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Mini-review

Aquaporins: Their role in gastrointestinal malignancies

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ABSTRACT

Aquaporins (AQPs) are small (~30 kDa monomers) integral membrane water transport proteins that allow water to flow through cell membranes in reaction to osmotic gradients in cells. In mammals, the family of AQPs has thirteen (AQP0-12) unique members that mediate critical biological functions. Since AQPs can impact cell proliferation, migration and angiogenesis, their role in various human cancers is well established. Recently, AQPs have been explored as potential diagnostic and therapeutic targets in gastrointestinal (GI) cancers. GI cancers encompass multiple sites including the colon, esophagus, stomach and pancreas. Research in the last three decades has revealed biological aspects and signaling pathways critical for the development of GI cancers. Since the majority of these cancers are very aggressive and rapidly metastasizes, identifying effective targets is crucial for treatment. Preclinical studies have utilized inhibitors of specific AQPs and knock down of AQP expression using siRNA. Although several studies have explored the role of AQPs in colorectal, esophageal, gastric, hepatocellular and pancreatic cancers, there is no comprehensive review compiling the available information on GI cancers as has been published for other malignancies such as ovarian cancer. Due to the similarities and association of various sites of GI cancers, it is helpful to consider these results collectively in order to better understand the role of specific AQPs in critical GI cancers. This review summarizes the current knowledge of the role of AQPs in GI malignancies with particular focus on diagnosis and therapeutic applications.

Introduction

Aquaporins (AQPs) are water permeable tetramers present in hydrophobic cell membranes [1]. Structurally, AQPs are a mixture of small (~30 kDa monomers), integral membrane water transport proteins. Studies of tissue localization and regulation have suggested that AQPs contribute to several physiological processes. AQPs are commonly expressed in cells physiologically involved in fluid secretion and absorption, such as in the eye, central nervous system, exocrine gland, kidney, and adipose tissue. Recent investigations show an important role of AQPs in cell proliferation, and its associated cell functions such as migration, invasion, wound healing and angiogenesis [2]. Since substantial water transport is required to fulfill the high catabolic energy demands for increased proliferation and metastasis of cancer cells, AQPs also play a central role in cancer metabolism [1]. AQPs are important in cell migration through facilitating change in cell shape under increased osmotic stress. AQPs are also involved in angiogenesis and tumor growth and are intensely expressed in a wide variety of cancer cells [1]. Using AQP1 null mice, AQP1 was shown to be required for angiogenesis [3]. Accumulating evidence suggests that AQPs are strongly associated with the histological tumor grade of gastrointestinal cancers [4]. In esophageal squamous cell carcinoma (ESCC) patients with high grade tumors have higher expression levels of AQP3 [5]. Likewise, AQP1, AQP3, and AQP5 expression was correlated with higher grade of tumors in colorectal cancer (CRC) patients [6,7]. In gastric cancer (GC), AQP5 expression significantly increased the proportion of differentiated cells with a spindle shape [8,9]. The co-expression of AQP3 and AQP5 was strongly associated to higher tumor grade in ESCC and in hepatocellular carcinoma (HCC) patients [10,11]. These observations suggest that the expression pattern of AQPs depend on type of cancer and tumor grade. Thirteen types of AQPs (AQP0 through AQP12) have been described and characterized in human tissues [12], and are subdivided into three categories based on absorptivity: (1) water only AQPs (AQP0-AQP6, and AQP8); (2) glycero-AQPs transport water, glycerol and urea (AQP3, AQP7, AQP9, and AQP10); and (3) super-AQPs or subcellular AQPs (AQP11 and AQP12) [13–15]. Of these thirteen types of AQP, nine are known to exist in the membrane of cells from the gastrointestinal tract (Table 1): AQP1, AQP3, AQP4, AQP5, AQP7, AQP8, AQP9, AQP10 and AQP11 [26,27].

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Table 1
Aquaporin's expression in gastrointestinal malignancies.

Cancer type	AQP type	Expression	References
Esophagus	3, 5	High	[5,16,17]
Gastric	4	Low	[18,19]
	3, 5	High	[8,9,20,21]
Colon	8	Low	[22]
	1, 3, 5	High	[6,23]
Pancreas	1, 5	High	[24]
Hepatocellular	8, 9	Low	[25]
	1, 3, 5	High	[10,11]

Of these, AQP4 and AQP8 are located in the gastric and colonic areas [27]. The objective of this manuscript is to review the role AQPs play in gastrointestinal cancers. Special focus will be to discuss the challenges that need to be overcome to clinically advance AQPs as biomarkers and therapeutic targets in gastrointestinal cancer.

Structure

Aquaporin (AQP) water channels, first identified in 1991, function to increase water flow across epithelial cell membranes [28,29]. AQPs are tetramers in cell membranes, including those of GI cells, with each monomer behaving as a water pore [30]. AQPs have differences in their protein sequence and channel size, which permits the transport of various sizes of particles and solvent [31]. AQPs are small proteins of about 270 amino acids and are usually expressed in the GI tract [32]. Both carboxy (–COOH) and amino (–NH2) termini are cytoplasmic. Hydropathy analyses revealed each 30 kDa monomer to comprise six α-helical transmembrane covering regions surrounding a selective aqueous pore [33]. The six transmembrane regions are coupled by three extracellular and two intracellular loops, A–E [34] (Fig. 1). Loop B is associated between helix 2 and 3, and loop E between helices 5 and 6. Loops B and E have highly conserved sequences (NPA motif; two repeating Asn–Pro–Ala sequences), which overlap the inner of the lipid bilayer forming a 3-D 'hourglass' shape, through which water passes [35]. However, unlike

