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Journal of Cancer Biology & Research

Review Article

Aquaporins in Colorectal Cancer: Exploring Their Role in Tumorigenesis, Metastasis, and Drug Response

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Submitted: 15 September 2023

Accepted: 31 October 2023

Published: 31 October 2023

ISSN: 2373-9436

OPEN ACCESS

Keywords

- Aquaporin
- Colorectal cancer
- Biomarkers
- Angiogenesis
- Metastasis
- Chemotherapy

Abstract

Aquaporins (AQPs) are small, integral proteins facilitating water transport across plasma cell membranes in reaction to osmotic gradients. This family has thirteen unique members (AQP0-12), which can also transport glycerol, urea, gases, and other salute small molecules. AQPs play a crucial role in the regulation of different cellular processes, including metabolism, migration, immunity, barrier function and angiogenesis. These proteins are found to aberrantly overexpress in various cancers, including colorectal cancer (CRC). Increasing evidence has been explored AQPs as a potential diagnostic biomarkers and therapeutic targets in different cancers. However, there is no comprehensive review compiling the available information on the crucial role of AQPs in the context of colorectal cancer. This review highlights the significance of AQPs as the biomarker and regulator of tumor cells metabolism. In addition, the proliferation, angiogenesis, and metastasis of tumor cells related to AQPs expression and function are discussed. Furthermore, understanding the AQPs prominent role in chemotherapy resistance is of great importance as a clinical point of view.

INTRODUCTION

Colorectal cancer (CRC) is known as the third most common cancer worldwide, and the second cause of death among cancer patients. In 2020, 10% of all cancer cases and 9.4% of cancer-related deaths were due to colorectal cancer, meaning about 1.9 million new cases and 0.9 million human deaths [1-3]. In recent years, regarding the high prevalence and mortality rate of patients with colorectal cancer, the world has faced challenges of serious need for medical services. The prevalence of colorectal cancer has increased, especially in people less than 50 years of age, and about 2% of patients with colorectal cancer have metastases at the time of diagnosis, and more than 50% of patients with primary tumors will develop metastases in the future. Although the prognosis of patients has significantly improved in the last twenty years, however, metastatic colorectal cancer is still an incurable disease in most cases [4-6].

Currently, the treatment methods are complicated and associated with serious challenges; that even may lead to death.

In metastatic types, the treatments are usually ineffective and are associated with the mortality and morbidity of the patients. Taken together, developing a new effective treatment for tumor cell growth and proliferation, invasion, and metastasis is of great importance [7].

Cell metabolism is dependent on different groups of molecules located in the cell membranes as transporters [8,9].

One group of molecules which are of special interest, are aquaporins (AQPs) [7]. Aquaporins are protein receptors, primarily known as water channels, with the ability to regulate the transport of water, glycerol, urea, and other small molecules. AQPs have been targeted in the treatment of different diseases such as diabetes insipidus and ADH syndrome. Recently, the role of these molecules in a much wider range of diseases has been identified, including cancer, heart failure, Sjögren's disease, etc [10]. The structure and function, role in water physiology and, solutes movement make AQPs as suitable drug targets [8-11]. Besides maintaining the water balance, AQPs have special roles in cancer, including cell migration, proliferation, and adhesion and they are overexpressed on tumor cells and there is often a significant association between the expression of AQPs and tumor grade [9-12,13]. Consequently, AQPs can be targeted in cancer therapy by means of regulators.

Aquaporins structure

AQPs are small water-channel trans-membrane proteins that assist in the bi-directional transport of the water through the cell membrane. In addition, AQP can act as the transporter of other small neutral molecules such as glycerol and urea, or certain gases like carbon dioxide and ammonia as osmotic gradients in all cells [14].

The human AQPs consist of 13 mammalian proteins (AQ0-AQ12), which have unique cellular distribution. Interestingly, six of the thirteen AQP isoforms (AQP1, AQP2, AQP4, AQP5, AQP7 and, AQP10) have high-resolution structures [15-21].

The structure of AQP is consisting of six alpha helices domains which are stretch through the membrane with both the amino acid and carboxylic terminals on the cytoplasmic side. This structure is highly conserved in all living organisms [22]. The alpha helixes are connected by five loops from A to E. Loops B (Cytosolic) and E (Non-cytosolic) contain a highly conserved Asparagine-Proline-Alanin (NPA) motif, which forms a barrel surrounding in the central pore-like region that contains additional protein density [14]. These motifs form a hourglass structure, making the water channel narrow in the middle (2.8 Å) and wider at each end.

The second section in non-cytosolic end of the pore is the "aromatic/Arginine (ar/R)" selectively filters. This filter consists of a cluster of amino acids, which enable the AQPs to selectively lead or block the transport of different molecules [22,23].Through the amino acid residues of the NPA and ar/R filters, alongside the other parts of the channel, AQP controls the substrate selection [14]. AQPs form a homo-tetramer structure in the cell membrane, in which all four monomers act as a water channel alone. The helices of each AQP monomer which are located on the outer part of the tetramer, are hydrophobic, while the helices that are near the center of the tetramer are hydrophilic [24]. Functionally, based on permeability characteristics, AQPs members are divided into two groups [25]. 1: water selective or classic AQPs (AQP 1, 2, 4, 5, and 8) which facilitate water transport exclusively. 2: Aquaglyceroporins, which have the additional ability to direct small carbohydrates, especially glycerol (AQP 3, 7, 9, and 10). The two subgroups can be distinguished based on amino acid sequences. The aquaglyceroporins group includes glycerol facilitators (GlpFs), from glycerol permease facilitators. The "signature" sequence for aquaglyceroporins is the aspartic acid residue (D) in the NPA motif, which enlarges the pore to accept larger molecules [26]. The schematic illustration of AQP is depicted in Figure 1.

