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Role of Aquaporins in Breast Cancer Progression and Metastasis

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<http://dx.doi.org/10.5772/64446>

Abstract

There are various limitations regarding the current pharmacological options for the treatment of breast cancer in terms of efficacy, target selectivity, side effect profile and survival. Endocrine-based therapy for hormone-sensitive cancers such as that of the breast is one of the most effective and well-tolerated therapeutic options but is hampered by either intrinsic or acquired resistance, resulting in a more aggressive form of the disease. It is generally agreed that this process occurs in parallel with cellular transition from epithelial to mesenchymal phenotype (EMT), with consequent enhancement of proliferative capacity, migrative ability and invasive potential. Aquaporins (AQPs) represent a large family of water channel proteins which are widely distributed in various tissues and which play a role in the physiological maintenance of the extracellular environment particularly to regulate electrolyte-water balance. Accumulating evidence shows that expression of several AQPs is modulated in cancer tissues, and this correlates with tumor grade. AQPs 1 and 3–5 are also involved in breast cancer invasion, through modulating the activity of various growth factors, signaling molecules and proteolytic enzymes. We review current data on the involvement of these proteins in processes associated with malignant progression and discuss possible applications of AQP-based therapy as an effective means of inhibiting cancer cells from metastasizing.

Keywords: breast cancer, metastasis, aquaporin, transport, ion channels

1. Introduction

Breast cancer remains the leading cause of tumor-associated mortality in women worldwide. Estrogen, acting through predominantly nuclear-located receptors (ER), has a significant

detrimental impact during its pathogenesis [1]. This forms the basis for endocrine therapy, with the application of pharmacological antagonists generally termed selective estrogen receptor modulators, such as tamoxifen. These have resulted in significant improvements in quality of life as well as improved prognosis [2] in a significant proportion of patients with clinically defined ER+ve status [3]. Unfortunately, *de novo* resistance to tamoxifen occurs in about 30–40% of patients (those with very low level of ER expression, clinically designated as ER–ve) and even in about 50% of the clinically defined ER+ve patients. Furthermore, almost all initially responsive patients with late stage metastatic disease eventually relapse due to the development of *acquired* resistance to anti-estrogen therapy. These forms of endocrine resistance invariably lead to a more aggressive form of resurgent disease [4], and occur in parallel with cellular transition from epithelial to mesenchymal phenotype (EMT). There is a strong association between the EMT process and metastasis, which involves detachment of individual epithelial cells from neighboring cells, loss of polarity, scattering, acquisition of enhanced motility and invasion into the extracellular matrix (ECM) before entering blood and lymphatic vessels. Many phenotypic changes occur during this process which includes the loss of cell-cell adhesion as a result of reduced E-cadherin and catenins expression in adherens junctions, reduced claudins and occludins expression at tight junctions and reduced expression of various epithelial cytokeratins such as KRT8, 18 and 19 which presumably aids in disruption of cytoskeletal connections that maintain tissue architecture. These changes are also paralleled with up-regulation of mesenchymal markers such as vimentin, fibronectin, alpha smooth muscle actin (ACTA2), N-cadherin and various matrix metalloproteinases (MMPs) [4, 5]. Attempts to overcome endocrine resistance include the use of pure estrogen antagonists such as fulvestrant (in place of tamoxifen, which is associated with some agonist actions with prolonged administration) or agents which inhibit peripheral extragonadal synthesis of estrogen (aromatase inhibitors such as anastrozole), which delays but does not resolve this problem [6, 7]. In addition, receptor tyrosine kinase (RTK) inhibitors have been used recently in the treatment of endocrine-resistant breast cancer [8], but they have limitations in terms of target specificity and clinical outcomes. For example, the reversible inhibitor of epidermal growth factor receptor (EGFR) erlotinib also blocks ERBB2 [9, 10], AKT (the downstream target of phosphatidylinositol 3-kinases; PI3K) and mitogen-activated protein kinase (MAPK) phosphorylation in breast cancer cells [11]. Furthermore, imatinib inhibits the activity of the tyrosine kinase domain of various targets such as ABL, KIT and platelet-derived growth factor receptor (PDGFR) [12, 13]. The lack of specificity of these agents might increase the risk of side effects and therefore limits their clinical usage and utility. Since the current therapeutic options for endocrine insensitive breast cancer patients have various limitations (including severe side effect profile and resistance), there is a need to find better therapeutic targets to control this condition and improve its prognosis.

Aquaporins (AQPs) represent a family of 13–14 small hydrophobic integral transmembrane water channel proteins which are widely distributed in various tissues in the body. Their function is to transport mainly water (through passive transport), glycerol, solutes (such as urea, carbon dioxide, ammonia and nitric oxide) [14–20], as well as larger polar solutes (such as sugars and hydrogen peroxide) [21–23]. The first discovered family member of these proteins was initially called CHIP28, but it is now known as AQP 1 [24, 25]. AQPs are classified

on the basis of their substrate permeability: (a) the classical water permeable AQPs 0, 1, 2, 4, 5, 6 and 8; (b) the water and small solute (e.g., glycerol and urea) permeable aquaglyceroporins AQPs 3, 7, 9, 10 and 12; (c) gas (carbon dioxide and nitric oxide) and ammonia permeable AQPs 1, 4 and 5; and (d) small ion (e.g., sodium and potassium) conducting AQP 1 [25]. Besides their main role in maintaining salt and water homeostasis, recent evidence suggests their involvement in various disease conditions including neoplasms such as breast cancer. These membrane channels have received much attention in recent years as potential novel drug targets for reducing cancer angiogenesis and metastasis. This chapter will provide evidence from recent studies regarding the involvement of various AQPs in breast cancer pathogenesis and will highlight their role in disease diagnosis, prognosis and treatment.

2. Structure of AQPs

Unlike other types of channels, AQPs do not show gating, saturation or membrane potential-dependent behavior. AQP family members share 25–60% protein sequence homology [14, 26, 27], and are assembled on the cell membrane and cytoplasmic compartments as homotetramers [28]. Each monomer is about 28–30 kDa in size and has its own water pore. Some members of this family such as AQPs 0 and 4 have unique features in that their tetramers assemble into higher order supramolecular structures described as orthogonal arrays of particles [29, 30]. The monomeric units of AQPs consist of six transmembrane α -helices (M 1, 2, 4–7 and 8), two half helices (M 3 and 7) and five connecting loops (a–e) [31]. Both the N- and carboxyterminal domains are present in the cytoplasmic compartment. Water movement occurs through a narrow pore (<0.3 nm) in which steric and electrostatic factors prevent the transport of protons and other small molecules [32]. Several studies have also indicated that the central pore allows the rapid transport of oxygen, carbon dioxide and nitric oxide (seen in AQPs 1, 4 and 5) [19, 33]. On the other hand, the aquaglyceroporins have a less constricted pore with a larger proportion of hydrophobic residues [34, 35]. **Figure 1** illustrates a schematic arrangement of an AQP channel.

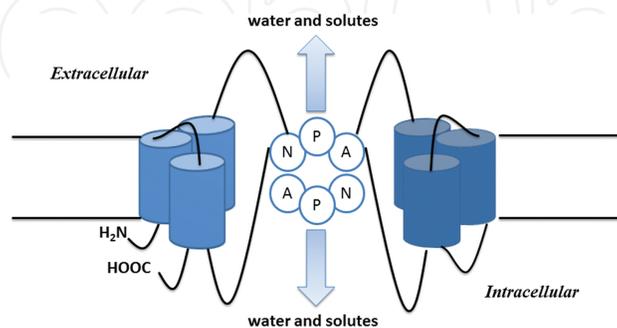


Figure 1. Schematic diagram of the aquaporin channel. The aquaporins are formed by two tandem repeats of three membrane-spanning helices. Two connecting loops, each containing a conserved sequence motif of Asn-Pro-Ala (NPA) on the loops, bend into molecules to pair with each other and form a channel in the plasma membrane through which water and solutes can pass between the cell and its environment.

3. Expression profile of AQPs

3.1. Normal tissues

These channel proteins exhibit a wide tissue distribution. Several AQPs (1–4) play a role in kidney function [36, 37]. For example, AQP 2 translocates from the intracellular vesicles to the apical plasma membrane of the collecting duct in response to vasopressin stimulation leading to water reabsorption by the kidney [37, 38]. AQP 1 allows carbon dioxide transport in the proximal tubules, for regulation of arterial pH during metabolic acidosis [39]. In the brain, AQP 4 is expressed in the perivascular astrocyte foot process region and plays a role in solute clearance from the interstitial fluid [40] and the neuro-excitatory processes [41]. In the skin, AQP 3 is expressed in the stratum corneum (SC) and plays a role in maintaining skin hydration and elasticity, and epidermal proliferation [42]. In the adipocytes, AQP 7 is involved in glycerol movement across the cell [36]. Several AQPs are expressed in various regions of the eye and play a role in ocular surface hydration, intraocular pressure regulation and visual signal transduction [43]. Other AQPs are expressed elsewhere but their physiological functions remain to be determined. For example, AQP 4 is expressed in the basolateral region of gastric parietal cells but its deletion in mice does not alter acid secretion [36, 44]. Furthermore, tissue-specific expression of AQP 4 in skeletal muscle [45], AQP 5 in sweat glands [46] and AQP 8 in various tissues [47] have not yet been linked with any specific physiological role.

