REVIEW ARTICLE

Aquaporin-5 in breast cancer

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Bystrup M, Login FH, Edamana S, Borgquist S, Tramm T, Kwon T-H, Nejsum LN. Aquaporin-5 in breast cancer. APMIS. 2022; 130: 253–260.

The water channel aquaporin-5 (AQP5) is essential in transepithelial water transport in secretory glands. AQP5 is ectopically overexpressed in breast cancer, where expression is associated with lymph node metastasis and poor prognosis. Besides the role in water transport, AQP5 has been found to play a role in cancer metastasis, migration, and proliferation. AQP5 has also been shown to be involved in the dysregulation of epithelial cell–cell adhesion; frequently observed in cancers. Insight into the underlying molecular mechanisms of how AQP5 contributes to cancer development and progression is essential for potentially implementing AQP5 as a prognostic biomarker and to develop targeted intervention strategies for the treatment of breast cancer patients.

Key words: aquaporin-5; biomarker; breast cancer; carcinoma; migration; proliferation.

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AQUAPORINS

Aquaporins (AQPs) are water channel proteins facilitating passive transport of water across cellular membranes driven by osmotic gradients [1]. Mammalian AQPs are expressed in a multitude of organs including brain, heart, skin, and kidneys, and have pivotal functions in physiological processes such as urinary concentration, skin hydration, salivary secretion, and adipocyte metabolism. AQP dysregulation has been shown to be linked to a variety of different pathophysiological conditions, including nephrogenic diabetes insipidus (NDI) [2], brain edema [3], Sjögren's syndrome [4], obesity [5], insulin resistance [6], and cancer [7–12].

Thirteen different mammalian AQPs (AQP0-12) are expressed in mammals. The AQPs form homotetramers where each monomer contains a pore permeable to water. The monomers consist of six transmembrane domains, which are connected by five loops (loop A to E), with the N- and C-termini located in the cytoplasm. Two highly conserved Asn–Pro–Ala (NPA) motifs in the cytoplasmic loop B and in the extracellular loop E [13], bend into the lipid bilayer to form the water-permeable pore (For schematics of AQP5 structure and topology, see Fig. 1A-D). The molecular weights of the AQP monomers range from 28.12 kDa to 37.2 kDa.