Aquaporins expression in normal and tumor tissues

AQP-0 is known as the major intrinsic protein (MIP) of the lens cells [27]. The mutations in MIP are predicted to disturb water flux across the lens cell membrane and causes cataracts [28]. Water transportation by AQP0 is at a slow rate; however, it has been demonstrated to play an important role in the cell-to-cell adhesion of the lens fiber. AQP1 as the most studied aquaporin is the first with a high-resolution structure. AQP1 is expressed in various tissues e.g., lungs, kidneys and, red blood cells. Mutations in AQP1 are shown to be related to urinary concentration deficiency or failure in peritoneal dialysis [29-30]. AQP1 is also up-regulated in different cancers, including multiple myeloma, lung, and colon and brain tumors, mammary carcinoma, choroid plexus tumors and hemangioblastoma [31-39].

AQP2 was found in renal collecting duct, and called as the water channel of the collecting duct [14]. AQP2 is shown as a diagnostic marker for pheochromocytoma and/or paraganglioma and is expressed in glioma cell lines [40-41].

AQP3 is expressed in the skin and provides cells glycerol. It is shown that the deletion of AQP has resulted in impairs of elasticity, skin hydration, barrier recovery and wound healing in which the administration of glycerol has reversed it [42]. It is found to abnormally express in various cancers including breast cancer, gastric cancer, colorectal cancer, and melanoma and plays a pivotal role in cancer metastasis [43]. AQP4 is the main water channel which express in the central nervous system [44]. In edematous astrocytomas and metastatic tumors, AQP4 is demonstrated to be up-regulated [45]. AQP5 controls the water flux throughout several body systems including digestive, respiratory, renal, integumentary, and reproductive systems and also in sense organs [13]. The AQP5 expression is associated with different cancers including lung, breast, colorectal and ovarian cancers [46-49]. AQP6 is found as anion permeation with marked specificity for nitrate which is shown to express in epithelial ovarian tumors [50-51]. AQP7 is localized to a wide range of tissues [52] and is shown to regulate the response to cellular stress in breast cancer [53]. AQP8 expression in various cancers is

