**Expression, Regulation and Function of Aquaporin-3 in colonic epithelial cells**

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**Highlights**

* Aquaporin 3 is expressed in colonic epithelial cells
* Altered aquaporin 3 expression affects epithelial tight junction integrity and permeability
* Colonic expression of aquaporin 3 is altered in various disease states, including inflammatory bowel disease, irritable bowel syndrome, and colorectal cancer

**Abstract**

The human colon balances water and electrolyte absorption and secretion while also forming a barrier protecting the body from the entry of harmful components. Aquaporin-3 (AQP3) is a water, glycerol and H2O2 transporting channel expressed in colonic epithelia. Although expression of colonic epithelial AQP3 is altered in several intestinal disorders, such as inflammatory bowel disease and irritable bowel syndrome, the regulation and specific roles of AQP3 remain to be fully defined. In this mini-review, we summarize the current understanding of the expression, regulation, and biological functions of AQP3 protein in colonic epithelia concerning intestinal absorption, secretion and barrier function.

**Keywords:** Aquaporin 3/Large intestine/Gene Expression Regulation/Water transport/Water-Electrolyte Balance/ Intestinal secretions

**1. Introduction**

 Aquaporins (AQPs) are transmembrane channels facilitating transport of water across biological membranes. Currently, the mammalian family of aquaporins consists of 13 members (AQP0-12). AQP3 belongs to the subfamily called aquaglyceroporins; it has the ability to transport water, glycerol, urea, and hydrogen peroxide (H2O2) [1].

 AQP3 is widely expressed in membranes of various epithelia. It is most prominently expressed in keratinocytes [2], colon [3, 4], and the basolateral membrane of kidney collecting duct cells [5]. However, AQP3 is also found in the upper part of the digestive tract, the respiratory tract [1] and in immune cells [6]. Such a widespread distribution of the channel suggests it has an important role in regulating many aspects of mammalian physiology and experimental models in which AQP3 is downregulated support this idea. Thus, AQP3 KO mice are defective in their ability to concentrate urine [7], display epidermal hydration and skin barrier defects [8], and are more prone to developing diarrhea and inflammation in experimental models of intestinal disease [9]. However, despite its important contribution to regulating many physiological and pathophysiological processes, the specific roles of AQP3 in many of these tissues are still poorly understood.

 Although AQPs are overlapping in function in terms of water transport, their subcellular localization varies, ranging from the plasma membrane to intracellular membranes, such as the mitochondrial membranes [1]. AQPs also differ in their capacity to transport various substances, such as urea, glycerol, H2O2, ions, and gas [10, 11]. Furthermore, some AQPs regulate aspects of epithelial cell biology other than transport, such as cell migration, morphogenesis and angiogenesis [12, 13]. Thus, although a number of AQPs are present in intestines [14], their roles and regulation may be distinct. In this mini review, we focus our attention on AQP3 in colon and aim to summarize current knowledge regarding its expression, regulation, and potential roles in physiology and in intestinal barrier function (a role for AQP3 in cancer has recently been reviewed elsewhere [11]). We also highlight some of the future challenges and questions to be resolved in dissecting the function of this aquaglyceroporin in colon.

**2. AQP3 expression and localization in colon**

One of the factors that has limited our understanding of the physiological roles of AQP3 in the colon has been the apparent variation in its expression between different experimental models. In rats, expression of AQP3 increases from proximal to distal colon, with a distinct basolateral plasma membrane localization in surface colonic epithelial cells (CECs) [4, 15]. In mice, basolateral membrane localization of AQP3 has been reported mainly at the base of the crypts (CD1 background) [9, 16]. Our own studies of isolated rodent CECs, found AQP3 to be expressed at both protein and mRNA levels in rats, but not in mice (C57BL/6 background) [15]. In contrast, we found mouse CECs to have abundant AQP4 labeling in the basolateral membrane of surface CECs and extending into the crypts [15]. Such differences might reflect significant variations in AQP expression between different strains of mice and/or imply overlapping functions for different members of the AQP family [17].