3.2. Tumors

There is accumulating evidence to suggest a role for several AQPs in cancer pathogenesis through their modulated expression profile in several tumors. It is speculated that AQPs facilitate water penetration into the growing tumor leading to its expansion through edema formation [48, 49]. They also appear to be involved in angiogenesis, tumor proliferation and migration/invasion [50–53]. About twenty types of tumors have been shown to express AQPs *in vivo*. For example, the expression level of AQPs 1, 4 and 9 are increased in astrocytoma [48, 54–57], while the level of AQP 1 was shown to be either increased [58] or decreased [59] in cholangiocarcinoma. Increased levels of AQPs 1, 3 and 5 [60–62] and decreased level of AQP 8 [63, 64] have been reported in colorectal cancer. In lung cancer, AQPs 1, 3, 4 and 5 were shown to be overexpressed [65–67]. Increased levels of AQPs 1, 3 and 5 were observed in cervical cancer [68, 69]. AQP 5 was increased in chronic myelogenous leukemia [70] and esophageal cancer [71]. In liver cancer, high levels of AQPs 3 and 5 [72] and low levels of AQPs 8 and 9 were observed [73].

There is a direct correlation between the expression level of several AQPs and tumor grade. High levels of AQPs 1, 4 and 9 were observed in astrocytoma correlating with advanced disease stage [48, 54–57]. Enhanced AQP 9 expression was evident in malignant compared to benign ovarian tissues and was positively correlated with tumor grade [74]. Furthermore, enhanced expression of AQP 1 was seen in lung adenocarcinoma and its inhibition reduced cell invasion [66].

4. Physiological role of AQP

4.1. Fluid transport and osmotic equilibrium

It has been suggested that at least eight (of the known 13) AQPs transport water, while others such as AQPs 3, 7, 9 and 10 are also able to transport glycerol (termed aquaglyceroporins) [44, 75]. Their expression in various organs such as the kidney tubules, lung and alveoli facilitate active fluid absorption and secretion by the creation of an osmotic gradient across the cell membrane and subsequent fluid movement through these channels. Genetic knockout of AQP 5 in mice resulted in impaired salivary [76, 77] and airway submucosal gland secretion [78]. In addition, tissue-specific knockout of AQP 1 in mice leads to impaired secretion of the cerebrospinal fluid [79] and ocular aqueous fluid [80], and inappropriate hypertonic fluid absorption in the proximal kidney tubules [81]. It should be noted, however, that other data suggest that knockout of various AQPs does not lead to impaired fluid absorption or secretion [82–86], suggesting that the requirement of AQPs to facilitate active fluid transport depends on the rate of such transport in each compartment. AQPs (specifically 1–4 and 7) are also involved in maintaining the osmotic equilibrium across the kidney tubules and the formation of concentrated urine. Marked polyuria and low urine osmolality was seen in AQPs 1 and 3 knockout mice, which led to severe dehydration [87, 88]. Reduced expression of AQP 2 also leads to acquired forms of nephrogenic diabetes insipidus (NDI) due to the inability of the kidneys to concentrate urine owing to the insensitivity of the distal nephron to the antidiuretic hormone arginine vasopressin [89]. AQP 4 is expressed in the glial cells of the brain and spinal cord, and plays an important role in water balance in the brain. A significant reduction in osmotic water permeability in glial cells was demonstrated in AQP-4-deficient mice which led to brain edema and swelling [90, 91]. In addition, several AQPs (0, 1, 3, 4 and 5) are expressed in various compartments of the eye and play an important role in the regulation of fluid movement and intraocular pressure [92–95].

4.2. CNS functions

AQP 4 was shown to be expressed in the glial cells in the brain particularly at astrocyte endfeet at the blood-brain barrier and the ependymal-cerebrospinal fluid barrier [96]. AQP 4 deficiency in mice resulted in reduced seizure susceptibility in response to pentylenetetrazol treatment [97], as well as in electrically-induced seizure following hippocampal stimulation [98]. Delayed potassium uptake from the brain extracellular space (ECS) [98, 99], and expanded ECS which dilutes the released potassium levels [100, 101], has been suggested to be responsible for the reduced seizure susceptibility in AQP-4-deficient mice. AQP 4 also increases water exit from the brain in vasogenic edema, as AQP-4-deficient mice show greater water accumulation in various models of brain edema [102–105]. Also, AQP 1 was shown to be expressed in the dorsal root ganglion neurons and nociceptive C-fibers, and AQP 1 deficiency in mice leads to reduced pain perception in response to thermal inflammatory pain in part through modulation of voltage gated sodium channel Nav 1.8 activity [105–107].

4.3. Glycerol transport

AQP 3 was shown to be expressed in the stratum corneum (SC) at the basal layer of the keratinocytes and plays a role in skin hydration. In AQP-3-deficient mice, SC hydration was significantly reduced due to reduced water content, decreased skin elasticity and wound healing [108]. An important factor which was also attributed to reduced skin hydration in AQP-3-deficient mice is the impaired glycerol transport from the blood to the epidermis through the basal keratinocytes, suggesting the importance of AQP 3 in glycerol transport. Dysregulated expression of AQP 3 has been found in various skin disorders associated with altered epidermal proliferation [109, 110]. In fact, topical or systemic replacement of glycerol prevented skin abnormalities (less hydration and elasticity and impaired barrier function) in the deficient mice [111].

4.4. Cell proliferation

A role for AQP 3 in cell proliferation has been suggested in various cell types. Using corneal epithelial cells, delayed restoration of full-thickness epithelia was seen in AQP-3-deficient mice after scraping. This was confirmed by reduction in proliferating BrdU-positive cells during healing [112]. Reduced keratinocyte cell proliferation was also evident in AQP-3-deficient mice or with siRNA-mediated knockout of AQP 3 in keratinocytes in part through reduction of p38 MAPK activity [113]. Furthermore, the proliferative rate of mouse colonic epithelial cells was significantly reduced in AQP-3-deficient mice, which might explain the enhanced colitis severity in these mice compared to WT mice in the dextran sulfate sodium model of colitis [114].

4.5. Cell adhesion

AQP 0 is thought to be involved in cell-cell adhesion. It has been found to be expressed in lens fiber cells in the eye and plays a role in maintaining their structure [115]. Loss-of-function mutation of AQP 0 in humans and mice resulted in congenital cataracts [34, 92]. In addition, AQP 4 was shown to mediate weak cell-cell interaction through its short helix in the extracellular loop [116]. Overexpression of AQP 4 in L-cells (which lack endogenous adhesion molecules) resulted in cell cluster formation, which supports the role of this AQP in intercellular adhesion.

4.6. Cell migration

Various AQPs have been shown to be involved in the cell migrative process. AQP 1 is expressed on the leading edge of migrating cultured endothelial cells in association with increased lamellipodia formation. AQP 1 deficiency in cultured endothelial cells results in significant reduction in their migration. Overexpression of AQP 1 or 4 enhanced cell migration along with prominent membrane ruffling at the leading edge [53]. The role of AQP 1 in cell migration was also confirmed using kidney proximal tubule cells where its deficiency reduced cell migration and its overexpression led to enhanced cell migration through the formation of lamella-like membrane protrusions at the cell leading edge [50]. Furthermore, AQP 4 was localized on the leading edge of migrating cultured astroglia cells, and its expression was increased by inducing

a small extracellular osmotic gradient. AQP 4 deficiency (by siRNA treatment or cell isolation from AQP-4-deficient mice) resulted in marked reduction in their migratory potential [51, 52]. AQP 3 deficiency in mammalian corneal epithelial cells [51], keratinocytes [113] and fibroblasts [117] also reduced their migrative ability both *in vitro* and *in vivo*.

AQPs enhance cell migration through various mechanisms. They facilitate rapid changes in cell volume and shape, which allows the cells to squeeze through the narrow and irregularly shaped extracellular space; this has been referred to as amoeboidal movement [118]. Also, they increase the local hydrostatic pressure (that push apart adjacent stationary cells), and actin repolymerization, to stabilize cell membrane protrusions at the leading edge which is required for the migratory process [119]. There is some evidence regarding the role of AQP 4 in regulating a complex of intracellular molecules such as alpha-syntrophin involved in membrane protrusions [120]. Some evidence also suggests a role for AQP 3 in reducing keratinocyte cell migration through reduced p38 MAPK activity [113]; this is generally recognized as an important signaling molecule for cell migration.

5. Involvement of AQPs in the etiology of cancer

There is accumulating evidence for the involvement of several forms of AQPs in various types of cancer which also correlates with tumor stage.