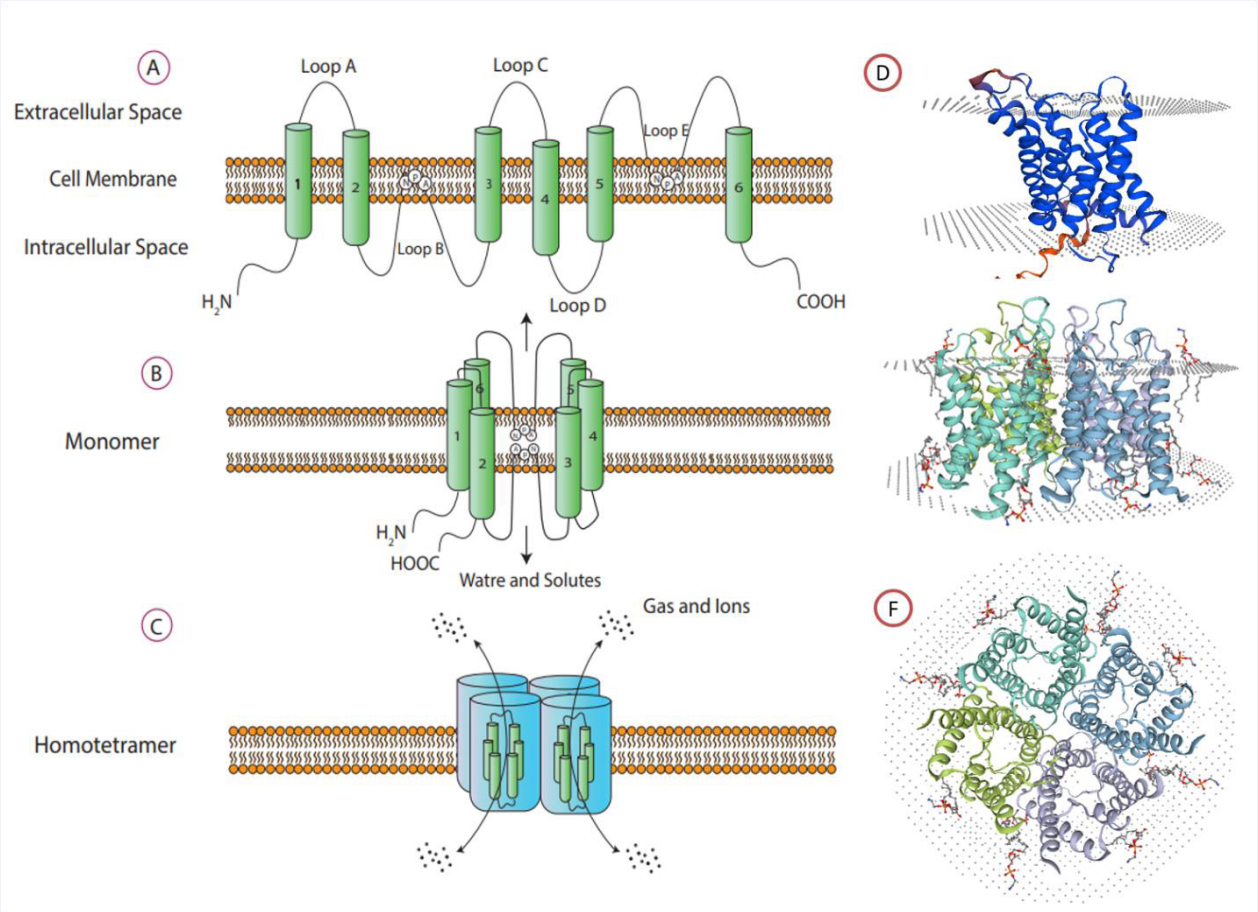

Figure 1 Schematic illustration of Aquaporin. A. Monomer topology of AQPs comprises of six tetramer helics 1-6, connected with loops A-E. Loops A, E and C are extracellular, loops B and E are fold back to the cell membrane (with N-P-A codes that shows the NPA motifs and ar/R) and the intracellular N and C terminals B. Monomer water channel formation by organization of helices and NPA motifs. C. Establishment of homotetramer with central pore, assembled by AQP monomers. D. Crystal structure of the monomer and E. Homotetramer of AQPs viewed from the side of membrane. F. Homotetramer crystal structure from top view revealing four water channel formed by each monomer and a central pore resulted of the homotetramer formation marked as X. Aquaporins: AQPs. Highl.y conserved Asparagine-Proline-Alanin: NPA.

associated with clinical significance [54-56]. AQP9 as the glycerol transporter is strongly expressed in human astrocytomas [12]. and its expression is correlated with tumor grade in epithelial ovarian cancer (57). AQP10-12 are shown to express in both the normal and tumor tissues, however, much more attention needs to clarify their role in the context of different diseases [58,59].

Role of aquaporins in cancer proliferation, angiogenesis, and metastasis of CRC

Currently, it has been revealed that the expression of AQPs is increased in cancerous cells of different origins, particularly in invasive malignancies, such as CRC [60]. In addition, it has been shown that AQPs play an important role in tumor cell proliferation, angiogenesis, and migration [39-60,61]. AQP1 is demonstrated to express in human HT20 colon cancer cells and play role in tumor cell migration. The elevated expression of this AQP in HT20 cells leads to increase plasma membrane water permeability and cell migration in both invasive trans-well and wound healing experiments, while, low expression level has reduced the plasma membrane water permeability and cell migration potency. Actin protein re-localization and RhoA and Rac activation were induced by AQP1 expression. Furthermore, following tail vein injection, AQP1 is shown to enhance the extravasation of HT20 cells in the lung of nude mice. In conclusion, the plasma membrane water permeability mediated by AQP, play crucial role in CRC metastasis and invasion [62].

In HT29 colon cancer cells with increased expression of AQP1, cell migration is significantly inhibited, while no effect was detected on the migration rate of HCT-116 cells with low expression of AQP1. In addition, HT29 cells treated with AQP1 inhibitor showed a significantly reduced rate of invasion. Furthermore, in angiogenesis assay, this treatment resulted in complete inhibition of endothelial tube formation [63].

Tissue microarray (TMA) analysis is used to evaluate the association of clinic-pathological findings with AQP1 expression. Analysis of 120 stage II and III colon cancer patients showed 35.8% AQP1-positive rate. There was an association between the expression of AQP1 and lymph node metastasis and lymphovascular and vascular invasion [64]. Moon et al., has

examined the expression of AQPs 1, 3, and 5 in seven colon and colorectal cancer cell lines using transcriptase–polymerase chain reaction analysis and in situ hybridization experiments. They found that in colorectal carcinogenesis, induction of AQPs 1 and 5 expressions was initiated in the early stage of the disease and maintained during the late stages of its development. Expression of these two AQPs was also maintained in metastasis to the liver. These results showed that AQPs 1 and 5 expressions are involved in the early stage of colorectal cancer development [65]. The Cancer Genome Atlas analysis has revealed that AQP1 expression was significantly decreased in CRC. However, following disease progression, the expression increased and was significantly elevated in stage IV compared to stages I and II, and also in patients with lymph node metastasis compared to those without [66]. The clinical significance of AQPs 1, 3, and 5 expressions in patients with CRC showed a significant correlation between these AQPs and lymph node metastasis in surgically resected CRC patients [49]. Li et al., examined whether AQP3 could increase the migration of human colorectal carcinoma cells or not. Additionally, its role in the prognosis of the disease was evaluated. Results revealed that AQP3 expression is up-regulated in CRC compared to the normal ones. In addition, there was a correlation between the levels of AQP3 expression and differentiation, lymph node, and distant metastasis of CRC. Human epidermal growth factor (hEGF), enhanced the expression of AQP3, followed by an increased migration rate of human colorectal cancer cells. Moreover, blocking of AQP3 and EGFR pathways inhibited the migration of CRC cells [67].

Different studies have shown that AQP5 has an important role in the proliferation and metastasis of CRC [65-68,69]. Clinical results indicate that enhanced expression of AQP5 in CRC is a predictor of tumor metastasis and is associated with poor prognosis [49-56,70,71]. CRC, the AQP5 overexpression is shown to be associated with increased circulating tumor cells and metastasis rate enhancement [71]. Moreover, it is reported that there is a close correlation between AQP5 expression and colorectal cancer cell differentiation, TNM stage, and metastasis. Over-expression of this AQP is associated with advanced TNM stage, lymph node metastasis, and poor prognosis [71].

The expression of AQP5 in 62.8% (59/94) of resected CRC tissue specimens and its relationship with liver metastasis is reported [69]. It is revealed that the increased expression of this AQP promotes the proliferation of CRC cells by induction of the Ras-MAPK pathway, whereas deletion of AQP5 leads to inhibition of cancer cell growth [69]. Deletion of AQP5 results in the inhibition of migration and invasion of CRC cells and modulates epithelial–mesenchymal transition (EMT) of CRC by suppressing the Wnt/b-catenin pathway [72]. Furthermore, the effects of AQP5 expression silencing in CRC cell lines resulted in decreased expression and secretion of vascular endothelial growth factor (VEGF)-A in these cells. Also, proliferation and angiogenesis of xenograft tumors were suppressed by inhibition of endogenous AQP5 expression in HT29 cells [73]. Examining the expressions of AQP5 mRNA and protein in CRC tissue and the effects of silencing protein expression of this AQP in the HT29 cell line showed that suppression of AQP5 can lead to inhibition of HT29 cell growth [74]. In another study, the knockdown of AQP5 decreased the proliferation of CRC [68]. These findings suggest that AQP5 may be involved in the detachment of tumor cells from the primary tumor to metastasize [75].