 Studies in humans also clearly demonstrate AQP3 to be expressed in the colon. mRNA transcripts for this AQP are abundant in biopsies from both proximal and distal colon [15, 18-20], where its localization to the epithelium is clearly demonstrated by its expression in organoids derived from these tissues [21, 22]. However, the precise localization of the protein in colonic epithelium is less clear. For example, basolateral plasma membrane localization in human distal colonic epithelia has been reported by Mobasheri et al. [23]. Supporting this observation is the finding of an AQP3 N-terminal motif (YRLL), conserved across humans, rats, mice, and dogs [24]. This motif consists of a tyrosine (Y) and two leucine residues (LL) that are involved in basolateral targeting [24, 25]. A basolateral localization for AQP3, albeit relatively weak, is also supported by immunohistochemical studies from our own lab [15]. In contrast, other studies have shown the protein to be expressed apically [20], or both apically and basolaterally on surface CECs [26]. The reasons for such variations in cellular localization of AQP3 in the colon are not yet clear but could reflect technical difficulties in protein labelling, for example, due to alternative processing of the protein or a high degree of brush border glycosylation interfering with antibody binding. Another, perhaps more likely, explanation could be that although the AQP3 mRNA is abundantly expressed in colonic epithelia, its protein levels may be limited by constitutively active post-transcriptional and/or post-translational mechanisms, thereby maintaining the protein at relatively low levels under normal circumstances [22]. Such mechanisms could constitute an “on/off” switch for AQP3 protein expression when it is required. This is an important area of research that needs to be addressed in future studies.

**3. Regulation of AQP3**

***3.1 Acute regulation of AQP3***

 As is the case with most transport proteins, acute regulation of AQPs (i.e. that occurring within seconds to hours), can occur via several mechanisms, such as changes in posttranslational modifications (PTMs), changes in protein conformation, or changes in subcellular distribution. In general, protein phosphorylation has proven to be an important regulatory PTM for AQPs. Phosphorylation is involved in gating (e.g. AQP0) and regulates membrane trafficking and localization (e.g. AQP2) [27]. However, regulation of AQP3 directly via phosphorylation has not yet been described. Although the physiological relevance is unclear, certain ions, such as Cu2+ [28] and Hg2+ [3], can directly inhibit AQP3 channel function. Channel regulation via alterations in gating might also occur, since reductions in external pH inhibit water and glycerol transport through the protein [29]. Acute regulation of AQP3 transport is suggested to be coupled to the activity of other membrane transporters. For example, in a human airway epithelial cell model, cAMP-induced activation of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) was found to be associated with enhanced cellular water and glycerol permeability by a mechanism most likely involving AQP3 [30]. Similar findings were reported in an oocyte model [31]. Since both CFTR and AQP3 are expressed in CECs, these findings may be relevant to the physiology of water transport in the colon. However, it is still not clear if increased AQP3 activity in response to activation of CFTR is due to altered channel gating or membrane trafficking. A role for regulated trafficking is highlighted from studies in Caco-2 cells showing rapid redistribution of the channel from an intracellular localization to the plasma membrane after treatment with the adrenergic agonist, epinephrine [32].

Another mode by which AQP3 channel function maybe acutely regulated involves post-transcriptional regulation of gene expression. Although not shown in colon, studies in a mouse model of acute intestinal ischemia have shown jejunal AQP3 levels to be decreased within an hour after ischemic damage. This effect was associated with increased levels of miR-874. In cell models, binding of miR-874 to the 3’UTR of the AQP3 gene was reported [33]. Other miRNAs may be involved in AQP3 regulation in various tissues (for review see [34]).

 Thus, a significant body of evidence suggests that, in the short term, AQP3 activity can be regulated at multiple levels that can involve alterations in gating, trafficking and expression. While the presence of such rapidly-acting regulatory mechanisms suggest an important role for the channel in gut homeostasis, considerable work is still required to more fully understand the molecular pathways involved and their contribution to intestinal epithelial physiology in conditions of health and disease.

