Roles of Cl^-/HCO_3^- anion exchanger 2 in the physiology and pathophysiology of the digestive system (Review)

HUI WANG[1,2], JIAXING AN[1], HAI JIN[1], SUYU HE[2], CHENGCHENG LIAO[3], JUAN WANG[1] and BIGUANG TUO[1]

[1]Department of Gastroenterology, Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou 563003;
[2]The Fourth Department of The Digestive Disease Center, Suining Central Hospital, Suining, Sichuan 629000;
[3]Special Key Laboratory of Oral Disease Research, Higher Education Institution in Guizhou Province, School of Stomatology, Zunyi Medical University, Zunyi, Guizhou 563006, P.R. China

Received January 19, 2021; Accepted April 14, 2021

DOI: 10.3892/mmr.2021.12130

Abstract. Cl^-/HCO_3^- anion exchangers (AEs), which are members of the solute carrier 4 family, contribute to the exchange of one intracellular HCO_3^- for one extracellular Cl^-. AE2, a vital subtype of the Cl^-/HCO_3^- exchangers, is expressed widely in various cells and tissues in mammals and serves essential roles in the pathophysiological processes of the cardiovascular system and renal tubular reabsorption. Recently, research on the function of AE2 in the digestive system shed new light on its roles in the regulation of cellular and organ physiology. AE2 not only participates in gastric acid secretion, but also mediates bile secretion and digestive cancer development. The aim of the present review was to describe the role of AE2 in the physiology and pathophysiology of the digestive system, with the aim of guiding clinical diagnosis and treatment.

Contents

Correspondence to: Professor Biguang Tuo, Department of Gastroenterology, Affiliated Hospital of Zunyi Medical University, 149 Dalian Road, Zunyi, Guizhou 563003, P.R. China
E-mail: tuobiguang@aliyun.com

Key words: Cl^-/HCO_3^- anion exchanger, digestive system diseases, ion channel, chloride, bicarbonate

1. Introduction

Cl^- and HCO_3^- are important anions in the body and they are involved in cellular processes associated with various physiological functions and disease development, including cell cycle, proliferation, differentiation, membrane potential, reactive oxygen species (ROS) levels, and intracellular pH (pHi) and extracellular pH (pHo) regulation (1,2). HCO_3^- is the second most abundant anion in bodily fluids after Cl^- and has an important role in human physiology (3,4). Cl^- regulates the functions of different organelles, including endosomes, phagosomes, lysosomes, endoplasmic reticulum, and mitochondria (1). In addition to its main role as a pHi regulator, HCO_3^- controls the activity and stability of dissolved proteins in bodily fluids, such as saliva, pancreatic juice, intestinal juice and airway surface fluid. HCO_3^- exists in balance with CO_2 produced by mammalian cell metabolism, and they are interconverted in a pH-dependent manner to compose the main buffer system that regulates human pH (4). The Cl^- and HCO_3^- levels in the cells are regulated by ion channels and exchangers, including cystic fibrosis transmembrane conductance regulator (CFTR), bicarbonate transport proteins, and members of the solute carrier (SLC) 4 and SLC26 families. The SLC4 family consists of 10 transmembrane proteins, including two electrogenic Na^+-HCO_3^- cotransporters [SLC4A4 (NBCe1) and SLC4A5 (NBCe2)], two electroneutral [SLC4A7 (NBCn1) and SLC4A10 (NBCn2)], three electroneutral anion exchangers [AEs; SLC4A1 (AE1), SLC4A2 (AE2), SLC4A3 (AE3)], the Na^+-dependent Cl^-/HCO_3^- exchanger SLC4A8 (NDCBE), and two other uncommon members [SLC4A9 (AE4) and SLC4A11 (BTR1] (5). Of these, AEs include AE1-3, which are 53-56% identical at the amino acid level. AE1 is expressed in the kidney and the erythrocyte membrane (6), AE2 is widely distributed in tissues and organs, and AE3 is expressed in the central nervous system (7) and heart specimens (8). The expression distribution of AE members is listed in Table I. The majority of AEs are located basolaterally in polarized cells. Under physiological conditions, all three AEs undergo Na^+-independent, electron-neutralized Cl^-/HCO_3^- exchange (5,9,10). Numerous studies have shown that AE2 is involved in a variety of physiological and pathophysiological processes. For example, in ameloblasts, AE2 mediates the

efflux of HCO_3^- across the basement membrane, supports H^+ secretion across the apical membrane, and contributes to tooth remodeling (11). In B lymphoid neoplasms, AE2 can be used as a target for specific peptide-targeted therapy (12). In human nucleated cells, AE2 mediates the transport of abscisic acid, which is a hormone involved in inflammatory response and glycemic control (13). In osteoclasts, AE2-mediated efflux of HCO_3^- across the basement membrane serves a key role in bone resorption activity, suggesting that the loss of AE2 function shifts bone balance toward ossification or pathological high bone density (14). Loss of AE2 function in AE2 (-/-) mice is lethal to embryos, as it leads to emaciation, hypodontia or edentulism and severe growth retardation, and most of them die during weaning (15). Recent studies have found that AE2 is expressed in most organs of the digestive system and has an important role in the physiological processes of the digestive system and digestive system diseases, such as gastric cancer (16) and primary biliary cholangitis (PBC) (17). However, the mechanism and function of AE2 in the digestive system, particularly in certain digestive system diseases and tumors, have not been fully elucidated. The aim of the present review was to describe the role of AE2 in the physiology and pathophysiology of the digestive system and the current use of AE2-based treatments.

2. Structural features of AE2

AE2 is a transmembrane bidirectional transporter that forms a homodimer in the membrane. AE2 has a two-domain structure, including an N-terminal cytosolic domain and a C-terminal membrane domain, consisting of 14 transmembrane segments (18,19). The human AE2 gene contains three alternative promoters, resulting in at least three different transcripts (AE2a, b and c); the human AE2a protein contains 1,240 residues with a molecular weight of 137 kDa, while the mouse AE2 gene encodes five N-terminal variant polypeptides (20). The N-terminal cytosolic domain and putative re-entrant loop 1 of the transmembrane domain of AE2 also contain structural determinants of pH sensitivity (Fig. 1) (21-23). AE2 has a carbonic anhydrase (CA) II-binding site in its C-terminal tail that can localize the enzyme to the basolateral membrane (BLM). The activity of AE2 is stimulated by both alkaline pH and hyperosmosis through a conserved motif in the N-terminal cytoplasmic domain, although the membrane domain also plays a role in AE2 anion permeability (20).