Investigating AQP8 expression in CRC revealed that the increased expression of this AQP significantly reduced the proliferation, aggressiveness, and colony formation of the SW480 and HT-29 cells following inactivation of PI3K/AKT signaling and inhibition of PCDH7 expression. In addition, studies on nude mice xenograft and metastasis models showed an important role for AQP8 in CRC cell proliferation and metastasis [76]. AQP8 is significantly expressed by para-neoplastic normal tissues and rarely by colorectal carcinoma cells; demonstrating downregulation of AQP8 in CRC tissues [56]. Recently, AQP9 is shown to highly express in metastatic CRC and causes Wnt/β-catenin pathway activation through Dishevelled 2 (DVL2). This pathway eventually leads to EMT activation and invasion and migration of CRC cells [77]. Figure 2 is representative of AQPs role in tumor progression.

Aquaporins in cellular metabolism

It is well-accepted that the growth, development, invasion, and metastasis of cancerous cells are closely related to the tumor microenvironment and cell metabolism. In addition, it has been shown that water molecules play crucial role in development of tumor cells and open insights into new strategies for cancer treatment. Increased water transport is required to meet the high metabolic demands of rapidly proliferating tumor cells. AQPs are involved in several physiological and metabolic mechanisms related to water and glycerol transport, and lipid metabolism. Additionally, as mentioned before, in tumors, the level of AQPs expression is high and abnormal. Therefore, AQPs via transfer of water and other molecules or small solutes, have a crucial role in different cellular functions such as regulation of the size and shape, energy metabolism, migration, adhesion, proliferation, and differentiation. Any disorders lead to edema, proliferation and migration of tumor cells as well as tumor angiogenesis and metastasis. Therefore, identifying the role of AQP as a transporter in homeostasis and cellular environments of tumors seems to be necessary, especially as a clinical point of view [78-81].

The pathological conditions of many diseases are associated with altered aquaporin function and abnormal AQP expression [82]. Aquaglyceroporins are involved in metabolic disorders such as obesity, diabetes and, etc. Disturbance in the ability of aquaglyceroporins for glycerol penetration, as well as their abnormal expression in certain tissues, is demonstrated to result in fat metabolism, cell proliferation, and epidermal water retention [83-84]. Orthodox aquaporins and aquaglyceroporin isoforms are associated with various cancers, often revealing a strong correlation between these AQPs expression levels and tumor grade [12-85]. In addition, the role of aquaglycerolporins in glycerol transport and its importance in cell proliferation and

fat metabolism, and cancer progression make aquaglycerolporins a potential therapeutic target [86]. Aquaglycerolipins (AQPs 3, 7, 9, 10, and 11) affect the metabolic pathways and are highly expressed in tissues involved in glycerol metabolism, such as fat cells and the liver [87].

Collectively, increased aquaglyceroporin expression in tumors suggests that glycerol contributes to tumor growth and proliferation in two ways: first as a primary material in the synthesis of the phospholipids, and/or second as a mediator or regulator of ATP production [11]. For rapid cell proliferation in cancer, both pathways are necessary [86]. AQP3 is the most studied one among the four human aquaglyceroporin isoforms in cancers, which is expressed in different epithelial cells [88-89]. Inhibiting the AQP3 expression leads to a decrease in cell proliferation of different cell lines and silencing the AQP3 reduces some lipid synthases in gastric cancer cells. Therefore, the disturbance in lipid synthesis due to the reduction of AQP3 is not only due to the reduction of glycerol absorption but also related to the inhibition of the lipid synthesis system. The PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase)/Akt (protein kinase B) signaling pathway was also inhibited following destruction of AQP3. This pathway is involved in impaired lipid and ATP production [90].

Since tumor cells divide more rapidly, they need more lipids for membrane synthesis and more energy to continue their aggressive proliferation and malignancy (91, 92). Phospholipids are necessary for plasma membrane formation, but they can also be catabolized by β-oxidation to produce ATP. Triglycerides (TAG) produced by glycerol metabolization are vital for cell survival and proliferation. By lipolytic processes, TAG can convert into free fatty acid (FFA) in tumor cells. Fatty acid oxidation (FAO) for ATP production, promotes cancer development. Therefore, inhibiting or down-regulating aquaglyceroporins may lead to defects in supplying materials and energy for cancer cell growth following the inhibition of lipid synthesis [86-93]. Another hypothesis for the function of AQP3 in tumor cell development is overexpression of AQP3 in which causes greater permeability to glycerol and more ATP [94]. which is necessary for biosynthesis and tumor genesis. The positive correlation of glycerol level with the amount of ATP is evidence of this hypothesis [95]. Glycerol could be considered as an important regulator of ATP in cells which give the reason for the AQP3 overexpression in several tumors [86].Tumors use an un-regulated metabolism for cell proliferation and survival due to high nutrient requirements as sustaining a balanced redox state. Glucose and glutamine are usually important sources of energy in cancer. Warburg, in the 20s, proposed the theory that disruption of mitochondrial respiration causes tumor formation and increased glucose consumption, which resulted in high production of lactate, i.e., aerobic fermentation known as the Warburg effect [96]. The primary material for tumor cell proliferation can be prepared following the catabolism of