With respect to tumor proliferation, AQP 5 interacts with the Ras-MAPK pathway and cyclin D1/CDK4 complexes in colon cancer [121] and with the EGFR/ERK1/2/p38 MAPK signaling cascade in lung cancer [122], resulting in enhanced proliferation, differentiation and survival. A role for AQP 3 has also been suggested for controlling proliferation of epidermal cancer cells through the facilitation of glycerol transport and increase in ATP generation [123]. In non-small-cell lung cancer cells, its effects appear to be associated with enhancement of the expression of p53, increase in the ratio of cleaved to procaspase 3 and reduction in the expression of proliferating cell nuclear antigen and B-cell lymphoma-2 (Bcl-2) [124]. AQP 4 is involved in glioblastoma cell proliferation; siRNA-mediated knockdown of AQP 4 induced cell apoptosis in part through modulation of key proteins involved in this process such as cytochrome c, Bcl-2 and Bad [125].

With regard to tumor migration/invasion and angiogenesis, AQP 3 silencing in non-small lung cancer cells resulted in significant inhibition of cell invasion through reduction of the activity of matrix metalloproteinases (MMPs) 2 and 9 and AKT phosphorylation, as well as reduction in angiogenesis through interaction with the HIF-2 α -VEGF pathway [124]. Overexpression of AQP 1 in B16F10 melanoma cells and 4T1 breast cancer cells resulted in enhanced cell invasion and tumor spread when injected through the tail vein in mice [53, 126]. siRNA-mediated knockdown of AQP 1 in melanoma cells also resulted in reduced cell proliferation and invasion [127]. Overexpression of AQP 1 in colon cancer cells increased their invasive potential through actin relocalization and RhoA and Rac activation [128]. In glioma cells, AQP 1 facilitated the shunting of H⁺ from the intracellular to the extracellular compartment and the release of lactate dehydrogenase (LDH) and cathepsin B, which results in the acidification of the tumor

microenvironment leading to enhanced tumor angiogenesis and invasion [129]. AQP 4 also plays a role in glioblastoma cell migration and invasion through rearrangement of the actin cytoskeleton [130]. Furthermore, overexpression of AQP 5 in non-small lung cancer cells enhanced cell metastasis through c-Src activation and induction of the EMT process [122].

6. Role of AQPs in the pathogenesis of breast cancer

While AQPs have been shown to be involved in the delivery of water to the mammary glands which is critical for milk production and secretion during lactation [131], their expression in breast tumors is modified and correlates with tumor grade.

6.1. AQP 1

Immunostaining indicates a predominantly membranous localization with some presence in the cytoplasm in large tumor cells (more pronounced at the tumor invasion front), but no expression was seen in smaller tumor cells. All of the AQP 1 positive invasive carcinomas are found to be of ductal type, ER-ve and HER2/neu -ve (triple -ve form), and its expression was significantly associated with poor clinical prognosis [132, 133]. A recent report suggested that the cytoplasmic expression of AQP 1 promotes breast cancer progression and was associated with a shorter survival rate especially in luminal subtype patients [134]. Its cytoplasmic expression was positively correlated with advanced pathological features of invasive ductal carcinoma and lymph node metastasis [134]. Another study reported that AQP 1 was highly expressed in blood vessels (mainly in CD31+ve endothelial cells) of human breast and endometrial carcinoma tissues, suggesting a role in tumor angiogenesis [135]. Using human umbilical vein endothelial cells (HUVECs), Zou et al. [135] showed that estrogen treatment significantly up-regulated AQP 1 expression in a time- and dose-dependent fashion, which was mediated through a functional estrogen response element motif in the promoter region of the AQP1 gene. Estrogen treatment significantly increased HUVEC proliferation, migration, invasion and tubule formation; all of these effects were inhibited by pretreatment of cells with AQP1-specific siRNA. These data suggest an important role of AQP 1 in cell invasion in part through regulating actin stress fiber formation through colocalization with the ezrin/radixin/moesin protein complex [135]. Qin et al. [134] showed that overexpression of AQP 1 in MCF-7 and MDA-MB-231 cells significantly enhanced (by approximately 2 fold) cell proliferation and invasion. Epidermal growth factor (EGF) stimulation induced AQP 1 redistribution from the cytoplasm to the cell membrane, further supporting a role in promoting cell invasion. In the mouse mammary tumor virus-driven polyoma middle T oncogene (MMTV-PyVT) model (which spontaneously develops a well-differentiated luminal-type breast carcinoma with lung metastasis), AQP 1 deficiency significantly reduced the breast tumor mass (by 46%) and volume (by 50%), vessel density and the number of lung metastases compared to the control group [136]. This effect was in part due to decreased expression of vascular endothelial growth factor receptor-2 (VEGFR2) and increased levels of hypoxia inducible factor-1 α (HIF-1 α) in the AQP 1 knockout mice [136].

6.2. AQP 3

AQP 3 overexpression in early breast cancer patients was shown to be associated with worse prognosis in patients with HER2-overexpressing phenotype after curative surgery [137]. Its expression was correlated with advanced stage, large tumor size and lymphatic and vascular invasion, highlighting its role in angiogenesis and invasion. In addition, Huang et al. [138] showed higher AQP 3 protein expression in breast cancer tissues (mainly in the cell membrane and the cytoplasm) of premenopausal compared to postmenopausal patients, and was associated with higher histopathological grade and lymph node metastasis in ER+ve breast cancer patients. Estrogen stimulation significantly up-regulated AQP 3 expression in ER+ve breast cancer cells (MCF-7 and T47D) by activating the estrogen response elements (EREs) in the promoter region of the AQP 3 gene. siRNA mediated knockdown of AQP 3 in ER+ve breast cancer cells significantly reduced estrogen-induced cell migration (by 30–70%) and invasion (by 43–71%). Overexpression of AQP 3 in T47D cells significantly enhanced cell migration and invasion. The role of AQP 3 in cell invasion was suggested to be in part through mediating actin cytoskeleton rearrangement (by the formation of filopodia and stress fibers required for invasion) and EMT induction (evident by reduced expression of the epithelial marker E-cadherin, and increased levels of the mesenchymal markers N-cadherin and snail-1) [138]. Using breast cancer cell lines MDA-MB-231 and Bcap-37, Cao et al. [139] showed that fibroblast growth factor-2 (FGF-2) significantly increased AQP 3 expression, and lentivirus-mediated shRNA inhibition of AQP3 expression significantly reduced FGF-2 induced cell migration by approximately 50%. This effect was mediated through AQP-3-induced activation of Akt and ERK1/2. A recent report showed that AQP 3 expression in the triple negative breast cancer cell lines MDA-MB-231 and DU4475 (as well as in HUVEC) was required for the transport of extracellular hydrogen peroxide into the cells in response to CXCL-12 stimulation to induce directional cell migration [140]. AQP 3 silencing in these cells was associated with impaired CXCL-12 induced directional migration due to impaired F-actin polymerization, PTEN and PTP1B oxidation, Akt phosphorylation, and the accumulation of the intracellular hydrogen peroxide at the leading edge of migrating cells was needed for polarity sensing. Furthermore, the role of AQP3 in invasion was tested by the injection of fluorescently labeled breast cancer cells into severe combined immunodeficient (SCID) mice. Lung metastasis was significantly reduced in AQP-3-deficient breast cancer cells, whereas its overexpression significantly increased the number of cells migrating to the lungs [140]. In addition, the expression of AQP 3 was also increased in MCF-7 cells by treatment with the chemotherapeutic agent 5'-deoxy-5-fluorouridine (5'-DFUR) [141], which was required for the 5'-DFUR-induced cell cycle arrest (through its action on G1/S phase transition and up-regulation of p21 and FAS).

6.3. AQP 4

The role of this AQP is not well studied in breast cancer, however, one report showed that AQP 4 expression (at both mRNA and protein level) was significantly higher in normal compared to cancer tissue [133], and was mainly expressed in the cell membrane and the cytoplasmic compartments.

6.4. AQP 5

Immunohistochemical analysis shows significant overexpression of AQP 5 in breast tumors from early breast cancer patients, and was correlated with the disease prognosis particularly in patients with ER/PR+ve tumors [142]. This observation was also confirmed by another group who showed that AQP 5 was not detectable in normal breast tissues, but was expressed mainly in the cell membrane of mammary carcinoma and associated with cellular differentiation, lymph node invasion and tumor stage [133]. The 5-year survival rate was decreased from 80% in AQP 5 -ve patients to 50% in AQP5+ve patients, suggesting that its expression was associated with short overall survival [133]. In another report, AQP 5 expression was observed in the ductal epithelial cells of human breast tissues with significant overexpression in invasive compared to benign tumors [143]. It was also expressed in MCF7 and MDA-MB-231 breast cancer cell lines (at mRNA and protein level); shRNA, or hyperosmotic stress-induced reduction in AQP 5 expression significantly reduced cell proliferation and migration toward fetal bovine serum (FBS) gradient. Some reports have suggested that AQP 5 induces tumorigenesis (at least in lung epithelial cells) upon phosphorylation of the cAMP protein kinase consensus site located in its cytoplasmic loop [144, 145].