AQPs are classified based on their permeability. AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8 are called orthodox AQPs and are selective for water. AQP3, AQP7, AQP9, and AQP10 are called aquaglyceroporins and are selective for water, glycerol, and urea. Some AQPs, (orthodox and aquaglyceroporins) can also transport small neutral solutes such as hydrogen peroxide or carbon dioxide. Several reports indicate that some AQPs can regulate signaling pathways, through yet largely unknown mechanisms. These different features of AQPs (pathway regulation, transport of
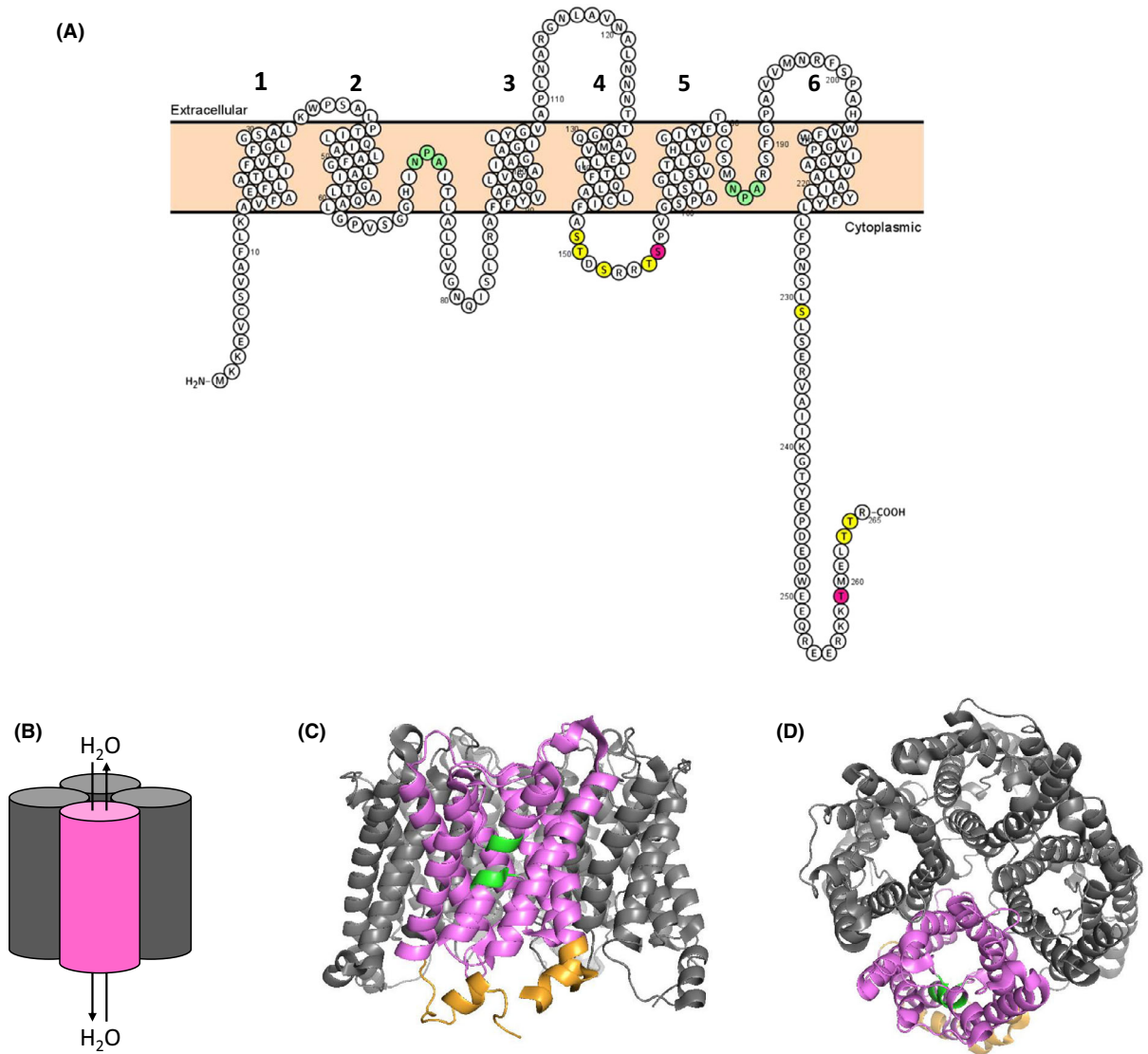
Fig. 1. Topology and aquaporin structure. (A) Membrane topology of a human AQP5 monomer (uniprot entry P55064). Each monomer contains six transmembrane domains (1 to 6) that are connected by Loops A to E with the N- and C-termini located in the cytoplasm. The two conserved asparagine–proline–alanine motifs (NPA motifs) are located in Loops B and D and are highlighted in green. Experimentally confirmed phosphorylation sites S156 and T259 are colored in red while other putative phosphorylation sites are shown in yellow. AQP5 topology schematic was created using Protter: interactive protein feature visualization and integration with experimental proteomic data [64]. (B) Schematic of an AQP5 homotetramer. One monomer is highlighted in pink. Each monomer contains a pore permeable to water. (C-D) AQP5 topology viewed from the side (C) and from the top (D). One monomer is highlighted in pink, NPA motifs in green, and N- and C-terminal domains in orange. C and D were generated using Pymol (PDB entry: 3D9S).

water, and relevant metabolic solutes) may affect AQP-dependent cancer phenotypes.

AQP5 EXPRESSION AND LOCALIZATION

AQP5 was first cloned from rat submandibular glands by Reina and colleagues in 1995 [14]. AQP5 is localized many tissues including in secretory glands like salivary [15, 16] and sweat glands [17, 18] and in the respiratory system [19] and the epithelial cells of male and female reproductive organs [20]. Studies with transgenic AQP5-null mice revealed decreased sweat secretion [17] and decreased saliva production [15]. Moreover, AQP5-null mice showed a tenfold decrease of alveolar

epithelial water permeability, but fluid clearance was unchanged [21].

