity.\textsuperscript{92} The cell cycle profile in the presence of butyrate showed that cells that overexpressed AQP1 are more resistant to being synchronized by this drug. The higher expression in these cells of cyclin E1, which regulates the checkpoint for G1-S transition,\textsuperscript{89-91} could support this response to butyrate treatment.\textsuperscript{24}

Figure 1. Analysis of cell cycle profiles by flow cytometry. Cell cycle profiles showing the distribution of cells in the different phases of the cell cycle of PC12-Wt, PC12-AQP1 and PC12-AQP3 cell lines. The analysis of propidium iodide-derived cell cycle profiles show a larger percentage of cells in the S and G2/M phases of PC12-AQP1 and PC12-AQP3 cells compare to wild type cells, indicative of a larger proliferative capacity when cells express AQPs. (Modified data from References 13 and 24).

Figure 2. Representative Western blots of protein levels (left panel) and summary of the quantification analysis (right panel) for comparison of wild type-PC12 cells vs AQP1-overexpressing PC12 cells. Significant differences are indicated as follows: *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001. Bars are mean ± SEM from N experiments where N = 4.
recently, we studied the cell cycle after nocodazole treatment to synchronize cells in the mitotic prophase. The analysis again showed clearly less synchronization in the G2/M phase for cells overexpressing AQP1 and AQP3, indicating that AQP overexpression made cells resistant to cell cycle arrest, probably by a mechanism that accelerates cell cycle progression and ultimately increases cell proliferation.

All these findings, together with data concerning the influence that AQPs have on cell volume and complexity, suggest that AQPs might have a relevant role during the G1 phase and the control of the G1-S transition, and thus provide an explanation for the presence of AQPs in many different tumors.

Cross-talk between transcription factors, cytokines and AQPs

Thanks to efforts dedicated to understand the proliferation of cancer cells, we know today that AQP1, AQP3 and AQP5 are the AQPs most commonly associated with human cancer. Several studies have indicated connections between AQPs and intracellular pathways, but a complete cascade of phosphorylation and activation of transcription factors and/or cytokines leading to promote cell proliferation has not yet been elucidated.

Expression of AQPs has been preferentially correlated, among signaling pathways, with activation of MAPK cascades which in turn, will lead to the transcription of genes associated with cell proliferation and thus, with various human carcinomas. For instance, expression of AQP5 is positively correlated with drug resistance factors, and silencing of AQP5 inhibits the cell proliferation at the same time that diminishes the phosphorylation of MAPK p38. In skin, AQP3 is important for the phosphorylation of p38, ERK and JNK in keratinocyte, and less phosphorylation of p38 and JNK was observed in epidermis from AQP3-null mice which exhibit clearly impaired wound healing capacity compared to wild-type animals. Further, the activation of GSK-3β, ERK, JNK, and p38 MAPK pathways has been associated with levels of AQ2 and proliferation in kidney collecting duct cells treated with lithium, while the anti-proliferative and anti-metastasis activity of anti-prostate cancer compounds such as Rg3 was associated with p38 MAPK-mediated downregulation of AQP1. On the other hand, NF-kβ seems to be the key transcription factor to which the actions of AQPs converge to produce a more proliferative phenotype. Moreover, this transcription factor seems to regulate the expression of some AQPs and, in turn, control cell proliferation. In addition, hypoxia-inducible transcription factors, HIF-1α or 2α, may take part in the proliferation mediated by AQPs. We have shown that stable overexpression of AQP1, 3 and 5 increases the stability of HIF-2α during chronic exposure to hypoxia thereby increasing the expression of many genes implicated in activities relevant for tumor growth, such as glucose uptake and metabolism, angiogenesis, cell proliferation and apoptosis. We also reported that AQP1 can be induced in hypoxia by HIF-1α, thus sustaining an auto-cycle effect that would contribute to cell proliferation.