7. AQPs: cancer diagnostic markers in breast cancer

There is no clinical data so far which confirms the use of AQPs as diagnostic markers for breast cancer. However, many reports suggest a strong correlation between the expression profile of certain types of AQPs and breast cancer pathogenesis and prognosis. For example, AQP 1 expression was associated with poor clinical prognosis in ductal type, ER -ve and HER2/neu -ve breast cancer patients [132]. The cytoplasmic expression of AQP 1 was also correlated with advanced pathological features of invasive ductal carcinoma, lymph node metastasis and shorter survival [134]. Overexpression of AQP 3 in HER2-overexpressing patients [137] as well as in premenopausal ER+ve breast cancer patients [138] was associated with advanced stage. AQP 5 expression was also shown to be associated with poor clinical prognosis [133], particularly in patients with ER/PR+ve tumors [142], and in the ductal epithelial cells of human breast tissues [143].

Detection of serum AQP 4 auto-antibodies has shown promising indication as a diagnostic tool in neuromyelitis optica (NMO), an inflammatory demyelinating disease that selectively affects optic nerves and spinal cord. It is claimed to be significantly associated with a higher number of relapses and longer disease duration [146, 147]. There are also reports suggesting a role for other AQPs: AQP 2 in determining the etiology of metabolic disorders dependent on the arginine vasopressin [148], AQP 3 in eczema [149] and AQP 4 in epilepsy [150].

8. AQPs: therapeutic targets for breast cancer

There appears to be potential for the use of AQP-based therapies (such as cysteine-reactive heavy metal-based inhibitors, AQP-induced water permeation, monoclonal AQP-specific

antibodies and AQP gene transfer) to treat various conditions including breast cancer. Several heavy metals have been shown to inhibit AQP 1. These include mercury II chloride (through covalent interaction with the Cys189 residue in the water pore of AQP 1) [151, 152] and silver and gold III compounds (through interaction with the cysteine residue near the conserved NPA domain) [153, 154]. Gold III compounds were also shown to inhibit AQP 3 through interaction with the Cys40 in its extracellular domain [154, 155]. Other nonmetal containing small molecule inhibitors include tetraethylammonium (TEA⁺), which reversibly inhibits AQP 1 through interaction with the Tyr186 site [156, 157]. The carbonic anhydrase inhibitor acetazolamide was also shown to inhibit AQPs 1 and 4 [158, 159]. Several antiepileptics, and the loop diuretic bumetanide, are reported to inhibit AQP 4 [159–161]. The other loop diuretic furosemide was also found to inhibit AQP 1 [162]. Furthermore, AQP gene transfer therapy is also in its early phases; AQP 1 cDNA transfer into the parotid glands for treating salivary gland hypofunction after radiation therapy is currently in phase I clinical trials [163–165].

In noncancerous conditions, some AQPs (1–4 and 7) are required for the formation of concentrated urine, which suggests that AQP-inhibitors might act as a unique form of diuretics to treat various disorders such as heart failure [87, 88]. Increased expression of AQP 4 exacerbated water accumulation in the brain, suggesting that AQP 4 inhibitors might be used to treat cytotoxic edema [90, 91]. Other potential therapeutic uses of AQP-therapies include treatment of various exocrine disorders, obesity and glaucoma [166].

AQP 1 is expressed on the endothelial cells of microvessels in various tumors including the breast [167], with a clear role in mediating angiogenesis and invasion through interaction with the actin cytoskeletal machinery, EGF, VEGF and HIF-1 α . It has been suggested that the carbonic anhydrase inhibitor acetazolamide, and the antiepileptic drug topiramate, suppress tumor invasion in part through inhibiting AQP 1 gene expression [168, 169]. AQP 3 was also shown to be involved in breast cancer cell invasion through interaction with the actin cytoskeleton proteins, ER, chemokines and growth factors (CXCL-12, FGF-2), downstream signaling molecules (ERK1/2, Akt, PTEN and PTP1B) and induction of the EMT process. Furthermore, AQP 5 also enhanced breast cancer invasion in part through interaction with cAMP. The chemotherapeutic drug cisplatin inhibits the expression of AQP 5 in ovarian cancer and leads to reduced lymph node metastasis [170]. Therefore [171], inhibitors of the above-mentioned AQPs may have potential applications in breast cancer therapy through their inhibitory actions on tumor angiogenesis and invasion.

9. Conclusion

There is growing evidence in several tumors (including that of the breast) to indicate that several growth factors (e.g., EGF, VEGF and FGF-2) which are known to enhance cell invasion, may do so, at least in part, through increasing expression of a number of AQPs, suggesting a prometastatic role for these channels. This is likely to be mediated by interaction with various signaling molecules involved in cell invasion such as Ras, MAPK and PI3K, leading to rearrangement of the actin cytoskeleton (through interaction with RhoA/Rac), extracellular

acidification (through interaction with LDH and HIF-1 α , which by itself enhances cell invasion), enhanced secretion of proteolytic enzymes needed to degrade the extracellular matrix (ECM) (e.g., MMP2/9 and cathepsin B) and induction of the EMT process. AQPs also enhance cell invasion through a 'rounding' of the cell to enable it to squeeze through the ECM (termed amoeboidal motility). **Figure 2** summarizes the putative role of AQPs in cancer pathogenesis.

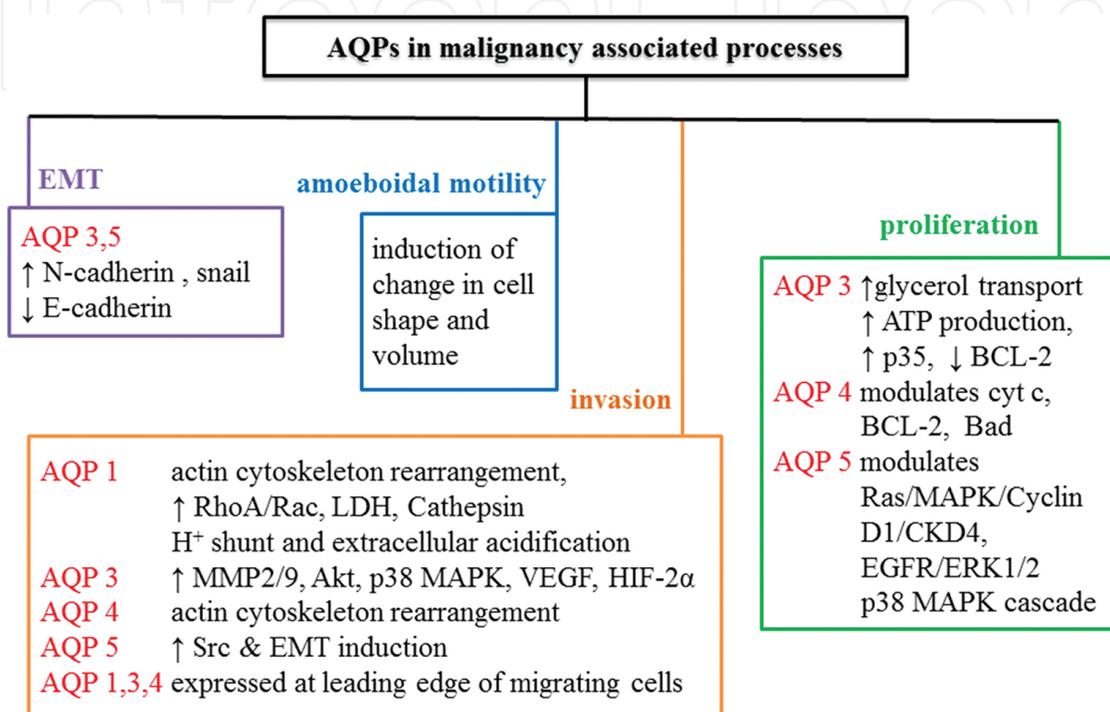


Figure 2. Role of AQPs in cancer pathogenesis. AQPs play an important role in cancer pathogenesis through enhancement of cancer cell proliferation, invasion and induction of epithelial to mesenchymal transition (EMT) as well as induction of amoeboidal motility. The mediators through which each AQP modulates these functions are elaborated in the scheme.

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References

- [1] Adamo V, Iorfida M, Montalto E, Festa V, Garipoli C, Scimone A, et al. Overview and new strategies in metastatic breast cancer (MBC) for treatment of tamoxifen-resistant patients. *Ann Oncol.* 2007;18(6):vi53-vi57.
- [2] Berry DA, Cronin KA, Plevritis SK, Fryback DG, Clarke L, Zelen M, et al. Effect of screening and adjuvant therapy on mortality from breast cancer. *N Engl J Med.* 2005;353(17):1784-1792.
- [3] Strasser-Weippl K, Goss PE. Advances in adjuvant hormonal therapy for postmenopausal women. *J Clin Oncol.* 2005;23(8):1751-1759.
- [4] Al Saleh S, Sharaf LH, Luqmani YA. Signalling pathways involved in endocrine resistance in breast cancer and associations with epithelial to mesenchymal transition (Review). *Int J Oncol.* 2011;38(5):1197-1217.
- [5] Luqmani YA, Al Azmi A, Al Bader M, Abraham G, El Zawahri M. Modification of gene expression induced by siRNA targeting of estrogen receptor alpha in MCF7 human breast cancer cells. *Int J Oncol.* 2009;34(1):231-242.
- [6] Massarweh S, Schiff R. Unraveling the mechanisms of endocrine resistance in breast cancer: new therapeutic opportunities. *Clin Cancer Res.* 2007;13(7):1950-1954.
- [7] Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. *Annu Rev Med.* 2011;62:233-247.
- [8] Normanno N, Morabito A, De Luca A, Piccirillo MC, Gallo M, Maiello MR, et al. Target-based therapies in breast cancer: current status and future perspectives. *Endocr Relat Cancer.* 2009;16(3):675-702.
- [9] Normanno N, Bianco C, De Luca A, Maiello MR, Salomon DS. Target-based agents against ErbB receptors and their ligands: a novel approach to cancer treatment. *Endocr Relat Cancer.* 2003;10(1):1-21.
- [10] Schaefer G, Shao L, Totpal K, Akita RW. Erlotinib directly inhibits HER2 kinase activation and downstream signaling events in intact cells lacking epidermal growth factor receptor expression. *Cancer Res.* 2007;67(3):1228-1238.
- [11] Guix M, Granja Nde M, Meszoely I, Adkins TB, Wieman BM, Frierson KE, et al. Short preoperative treatment with erlotinib inhibits tumor cell proliferation in hormone receptor-positive breast cancers. *J Clin Oncol.* 2008;26(6):897-906.
- [12] Gambacorti-Passerini C. Part I: Milestones in personalised medicine – imatinib. *Lancet Oncol.* 2008;9(6):600.
- [13] Deininger MW, Druker BJ. Specific targeted therapy of chronic myelogenous leukemia with imatinib. *Pharmacol Rev.* 2003;55(3):401-423.