3. AE2 and the esophagus

In the esophagus, AE2 localizes to the BLM and exports one HCO_3^- for every Cl^- that is imported. Therefore, Cl^- is transported across cells from the basal side into the lumen and is involved in esophageal fluid secretion (24,25).

The esophagus is a muscular tube that connects the hypopharynx to the gastric cardia; its main function is delivering food to the stomach. The ingested food enters the esophagus after a short stay in the oral cavity, so the upper part of the esophagus is exposed to the temperature, pH and osmotic pressure of the ingested food, while the lower end of the esophagus can be corroded by reflux of gastric contents (26). The most important defense mechanism of esophageal epithelial cells against reflux-induced injury is esophageal epithelial resistance consisting of functional and structural components including the following: i) A surface mucus and an unstirred aqueous layer containing HCO_3^-, which provides an alkaline environment; ii) cellular junctions (tight junctions) of the apical surface and BLM that prevent diffusion of H^+ into the intercellular space and cell, respectively; and iii) intracellular buffer systems, such as HCO_3^- or phosphate buffer systems (26). Among them, the secretion of HCO_3^- in the lumen is considered to protect the esophagus by neutralizing acidic reflux. Secretion of esophageal HCO_3^- is associated with the esophageal submucosal glands (SMG) (27). In the SMG, CFTR and SLC26A6 on the apical membrane and BLM, respectively, are mainly involved in HCO_3^- secretion, and apical Cl^- transport in the SMG can be linked to the CFTR Cl^- channel (Fig. 2) (27). Transcellular Cl^- transport, on the other hand, is particularly important in SMG, as fluid secretion from SMG is more significant. Transcellular Cl^- transport is mediated by AE2 localized at the basolateral side of the cell and, at the same time, AE2, independently of Na^+, mediates the entry of one Cl^- into the cell with exocytosis of one HCO_3^-, which acidifies the cell and prevents excessive alkalinization (24,25). In combination, these findings suggest that the role of AE2 in the esophagus may be to protect the esophageal mucosa by mediating Cl^- transcellular secretion into the lumen in the basal layer, further causing fluid secretion. Therefore, AE2 may serve as a therapeutic target for reflux esophagitis.

AE2 also has a role in the pathogenesis of esophageal cancer (28). In esophageal squamous cell carcinoma (ESCC), AE2 has been demonstrated to be upregulated and mainly located in the cell membrane or the cytoplasm of esophageal cancer cells. AE2 knockdown decreased apoptosis, and increased migration and invasion of ESCC cells through regulation of matrix metalloproteinase expression (28). The altered AE2 expression may affect tumorigenesis by altering the pHi. Decreased AE2 expression facilitates intracellular alkalinization, which promotes cancer cell metabolism (29). Clinically, by analyzing the clinicopathological characteristics of 61 patients with ESCC, it was found that the expression levels of AE2 in the whole tumor were not significantly associated with the overall 5-year survival rate, but the reduced expression of AE2 at the invasive front was correlated with poor prognosis (28); this finding requires further study.

4. AE2 and the stomach

The stomach serves as a center for the mechanical distribution of food and contains digestive glands; some of the chemical processes of digestion also occur in the stomach. Gastric mucosa secretes HCO_3^- with mucus, which creates a pH gradient in the mucus of surface cells and, although the lumen is acidic, the pH value near the surface cells is almost neutral. This 'mucus-bicarbonate diffusion barrier' is an important part of protection against gastric acid damage (30). AE2 serves an important role as a Cl^-/HCO_3^- exchanger. First, AE2 is localized at the basal side (31). Second, studies have shown that rabbit parietal cells and mucous cells derived from the same gastric stem cell population exhibited completely different AE2 isoform expression patterns (32). The isoform expression pattern in parietal cells is AE2b>>AE2c>or=AE2a,

Table I. Organizational characteristics and expression of human AEs.

Protein name	Alias	Chromosome localization	Gene length (kb)	Tissue distribution
AE1	SLC4A1	17q21-q22	~20	Erythrocyte membrane, kidney
AE2	SLC4A2	7q36.1	~17	Salivary gland, stomach, duodenal enterocytes, proximal colon, epididymal epithelium, ameloblasts, bile duct, hepatocyte, pancreas, airway, kidney
AE3	SLC4A3	2q36	~14	Central nervous system, heart

From refs. (5,7-11). AE, anion exchanger; SLC, solute carrier.

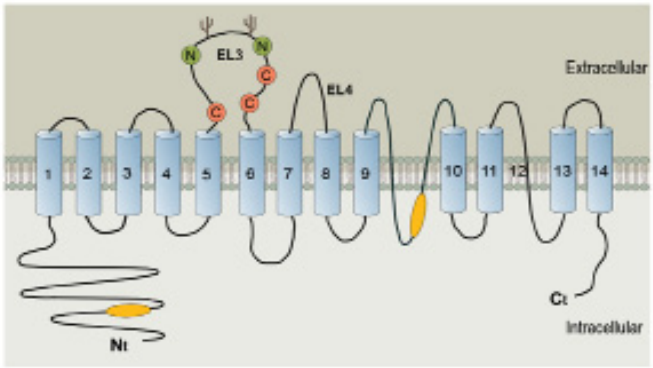

Figure 1. Putative structural model of AE2 transporter. AE2 has a large cytoplasmic Nt, followed by a TMD of >500 amino acids, as well as a Ct of 40 amino acids that is well conserved in AEs. The Nt and re-entrant loop 1 contain structural determinants of pH sensitivity (yellow regions). The third extracellular loop (EL3) between TMD 5 and 6 typically includes multiple Cs and Ns. Ct can bind to carbonic anhydrase II. AE2, anion exchanger 2; Nt, N-terminus; TMD, transmembrane domain; Ct, C-terminus; EL, extracellular loop; C, cysteine residue; N, N-glycosylation sites.