In an attempt to explore how overexpression of AQP1 affects cell proliferation, we used a bioinformatics approach to analyze the genome-wide consequences of AQP1 overexpression in a cell model. The microarray analysis showed that more than half the genes with altered expression in cells overexpressing AQP1 had cell proliferation-related functions (Fig. 3), and from the list of the 24 cellular processes or diseases with larger numbers of associated genes with altered expression, the 9 top-ranked and some others listed, are clearly associated with cell morphology, movement, growth, death, development, proliferation and survival (Fig. 3).

Further validation of microarray analysis results was performed using qPCR, and the expression profiles of 16 selected genes in cells with overexpression of AQP1 or AQP3 was verified. In AQP1-overexpressing cells, the analysis revealed upregulation of many transcription factors important in cell proliferation, such as ZEB2, JUN, JUNB and NF-kβ2 (Fig. 4). Moreover, it confirmed high expression of the chemokine TNFSF18 and the TNF receptor, able to activate the stabilization and translocation of NF-kβ from the cytoplasm to the nucleus. Higher levels of the proliferative NF-kβ target-genes such as ZEB2, cyclin D and the cytokines CXCL9 and CXCL10, were also found to be overexpressed in our analysis (Fig. 4).

CXCL9 and CXCL10, the two most upregulated genes in our analysis, both have important pro-tumor roles and can both be activated by the transcription factor NF-kβ, as suggested in our model (Fig. 4). Additionally, downregulation of ubiquitin peptidases...
such as USP 11 (Fig. 4), or USP13 and 51, whose repression leads to higher stabilization of NF-κB,\textsuperscript{118,119} would contribute to higher cell proliferation in AQP1-overexpressing cells.

In the microarray analysis, we also observed changes in the expression of many other important genes (ADAM 22 and 23, BCL2L1, and prolactin, among others) associated with proliferation, migration, metastasis or apoptosis,\textsuperscript{24} but the role and relative importance of each needs further investigation.

**Apoptosis and AQPs**

Many physiological processes including proper tissue development and homeostasis require a balance between apoptosis and cell proliferation. The mechanism of apoptosis is complex and involves many pathways.\textsuperscript{120} Defects can occur at any point along these pathways, leading to malignant transformation of the affected cells, tumor metastasis and resistance to anticancer drugs. Despite being the cause of problems, apoptosis also plays an important role in the treatment of cancer, as a target of many treatment strategies, raising the intriguing possibility that defects in apoptotic programs contribute to treatment failure.

An important process that cells undergo after entering the apoptotic pathway is loss of cell volume,\textsuperscript{121} or apoptotic cell shrinkage, and AQPs, with their high capacity to increase the speed of movement of water across cell membranes, have been associated with this process. Jablonski et al.\textsuperscript{122} were the first to show the importance of AQPs for apoptotic volume decrease (AVD) and subsequent apoptosis. They observed that inhibition of AQPs by HgCl$_2$ blocked AVD and apoptotic events such as cell shrinkage, changes in the mitochondrial membrane potential, DNA degradation, and caspase-3 activation in ovarian granulosa, thymocytes and CHO-AQP1 cells, after

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*Figure 3. Top-ranked processes and pathologies related to AQP1 overexpression according to Ingenuity Pathway Analysis (IPA) obtained after Affymetrix analysis performed in PC12-AQP1 vs PC12-wt cells. Data was listed according its p-value, in a decreasing order, and in red were highlighted those functions and pathologies related with cell proliferation.*
apoptotic stimulus. Moreover, they observed that CHO cells overexpressing AQP1 showed higher apoptotic induction than CHO wild-type cells, and described how apoptotic cells lose their water permeability despite the presence of AQP1 in the membrane remaining unaltered. In hepatic tumor cells, the same research group observed decreased expression of AQP8 and 9 and lack of water movement across the cellular membrane when compared to normal hepatic cells. These cancerous cells also exhibited inherent resistance to apoptotic stimuli after serum starvation or TGF-β treatment, again suggesting the need for a functional AQP channel during AVD.