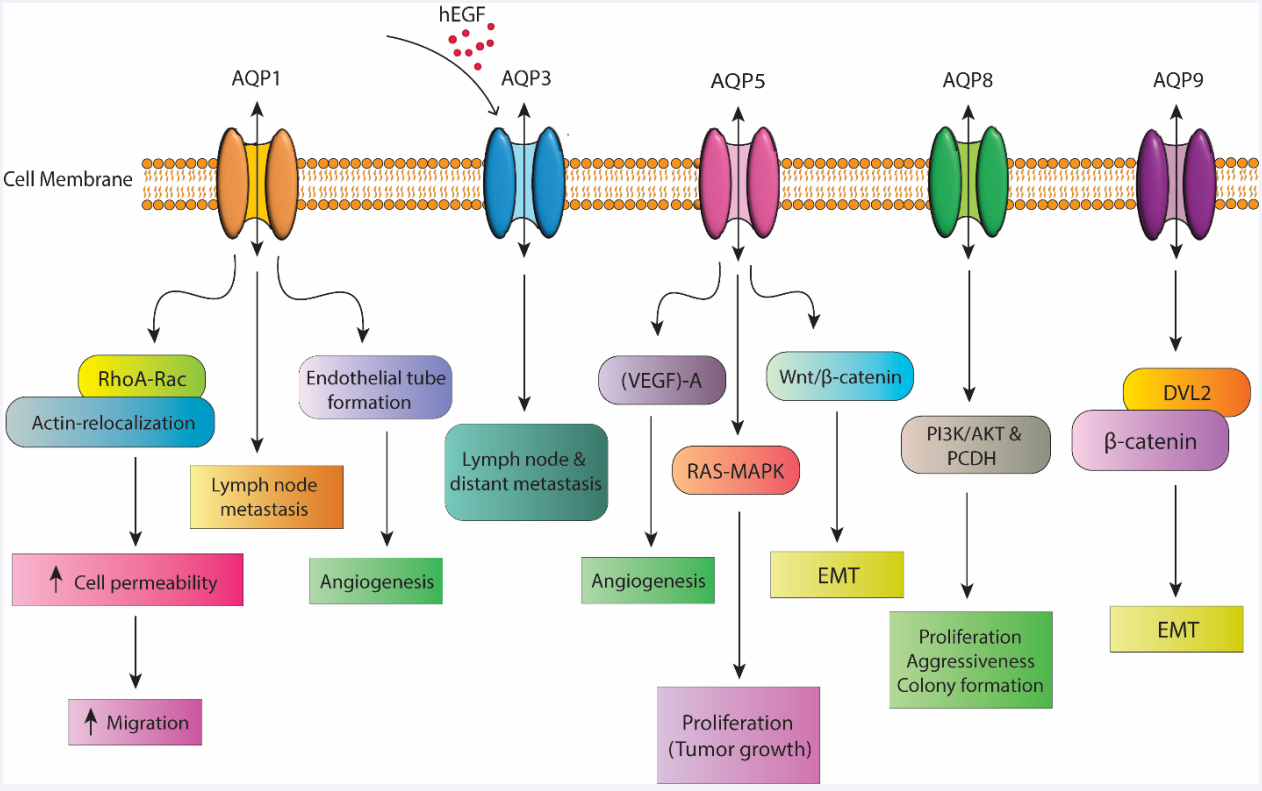

Figure 2 Aquaporins role in proliferation, angiogenesis, and metastasis of colorectal cancer.
Aquaporins: AQP. human Epidermal Growth Factor: hEGF. Vascular Endothelial Growth Factor: VEGF.Ras/Mitogen Activated Protein Kinase:Ras/MAPK. Epithelial–Mmesenchymal Transition: EMT. Dishevelled 2: DVL2.

glucose and glutamine [97]. Glucose induces carbon skeletons oxidation which causes electrons absorption by cells in the form of reduced nicotinamide. The malate-aspartate shuttle helps the adenine dinucleotide (NADH) to enter the mitochondrial electron transport chain. This process helps in cellular respiration and ATP production. Proliferating tumor cells promote glucose metabolism and convert excess pyruvate to lactate. This process, leads to maintaining the cytoplasmic level of the NAD+/NADH ratio [97-98]. For ATP production, glycerol can be used as an intermediate for pyruvate via the glycerol 3-phosphate shuttle (G-3-P shuttle) which results in tumor cell aquaglyceroporins overexpression. It should be noted that the cancer metabolism is constantly reprogrammed and optimized for available nutrient usage. Generally, glycerol as an intermediate of phospholipids can contribute to tumor cell proliferation, and its mutual effect on ATP production should be considered [86]. AQP9 is highly expressed in human glioblastoma, which reveals its role in the excretion of glycerol and lactate, indicating the involvement of lactic acidosis in glioma metabolism of energy. This confirms the important function of AQPs in glycerol absorption, as well as the regulation of its efflux [99]. It is shown that AQP9 is expressed in the basolateral membrane of benign and borderline tumor cells in ovarian cancer, whereas it is expressed widely in the membrane of malignant cells [57]. Additionally, it is demonstrated that a malignant ovary has more expression than a borderline which is more than benign/normal tissue. Also, AQP9 was overexpressed in mucinous tumors more than in serous ovarian tumors, and high-grade tumors revealed more expression than low grades [57-100], indicating that high expression of AQP9 could be correlated with poor prognosis. Consequently, this evidence reveals the importance of aquaglyceroporins and glycerol function in cancer development.