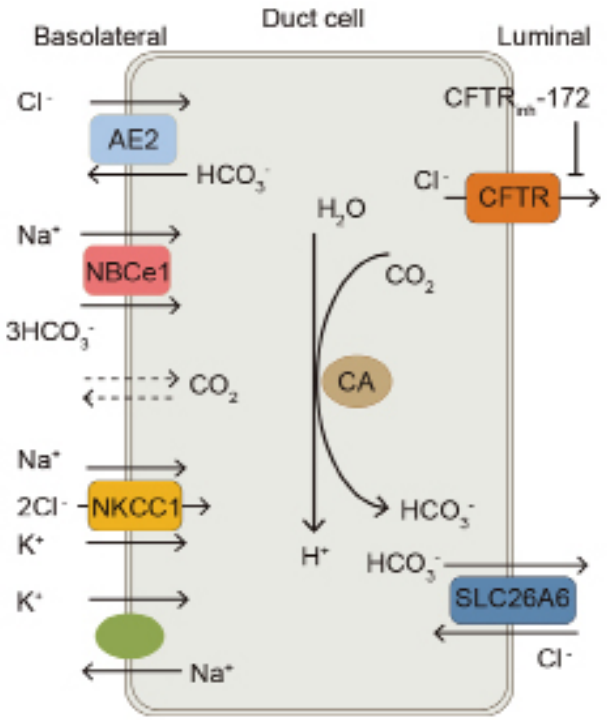

Figure 2. Model of a duct cell illustrating ion transport mechanisms involved in Cl^- and HCO_3^- transport in the esophageal submucosal gland. At the basolateral membrane, HCO_3^- entry is mediated by the NBCe1. AE2 mediates Cl^- uptake by the cell. NKCC1 mediates Na^+, K^+ and Cl^- entry into the cell. At the apical membrane, HCO_3^- efflux is mediated by Cl^-/HCO_3^- exchanger SLC26A6. Apical CFTR may be permeable to HCO_3^- or Cl^- efflux. Cl^- efflux drives apical SLC26A6, leading to HCO_3^- secretion. AE2, anion exchanger 2; NBCe1, Na^+-HCO_3^- cotransporter; NKCC1, Na^+-K^+-$2Cl^-$ cotransporter 1; CA, carbonic anhydrase; CFTR, cystic fibrosis transmembrane conductance regulator; CFTRinh-172, glibenclamide, cystic fibrosis transmembrane conductance regulator inhibitor; SLC26A6, solute carrier family 26 member 6.

and the isoform expression pattern in mucous cells is AE2a>AE2b>>AE2c (32). Parietal cells are activated by secreting H^+ into the lumen, inducing pHi increase and intracellular alkalinization (31). AE2, which is localized in the BLM and acts as an output of HCO_3^-, is activated and pumps HCO_3^- to the basolateral side, which can promote cell recovery from alkaline pH, reabsorb HCO_3^- into the blood, avoid excessive alkalinization of cells and further provide Cl^- into the lumen by pumping Cl^- into cells. The activity of AE2 is stimulated by both alkaline pH and hyperosmosis, with the hypertonic activation of AE2 being secondary to the hypertonic activation of Na^+/H^+ exchange (31). AE2-mediated anion exchange is also stimulated by ammonium and hyperosmolarity, and the mechanism involves intracellular Ca^{2+} chelation and calmidazolium-sensitive inhibition of anion exchange (33). Both gastric acid secretion and osmolality in the gastric cavity are increased following the ingestion of food, which indicates that AE2 serves a vital role in assisting parietal cells to excrete HCO_3^- and supply the gastric lumen with Cl^-. AE2 is essential for the secretion of acid in acid-secreting parietal cells. Accordingly, a previous study identified gastric acid deficiency, moderate dilatation of the gastric glandular lumen and a reduced number of parietal cells in AE2 (-/-) mice (15). Ultrastructural analysis revealed abnormal parietal cell architecture, severely impaired secretory tubule development and few tubular vesicles, but normal apical microvilli, indicating that normal AE2 function is required in mouse parietal cells, particularly for normal gastric acid secretion and normal development of secretory tubules and canalicular vesicle membranes (15). In humans, the complete loss of AE2 function may be lethal to embryos (15). Of note, in AE2 (a and b) (-/-) mice, basal acid secretion was normal and no parietal cells were hypoplastic, whereas carbachol/histamine-stimulated acid secretion was impaired by 70%. These results indicated the key role of the AE2 a and b isoforms in

gastric acid secretion. Gastric expression of the residual AE2c isoform was low and mice deficient in AE2c did not exhibit impaired acid-stimulated secretion (34). Further studies are required to explain the differences between the physiological and pathophysiological functions.

During the stimulation of gastric acid secretion, parietal cells alkalinize the mucosal interstitial fluid. The expression of AE2 variants with overlapping pHo sensitivities in the parietal cell BLM serves to extend the range of pHo. Under this range, pHo regulates parietal cell basolateral Cl^-/HCO_3^- exchange, while allowing other mechanisms of pHi homeostasis (35). Maintaining the pHi in the optimal range is essential for cell metabolism and survival. In addition, secretion leads to the decrease in cell volume which, in turn, leads to further changes in volume regulation to stabilize the shape of the cell, subcellular structure and concentration of cytoplasmic components, involving Na^+/H^+ exchange and Cl^-/HCO_3^- exchange, Na^+/HCO_3^- cotransport and $Na^+/K^+/2Cl^-$ cotransport (NKCC) (30).

AE2 may have a role in cell proliferation and apoptosis. AE2 increases intracellular chloride and mediates high glucose-induced apoptosis of umbilical vein endothelial cells in a time- and concentration-dependent manner through the mitochondrial permeability transition pore/ROS/caspase-3-dependent pathway (36). Studies on wound repair in rat gastric epithelial cells have shown that 4.4'-diisothiocyano-2,2'-stilbene disulfonic acid (DIDS), or the removal of Na^+, Cl^- and/or HCO_3^-, inhibit the key processes involved in wound healing, such as cell migration. The transport processes responsible for these phenomena remain unclear. However, NBCe1 and AE2 transcripts are detected in these cells.