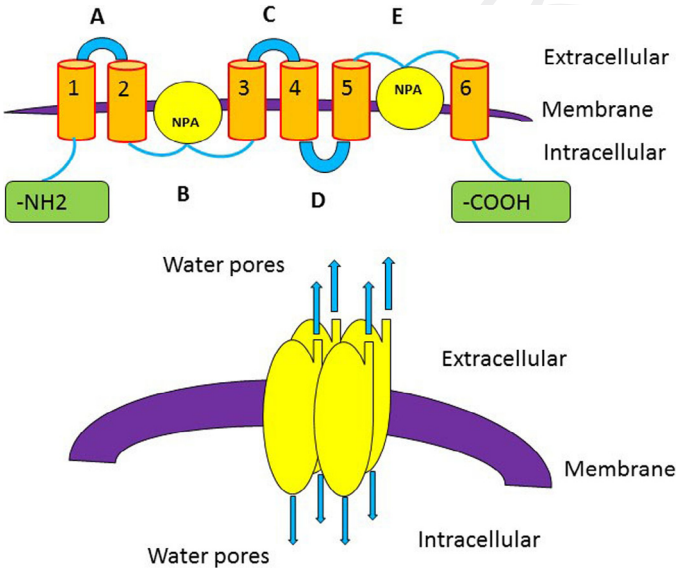

Fig. 1. AQP monomer comprises six membrane spanning helices connected by five loops. In the membrane, the six alpha helices forms a right-handed twisted arrangement. B and E loops meet in the middle to form a functional water pore, that contains two highly conserved NPA domain (Asn, Pro, Ala).Tetrameric assembly of AQP in a membrane in which individual monomers contain water pores. "↑" indicate water movement through the narrow selectivity filter of the aquaporin channel.

most AQPs, AQP5 cytoplasmic loop D has a cAMP-kinase phosphorylation (PKA) site (Fig. 1) [36,37]. Mutation of the PKA site of AQP5 does not affect its expression or water flow [38,39]. Further, mutation of the E-loop NPA domain results in the localization of AQP5 to the intracellular cytoplasm [39].

Esophageal squamous cell carcinoma

This year, an estimated 16,980 adults in the United States will be diagnosed with esophageal cancer. It is estimated that 15,590 deaths will occur [40]. In particular, the prevalence rates differ globally by close to 16-fold, with the highest rates found in Eastern Asia and Southern and Eastern Africa and lowest rates in Central America, and Western and Middle Africa [41]. In the greatest-risk areas, extending from North-Central China to northern Iran through the central Asian republics, which has been titled the "EC belt", 90% of patients have ESCC [42,43]. Generally, EAC is more prevalent in developed countries, due to lack of exercise, smoking, induced obesity and chronic gastro-esophageal reflux disease, which is believed to initiate Barrett's esophagus (BE) [44,45]. In ESCC patient biopsies, a higher expression level of AQP3 is observed in tumor areas as compared to adjacent normal tissue [16]. Knockdown of AQP3 inhibits the growth and adhesion of ESCC cell lines mediated through the FAK-MAPK pathway [5]. Co-expression of AQP3 and AQP5 has been observed in the membrane of tumor cells in ESCC tissues at considerably greater levels than in adjacent normal areas [16]. Furthermore, co-expression of these AQPs is correlated with increased invasion depth, aggressive lymph node (LN) prominence, and positive metastasis (both $P = 0.01$). The overexpression of AQP3 and AQP5 alone did not influence tumor progression [16]. Multivariate analysis indicates that the co-expression of AQP3 and AQP5 is an independent prognostic factor for overall and disease-free survival in ESCC. Combined measurement of the expression of these two proteins may provide useful biomarkers for diagnosis and prediction of ESCC patients.

A higher level of AQP5 expression is observed in ESCC cell lines TE2 and TE5 [17]. Knockdown of AQP5 (using siRNA) inhibited TE2 and TE5 cell proliferation and induced G1-S stage arrest and apoptosis [17], indicated by a significant increase in p21 and a significant decrease in CCND1 mRNA expression. The expression pattern of AQP5 and CCND1 is similar in ESCC tissue. Furthermore, immunohistochemistry (IHC) for AQP5 in samples from 68 patients with ESCC revealed that the expression of AQP5 is correlated with tumor growth, histological type and tumor recurrence [17]. Altogether, these investigations suggest that AQP5 may be a useful prognostic biomarker and a potential therapeutic target for drug development in ESCC.

Chang et al. [46] demonstrated that EGF induced AQP8 expression and migration in Eca-109 cells via the EGFR/ERK1/2 signaling pathway (Table 2). These observations suggest that AQP8 is involved in tumor development. This study is contrary to reports in other GI cancers such as colorectal cancer and hepatocellular carcinoma [22,25].

Gastric cancer

Gastric cancer (GC) is the second most common cause of cancer-associated mortality in the world [40]. For the past two decades, many reports have shown that ion channels and water carriers play important roles in GC. Screening of the expression profile of AQPs (AQP0-AQP12) by RT-PCR in GC tissues and corresponding normal mucosa from 89 patients with GC revealed that of 13 AQPs examined, AQP-1, -3, -4, -5 and -11 are expressed in GC or normal gastric tissues (Table 1), and AQP-3, -4 and -5 exhibit differential expression between GC and corresponding normal tissues [8]. The expression of AQP3 is higher in undifferentiated GC tumors than

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