Although, AQP9 has lower expression in hepatocellular carcinoma [101], but its high expression can inhibit cell invasion and cancer development. Therefore, different roles and function of aquaglyceroporins may be seen in various cancers [86]. The tumor requirement of glycerol is associated to the type and stage of the cancer. In melanoma, the malignant phenotype is correlated with low AQP3 expression compared to normal skin and benign nevi. This shows that the transition from tumor proliferation to migration reduces the glycerol requirement [86]. Glycerol consumption increases in several non-metastatic cancer cell lines in contrast to metastatic ones [86]. AQP-5 can change the microenvironment and metabolism of tumor cells to increase the volume of liquid secretion from different tissues and result in the decrease in protein and salt concentration [84], and the proliferation of tumor cells. Collectively, it should be mentioned that glycerol requirement is different and depend on various tissue origins, malignancies, grades, and metastatic characteristics, which challenge aquaporin's function and role. Additionally, due to the various role of AQPs, cancer requirement to control glycerol amount may lead to low expression of special isoforms [86].

Aquaporin role in CRC chemotherapy resistance

One main approach in CRC therapy is chemotherapy, especially for invasive types; however, the efficacy is affected by several variables. Cancer cells resistance to chemotherapy is one of the major reasons for its ineffectiveness. Chemotherapy resistance can be induced by several cellular factors and complex pathways [102-104]. Therefore, identifying the genes and mechanisms related to the development of multidrug resistance (MDR) is very important and can contribute to the treatment of CRC. Multidrug resistance can be classified into two groups. First, the MDR mechanism which consists of energy-dependent drug pumps. This MDR mechanism is mediated by special membrane glycoproteins, including P-glycoprotein 1 (P-gp), multidrug resistance-associated protein (MRP), and lung resistance-related protein (LRP). The second MDR mechanism consist of enzymes, such as DNA topoisomerase II (TOPO II), thymidylate synthase (TS), and protein kinase C [105-106]. AQPs are suggested to play a crucial role in MDR. Following assessment of the AQP5 expression levels and multidrug resistance genes, such as P-glycoprotein (P-gp), topoisomerase (TOPO), glutathione S-transferase-π (GST-π) and thymidylate synthase, it has been revealed that, the AQP5 can acts as a crucial regulator in multidrug resistance development in CRC. In addition, RNA interference technology is used to silence the AQP5 expression in differentiated human colon adenocarcinoma cell lines. Results revealed the crucial role of AQP5 in MDR development. These findings can be the basis for a potential effect of pharmacological AQP5 inactivation on chemotherapy sensitivity for colon cancer treatment [74].The sensitivity of HT-29 cells to the chemotherapeutic drugs including, cisplatin (DDP) and 5-fluorouracil (5-FU) is demonstrated to be increased following AQP5 silencing using siRNA. In addition, the higher levels of AQP5 expression are demonstrated to be associated with resistance to the imatinib mesylate, as a tyrosine kinase inhibitor, which is used in treatment of Chronic Myeloid Leukemia (CML) in the chronic phase. Blocking of AQP5, also reduced the cell proliferation rate in CML cells [107].MAPK [108-111], and PI3K/Akt [112]. signaling pathways are involved in MDR. MAPK, serine/threonine protein kinase in eukaryotic cells, sends stimulating signals to the cells and induces reactions after activation. MAPK signaling pathways are composed of ERK, JNK/stress-activated protein kinase (SPAK), and p38 MAPK [113]. PI3K/Akt is a crucial pathway for cell growth regulation [113]. AQP5 functions in the CRC MDR via controlling the resistance proteins. To determine the special signaling pathways that cause MDR by AQP5 in CRC, MAPK phosphorylation levels were measured in transfected HT-29 cells. It was shown that silencing of AQP5 expression reduces p38 MAPK phosphorylation and activation, indicating that, MAPK phosphorylation serves as a signaling pathway leading to MDR by AQP5 in CRC. Therefore, AQP5 inactivation may be suitable for drug-resistance treatment in CRC [74]. Recently, it has been shown that several AQPs induce diffusion of H2O2 through lipid cell membranes; including peroxyporins (AQP1, AQP3, AQP5, AQP8, AQP9 and, AQP11) [114-116]. The correlation between peroxyporins and cancer development has been shown in various studies [117]. Oxidative stress can cause genetic instability, and by changing cellular processes, CRC will eventually occur. On the other hand, adaptive