AQP5 IN PATHOPHYSIOLOGICAL CONDITIONS

AQP5 was found to be dysregulated in several pathophysiological conditions including Sjögren's syndrome, which is a systemic autoimmune disease with reduced salivary secretion due to glandular dysfunction [22–24]. In a non-obese diabetic (NOD) mouse model for Sjögren's syndrome, AQP5 localized to both apical and basolateral plasma membranes of acinar cells compared with an exclusive apical localization observed in the control mice [25], implying a dysregulation of AQP5 subcellular localization in Sjögren's syndrome [25]. Moreover, *AQP5* polymorphisms were associated with the rate of decline in lung function in continuous smokers with chronic obstructive pulmonary disease [26] and in mice, AQP5 mRNA and protein levels were decreased after acute lung infection with adenovirus [27]. Also, AQP5 is upregulated in multiple cancers, including lung adenocarcinoma [28], cervical cancer [29], gastric cancer [30], pancreatic adenocarcinoma [8, 31], and breast ductal adenocarcinoma [32].

BREAST CANCER

According to estimates by the International Agency for Research on Cancer, female breast cancer is the leading cause of cancer incidents worldwide in 2020 with nearly 2.3 million incident cases representing 11.7% of all cancer cases and 1 in 4 cancer cases in women [33]. It is the fifth leading cause of cancer mortality worldwide with around 685,000 deaths in 2020 [33]. The survival rate has been steadily increasing over the last 50 years primarily due to development of systemic screening and earlier detection at lower stages as well as improved treatment. In Denmark, the overall survival rate has increased from 46% for early breast cancer patients treated in 1978-1987 compared to 72% for patients treated in 2008-2012 [34].

Early breast cancer is treated according to clinico-pathological parameters encompassing tumor size (T-stage), histological type and malignancy grade of the tumor, metastatic spread to lymph nodes (N-stage) and according to the two targetable receptors, the estrogen receptor (ER) and the human epidermal growth factor (HER2)-receptor. Age of the patient, menopausal status, and status of the resection margins are also important for treatment decisions.

Neoplastic lesions in the breast can be divided into invasive carcinoma or non-invasive carcinoma (*in situ*). *In situ* carcinomas are characterized by neoplastic cells localized to the epithelium of ducts and/or acini and surrounded by an intact basement membrane without invasion and hence without the capacity for metastatic spread. *In situ* carcinomas in the breast are separated into ductal carcinomas in situ (DCIS) and lobular carcinoma in situ (LCIS) on a morpho-molecular base combining the histo-morphological appearance and expression of adhesion molecules, for example, E-cadherin [35,6]. Invasive breast cancer occurs when the neoplastic cells obtain the ability to break through the basement membrane and invade the surrounding breast tissue. The invasive cancer cells may further have, or may obtain, metastatic potential and may spread either through the lymphatic system to regional lymph nodes or hematogenously to remote organs, for example, brain, bones, lungs, and liver [37]. The most frequent histological types of breast cancer are invasive ductal carcinomas (IDC) (75%) and invasive lobular carcinoma (ILC) (10%) [36]. Invasive lobular carcinomas are composed of dis-cohesive tumor cell populations infiltrating the surroundings as single tumor cells or tumor cells in single filing. The diffuse growth pattern is promoted by the loss of E-cadherin, which may be visualized by immunohistochemical staining [38]. Loss of E-cadherin expression may, however, also be seen in IDC [39].