Additionally, in a collecting duct cell line, downregulation of AQP2 protein levels by chronic alkalosis together with a G2/M arrest was paralleled by changes consistent with apoptosis. It has been proposed that AQP2 expression facilitates changes in cell volume and the activation of channels or transporters necessary for the control of cell growth and cell death, resulting in a more rapid AVD and more rapid achievement of adequate levels of ions necessary to activate the enzymatic apoptotic cascade.

In cortical neurons of the central nervous system, Jessica et al. analyzed the expression of different AQPs while cells undergo apoptosis induced by treatment with lactacystin, a specific proteasome inhibitor. These authors found that AQP4 was highly downregulated, suggesting that this aquaporin, considered to be the main water channel in the brain, does not play an important role in the loss of water during AVD. In contrast, overexpression of AQP1, AQP8 and AQP9 was observed after lactacystin treatment. However, favoring a role for AQP4 in apoptosis, Kong et al. observed increased basal apoptosis in adult neural stem cells obtained from KO AQP4 −/− mice.

In general, the aforementioned studies suggest that the presence of AQPs is needed to enable the water movement necessary for the apoptotic loss of cell

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**Figure 4.** Summary of metabolic routes “putatively” altered by overexpression of AQP1 according with the comparison, by Affymetrix analysis and western blot assays, of the 2 groups of cell lines analyzed, PC12-Wt and PC12-AQP1. In the scheme it is shown that different cytokines among which TNFSF18 (in red, overexpressed) can activates the IKK complex via phosphorylation by a membrane receptor such as TNFR or others (IL6R, GP130, IFNγR) and by doing that will promote the dissociation of N-Fkβ from Ikβ. In turn, released-NF-kβ will translocate to the nucleus and acting as a transcription factor will activate many genes (ZEB2, Bcl-XL, CyclinD1, CXCL10 and CXCL9), all of them overexpressed and shown in red, implicated in cell proliferation and tumor progression. Down regulation (in green) of USP11, a de-ubiquitylase of Ikβ, will favor its proteasome degradation and this will also increase levels of free-activated NF-kβ. Another important effect of NF-kβ activation would be the repression of E-cadherin. In the absence of E-cadherin, β-catenin will lose its anchor to the cell membrane and will translocate to the nucleus and then co-activates the transcription factor TCF/LEF, thus promoting expression of pro-proliferative genes as MMP19 and Cyclin D1, or anti-apoptotic genes such as COX2 and Survivin. Cytoplasmic β-catenin, in the absence of signals, would remain inactivated forming a complex with Axin, APC and GSK-3β.
volume or cell shrinkage. However, somewhat contrasting results have been reported recently showing that overexpression of AQP1 and AQP3 confers PC12 cells a higher resistance to synchronized by nocodazole, a reagent that arrests cells in the mitotic prophase and induces apoptotic cell death. Analysis of Annexin V labeling and cleaved PARP protein clearly indicated that cells overexpressing AQPs are more resistant to apoptosis induced by nocodazole. Consistent with this, in AQP-overexpressing cells, we found high levels of prolactin and of Bcl-2 genes associated with apoptosis resistance.

In agreement with our work, expression of lung AQP1 and 5 were significantly decreased in mice with acute lung injury together with increased inflammatory response and apoptosis of alveolar epithelial and vascular endothelial cells. Additionally, in osteosarcoma cells (MG63 and U2OS) interference of AQP1 expression induces cell apoptosis evaluated by double Annexin V/propidium iodide staining. These cells exhibited higher levels of cleaved caspase-3 and Bax protein, while the protein levels of Bcl-2, that negatively regulates caspase activation and controls the G2 checkpoint decreases.