oxidative defense induces therapeutic resistance, which causes failure in cancer treatment. Peroxyporins are known as aquaporin membrane channels that facilitate membrane permeation of H2O2 and are crucial for regulating cell proliferation and antioxidant defense. In a study, several colon cancer cell lines were treated with H2O2 and the sensitivity of cells to H2O2, cellular antioxidant status, intracellular ROS accumulation and the expression of AQP1, AQP3, and AQP5 peroxyporins were investigated. Results showed that HT-29 cells were the most resistant cell line and showed highly expression of peroxyporins and low levels of intracellular ROS, but in GSH levels, catalase activity, NF2, and PPARγ levels did not show any differences. This study shows that resistance to oxidative stress can be induced by different strategies except the antioxidant defense system. Peroxyporin can change the cellular antioxidant defense system and causes resistance to oxidative stress. Since regulation of H2O2 permeation can contribute to the tumor-resistant phenotype, controlling the intracellular ROS via regulating peroxyporin expression may be an important approach against MDR in cancer [118]. Little is known about the mechanism of AQPs as regulators of apoptotic genes. In addition, it is ambiguous whether the AQPs is located in the mitochondrial membrane and is involved in the apoptosis process by modulating water movement [119-120]. Several members of the AQP family are shown to aberrantly express in melanoma. AQP3 and AQP9 prevent the therapeutic effect of arsenite in melanoma by increasing the expression of anti-apoptotic genes including, Bcl-2 and XIAP while decreasing the expression of pro-apoptotic genes including, P53 and Bax; implying their anti-apoptotic role [106]. The presence of AQP3 in the nucleus confirms its association with apoptotic genes, which is probably different from their basic role as water transporters [106]. Although Cisplatin (cDDP) is in first-line of chemotherapy drug for the treatment of gastric cancer (GC), but it faces different challenges of resistance. There are different and complex mechanisms for cDDP resistance. AQP3 is overexpressed in various cancers such as GC and is thought to have a function in GC development and progression. It is shown that AQ3 mediates cisplatin resistance in gastric cancer via autophagy. Therefore, AQP3-targeted therapies can be a suitable approach for GC treatment [121]. Autophagy is a special cell strategy with catabolic functions. This highly regulated mechanism induces the degradation of different intracellular components such as toxic aggregates, misfolded proteins, and damaged organelles, resulting in oxidative stress decrease and cell damage prevention [122]. Moreover, autophagy is induced in various situations, such as hypoxia, metabolic stress, nutrient deprivation, cancer treatments, and radiation therapy, and also for cellular adaptation and growth [123]. Autophagy may have a tumor suppressor role in normal cells, and dysregulation of this process may induce tumor development. In cancers, autophagy may lead to tumor progression due to its role in cellular adaption and survival. Autophagy is shown to induce chemoresistance in different cancers. In GC, autophagy which induces via AQP3 can lead to cDDP resistance [121-124]. Approximately, half of the breast cancer patients do not respond to initial chemotherapy treatments, which could be due to drug resistance. The effects of AQP-5 on drug resistance in breast cancer cells revealed enhanced drug sensitivity [125]. Additionally, more studies have confirmed the association of AQPs with sensitivity to chemotherapy drugs and better prognosis. The expression of AQP1 can be a predictive biomarker of response to chemotherapy in patients with CRC, and chemotherapy is ineffective in CRC patients without AQP1 expression. AQP1 has also been shown to be involved in the development, progression, and metastasis of various types of cancers, including CRC, which is associated with invasion and metastasis. However, its expression may be related to sensitivity to chemotherapy [126]. The role of AQP1 as a predictor of response to chemotherapy in breast cancer treatment is assessed. Results revealed that breast cancer patients, who gave anthracycline treatment in accordance with high expression of AQP1, had better responses in comparison to those with low expression of AQP1. Moreover, miR-320a-3p is shown to inhibit the expression of AQP1 and reduce the sensitivity to anthracycline drugs [127].

Aquaporins as biomarker in CRC

Biomarkers play significant roles in cancer diagnosis, prognosis, prediction, pharmacodynamics, and recurrence. AQPs have been identified in different cancers as biomarker with various roles [69,128-131]. In CRC, different groups of all thirteen AQPs isoforms are shown to be expressed [65,132-134]. It is proposed that, AQP5 is a prognostic biomarker for CRC regarding its high expression in 40 colorectal cancer tissues; related to tumor-nodes-metastasis (TNM), stage, and differentiation in comparison to para-neoplastic tissues [56]. The AQP5 overexpression in specimens, from 45 colorectal cancer patients, can act as a biomarker related to TNM stage, circulating tumor cells (CTCs), metastasis, aggressiveness and predictive marker of prognosis [71-135]. High expression of AQP1 in resected cancer specimens of CRC patients is seen. Evaluation of 268 CRC specimens with different stages (0-IV) has revealed the high expression of AQP1 in about 41% of patients. Moreover, AQP1 expression also is significantly accompanied by multiple invasive features including; vessel and lymphatic invasion, lymph node metastasis, greater invasive depth, and also the differentiation level. However, the expression of AQP1 is not associated with hepatic metastasis [126]. AQP1 is suggested as a predictive biomarker to respond to 5-fluorouracil-based adjuvant chemotherapy in CRC patients with stage II and III cancer [126]. Therefore, adjuvant chemotherapy in patients with CRC is recommended based on the expression pattern of AQP1, suggesting it as an ideal target for patients with CRC in stages II and III of the disease [136]. The association of AQP1 expression with a lower 5-year survival rate and higher invasiveness in 120 CRC patients in stages II and III is indicative of AQP1 as a poor prognostic biomarker [64]. Lower expression of AQP9 has been proposed as a predictive biomarker in response to adjuvant chemotherapy in patients with CRC in stage III [137]. Furthermore, AQP 1 and 5 are found in the early stage of CRC development; suggesting their role as progression biomarkers [65].

Therapeutic Potential of Aquaporin in CRC

AQPs are shown to play a role in tumor invasiveness. Table

Table 1: Therapeutic potential of aquaporins (AQPs) inhibition

Mediator	Targeted AQP	Cell line/mouse	Result	Reference
AqB013	AQP1	HT29	Significant suppression in migration, invasion and complete angiogenesis blockade in dose-dependent manner	(63)
Acetazolamide	AQP1	CRC xenograft of nude mouse	Tumor growth reduction, inhibition of VEGF expression and angiogenesis	(145)
Bumetanide derivatives (AqB001, AqB006, AqB007, and AqB011)	AQP1	HT29	Inhibition of ion conductance, reduction of migration and metastasis	(143)
AqB011 and bacopaside II	AQP1	CRC cell lines	AQP1 ion channel and water channel blockade cause amplification inhibition of cell migration	(146)
Mir-133a-3p	AQP1	Different CRC cell lines	Decrease of cell proliferation, migration and invasion	(142)
Nanoformulations of a potent copperbased aquaporin inhibitor	AQP3	C26 (murine cell line)	Potent cytotoxic effects toward tumor cell	(151)
CuSO4	AQP3	HCT116 cell	Blockade the enhanced migration ability	(67)
AQP5-specific shRNA	AQP5	CRC cell lines	Impaired migration and invasion of colorectal cancer, reduced expression of Matrix Metallopeptidase (MMP)-2 and MMP-9, markedly decrease of Wnt1 and β-catenin expression, revealing epithelial–mesenchymal transition (EMT)	(72)
Cairicoside E	AQP5	Different CRC cell lines	Suppression of EMT and p-Smad2/3 induced by TGF-β1.	(152)
AQP5-specific shRNA	AQP5	HCT116 or HT29 cells	Chemosensitivity enhancement of CRC cells to 5-FU. Facilitating the 5-FU-mediated apoptosis and tumor growth suppression. Reduction of 5-FU chemoresistance related to inhibition of the Wnt–β-catenin pathway, in vivo.	(153)
Berberine	AQP 1,3,5	Different CRC cell lines	Inhibit the growth, migration and invasion of tumor, up regulating PTEN which inhibit the PI3K/AKT pathway	(35)