In conclusion, AE2 has an important physiological function in the stomach, and the abnormality of AE2 may also lead to the occurrence of tumors. AE2 was found to be downregulated in gastric cancer cells (16), and its downregulation was correlated with poor differentiation and prognosis of gastric cancer (37). The lower expression of AE2 in gastric cancer was partly due to the promotion of ubiquitin-mediated rapid degradation in the presence of p16, whereas gastrin inhibited the growth of gastric cancer cells, at least partly by upregulating AE2 (37). The potential underlying mechanism is the stimulation of AE2 expression in gastric cancer cells through the transcription factor early growth response 1 (EGR1) in a cholecystokinin B receptor-dependent manner (38). It was previously reported that the combination of trastuzumab and gastrin exerted a synergistic inhibitory effect on human epidermal growth factor receptor 2-negative gastric cancer cells by targeting cytoplasmic AE1 and p16 inhibition, and that AE2 expression, which was originally decreased in gastric cancer tissues, was upregulated during combination therapy (39). It can be concluded that AE2 participates in the occurrence and development of gastric cancer, suggesting that AE2 may be a potential target for the treatment of gastric cancer.

5. AE2 and the intestine

AE2 and the small intestine. AE2 protein is expressed in the small intestine and colon, and is mainly localized in the BLM, exchanging HCO_3^- and Cl^- in an electroneutral manner. It is involved in physiological regulatory mechanisms, such as HCO_3^- secretion and Cl^- excretion. Ion secretion in the duodenum contributes to the formation of a liquid, buffered intraluminal environment with an optimized pH, protects the intestinal epithelium from gastric acid and promotes digestion in the proximal intestine (40). In the intestine, the osmotic gradient that pulls water into the intestine is mainly produced by Cl^- and, to a lesser extent by HCO_3^- secretion, with Na^+ passively following from the paracellular space (41). On the apical membrane, Cl^- is secreted into the intestinal lumen through three pathways, CFTR, calcium-activated Cl^- channel and the voltage-gated chloride channel protein 2, which further produces a driving osmotic gradient for fluid secretion (41). In the basolateral membrane, the ability of the intestine to secrete fluid depends on the NKCC cotransporter which mediates Cl^- uptake at the basolateral side of enterocytes, thus providing a substrate for apical Cl^- secretion (42). However, no significant manifestations of constipation were observed in patients treated with ring diuretics compared with the placebo group in a previous study (43), indicating that an alternative pathway for Cl^- uptake by enterocytes exists. AE2 may be a protein that assists Cl^- uptake in the mouse duodenum (44). Similarly, the phenomenon of AE2 acting as an assistant Cl^- uptake channel has already been observed in submandibular acinar cells involved in salivary gland fluid secretion (45). The duodenal mucosa senses luminal acidity through epithelial ion transporters and neuronal acid receptors, further increasing the absorption of luminal acid and HCO_3^- secretion, thereby maintaining the acid-base balance between the stomach and the duodenum (46). Chloride uptake via AE2 is coupled to basolateral $NaHCO_3$ cotransport to support CFTR-mediated Cl^- and HCO_3^- secretion (44). Both endogenous prostaglandin and NO are involved in the local regulation of acid-induced duodenal HCO_3^- secretion; NO stimulates HCO_3^- secretion by increasing prostaglandin production, and prostaglandin E2 stimulates HCO_3^- secretion by activating prostaglandin receptor subtypes EP3 and EP4 (47). In addition, estrogen can regulate HCO_3^- secretion in the duodenal mucosa of mice (48), but whether these regulatory factors regulate AE2 expression and function requires further study.

AE2 and the colon. The colon is responsible for regulating the electrolyte and water content of stool. In the human intestine, AE2 and bAE3 (the brain subtype) are expressed throughout the intestine, and both localize basolaterally in epithelial cells. Their expression is higher in the colon compared with the ileum and jejunum (49). The expression of AE2 and bAE3 in the mouse intestine is the same as that in the human intestine (50). AE2 polypeptides were found to be more abundant in colonic surface cells compared with crypt cells, whereas ileal crypts and villi exhibited a similar abundance of AE2 (50). AE2 has also been observed in the mural and vascular smooth muscle of the murine intestine (50). As previously reported, the osmotic gradient for the secretion of fluid into the intestine is mainly produced by Cl^- on the apical surface of epithelial cells and to a lesser extent by HCO_3^- secretion, with Na^+ passively following through the paracellular space (41). In the apical membrane, the three routes of HCO_3^- extrusion into the lumen are the Cl^-/HCO_3^- exchanger SLC26A3, CFTR

and the short chain fatty acid/HCO_3^- exchanger (51). On the basement membrane, HCO_3^- is transported into cells through Na^+/H^+ exchange (SLC family 9 member A1), as well as carbonic anhydrase and Na^+/HCO_3^- cotransporter (51). NKCC1 takes up Cl^- at the BLM (51). There is a carrier-mediated electroneutral Cl^-/HCO_3^- exchange in the BLM, which can function as a Cl^-/OH^- exchanger in the absence of HCO_3^- and modulate pHi in the BLM of the rat distal colon (52). A study on intestinal organoids showed that the CFTR knockout crypt epithelium maintains an alkaline pHi due to loss of Cl^- and HCO_3^- efflux, which impairs pHi regulation by AE2 (44). In the AE2 (-/-) colon, basolateral NKCC1-supported Cl^- secretion was increased, whereas HCO_3^- secretion was reduced (53). Spinophilin and CA XII enhanced the Cl^-/HCO_3^- exchange activity of AE2. Spinophilin bound to AE2 and significantly increased its anion exchange activity; in particular, the spinophilin 1-480 domain was required to enhance AE2 activity (54). The BLM-associated CA isoform CAXII colocalized with AE2 to the plasma membrane and significantly increased the activity of AE2 (54). Activators of Cl^- secretion include growth hormone, neuropeptides, opioids, norepinephrine and autocrine survival factors (55).