- [14] Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, et al. Aquaporin water channels – from atomic structure to clinical medicine. *J Physiol*. 2002;542(Pt 1): 3-16.
- [15] Verkman AS, Mitra AK. Structure and function of aquaporin water channels. *Am J Physiol Renal Physiol*. 2000;278(1):F13-F28.
- [16] Wang Y, Tajkhorshid E. Nitric oxide conduction by the brain aquaporin AQP4. *Proteins*. 2010;78(3):661-670.
- [17] Herrera M, Hong NJ, Garvin JL. Aquaporin-1 transports NO across cell membranes. *Hypertension*. 2006;48(1):157-164.
- [18] Holm LM, Jahn TP, Moller AL, Schjoerring JK, Ferri D, Klaerke DA, et al. NH₃ and NH₄⁺ permeability in aquaporin-expressing *Xenopus* oocytes. *Pflugers Arch*. 2005;450(6):415-428.
- [19] Musa-Aziz R, Chen LM, Pelletier MF, Boron WF. Relative CO₂/NH₃ selectivities of AQP1, AQP4, AQP5, AmtB, and RhAG. *Proc Natl Acad Sci U S A*. 2009;106(13): 5406-5411.
- [20] Hub JS, Grubmuller H, de Groot BL. Dynamics and energetics of permeation through aquaporins. What do we learn from molecular dynamics simulations? *Handb Exp Pharmacol*. 2009;190:57-76.
- [21] Miller EW, Dickinson BC, Chang CJ. Aquaporin-3 mediates hydrogen peroxide uptake to regulate downstream intracellular signaling. *Proc Natl Acad Sci U S A*. 2010;107(36): 15681-15686.
- [22] Hara-Chikuma M, Chikuma S, Sugiyama Y, Kabashima K, Verkman AS, Inoue S, et al. Chemokine-dependent T cell migration requires aquaporin-3-mediated hydrogen peroxide uptake. *J Exp Med*. 2012;209(10):1743-1752.
- [23] Tsukaguchi H, Weremowicz S, Morton CC, Hediger MA. Functional and molecular characterization of the human neutral solute channel aquaporin-9. *Am J Physiol*. 1999;277(5 Pt 2):F685-F696.
- [24] Preston GM, Carroll TP, Guggino WB, Agre P. Appearance of water channels in *Xenopus* oocytes expressing red cell CHIP28 protein. *Science*. 1992;256(5055):385-387.
- [25] Agre P, Preston GM, Smith BL, Jung JS, Raina S, Moon C, et al. Aquaporin CHIP: the archetypal molecular water channel. *Am J Physiol*. 1993;265(4 Pt 2):F463-F476.
- [26] Fujiyoshi Y, Mitsuoka K, de Groot BL, Philippsen A, Grubmuller H, Agre P, et al. Structure and function of water channels. *Curr Opin Struct Biol*. 2002;12(4):509-515.
- [27] Takata K, Matsuzaki T, Tajika Y. Aquaporins: water channel proteins of the cell membrane. *Prog Histochem Cytochem*. 2004;39(1):1-83.

- [28] Walz T, Fujiyoshi Y, Engel A. The AQP structure and functional implications. *Handb Exp Pharmacol*. 2009;190:31-56.
- [29] Crane JM, Verkman AS. Determinants of aquaporin-4 assembly in orthogonal arrays revealed by live-cell single-molecule fluorescence imaging. *J Cell Sci*. 2009;122(Pt 6): 813-821.
- [30] Rash JE, Yasumura T, Hudson CS, Agre P, Nielsen S. Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. *Proc Natl Acad Sci U S A*. 1998;95(20):11981-11986.
- [31] Verkman AS, Anderson MO, Papadopoulos MC. Aquaporins: important but elusive drug targets. *Nat Rev Drug Discov*. 2014;13(4):259-277.
- [32] Hub JS, de Groot BL. Mechanism of selectivity in aquaporins and aquaglyceroporins. *Proc Natl Acad Sci U S A*. 2008;105(4):1198-1203.
- [33] Herrera M, Garvin JL. Aquaporins as gas channels. *Pflugers Arch*. 2011;462(4):623-630.
- [34] Chepelinsky AB. Structural function of MIP/aquaporin 0 in the eye lens; genetic defects lead to congenital inherited cataracts. *Handb Exp Pharmacol*. 2009;190:265-297.
- [35] Yang B, Brown D, Verkman AS. The mercurial insensitive water channel (AQP-4) forms orthogonal arrays in stably transfected Chinese hamster ovary cells. *J Biol Chem*. 1996;271(9):4577-4580.
- [36] Verkman AS. Aquaporins in clinical medicine. *Annu Rev Med*. 2012;63:303-316.
- [37] Noda Y, Sohara E, Ohta E, Sasaki S. Aquaporins in kidney pathophysiology. *Nat Rev Nephrol*. 2010;6(3):168-178.
- [38] Deen PM, Verdijk MA, Knoers NV, Wieringa B, Monnens LA, van Os CH, et al. Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science*. 1994;264(5155):92-95.
- [39] Skelton LA, Boron WF, Zhou Y. Acid-base transport by the renal proximal tubule. *J Nephrol* 2010(23):S4-S18.
- [40] Yang L, Kress BT, Weber HJ, Thiyagarajan M, Wang B, Deane R, et al. Evaluating glymphatic pathway function utilizing clinically relevant intrathecal infusion of CSF tracer. *J Transl Med*. 2013;11(107):1479-5876.
- [41] Verkman AS, Ratelade J, Rossi A, Zhang H, Tradtrantip L. Aquaporin-4: orthogonal array assembly, CNS functions, and role in neuromyelitis optica. *Acta Pharmacol Sin*. 2011;32(6):702-710.
- [42] Hara-Chikuma M, Verkman AS. Roles of aquaporin-3 in the epidermis. *J Invest Dermatol*. 2008;128(9):2145-2151.
- [43] Verkman AS, Ruiz-Ederra J, Levin MH. Functions of aquaporins in the eye. *Prog Retin Eye Res*. 2008;27(4):420-433.

- [44] Rojek A, Praetorius J, Frokiaer J, Nielsen S, Fenton RA. A current view of the mammalian aquaglyceroporins. *Annu Rev Physiol.* 2008;70:301-327.
- [45] Yang B, Verbavatz JM, Song Y, Vetrivel L, Manley G, Kao WM, et al. Skeletal muscle function and water permeability in aquaporin-4 deficient mice. *Am J Physiol Cell Physiol.* 2000;278(6):C1108-C1115.
- [46] Song Y, Sonawane N, Verkman AS. Localization of aquaporin-5 in sweat glands and functional analysis using knockout mice. *J Physiol.* 2002;541(Pt 2):561-568.
- [47] Yang B, Song Y, Zhao D, Verkman AS. Phenotype analysis of aquaporin-8 null mice. *Am J Physiol Cell Physiol.* 2005;288(5):12.
- [48] Saadoun S, Papadopoulos MC, Davies DC, Krishna S, Bell BA. Aquaporin-4 expression is increased in oedematous human brain tumours. *J Neurol Neurosurg Psychiatry.* 2002;72(2):262-265.
- [49] Warth A, Simon P, Capper D, Goepfert B, Tabatabai G, Herzog H, et al. Expression pattern of the water channel aquaporin-4 in human gliomas is associated with blood-brain barrier disturbance but not with patient survival. *J Neurosci Res.* 2007;85(6):1336-1346.
- [50] Hara-Chikuma M, Verkman AS. Aquaporin-1 facilitates epithelial cell migration in kidney proximal tubule. *J Am Soc Nephrol.* 2006;17(1):39-45.
- [51] Auguste KI, Jin S, Uchida K, Yan D, Manley GT, Papadopoulos MC, et al. Greatly impaired migration of implanted aquaporin-4-deficient astroglial cells in mouse brain toward a site of injury. *Faseb J.* 2007;21(1):108-116.
- [52] Saadoun S, Papadopoulos MC, Watanabe H, Yan D, Manley GT, Verkman AS. Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. *J Cell Sci.* 2005;118(Pt 24):5691-5698.
- [53] Saadoun S, Papadopoulos MC, Hara-Chikuma M, Verkman AS. Impairment of angiogenesis and cell migration by targeted aquaporin-1 gene disruption. *Nature.* 2005;434(7034):786-792.
- [54] Jelen S, Parm Ulhoi B, Larsen A, Frokiaer J, Nielsen S, Rutzler M. AQP9 expression in glioblastoma multiforme tumors is limited to a small population of astrocytic cells and CD15(+)/CalB(+) leukocytes. *PLoS One.* 2013;8(9):2013.
- [55] Saadoun S, Papadopoulos MC, Davies DC, Bell BA, Krishna S. Increased aquaporin 1 water channel expression in human brain tumours. *Br J Cancer.* 2002;87(6):621-623.
- [56] Zhu SJ, Wang KJ, Gan SW, Xu J, Xu SY, Sun SQ. Expression of aquaporin8 in human astrocytomas: correlation with pathologic grade. *Biochem Biophys Res Commun.* 2013;440(1):168-172.