***3.2 Long-term regulation of AQP3***

 Long-term regulation of AQP3 expression involves transcriptional and translational regulation of the gene and its mRNA in addition to mechanisms that control degradation of the protein.

 The 5’-flanking region of the AQP3 gene harbors promotor activity and binding sites for a number of transcription factors, which have been the focus of several studies [21, 35]. One important transcriptional regulator of AQP3 appears to be cAMP response element-binding protein (CREB), for which two binding sites have been identified in the promotor region of the gene. Studies in Caco-2 cells have shown that upregulation of AQP3 expression by Mg2+ is prevented by siRNA for CREB and by blocking components of the cAMP-dependent signaling pathway, such as adenylyl cyclase (AC) and protein kinase A [35]. Similarly, vasoactive intestinal polypeptide (VIP) has been shown to upregulate AQP3 expression in HT29 cells in a cAMP-dependent manner [36]. Thus, similar to other extraintestinal epithelial cells [37, 38], hormones, neurotransmitters and immune mediators that elevate intracellular levels of cAMP in colonic epithelial cells are likely to play an important role in regulating the overall tone of AQP3 expression and activity.

In addition to CREB, other transcription factors are also likely to be important in regulating AQP3 expression. For example, a recent study found that the short proximal fragment of the AQP3 promotor (containing the TATA-box and an SP1-site) was sufficient for “constitutive transcription” of the gene [39]. Transcription of the AQP3 gene can be suppressed by binding of Specificity Protein (SP) 3 to this SP1-site and this interaction appears to be a target for gene regulation in the context of inflammation, since the inflammatory cytokine, TNFα, was found to enhance SP3-induced gene suppression. The mechanisms involved remain to be elucidated but do not appear to involve increased levels of SP3 binding to the AQP3 promoter [39]. The mechanisms by which another important proinflammatory cytokine, IFNγ, regulate AQP3 expression have also been studied in CECs. In these studies, IFNγ was found to suppress AQP3 mRNA-expression in HT29 cells and human enteroids [21]. In this case, the inhibitory effects of IFNγ appear to be mediated by STAT1 [21].

 In addition to cytokines, growth factors and hormones also appear to contribute to transcriptional regulation of colonic epithelial AQP3. Using the human colorectal carcinoma cell line, HCT116, epidermal growth factor (EGF) has been shown to upregulate AQP3 expression. This response appears to be mediated by the phosphatidylinositol 3-kinase/AKT-dependent signaling pathway, but is independent of ERK MAP kinase [40]. In contrast, studies in Caco-2 cells demonstrate that insulin decreases expression of AQP3 at both the mRNA and protein level; an effect which was shown to be mediated by the transcription factor, forkhead box a2 (Foxa2) [41].

 In summary, the AQP3 promoter region contains numerous sites for interaction with transcription factors, enabling expression of the protein to be regulated by a range of diverse stimuli, including hormones, inflammatory mediators, and neurotransmitters. However, it is not just endogenous signals that can regulate expression of AQP3 but also those arising from within the gut lumen. Many studies have documented how dietary components and microbes, likely through the production of toxins and metabolites, regulate expression of the channel [15, 42]. Such complex regulatory mechanisms indicate important physiological roles for AQP3 in the colonic epithelium and a need for its levels to be closely controlled.