The over-expression of AQP3 and AQP9 in human melanoma cells revealed that both AQPs significantly increased the chemoresistance of these cells to apoptosis induced by arsenite, through a mechanism that involved downregulation of p53 and upregulation of Bcl-2 and XIAP. This implicates AQPs in melanoma progression and resistance to apoptosis. Similarly, in primary squamous cell carcinoma, which shows high levels of AQP3, treatment with CuSO4, a pan-AQP inhibitor, caused apoptotic cell death in a concentration-dependent manner, and in ovarian cancer cell line, epigallocatechin gallate, a potential anti-cancer drug, showed strong anti-proliferative effects and apoptosis induction accompanied by a downregulation of AQP5 expression. Furthermore, interference of AQP5 induces not only decreased cell proliferation but also enhanced apoptosis in human chronic myelogenous leukemia cells. These findings indicate that AQP5 expression may play a role in inhibiting apoptosis, possibly through the caspase 9 pathway. Similar results were found by Shimizu et al. in esophageal squamous cell carcinoma in which AQP5 inhibition promoted cell apoptosis.

These mixed findings together indicate that the participation of AQPs in apoptosis may be directly associated with the cell volume decrease necessary to enter in apoptosis, but at the same time, high overexpression of AQPs could favor increments in the cell volume that could even counteract the AVD process and make cells resistant to apoptosis, thereby allowing cell survival and sustaining further the cell proliferation.

### AQPs and cell proliferation in physiological processes unrelated to cancer

During the last decade, the most frequent scenario in which AQPs have been associated with proliferation is in human cancer, as indicated already. However, it is important to note that expression levels of specific AQPs have been demonstrated to account for higher water transport and fluid clearance from specific compartments not only under the pathophysiological conditions of cancer, but also under many other normal physiological conditions that imply changes in the hydraulic conductivity of tissues and organs (such as those that occur in the lung, kidney, skin or brain during fetal development and organ regeneration).

Upregulation of AQP expression has been observed under stimuli such as dehydration in the kidney leading to overexpression of AQP2 and AQP3, or hypoxia in the lung producing overexpression of AQP1 and in none of these cases have the proposals to explain AQPs overexpression been at all related to tumor proliferation. More recent studies have demonstrated involvement of AQP1 in the differentiation of stem-like cells in rat bile duct formation, development of human corneal keratocytes and renewal of limbal basal epithelium from a corneal epithelial stem cell niche, all important roles associated with cell proliferation and differentiation. Moreover, impaired proliferation, migration and neuronal differentiation has been demonstrated in adult neural stem cells derived from AQP4 knockout mice indicating a role for AQP4 in the activation and differentiation of the mice SVZ neurogenic niche.

In a very recent study, we confirmed that the presence of AQP1 favors the proliferation process produced by hypoxia in the carotid body (CB) in wild-type when compared to AQP1-knockout mice. Quantification of the total number of new BrdU+ cells showed less proliferation in the absence of AQP1 (Fig. 5), and the smaller number of new BrdU+/TH+...
cells measured after hypoxia treatment evidenced specific impairment in the proliferation of CB glomus cells. Previously, we demonstrated that AQP1 is present not only in type I but also in type II CB cells, now known to be CB stem cells that form the neurogenic niche in this organ. Hence, our recent results are compatible with the idea that the presence of AQP1 in the CB favors differentiation of glia-like stem cells elicited by hypoxia, to give new BrdU+/TH+ cells, concomitant to the observed hypertrophic growth of this organ.

More experiments are necessary to specifically investigate the participation of AQP1 in cell differentiation/proliferation in this neurogenic region in adults, and it would be interesting to explore its role in other known neurogenic niches. Likewise, it would not be surprising to find that other AQPs play a role in the differentiation/proliferation process of other stem-cell niches.

**Final remarks**

As it has been indicated, a broad range of evidence demonstrates that Aquaporins have a role in cell proliferation by acting through various different mechanisms, specifically, by allowing fast cell volume regulation during cell division; by affecting progression of cell cycle and helping maintain the balance between proliferation and apoptosis, and by crosstalk with other cell membrane proteins or transcription factors that, in turn, modulate progression of the cell cycle or by regulating biosynthesis pathways of cell structural components. In any of these functions complete independence of its canonical water permeability feature has not yet been demonstrated. Thus given the versatile functions of aquaporins, additional and unexpected roles of these channels are sure to emerge in the coming years. Still, further investigations are needed to broaden our understanding of the implications of AQPs in cell proliferation.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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