AE2 expression is increased in colon tumor tissue compared with adjacent non-cancerous tissue. In addition, the inhibition of AE2 expression in colorectal cancer cells has been found to decrease cell proliferation, indicating that AE2 may have a role in promoting colorectal cancer growth (56). Increased metabolism accelerates local acid production in cancer tissue. In one study, it was found that acid uptake was inhibited in colorectal cancer cells, whereas cellular acid uptake increased together with AE2 expression upon TGF-β1 stimulation (57). However, no acid transmission was observed between colorectal cancer cells with or without TGF-β1 treatment (57). Whether AE2 regulates pH in tumor tissue by assisting in the expulsion of HCO_3^-, and thus affecting the biological behavior of tumors, requires further study. Clinically, in a survival analysis of 57 patients with colon malignancies, AE2 expression was associated with poor prognosis (56). In colon cancer, AE2 expression promoted the proliferation of colon cancer cells, AE2 overexpression was correlated with Ki67 expression (a nuclear proliferation marker) and gastrin inhibited the proliferation of colon cancer cells by inhibiting the expression of EGR1 and AE2 and blocking extracellular signal-regulated kinase phosphorylation (56). The mechanism of AE2 function in colon cancer and its clinical significance require further study.

6. AE2 and the pancreas

The pancreas is a complex endocrine and exocrine organ; the exocrine pancreas is composed of acinar and ductal cells. AE2 is expressed in the BLM of acinar cells in the pancreas (58). It is also expressed in the pancreatic duct cells (59,60), but in low quantities (33). Acinar cells initially secrete an isotonic Cl^--rich fluid. Subsequently, pancreatic ductal cells alter the ionic composition of pancreatic juice and secrete large amounts of pancreatic juice and HCO_3^-. In humans, under conditions of pH 7.4 and 5% CO_2, the HCO_3^- equilibrium concentration is ~25 mM, and the HCO_3^- concentration in stimulated pancreatic juice can reach 140 mM (4), which is necessary for normal digestion. Since AE2 is minimally expressed in the duct, it is reasonable to hypothesize that the function of AE2 in the duct is very limited. Of note, previous data suggest that the chloride channel CFTR in pancreatic ductal cells may switch to the HCO_3^--conducting channel by activating with-no-lysine kinase 1 and the downstream kinases oxidative stress-responsive kinase1 and sterile 20/SPS1-related proline/alanine-rich kinase (61). The increase of CFTR HCO_3^-/Cl^- permeability from 0.4 to 1.0 has little effect on the secreted HCO_3^- concentration or volume flow. However, the model showed that the ~80% reduction in basolateral AE2 activity was essential for minimizing the intracellular Cl^- concentration following cyclic adenosine monophosphate (cAMP) stimulation, and thus maximized the secreted HCO_3^- concentration (62). Conversely, in the pancreas, whether AE2 is involved in HCO_3^- secretion and whether its regulation occurs in a similar manner has not yet been demonstrated. However, it has been experimentally shown that the knockdown of AE2 and CAXII inhibits AE2 activity and fluid secretion in the pancreatic and salivary ducts (63). Furthermore, the exchange activity of AE2 increased significantly following binding to spinophilin. In particular, the spinophilin1-480 domain was required to enhance AE2 activity. The BLM-associated CA isoform, CAXII, was found to colocalize with AE2 in the plasma membrane and to significantly increase the activity of AE2 (54). In conclusion, studies on the AE family's role in the process of Cl^-/HCO_3^- exchange in the pancreas are limited, and further research is required.

7. AE2 and the liver

AE2 is located in the extrahepatic bile duct and cholangiocytes (64), and its distribution is different from that of the aforementioned digestive organs. Subapical (65) or apical distribution (66) of AE2 has been observed in hepatobiliary epithelial cells. In addition, both AE2 and CFTR coexisted with water channel protein aquaporin 1 (AQP1) in the vesicles of cholangiocytes, which were concentrated in the apical membrane of cholangiocytes stimulated by cAMP and secretin (65). AE2 mRNA signals were detectable in the cytoplasm of some hepatocytes (mainly periportal) (67). Even at the apical membrane, the direction of AE2-driven ion transport was found to remain unchanged, with AE2 still pumping in one Cl^- and pumping out one HCO_3^-, demonstrating that AE2 can participate in the secretion of HCO_3^-. Collectively, these results demonstrated that AE2 is involved in the regulation of pHi homeostasis and secretin-stimulated biliary HCO_3^- secretion. Biliary HCO_3^- is largely dependent on apical HCO_3^- release. The main exchangers involved in Cl^-/HCO_3^- exchange in apical bile duct membranes are AE2, cAMP-regulated Cl^- channels and Ca^{2+}-regulated Cl^- channels (68). AE2 contributes to the creation of an alkaline protective environment in the bile duct lumen through Cl^-/HCO_3^- exchange at the apical membrane and HCO_3^- secretion (69), thus protecting the liver from bile acid attack (5). An intact glycocalyx is present on the apical membrane of cholangiocytes, and the intracellular HCO_3^- sensor soluble adenylyl cyclase (sAC) and functional AE2 are crucial for maintaining biliary HCO_3^- concentration and protecting cholangiocytes from toxic bile acid-induced apoptosis (70-72). The bile duct requires biliary cellular