- [57] El Hindy N, Bankfalvi A, Herring A, Adamzik M, Lambertz N, Zhu Y, et al. Correlation of aquaporin-1 water channel protein expression with tumor angiogenesis in human astrocytoma. *Anticancer Res.* 2013;33(2):609-613.
- [58] Mazal PR, Susani M, Wrba F, Haitel A. Diagnostic significance of aquaporin-1 in liver tumors. *Hum Pathol.* 2005;36(11):1226-1231.
- [59] Aishima S, Kuroda Y, Nishihara Y, Taguchi K, Iguchi T, Taketomi A, et al. Down-regulation of aquaporin-1 in intrahepatic cholangiocarcinoma is related to tumor progression and mucin expression. *Hum Pathol.* 2007;38(12):1819-1825.
- [60] Yoshida T, Hojo S, Sekine S, Sawada S, Okumura T, Nagata T, et al. Expression of aquaporin-1 is a poor prognostic factor for stage II and III colon cancer. *Mol Clin Oncol.* 2013;1(6):953-958.
- [61] Moon C, Soria JC, Jang SJ, Lee J, Obaidul Hoque M, Sibony M, et al. Involvement of aquaporins in colorectal carcinogenesis. *Oncogene.* 2003;22(43):6699-6703.
- [62] Shi X, Wu S, Yang Y, Tang L, Wang Y, Dong J, et al. AQP5 silencing suppresses p38 MAPK signaling and improves drug resistance in colon cancer cells. *Tumour Biol.* 2014;35(7):7035-7045.
- [63] Wang W, Li Q, Yang T, Bai G, Li D, Sun H. Expression of AQP5 and AQP8 in human colorectal carcinoma and their clinical significance. *World J Surg Oncol.* 2012;10(242):1477-7819.
- [64] Fischer H, Stenling R, Rubio C, Lindblom A. Differential expression of aquaporin 8 in human colonic epithelial cells and colorectal tumors. *BMC Physiol.* 2001;1(1):23.
- [65] Machida Y, Ueda Y, Shimasaki M, Sato K, Sagawa M, Katsuda S, et al. Relationship of aquaporin 1, 3, and 5 expression in lung cancer cells to cellular differentiation, invasive growth, and metastasis potential. *Hum Pathol.* 2011;42(5):669-678.
- [66] Hoque MO, Soria JC, Woo J, Lee T, Lee J, Jang SJ, et al. Aquaporin 1 is overexpressed in lung cancer and stimulates NIH-3T3 cell proliferation and anchorage-independent growth. *Am J Pathol.* 2006;168(4):1345-1353.
- [67] Xie Y, Wen X, Jiang Z, Fu HQ, Han H, Dai L. Aquaporin 1 and aquaporin 4 are involved in invasion of lung cancer cells. *Clin Lab.* 2012;58(1-2):75-80.
- [68] Chen R, Shi Y, Amiduo R, Tuokan T, Suzuk L. Expression and prognostic value of aquaporin 1, 3 in cervical carcinoma in women of Uygur ethnicity from Xinjiang, China. *PLoS One.* 2014;9(2):2014.
- [69] Zhang T, Zhao C, Chen D, Zhou Z. Overexpression of AQP5 in cervical cancer: correlation with clinicopathological features and prognosis. *Med Oncol.* 2012;29(3):1998-2004.
- [70] Chae YK, Kang SK, Kim MS, Woo J, Lee J, Chang S, et al. Human AQP5 plays a role in the progression of chronic myelogenous leukemia (CML). *PLoS One.* 2008;3(7):0002594.

- [71] Liu S, Zhang S, Jiang H, Yang Y, Jiang Y. Co-expression of AQP3 and AQP5 in esophageal squamous cell carcinoma correlates with aggressive tumor progression and poor prognosis. *Med Oncol*. 2013;30(3):013-0636.
- [72] Guo X, Sun T, Yang M, Li Z, Gao Y. Prognostic value of combined aquaporin 3 and aquaporin 5 overexpression in hepatocellular carcinoma. *Biomed Res Int*. 2013;2013(206525):9.
- [73] Jablonski EM, Mattocks MA, Sokolov E, Koniaris LG, Hughes FM, Jr., Fausto N, et al. Decreased aquaporin expression leads to increased resistance to apoptosis in hepatocellular carcinoma. *Cancer Lett*. 2007;250(1):36-46.
- [74] Yang JH, Yan CX, Chen XJ, Zhu YS. Expression of aquaglyceroporins in epithelial ovarian tumours and their clinical significance. *Int Med Res*. 2011; 39(3):702-711.
- [75] Verkman AS. More than just water channels: unexpected cellular roles of aquaporins. *J Cell Sci*. 2005;118(Pt 15):3225-3232.
- [76] Ma T, Song Y, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. *J Biol Chem*. 1999;274(29):20071-20074.
- [77] Krane CM, Melvin JE, Nguyen HV, Richardson L, Towne JE, Doetschman T, et al. Salivary acinar cells from aquaporin 5-deficient mice have decreased membrane water permeability and altered cell volume regulation. *J Biol Chem*. 2001;276(26):23413-23420.
- [78] Song Y, Verkman AS. Aquaporin-5 dependent fluid secretion in airway submucosal glands. *J Biol Chem*. 2001;276(44):41288-41292.
- [79] Oshio K, Watanabe H, Song Y, Verkman AS, Manley GT. Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel Aquaporin-1. *Faseb J*. 2005;19(1):76-78.
- [80] Zhang D, Vetrivel L, Verkman AS. Aquaporin deletion in mice reduces intraocular pressure and aqueous fluid production. *J Gen Physiol*. 2002;119(6):561-569.
- [81] Schnermann J, Chou CL, Ma T, Traynor T, Knepper MA, Verkman AS. Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc Natl Acad Sci U S A*. 1998;95(16):9660-9664.
- [82] Bai C, Fukuda N, Song Y, Ma T, Matthay MA, Verkman AS. Lung fluid transport in aquaporin-1 and aquaporin-4 knockout mice. *J Clin Invest*. 1999;103(4):555-561.
- [83] Ma T, Fukuda N, Song Y, Matthay MA, Verkman AS. Lung fluid transport in aquaporin-5 knockout mice. *J Clin Invest*. 2000;105(1):93-100.
- [84] Song Y, Fukuda N, Bai C, Ma T, Matthay MA, Verkman AS. Role of aquaporins in alveolar fluid clearance in neonatal and adult lung, and in oedema formation following acute lung injury: studies in transgenic aquaporin null mice. *J Physiol*. 2000;3:771-779.

- [85] Song Y, Jayaraman S, Yang B, Matthay MA, Verkman AS. Role of aquaporin water channels in airway fluid transport, humidification, and surface liquid hydration. *J Gen Physiol.* 2001;117(6):573-582.
- [86] Yang B, Folkesson HG, Yang J, Matthay MA, Ma T, Verkman AS. Reduced osmotic water permeability of the peritoneal barrier in aquaporin-1 knockout mice. *Am J Physiol.* 1999;276(1 Pt 1):C76-C81.
- [87] Ma T, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. *J Biol Chem.* 1998;273(8):4296-4299.
- [88] Ma T, Song Y, Yang B, Gillespie A, Carlson EJ, Epstein CJ, et al. Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. *Proc Natl Acad Sci U S A.* 2000;97(8):4386-4391.
- [89] Khanna A. Acquired nephrogenic diabetes insipidus. *Semin Nephrol.* 2006;26(3):244-248.
- [90] Thiagarajah JR, Papadopoulos MC, Verkman AS. Noninvasive early detection of brain edema in mice by near-infrared light scattering. *J Neurosci Res.* 2005;80(2):293-299.
- [91] Papadopoulos MC, Verkman AS. Aquaporin-4 gene disruption in mice reduces brain swelling and mortality in pneumococcal meningitis. *J Biol Chem.* 2005;280(14):13906-13912.
- [92] Berry V, Francis P, Kaushal S, Moore A, Bhattacharya S. Missense mutations in MIP underlie autosomal dominant 'polymorphic' and lamellar cataracts linked to 12q. *Nat Genet.* 2000;25(1):15-17.
- [93] Thiagarajah JR, Verkman AS. Aquaporin deletion in mice reduces corneal water permeability and delays restoration of transparency after swelling. *J Biol Chem.* 2002;277(21):19139-19144.
- [94] Li J, Patil RV, Verkman AS. Mildly abnormal retinal function in transgenic mice without Muller cell aquaporin-4 water channels. *Invest Ophthalmol Vis Sci.* 2002;43(2):573-579.
- [95] Levin MH, Verkman AS. Aquaporin-dependent water permeation at the mouse ocular surface: in vivo microfluorimetric measurements in cornea and conjunctiva. *Invest Ophthalmol Vis Sci.* 2004;45(12):4423-4432.
- [96] Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP. Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J Neurosci.* 1997;17(1):171-180.
- [97] Binder DK, Oshio K, Ma T, Verkman AS, Manley GT. Increased seizure threshold in mice lacking aquaporin-4 water channels. *Neuroreport.* 2004;15(2):259-262.