**4. Functional roles of AQP3 in colon**

***4.1 AQP3 in regulation of water transport***

 One of the primary functions of the intestinal epithelium is to absorb water from the luminal contents and on a daily basis the absorption of approximately 2 L of water occurs in the colon [14]. Fluid absorption is driven by the osmotic gradients established by active solute and nutrient absorption with water following passively, either through the paracellular pathway via tight junctions or by the transcellular route through aquaporins [43]. In several disease conditions water absorption can become dysregulated, manifesting clinically either as diarrhea or constipation. Numerous studies suggest a role for AQP3 in promoting colonic water absorption and there has been a great deal of research interest in investigating changes in its expression in various intestinal disorders. For example, studies carried out by Camilleri et al. found that there was decreased abundance of AQP3 mRNA and protein in rectosigmoid biopsies from patients with diarrhea-predominant irritable bowel syndrome (IBS-D), with or without bile acid malabsorption (BAM), compared to healthy controls [19]. In line with this, our own studies in CECs isolated from rats fed with cholic acid also showed AQP3 protein levels to be reduced [15]. However, in the rat study, mRNA expression of AQP3 was increased, despite an apparent reduction in AQP3 protein. The differences in APQ3 mRNA expression between the two studies may stem from species differences in AQP3 regulation. However, an important difference between the studies lies in the procedures used for sample preparation. In the rat study the tissue examined represents isolated epithelial fractions whereas the human study is based on examining the mixed tissue of the mucosal biopsies. As such, the studies are not directly comparable. While further work is required, these data may indicate differences in the molecular pathways by which AQP3 is regulated by bile acids in rodents and humans.

 Other in vivo and in vitro studies also point to a role for AQP3 in regulation of intestinal fluid transport. For example, in a rat model of morphine-induced constipation, AQP3 expression was found to be increased [44]. In these studies, increased AQP3 expression and constipation were sensitive to the Selective Serotonin Reuptake Inhibitor (SSRI), fluoxetine, suggesting a possible involvement of paracrine regulation of AQP3 expression by 5-HT. Other studies suggest that AQP3 expression might also be under paracrine regulation by PGE2 release from mucosal macrophages [45].

 While there is considerable data demonstrating that changes in AQP3 expression are associated with alterations in colonic fluid transport, there have still been relatively few studies directly assessing the functional role of AQP3. However, in one such study, where the authors employed rectal infusion of either HgCl2 or CuSO4 to directly inhibit AQP3 channels in rats, increased fecal water content and diarrhea were observed, without alterations in AQP3 protein abundance [46]. However, it should be also be noted that some studies have demonstrated upregulation of AQP3 expression to occur in response to agents which promote fluid loss, e.g. the prosecretory neurotransmitter, vasoactive intestinal polypeptide (VIP), or the laxative, magnesium sulfate [35, 36]. Whether increased AQP3 expression in these models contributes to fluid loss, or conversely, occurs as a compensatory mechanism to prevent water loss is not yet clear.

In summary, alterations in AQP3 expression are clearly associated with intestinal disorders related to dysregulated colonic fluid transport. However, the precise roles that the channel plays under physiological and pathophysiological conditions still need to be more fully defined. Such roles may differ in different disease states and may overlap with additional functions of the channel in regulating other important aspects of intestinal homeostasis, such as barrier function.

***4.2 AQP3 in regulation of intestinal barrier function***

While a primary function of the gastrointestinal tract is to absorb nutrients and fluid from digested food, at the same time it must also form a protective barrier between the luminal contents and the internal milieu. This barrier consists of physical and biochemical components and includes the outer mucus layer, the epithelial cell layer, and the immune cells present in the inner connective tissue lamina propria [47], see fig. 1. Also an important component of the barrier are the structures which hold the epithelial cells together; the tight junctions (TJ), adherens junctions (AJ) and desmosomes. The intestinal barrier is under regulation by many factors, both endogenous and exogenous, and defects in this barrier are associated with the onset of several disease conditions, including inflammatory bowel disease, irritable bowel syndrome, and colon cancer [47].



**Figure 1. Schematic representation and hypothetical model of AQP3 regulation of intestinal barrier function.** AQP3 localizes to the basolateral membrane of colonocytes, where induces the organization of occludin and claudin into tight junctions. During inflammation, proinflammatory cytokines, such as IFNγ, released by T-cells, or TNFα, released by macrophages (Mφ), inhibit the transcription of AQP3. Without the influence of AQP3 in organization of the tight junction proteins, the paracellular pathway becomes leaky allowing entry of opportunistic pathogens into the mucosa, thus perpetuating the inflammatory state. ZO-1: zonula occludens protein 1, DC: dendritic cell.