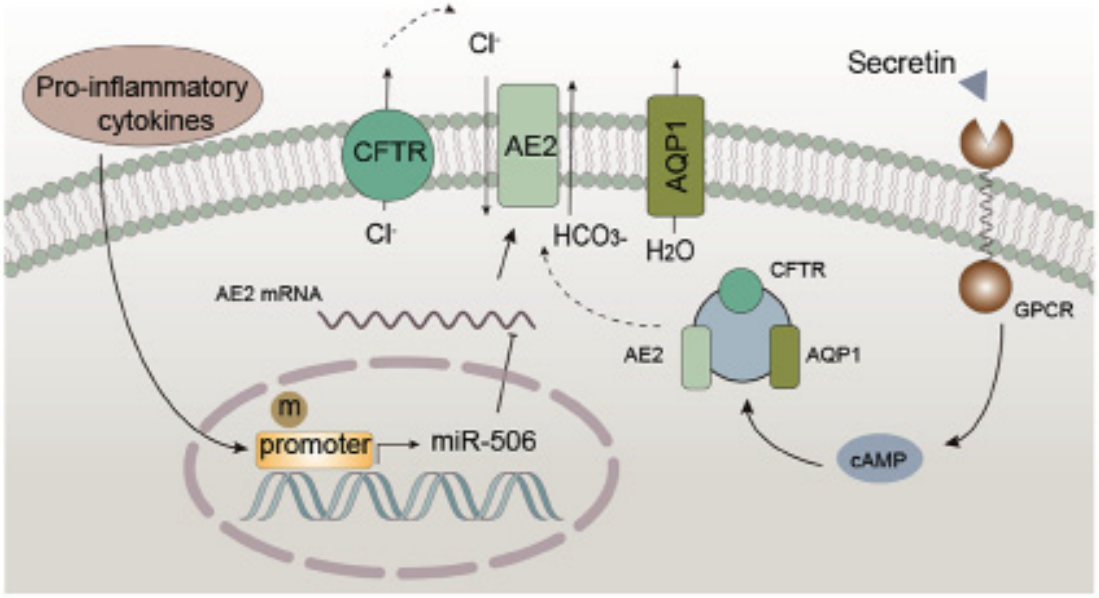

Figure 3. Regulatory mechanism of AE2 in cholangiocytes. Pro-inflammatory cytokines (IL-8, IL-12, IL-17, IL-18 and TNF-α) enhance the expression of miR-506, which directly targets AE2, thereby inhibiting the expression of AE2. In addition, the hypermethylation of the AE2 promoter region inhibits AE2 expression. Under physiological conditions, secretin binds to receptors and promotes cAMP/protein kinase C-dependent exocytosis of vesicles containing CFTR, AE2 and AQP1 in the apical membrane of cells. This results in the secretion of Cl^- via CFTR and further exchange with HCO_3^- via AE2, creating a luminal osmotic gradient for AQP1 to move water, leading to biliary secretion. AE2, anion exchanger 2; miR, microRNA; cAMP, cyclic adenosine monophosphate; CFTR, cystic fibrosis transmembrane conductance regulator; AQP1, aquaporin 1; GPCR, G protein-coupled receptor.

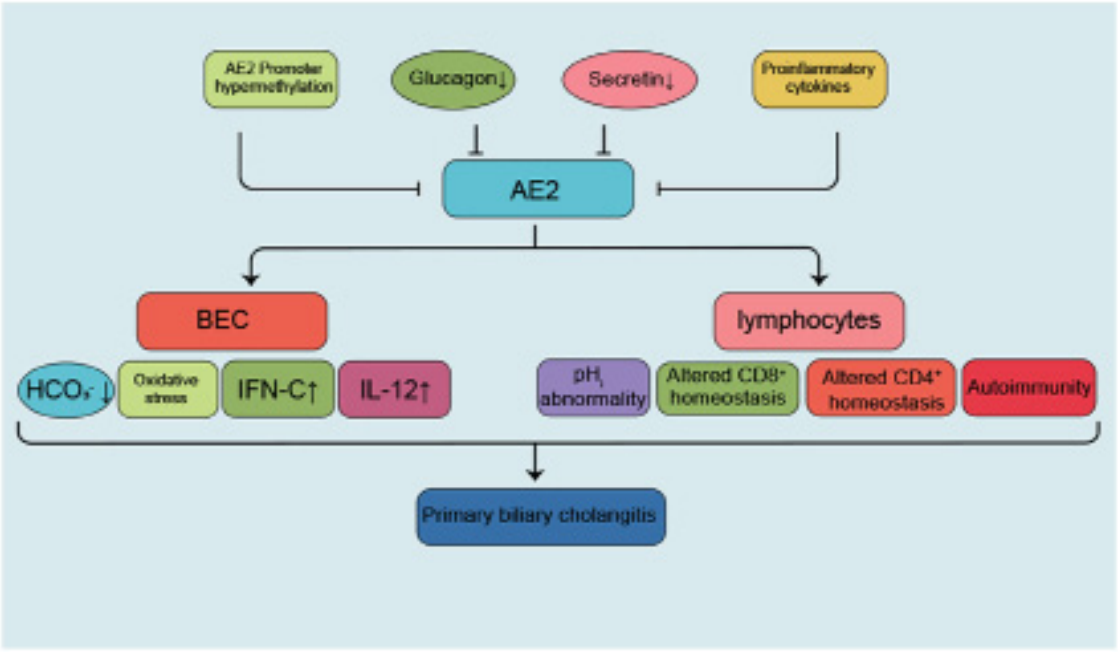

Figure 4. AE2-associated pathogenesis of PBC. In PBC, various factors, such as insufficient secretion of glucagon or secretin and pro-inflammatory cytokines (IL-8, IL-12, IL-17, IL-18 and TNF-α), lead to reduced expression and/or function of AE2, resulting in impaired bile HCO_3^- secretion, followed by bile duct injury and cholestasis. Finally, the destruction of cholangiocyte autoimmune response and its sensitivity to apoptotic injury result in PBC. AE2, anion exchanger 2; PBC, primary biliary cholangitis; BEC, bile duct cell; IFN, interferon.

HCO_3^- secretion to maintain the ionization of toxic bile acids and, thus, make them membrane-impermeable (68,70). Therefore, the expression of AE2 affects bile acid uptake in immortalized non-malignant human intrahepatic cholangial and human cholangiocarcinoma cells. In addition, the bile acid uptake rate and toxicity are high in the presence of decreased AE2 expression in those cells (73). AE2 internalization in hepatocytes may result in decreased tubular HCO_3^- output and decreased bile flow (73). In addition, AE2 is responsible for maintaining pHi in cholangiocytes through Cl^-/HCO_3^- exchange (69). Mouse cholangiocytes express NBCe1, which may compensate for the lack of AE2 (74). As illustrated in Fig. 3, AE2 is known to be

Table II. Physiology and pathophysiology characteristics of AE2.