1 demonstrates the therapeutic potential of AQPs inhibition. Given that overexpression of AQP1 is related to proliferation, angiogenesis, and lymph node metastasis of cancer cells in CRC, it could be considered as an appropriate therapeutic target. Pharmacologically and/or genetically inhibition of AQP1 could suppress the invasion, proliferation, and growth of other types of cancer cells such as breast, prostate, thyroid, bladder, and ovarian cancers [138-141]. AQP1 has been targeted in various cancers by miR-223 [139], miR-133a-3p [142], Rg3 [140], AqB007, AqB011 [143], AqB013 [63]. and, J82 [144]. AqB013 is one example of drug used to evaluate the effect of AQP1 blockade in the treatment of CRC. Treatment of HT29 colon cancer cells, expressing a high level of AQP1 with AqB013 have demonstrated significant suppression in migration, invasion, and complete angiogenesis blockade in a dose-dependent manner, while, it has no effect on migration of HCT-116 cells with low AQP1 expression [63]. Following the assessment of the effect of Acetazolamide in CRC xenograft of nude mouse, it is found that this drug is able to reduce tumor growth by decreasing the expression of AQP1 at the level of RNA and protein. It is noteworthy that, inhibiting AQP1 expression was related to inhibition of VEGF expression as a known angiogenic factor in cancer [145]. The effect of aquaporin ligand; bumetanide derivatives including AqB001, AqB006, AqB007, and AqB011 were evaluated following AQP1 obstruction in the human HT29 cell line [143]. AqB011 and AqB007 by interacting with D loop domain in the AQP1 structure revealed the greatest inhibition of ion conductance of AQP1 which led to the reduction of migration and metastasis of CRC cell lines [143]. It has been revealed that AqB011 and bacopaside II by inhibition of ion conductance and water channel activity of AQP1 (respectively) lead to augment the inhibition of colon cancer cell migration [146]. Targeting microRNAs tend to be another method of inhibiting protein expressions in different cancers [147-149]. It has been stated that AQP1 overexpresses in colon cancer cells [142] can serve as one of the miR-133-39p targets [150]. miR-133a-3p overexpression significantly suppresses AQP1 expression which lead to a decrease in cell proliferation, migration, and invasion in CRC cells [142]. Inhibition of AQP3 using nano-formulations of a potent copper-based aquaporin inhibitor have shown to increase the cytotoxic effect on tumor cells of C26 murine cells [151]. The blockade of AQP3 expression using CuSO4 is resulted in enhanced migration of HCT116 cells. Additionally, the hEGF induction of AQP3 overexpression is demonstrated to be suppressed using PI3K/AKT inhibitor, LY294002. However, U0126 as the inhibitor of ERK caused a minor effect on the hEGF-induced AQP3 up-regulation [67]. Silencing the AQP5 in CRC cell lines is shown to be related to impaired migration and invasion of colorectal cancer cells. In addition, the epithelial–mesenchymal transition (EMT) markers were altered as the expression of E-cadherin, Tissue Inhibitor of Metalloproteinases (TIMP)-1 and TIMP-2 were increased, while the Vimentin, N-cadherin, Plasminogen Activator, Urokinase (uPA) and Snail were down-regulated. The Wnt/β-catenin pathway was markedly changed toward reduction, indicating the effect of AQP5 expression on

invasiveness and metastasis of colon cancer [72]. Cairicoside E, a natural resin glycoside compound is revealed to suppress the AQP5 expression which results in EMT inhibition and a further reduction in p-Smad2/3 induced by TGF-β1. It is well confirmed that the effect of Cairicoside E is on AQP5 expression in which the CE had no significant effect on EMT markers and p-Smad2/3 induced by TGF-β1 in the condition of AQP5 silencing, separately [152]. Silencing the AQP5 using a specific short hairpin RNA construct could increase the chemosensitivity of CRC cells to 5-fluorouracil, mediating the apoptosis and suppression of tumor growth. The 5-fluorouracil (5-FU) chemosensitivity is mediated by the Wnt–β-catenin pathway suggesting AQP5 as a useful therapeutic target for CRC [153]. Berberine as a natural isoquinoline alkaloid is shown to have anti-cancer properties. Findings indicate that, the administration of this molecule lead to inhibition of the migration, invasion and the growth of CRC cells via down-regulation of AQP 1, 3 and, 5 expressions. This process is mediated following up-regulation of PTEN which inhibits the PI3K/AKT pathway, both at the gene and protein levels [35].

CONCLUSION

Aquaporins tend to be considered crucial in the development, angiogenesis, and invasiveness of colorectal cancer as one of the major concerns of the world. Among the thirteen isoforms, AQP1, 3 and, 5 seem to be related to poor prognosis of CRC, and their aberrant expression is associated with high invasiveness and increased tumor cell proliferation, angiogenesis, and metastasis. They also can serve as the reason for chemotherapy ineffectiveness. In addition, by regulating the cellular metabolism, the AQP3 and 9 play a crucial role in tumor genesis. Taken together, a better understanding of aquaporin role in colorectal carcinogenesis leads to better identification of novel AQP inhibitors to target specific AQPs associated with CRC cancers.

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