- [98] Binder DK, Yao X, Zador Z, Sick TJ, Verkman AS, Manley GT. Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels. *Glia*. 2006;53(6):631-636.
- [99] Padmawar P, Yao X, Bloch O, Manley GT, Verkman AS. K⁺ waves in brain cortex visualized using a long-wavelength K⁺-sensing fluorescent indicator. *Nat Methods*. 2005;2(11):825-827.
- [100] Binder DK, Papadopoulos MC, Haggie PM, Verkman AS. In vivo measurement of brain extracellular space diffusion by cortical surface photobleaching. *J Neurosci*. 2004;24(37):8049-8056.
- [101] Zador Z, Magzoub M, Jin S, Manley GT, Papadopoulos MC, Verkman AS. Microfiber-optic fluorescence photobleaching reveals size-dependent macromolecule diffusion in extracellular space deep in brain. *Faseb J*. 2008;22(3):870-879.
- [102] Papadopoulos MC, Manley GT, Krishna S, Verkman AS. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. *Faseb J*. 2004;18(11):1291-1293.
- [103] Bloch O, Papadopoulos MC, Manley GT, Verkman AS. Aquaporin-4 gene deletion in mice increases focal edema associated with staphylococcal brain abscess. *J Neurochem*. 2005;95(1):254-262.
- [104] Tait MJ, Saadoun S, Bell BA, Verkman AS, Papadopoulos MC. Increased brain edema in aqp4-null mice in an experimental model of subarachnoid hemorrhage. *Neuroscience*. 2010;167(1):60-67.
- [105] Bloch O, Auguste KI, Manley GT, Verkman AS. Accelerated progression of kaolin-induced hydrocephalus in aquaporin-4-deficient mice. *J Cereb Blood Flow Metab*. 2006;26(12):1527-1537.
- [106] Saadoun S, Bell BA, Verkman AS, Papadopoulos MC. Greatly improved neurological outcome after spinal cord compression injury in AQP4-deficient mice. *Brain*. 2008;131(Pt 4):1087-1098.
- [107] Kimura A, Hsu M, Seldin M, Verkman AS, Scharfman HE, Binder DK. Protective role of aquaporin-4 water channels after contusion spinal cord injury. *Ann Neurol*. 2010;67(6):794-801.
- [108] Ma T, Hara M, Sougrat R, Verbavatz JM, Verkman AS. Impaired stratum corneum hydration in mice lacking epidermal water channel aquaporin-3. *J Biol Chem*. 2002;277(19):17147-17153.
- [109] Kim NH, Lee AY. Reduced aquaporin3 expression and survival of keratinocytes in the depigmented epidermis of vitiligo. *J Invest Dermatol*. 2010;130(9):2231-2239.
- [110] Nakahigashi K, Kabashima K, Ikoma A, Verkman AS, Miyachi Y, Hara-Chikuma M. Upregulation of aquaporin-3 is involved in keratinocyte proliferation and epidermal hyperplasia. *J Invest Dermatol*. 2011;131(4):865-873.

- [111] Hara M, Verkman AS. Glycerol replacement corrects defective skin hydration, elasticity, and barrier function in aquaporin-3-deficient mice. *Proc Natl Acad Sci U S A*. 2003;100(12):7360-7365.
- [112] Levin MH, Verkman AS. Aquaporin-3-dependent cell migration and proliferation during corneal re-epithelialization. *Invest Ophthalmol Vis Sci*. 2006;47(10):4365-4372.
- [113] Hara-Chikuma M, Verkman AS. Aquaporin-3 facilitates epidermal cell migration and proliferation during wound healing. *J Mol Med*. 2008;86(2):221-231.
- [114] Thiagarajah JR, Zhao D, Verkman AS. Impaired enterocyte proliferation in aquaporin-3 deficiency in mouse models of colitis. *Gut*. 2007;56(11):1529-1535.
- [115] Sindhu Kumari S, Gupta N, Shiels A, FitzGerald PG, Menon AG, Mathias RT, et al. Role of Aquaporin 0 in lens biomechanics. *Biochem Biophys Res Commun*. 2015;462(4):339-345.
- [116] Hiroaki Y, Tani K, Kamegawa A, Gyobu N, Nishikawa K, Suzuki H, et al. Implications of the aquaporin-4 structure on array formation and cell adhesion. *J Mol Biol*. 2006;355(4):628-639.
- [117] Cao C, Sun Y, Healey S, Bi Z, Hu G, Wan S, et al. EGFR-mediated expression of aquaporin-3 is involved in human skin fibroblast migration. *Biochem J*. 2006;400(2):225-234.
- [118] Sahai E, Marshall CJ. Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. *Nat Cell Biol*. 2003;5(8):711-719.
- [119] Condeelis J. Life at the leading edge: the formation of cell protrusions. *Annu Rev Cell Biol*. 1993;9:411-444.
- [120] Neely JD, Amiry-Moghaddam M, Ottersen OP, Froehner SC, Agre P, Adams ME. Syntrophin-dependent expression and localization of Aquaporin-4 water channel protein. *Proc Natl Acad Sci U S A*. 2001;98(24):14108-14113.
- [121] Kang SK, Chae YK, Woo J, Kim MS, Park JC, Lee J, et al. Role of human aquaporin 5 in colorectal carcinogenesis. *Am J Pathol*. 2008;173(2):518-525.
- [122] Zhang Z, Chen Z, Song Y, Zhang P, Hu J, Bai C. Expression of aquaporin 5 increases proliferation and metastasis potential of lung cancer. *J Pathol*. 2010;221(2):210-220.
- [123] Hara-Chikuma M, Verkman AS. Prevention of skin tumorigenesis and impairment of epidermal cell proliferation by targeted aquaporin-3 gene disruption. *Mol Cell Biol*. 2008;28(1):326-332.
- [124] Xia H, Ma YF, Yu CH, Li YJ, Tang J, Li JB, et al. Aquaporin 3 knockdown suppresses tumour growth and angiogenesis in experimental non-small cell lung cancer. *Exp Physiol*. 2014;99(7):974-984.

- [125] Ding T, Zhou Y, Sun K, Jiang W, Li W, Liu X, et al. Knockdown a water channel protein, aquaporin-4, induced glioblastoma cell apoptosis. *PLoS One*. 2013;8(8):2013.
- [126] Hu J, Verkman AS. Increased migration and metastatic potential of tumor cells expressing aquaporin water channels. *Faseb J*. 2006;20(11):1892-1894.
- [127] Nicchia GP, Stigliano C, Sparaneo A, Rossi A, Frigeri A, Svelto M. Inhibition of aquaporin-1 dependent angiogenesis impairs tumour growth in a mouse model of melanoma. *J Mol Med*. 2013;91(5):613-623.
- [128] Jiang Y. Aquaporin-1 activity of plasma membrane affects HT20 colon cancer cell migration. *IUBMB Life*. 2009;61(10):1001-1009.
- [129] Hayashi Y, Edwards NA, Proescholdt MA, Oldfield EH, Merrill MJ. Regulation and function of aquaporin-1 in glioma cells. *Neoplasia*. 2007;9(9):777-787.
- [130] Ding T, Gu F, Fu L, Ma YJ. Aquaporin-4 in glioma invasion and an analysis of molecular mechanisms. *J Clin Neurosci*. 2010;17(11):1359-1361.
- [131] Mobasheri A, Barrett-Jolley R. Aquaporin water channels in the mammary gland: from physiology to pathophysiology and neoplasia. *J Mammary Gland Biol Neoplasia*. 2014;19(1):91-102.
- [132] Otterbach F, Callies R, Adamzik M, Kimmig R, Siffert W, Schmid KW, et al. Aquaporin 1 (AQP1) expression is a novel characteristic feature of a particularly aggressive subgroup of basal-like breast carcinomas. *Breast Cancer Res Treat*. 2010;120(1):67-76.
- [133] Shi Z, Zhang T, Luo L, Zhao H, Cheng J, Xiang J, et al. Aquaporins in human breast cancer: identification and involvement in carcinogenesis of breast cancer. *J Surg Oncol*. 2012;106(3):267-272.
- [134] Qin F, Zhang H, Shao Y, Liu X, Yang L, Huang Y, et al. Expression of aquaporin1, a water channel protein, in cytoplasm is negatively correlated with prognosis of breast cancer patients. *Oncotarget*. 2016; 7(7):8143-8154.
- [135] Zou LB, Shi S, Zhang RJ, Wang TT, Tan YJ, Zhang D, et al. Aquaporin-1 plays a crucial role in estrogen-induced tubulogenesis of vascular endothelial cells. *J Clin Endocrinol Metab*. 2013;98(4):2012-4081.
- [136] Esteva-Font C, Jin BJ, Verkman AS. Aquaporin-1 gene deletion reduces breast tumor growth and lung metastasis in tumor-producing MMTV-PyVT mice. *Faseb J*. 2014;28(3):1446-1453.
- [137] Kang S, Chae YS, Lee SJ, Kang BW, Kim JG, Kim WW, et al. Aquaporin 3 Expression Predicts Survival in Patients with HER2-positive Early Breast Cancer. *Anticancer Res*. 2015;35(5):2775-2782.
- [138] Huang YT, Zhou J, Shi S, Xu HY, Qu F, Zhang D, et al. Identification of estrogen response element in aquaporin-3 gene that mediates estrogen-induced cell migration and invasion in estrogen receptor-positive breast cancer. *Sci Rep*. 2015;5:12484.