Digestive organ	Distribution	Physiological function	Pathophysiology
Esophagus	Basolateral membrane	AE2 may further cause fluid secretion by mediating Cl^- secretion into the lumen across cells in the basal layer.	1. In esophageal squamous cell carcinoma, AE2 is upregulated. 2. Low-grade expression of AE2 at the invasive front is associated with shorter postoperative survival (sample size, 61).
Stomach	Basolateral membrane	AE2 assists parietal cells to expel HCO_3^- and import Cl^-. Maintains pHi within optimum range.	1. In gastric cancer, AE2 is downregulated. 2. AE2 expression is associated with poor prognosis.
Small intestine	Basolateral membrane	Bicarbonate secretion and Cl^- excretion provide an appropriate pH environment, protect the intestinal epithelium from gastric acid, and promote digestion in the intestine.	Unknown
Colon	Basolateral membrane	1. AE2 binding to SLC9A1 constitutes a minor component of colonic Cl^- uptake. 2. AE2 is involved in regulating pHi in the basolateral membrane of the rat distal colon.	1. In colorectal cancer, AE2 is upregulated. 2. AE2 expression is associated with poor prognosis (sample size, 57).
Pancreas	Basolateral membrane of acinar cells. Pancreatic duct cells, but in limited quantities.	An ~80% reduction in basolateral AE2 activity is obtained by maximizing the secreted HCO_3^- concentration.	Unknown
Liver	Subapical or apical membrane of hepatobiliary epithelial cells. Apical membrane of cholangiocytes.	AE2 is involved in the regulation of pHi homeostasis and secretin-stimulated biliary bicarbonate secretion.	1. Decreased expression of AE2 leads to insufficient biliary bicarbonate secretion under secretin stimulation. 2. In primary biliary cholangitis, AE2 expression and/or function are reduced, leading to bile duct injury. 3. In hepatocellular carcinoma, AE2 is upregulated.

From Refs (15-17,28,29,38,41,52,53,57,63,70,72,74,84,94,95). AE2, anion exchanger 2; pHi, intracellular pH; SLC9A1, solute carrier family 9 member A1.

regulated by glucagon, secretin, and the microRNA (miR)-506 which is located on the X chromosome (75,76). The target organs of glucagon and secretin are hepatocytes and cholangiocytes, respectively. These two hormones appear to be quite similar and to interact with their specific G protein-coupled receptors, causing an increase in the intracellular cAMP level and activating cAMP-dependent Cl^- and HCO_3^- secretion. Both hepatocytes and cholangiocytes appear to have cAMP-responsive intracellular vesicles (76). Glucagon may induce pericanalicular vesicles which contain AE2 and the water channel AQP8 and the glutathione carrier ATP binding cassette subfamily C member 2 to the canalicular membrane in hepatocytes, leading to canalicular hypersecretion of HCO_3^--rich bile; secretin may also stimulate ductal HCO_3^- secretion by interacting with secretin receptors that activate cAMP/CFTR/AE2 signaling, which is increased by biliary hyperplasia (77). The proinflammatory cytokines interleukin (IL)-8, IL-12, IL-17, IL-18 and tumor necrosis factor (TNF)-α enhance the expression of miR-506 in the biliary epithelium, which inhibits AE2 expression by directly targeting AE2 mRNA, and sensitizes cholangiocytes to bile salt-induced apoptosis (BSIA) (78). In addition, miR-506 is located on the X chromosome (79), which explains the fact that PBC mainly affects women and emphasizes the role of the X chromosome in PBC. Increased AE2 protein expression and activity are the main reasons for the increase in rifampicin-induced bile flow (80). AE2 knockdown sensitized immortalized H69 human cholangial cells to not only etoposide-induced apoptosis, but also BSIA. Mechanistically, AE2 downregulation mediates apoptosis through the activation of sAC and then through the intrinsic apoptotic pathway, since sAC is an evolutionarily conserved HCO_3^- sensor that regulates

apoptosis, barrier function and TNF signaling. In addition, sAC inhibition prevents Bax phosphorylation at Thr167, as well as Bax translocation into mitochondria and cytochrome c release; these molecules are involved in endogenous apoptosis during BSIA in cholangiocytes (71). This process depends on intracellular Ca^{2+} storage (71). Insufficient AE2 function in lymphocytes may interfere with pHi regulation in cells and alter immune homeostasis, leading to autoimmunity (81). On the other hand, as aforementioned (71), AE2 downregulation sensitized immortalized human cholangiocytes to BSIA which, together with alterations in immune homeostasis, may favor the production of antimitochondrial antibodies and autoimmune attack in the bile duct (71).

AE2 and primary biliary cholangitis. PBC and liver cancer are the main liver diseases associated with AE2. Biliary atresia-specific induced pluripotent stem cells were found to exhibit reduced AE2 expression (82). Similarly, in patients with PBC, the expression of AE2 in liver biopsies was significantly reduced compared with healthy controls (83), and AE2 activity was also decreased (84). Decreased AE2 expression is associated with dysregulated autophagy, abnormal pyruvate dehydrogenase complex, E2 component expression, cellular senescence, as well as hypermethylation of the AE2 promoter region in PBC bile duct lesions, followed by the insufficiency of secretin-stimulated bile bicarbonate secretion (17,83). In addition, secondary inflammatory and pro-inflammatory cytokine-mediated injury could lead to a decrease in AE2-mediated bile secretion (85). AE2 deficiency of PBC immunocytes may have an essential role in autoimmune phenomena (86,87). Furthermore, AE2 downregulation was found to sensitize cholangiocytes to apoptotic damage (71). Consistently, AE2 (a and b) (-/-) mice displayed PBC-like features, including extensive portal inflammation, bile duct injury and infiltration by surrounding $CD4^+$ and $CD8^+$ T lymphocytes, increased oxidative stress in cholangiocytes, liver fibrosis, increased production of interferon-c and IL-12, increased serum IgM, IgG and liver alkaline phosphatase levels, and serum detectable antimitochondrial antibodies (88). Among them, $CD8^+$ T cells were found to rely on AE2 to maintain pHi at physiological levels (89). Therefore, AE2 (a and b) (-/-) mice exhibited a higher number of intrahepatic cytotoxic $CD8^+$ T cells, which inhibited programmed cell death-1 (PD-1) expression, accompanied by a reduction in apoptosis, thus favoring chronic immune-mediated cholangitis (87). Early in the life of mice, AE2 deficiency leads to intrahepatic T-cell activation and PD-1/programmed cell death ligand 1-mediated deletion. With age, intrahepatic $CD8^+$ T cells epigenetically inhibit PD-1, and their corresponding expansion and further activation favor autoimmune cholangitis (87). In conclusion, the possible mechanism through which AE2 mediates PBC is that, in PBC, various factors, such as glucagon, insufficient secretion of secretin and pro-inflammatory cytokines (IL-8, IL-12, IL-17, IL-18 and TNF-α), lead to a reduced expression and/or function of AE2. This leads to impaired bile HCO_3^- secretion, followed by bile duct cell injury and cholestasis, as well as destruction of cholangiocyte autoimmune response and increased sensitivity to apoptotic injury, finally resulting in bile duct injury (Fig. 4). In a clinical study of 258 patients with PBC, and two independent groups of 286 and 269 healthy controls, AE2 variants were shown to be an independent prognostic factor for PBC by multivariate Cox regression analysis that included clinical and biochemical parameters (90); the progression of liver disease under ursodeoxycholic acid (UDCA) treatment was found to be significantly associated with single-nucleotide polymorphisms in the TNF-α and AE2 genes (90). UDCA can upregulate the expression of bile salt export pump, but not that of AE2 (91). UDCA is conjugated to promote secretin-stimulated hydrocholeresis through AE2, intracellular Ca^{2+}, microtubules, protein kinase C-α, PI3K, protein kinase A and mitogen-activated protein kinase kinase (92). Similarly, the secretion of carbonate-rich bile was significantly enhanced in rats treated with UDCA (92). These data strongly suggest that AE2 may serve as a novel target for the diagnosis and treatment of PBC.