Numerous studies suggest that AQP3 has an important role to play in the maintenance of intestinal epithelial barrier function. For example, inhibition of channel expression results in downregulation of the TJ proteins, claudin-1 and occludin, decreased transepithelial resistance (TEER), and increased permeability to macromolecules in cultured CECs [48]. Thus, in vivo, decreases in AQP3 expression would be expected to facilitate luminal bacteria, toxins, and antigens gaining access to the mucosa via the paracellular route, thereby potentially promoting inflammation. Other studies suggest a role for AQP3 in maintaining barrier function through its capacity to transport bioactive molecules, such as glycerol and hydrogen peroxide (H2O2). Administration of glycerol has been shown to be protective against development of colitis in mice, an effect that may be due to altered cellular metabolism [49]. H2O2 can be generated at the luminal membrane of epithelial cells either endogenously through the cells own nitric oxide synthase activity (via activation of NOX1) or via its release from gut bacteria. AQP3 mediates influx of extracellular H2O2, triggering cytoprotective pathways, including epidermal growth factor receptor (EGFR) [50, 51]. Mice deficient in AQP3 display altered H2O2 signaling, impaired formation of lamellopodia and focal adhesions, impaired wound healing, and increased inflammation in response to intestinal injury [52]. These findings suggest that AQP3-mediated H2O2 uptake is an important regulator of the epithelial barrier by promoting epithelial restitution in response to injury. Further evidence supporting this idea comes from studies demonstrating that blockade of the AQP3 channel prevents colonic epithelial cell migration in vitro, an essential part of the restitution process [40].

 Studies of AQP3 expression in biopsies from patients with IBD have shown its expression to be altered during the course of intestinal inflammation. Thus, in patients with Crohn’s disease, mucosal levels of AQP3 mRNA and protein, along with several other AQPs, were found to be downregulated [20]. In contrast, studies from biopsies of patients with ulcerative colitis have shown levels of AQP3 mRNA to be upregulated [18]. While the reasons for such differing observations are unclear, they could reflect differences in tissue sampling sites or the degree of inflammation at the time of sampling. Nevertheless, both studies point to changes in epithelial turnover of AQP3 in conditions of intestinal inflammatory disease. Further evidence for an important role for AQP3 in protecting against development of intestinal inflammation comes from in vivo studies of intestinal inflammation. Similar to that seen in IBD patients, colonic epithelial AQP3 expression is reduced in rodent models of chemically-induced colitis [53], whereas knockdown of the channel predisposes the animals to more severe colitis [53] [9]. While the mechanisms underlying downregulated expression of AQP3 in intestinal inflammation have not yet been elucidated, it appears to occur as a consequence of epithelial exposure to proinflammatory cytokines, such as TNFα and IFNγ [21, 39].

 In summary, in vitro, in vivo, and clinical studies support a role for AQP3 in promoting intestinal epithelial barrier function and thus its reduced expression may contribute, at least in part, to enhanced epithelial permeability and consequently the progression of mucosal inflammation.

**5. Conclusion and future challenges**

Research into AQP3 expression in the colon indicates a significant role for the channel in regulating the fluid transport and barrier functions of the epithelium and, as such, it likely has important roles to play in development of pathological conditions, such as diarrhea or constipation, IBS, and IBD. However, there is still significant work to be done in order to fully understand the specific roles that AQP3 plays under normal and pathophysiological conditions. One important area of research that needs to be more fully addressed is the precise localization of AQP3 in the colon, including its cellular distribution, expression along the crypt-villus axis, and expression in mucosal cells other than CECs (e.g. immune cells). Future studies should also aim to more fully address the functional roles of AQP3, define the molecular mechanisms by which it contributes to water transport and barrier function, and to elucidate how these pathways become dysregulated during disease conditions. Through this research, we will gain a better understanding of fundamental roles that AQP3 plays in regulating colonic epithelial physiology in health and disease, which in turn may lead to new therapeutic approaches for intestinal disorders.

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