- [139] Cao XC, Zhang WR, Cao WF, Liu BW, Zhang F, Zhao HM, et al. Aquaporin3 is required for FGF-2-induced migration of human breast cancers. *PLoS One*. 2013;8(2):28.
- [140] Satooka H, Hara-Chikuma M. Aquaporin-3 controls breast cancer cell migration by regulating hydrogen peroxide transport and its downstream cell signaling. *Mol Cell Biol*. 2016;36(7):1206-1218.
- [141] Trigueros-Motos L, Perez-Torras S, Casado FJ, Molina-Arcas M, Pastor-Anglada M. Aquaporin 3 (AQP3) participates in the cytotoxic response to nucleoside-derived drugs. *BMC Cancer*. 2012;12(434):1471-2407.
- [142] Lee SJ, Chae YS, Kim JG, Kim WW, Jung JH, Park HY, et al. AQP5 expression predicts survival in patients with early breast cancer. *Ann Surg Oncol*. 2014;21(2):375-383.
- [143] Jung HJ, Park JY, Jeon HS, Kwon TH. Aquaporin-5: a marker protein for proliferation and migration of human breast cancer cells. *PLoS One*. 2011;6(12):1.
- [144] Woo J, Lee J, Chae YK, Kim MS, Baek JH, Park JC, et al. Overexpression of AQP5, a putative oncogene, promotes cell growth and transformation. *Cancer Lett*. 2008;264(1):54-62.
- [145] Sidhaye V, Hoffert JD, King LS. cAMP has distinct acute and chronic effects on aquaporin-5 in lung epithelial cells. *J Biol Chem*. 2005;280(5):3590-3596.
- [146] Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med*. 2005;202(4):473-477.
- [147] Mader S, Lutterotti A, Di Pauli F, Kuenz B, Schanda K, Aboul-Enein F, et al. Patterns of antibody binding to aquaporin-4 isoforms in neuromyelitis optica. *PLoS One*. 2010;5(5):0010455.
- [148] Ishikawa S. Urinary excretion of aquaporin-2 in pathological states of water metabolism. *Ann Med*. 2000;32(2):90-93.
- [149] Olsson M, Broberg A, Jernas M, Carlsson L, Rudemo M, Suurkula M, et al. Increased expression of aquaporin 3 in atopic eczema. *Allergy*. 2006;61(9):1132-1137.
- [150] Lee TS, Eid T, Mane S, Kim JH, Spencer DD, Ottersen OP, et al. Aquaporin-4 is increased in the sclerotic hippocampus in human temporal lobe epilepsy. *Acta Neuropathol*. 2004;108(6):493-502.
- [151] Zhang R, van Hoek AN, Biwersi J, Verkman AS. A point mutation at cysteine 189 blocks the water permeability of rat kidney water channel CHIP28k. *Biochemistry*. 1993;32(12):2938-2941.
- [152] Preston GM, Jung JS, Guggino WB, Agre P. The mercury-sensitive residue at cysteine 189 in the CHIP28 water channel. *J Biol Chem*. 1993;268(1):17-20.

- [153] Niemietz CM, Tyerman SD. New potent inhibitors of aquaporins: silver and gold compounds inhibit aquaporins of plant and human origin. *FEBS Lett.* 2002;531(3):443-447.
- [154] Martins AP, Ciancetta A, de Almeida A, Marrone A, Re N, Soveral G, et al. Aquaporin inhibition by gold(III) compounds: new insights. *ChemMedChem.* 2013;8(7):1086-1092.
- [155] Martins AP, Marrone A, Ciancetta A, Galan Cobo A, Echevarria M, Moura TF, et al. Targeting aquaporin function: potent inhibition of aquaglyceroporin-3 by a gold-based compound. *PLoS One.* 2012;7(5):18.
- [156] Brooks HL, Regan JW, Yool AJ. Inhibition of aquaporin-1 water permeability by tetraethylammonium: involvement of the loop E pore region. *Mol Pharmacol.* 2000;57(5):1021-1026.
- [157] Detmers FJ, de Groot BL, Muller EM, Hinton A, Konings IB, Sze M, et al. Quaternary ammonium compounds as water channel blockers. Specificity, potency, and site of action. *J Biol Chem.* 2006;281(20):14207-14214.
- [158] Ma B, Xiang Y, Mu SM, Li T, Yu HM, Li XJ. Effects of acetazolamide and anordiol on osmotic water permeability in AQP1-cRNA injected *Xenopus* oocyte. *Acta Pharmacol Sin.* 2004;25(1):90-97.
- [159] Gao J, Wang X, Chang Y, Zhang J, Song Q, Yu H, et al. Acetazolamide inhibits osmotic water permeability by interaction with aquaporin-1. *Anal Biochem.* 2006;350(2):165-170.
- [160] Huber VJ, Tsujita M, Yamazaki M, Sakimura K, Nakada T. Identification of arylsulfonamides as Aquaporin 4 inhibitors. *Bioorg Med Chem Lett.* 2007;17(5):1270-1273.
- [161] Huber VJ, Tsujita M, Kwee IL, Nakada T. Inhibition of aquaporin 4 by antiepileptic drugs. *Bioorg Med Chem.* 2009;17(1):418-424.
- [162] Ozu M, Dorr RA, Teresa Politi M, Parisi M, Toriano R. Water flux through human aquaporin 1: inhibition by intracellular furosemide and maximal response with high osmotic gradients. *Eur Biophys J.* 2011;40(6):737-746.
- [163] Baum BJ, Zheng C, Cotrim AP, Goldsmith CM, Atkinson JC, Brahim JS, et al. Transfer of the AQP1 cDNA for the correction of radiation-induced salivary hypofunction. *Biochim Biophys Acta.* 2006;8(7):5.
- [164] Gao R, Yan X, Zheng C, Goldsmith CM, Afione S, Hai B, et al. AAV2-mediated transfer of the human aquaporin-1 cDNA restores fluid secretion from irradiated miniature pig parotid glands. *Gene Ther.* 2011;18(1):38-42.
- [165] Baum BJ, Alevizos I, Zheng C, Cotrim AP, Liu S, McCullagh L, et al. Early responses to adenoviral-mediated transfer of the aquaporin-1 cDNA for radiation-induced salivary hypofunction. *Proc Natl Acad Sci U S A.* 2012;109(47):19403-19407.

- [166] Wang F, Feng XC, Li YM, Yang H, Ma TH. Aquaporins as potential drug targets. *Acta Pharmacol Sin.* 2006;27(4):395-401.
- [167] Endo M, Jain RK, Witwer B, Brown D. Water channel (aquaporin 1) expression and distribution in mammary carcinomas and glioblastomas. *Microvasc Res.* 1999;58(2):89-98.
- [168] Pedersen SF, Hoffmann EK, Mills JW. The cytoskeleton and cell volume regulation. *Comp Biochem Physiol A Mol Integr Physiol.* 2001;130(3):385-399.
- [169] Ma B, Xiang Y, Li T, Yu HM, Li XJ. Inhibitory effect of topiramate on Lewis lung carcinoma metastasis and its relation with AQP1 water channel. *Acta Pharmacol Sin.* 2004;25(1):54-60.
- [170] Yang J, Yan C, Zheng W, Chen X. Proliferation inhibition of cisplatin and aquaporin 5 expression in human ovarian cancer cell CAOV3. *Arch Gynecol Obstet.* 2012;285(1):239-245.
- [171] Khajah MA, Mathew PM, Alam-Eldin NS, Luqmani YA. Bleb formation is induced by alkaline but not acidic pH in estrogen receptor silenced breast cancer cells. *Int J Oncol.* 2015;46(4):1685-1698.