AQP5 IN BREAST CANCER

Although the AQP5 transcript is minimally expressed in normal human breast tissue, AQP5 protein expression was observed in breast tissue from 10 cases of benign breast tumors (Fibrocystic change (n = 6), Fibroadenoma (n = 2), Intraductal papilloma (n = 1), and Gynecomastia (n = 1)), where AQP5 localized to the apical plasma membrane of the ductal luminal epithelial cells [32]. In contrast, AQP5 immunolabeling was not associated with acinar cells where the milk is produced [32]. In 20 cases of IDC AQP5 immunolabeling was strongly observed intracellularly in the invasive cancer cells and interestingly, AQP5 expression correlated with spread to lymph nodes and poor prognosis [32]. In another study of tissue samples from 78 breast cancer patients with IDC, infiltrating lobular carcinoma and mucinous adenocarcinoma [40], AQP5 staining intensity was significantly higher in 74 cases of IDC with metastasis to lymph nodes compared to cases without spread [40]. Moreover, the AQP5-positive staining of samples from

stage I and II tumors was significantly lower compared with the staining of stage III tumors [40]. Lee and colleagues also found a correlation between high levels of AQP5 and poor prognosis with lower survival compared to patients with low or no AQP5 expression in a total of 447 cases of early breast cancer patients [41]. Immunohistochemistry analysis of 96 samples from triple-negative breast cancer patients revealed that both AQP3 and AQP5 expression were increased, which correlated with reduced 5-year disease-free survival and overall survival [42]. These observations support that AQP5 expression in breast cancer correlates with spread to lymph nodes and poor prognosis. AQP5 expression in breast cancer is summarized in Fig. 2. It is important to notice that AQP1 and AQP3 are also expressed in healthy breast tissue and up-regulated in breast cancer tissue [40]. These AQPs can actually be co-expressed in the same tumor. For instance, in triple-negative breast cancer, increased expression levels were detected for both AQP3 and AQP5 [42]. High AQP1 expression was observed in basal-like carcinomas, which is a sub-class of triple-negative breast cancer [43]. Further investigation is needed to determine the interplay between AQPs in tumor biology and how this interplay is affected by tumor-specific molecular markers.

AQP5 in cancer cell proliferation

A main characteristic of cancer cells is autonomous increased proliferation, independently of normal growth regulation. In various cancer cell lines (e.g., lung, ovarian, and colorectal cells), AQP5 expression has been found to correlate with increased proliferation [30, 44–46,]. Jung and colleagues demonstrated that lentivirus-mediated shRNA AQP5-knockdown resulted in a significant reduction of proliferation of the human breast cancer cell line MCF7, and also found that hyperosmotic stress induced by sorbitol-containing medium decreased AQP5 expression and reduced the proliferation of MCF7 cells [32]. Moreover, AQP5 overexpression is correlated to higher expression of the proliferation marker Ki67 using Spearman's rank correlation coefficient method in triple-negative breast cancer patients [42].

AQP5 in cancer cell migration and invasion

Cancer cell migration is an essential part of cancer metastasis. AQPs have been suggested to facilitate cell migration by allowing water influx at the leading lamellipodium, resulting in a slight
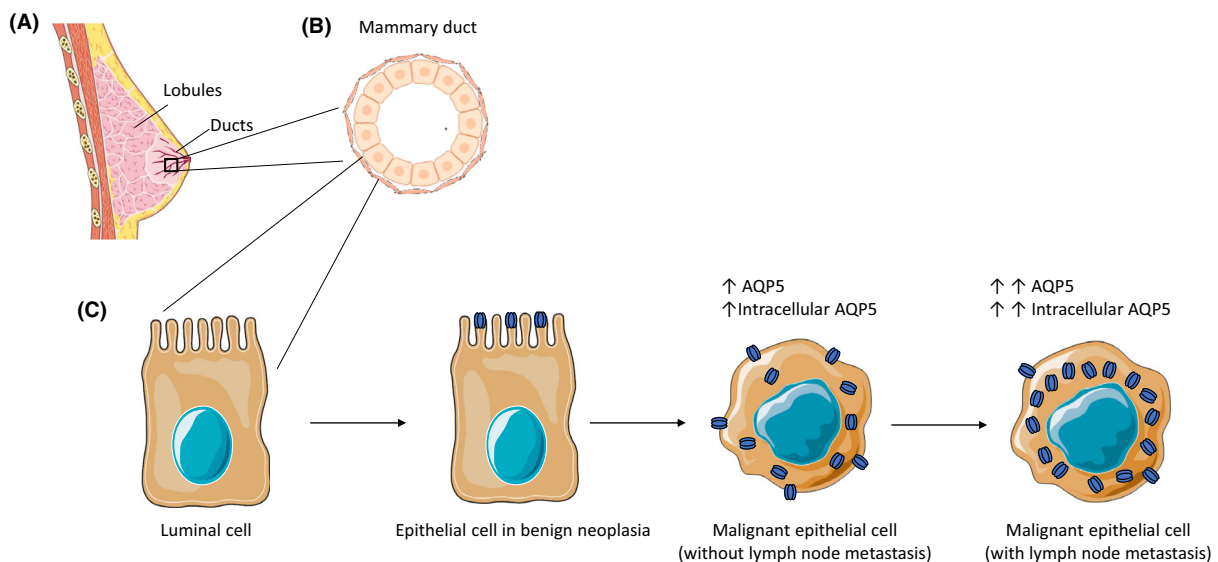

Fig. 2. Expression profile of AQP5 in the progression of breast cancer. (A) Schematic showing a female breast with lobules and milk ducts. (B) Schematic of a zoom of a mammary duct which is lined with a single luminal layer of secretory epithelial cells surrounded by a continuous layer of contractile myoepithelial cells. (C) Schematic illustrating the changes in AQP5 expression and subcellular localization in breast cancer development. AQP5 is not expressed in luminal cells but is overexpressed in epithelial cells in benign neoplasia. In malignant epithelial cells without lymph node metastasis, AQP5 is overexpressed and localize both in the plasma membrane and intracellularly. In malignant epithelial cells with lymph node metastasis, both plasma membrane and cytoplasmic overexpression of AQP5 are further increased. Figures are created using images from smart.servier.com.

swelling that allows increased actin network formation [47].

The role of AQP5 in cell migration has been investigated in both non-cancerous [48] and cancerous cell lines [32]. In MCF7 cells, AQP5-knockdown significantly decreased MCF7 cell migration compared with the control [32]. Moreover, AQP5 silencing in adriamycin-resistant MCF7 breast cancer cells resulted in reduced migration and invasion in addition to increasing chemosensitivity of the breast cancer cells [49]. AQP5 overexpression in the lung cancer cell lines SPC-A1 and PC-9 significantly increased cell migration and invasion compared with control cells [45]. AQP5-knockdown via siRNA in the LTEP-A2 lung cancer cell line resulted in decreased cell migration, invasion, and significantly decreased wound closure in an *in vitro* wound-healing assay compared with control [50]. In addition, exosome-mediated delivery of AQP5-targeting miRNAs decreased AQP5 protein expression and attenuated cell migration in the human breast cancer cell line MDA-MB-231 [51].

In contrast, in normal Madin–Darby canine kidney (MDCK) cells, AQP5 overexpression resulted in decreased collective migration with reduced cell–cell coordination [48]. Interestingly, AQP5 activation of the Ras signaling pathway induced detachment of cells located at the front of the migrating cell sheet [48]. Thus, AQP5 seems to differentially affect the migration of normal and cancer cells.

Compromised cell–cell adhesion in cancer is associated with increased cell migration, invasion, and metastasis. Several AQPs, including AQP5, have been shown to affect junctional proteins [7, 48, 52]. In sections from benign and invasive breast tumors, high AQP5 expression correlated with low γ-catenin levels, but no significant correlation between the levels of AQP5 and the tight junction protein ZO-1 was observed [48]. Correlation between AQP5 expression and levels of junctional proteins has also been observed in samples from pancreatic ducal adenocarcinoma where high AQP5 expression correlated with low E-cadherin levels and high vimentin levels [8, 31]. In salivary glands from AQP5-null mice, tight junction proteins occludin, claudin-3, and claudin-7 were downregulated in a gender-specific manner, with female AQP5 null mice having a higher decrease in expression compared with male AQP5 null mice [53]. Expression of other tight junction proteins, like ZO-1, was unaltered [53].

Overexpression of AQP5 in MDCK cells was shown to decrease the levels of several junctional proteins at cell–cell junctions; namely β-catenin, γ-catenin, ZO-1, and p120-catenin [48]. Moreover, a dispase-based dissociation assay revealed decreased strength of cell–cell adhesion [48]. The effect of AQP5 on cell–cell junction proteins could partially be recapitulated by the expression of the COOH-terminal tail of AQP5 targeted to the plasma membrane [52], indicating that the effect on junctional proteins is independent of AQP5 water transport capacity.

AQP5 and signaling in cancer

AQP5 is associated with several signaling pathways involved in cancer [54]. In cell cultures of mouse fibroblast NIH 3T3, AQP5 has been shown to activate the Ras pathway via serine 156 phosphorylation [55]. However, in breast cancer tissue from human patients with 10 cases of benign tumors and 20 cases of invasive breast cancer, high AQP5 expression did not correlate with activated Ras [56]. A positive tendency for activated Ras and activated Rac1, known for involvement in cell motility, was however found in grade 3 tumors [56]. In human colon adenocarcinoma HT-29 cells, siRNA-mediated silencing of AQP5 significantly decreased p38 mitogen-activated protein kinase (MAPK) signaling which coincided with lower expression of drug resistance genes and increased sensitivity to drugs [57]. Interestingly, p38 MAPK signaling was also implicated in breast cancer metastasis [58]. However, a link between AQP5 expression levels and p38 MAPK signaling in breast cancer has yet to be described.

In the human colorectal cancer cell lines SW480 and HCT-116, transforming growth factor β1 (TGF-β1) increased AQP5 protein and mRNA levels, and the upregulation of AQP5 led to an enhanced p-Smad2/3 expression. AQP5 silencing with AQP5 siRNA downregulated the p-Smad2/3 levels, suggesting that AQP5 may promote epithelial to mesenchymal transition (EMT) through activation of Smad2/3 [59]. The effect of altered AQP5 expression on the EMT process was further supported since AQP5-knockdown in colorectal cancer cells caused a significant decrease of EMT-related proteins via downregulation of the Wnt/β-catenin pathway [60]. Moreover, activation of the Wnt signaling pathway is correlated with AQP5 expression in gastric stem cells and cancer stem-like cells [54, 61]. In lung cancer cells, AQP5 expression levels were positively correlated with the proliferative ability and the expression levels of the proliferative marker proteins, where proliferating cell nuclear antigen (PCNA) and c-myc were increased [50]. Lung cancer cells overexpressing AQP5 showed a significant increase in activated epidermal growth factor receptor (EGFR) phosphorylation (p-EGFR) and extracellular signal-regulated kinase (ERK1/2) and p38 MAPK [50]. In contrast, AQP5 silencing

decreased the activity of the EGFR/ERK/p38-MAPK pathways in lung tissue [50]. Moreover, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and AQP5 expression correlated negatively in normal tissue, whereas in the ovarian cancer cell lines, SKOV3 and CAOV3, inhibition of NF-κB by pyrrolidine dithiocarbamate (PDTC) downregulated the expression of NF-κB, resulting in a decrease of mRNA and protein levels of AQP5 and tumor cell proliferation [62].

AQP5 Intervention

A recent study identified novel AQP5-targeting microRNAs (miR-1226-3p, miR-19a-3p, and miR-19b-3p) and demonstrated their role in the regulation of AQP5 expression and migration of MDA-MB-231 breast cancer cells [51]. The miRNA-mediated downregulation of AQP5 in breast cancer cells led to significantly reduced cell migration and proliferation through impeded gap junction pathways [51]. Also, a compound isolated from insect pathogenic fungi, Fumosorinone (FU), was found to decrease AQP5 expression and inhibit proliferation, migration, and invasion of human breast cancer MDA-MB-231 cells [63].

PERSPECTIVES

AQP5 is overexpressed in breast cancer where expression correlates with spread to lymph nodes and poor prognosis. Thus, AQP5 could be a potential diagnostic and prognostic biomarker and a potential intervention target to inhibit breast cancer progression and spread. Thus, a detailed understanding of the molecular and cellular effects of AQP5 overexpression in breast cancer is critical.

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