AE2 and liver cancer. AE2 was found to be overexpressed in hepatocellular carcinoma (93,94). AE2 knockdown significantly reduced the viability of poorly differentiated HA22T/VGH cells and arrested the cell cycle in the sub-G1 phase. In addition, treatment with inhibitor DIDS significantly inhibited cell proliferation and induced apoptosis of the poorly differentiated HA22T/VGH cells (94,95). It has been found that CA IX interacts with AE2 and Na^+-HCO_3^- cotransporter (NBCe1) in lamellipodia and increases cell migration through its ability to facilitate ion transport and pH control at the protruding front of moving cells (96). Whether AE2 is involved in other biological behaviors of liver cancer requires further study.

8. Conclusion

In conclusion, AE2 is involved in the development and progression of digestive system diseases by regulating intracellular and extracellular Cl^-/HCO_3^- exchange and further regulating cellular function. The physiology and pathophysiology of AE2 are summarized in Table II. The role of AE2 in digestive system diseases, such as PBC, and digestive tract tumors, is set to become a new research hotspot. AE2 may become a novel molecular marker for the diagnosis and treatment of digestive system diseases, and drug development for AE2 may open up a new direction for the diagnosis and treatment of digestive system diseases in the future.

Acknowledgements

Not applicable.

Funding

The present study was supported by grants from the National Natural Science Foundation of China (grant no. 82073087), National Natural Science Foundation of China (grant no. 81960507), the Guizhou Provincial Department of Education Youth Science and Technology Talents Growth Project [grant no. QIAN-JIAO-HE KY ZI (2018)236], the Zunyi Medical University 2017 New Academic Cultivation and Innovation Exploration Special Project [grant no. Qian-Ke-He-Ping-Tai-Ren-Cai (2017)5733-072].

Availability of data and materials

Not applicable.

Authors' contributions

HW wrote the manuscript; JA, HJ and CL collected, analyzed and organized the relevant literature; SH and JW revised the grammar of the manuscript; BT revised the manuscript for clarity and style. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Valdivieso ÁG and Santa-Coloma TA: The chloride anion as a signalling effector. Biol Rev Camb Philos Soc 94: 1839-1856, 2019.
2. Trampert DC, van de Graaf SFJ, Jongejan A, Oude Elferink RPJ and Beuers U: Hepatobiliary acid-base homeostasis: Insights from analogous secretory epithelia. J Hepatol 74: 428-441, 2020.
3. Jun I, Cheng MH, Sim E, Jung J, Suh BL, Kim Y, Son H, Park K, Kim CH, Yoon JH, *et al*: Pore dilatation increases the bicarbonate permeability of CFTR, ANO1 and glycine receptor anion channels. J Physiol 594: 2929-2955, 2016.
4. Shin DH, Kim M, Kim Y, Jun I, Jung J, Nam JH, Cheng MH and Lee MG: Bicarbonate permeation through anion channels: Its role in health and disease. Pflugers Arch 472: 1003-1018, 2020.
5. Parker MD and Boron WF: The divergence, actions, roles, and relatives of sodium-coupled bicarbonate transporters. Physiol Rev 93: 803-959, 2013.
6. Bertocchio JP, Genetet S, Da Costa L, Walsh SB, Knebelmann B, Galimand J, Bessenay L, Guitton C, De Lafaille R, Vargas-Poussou R, *et al*: Red blood cell AE1/band 3 transports in dominant distal renal tubular acidosis patients. Kidney Int Rep 5: 348-357, 2020.
7. Casey JR, Sly WS, Shah GN and Alvarez BV: Bicarbonate homeostasis in excitable tissues: Role of AE3 Cl^-/HCO_3^- exchanger and carbonic anhydrase XIV interaction. Am J Physiol Cell Physiol 297: C1091-C1102, 2009.
8. Sowah D, Brown BF, Quon A, Alvarez BV and Casey JR: Resistance to cardiomyocyte hypertrophy in $ae3^{-/-}$ mice, deficient in the AE3 Cl^-/HCO_3^- exchanger. BMC Cardiovasc Disord 14: 89, 2014.
9. Kopito RR, Lee BS, Simmons DM, Lindsey AE, Morgans CW and Schneider K: Regulation of intracellular pH by a neuronal homolog of the erythrocyte anion exchanger. Cell 59: 927-937, 1989.
10. Linn SC, Kudrycki KE and Shull GE: The predicted translation product of a cardiac AE3 mRNA contains an N terminus distinct from that of the brain AE3 Cl^-/HCO_3^- exchanger. Cloning of a cardiac AE3 cDNA, organization of the AE3 gene, and identification of an alternative transcription initiation site. J Biol Chem 267: 7927-7935, 1992.
11. Bronckers AL, Jalali R and Lytton J: Reduced protein expression of the $Na^+/Ca^{2+}+K^+$-exchanger (SLC24A4) in apical plasma membranes of maturation ameloblasts of fluorotic mice. Calcif Tissue Int 100: 80-86, 2017.
12. Celay J, Lozano T, Concepcion AR, Beltrán E, Rudilla F, García-Barchino MJ, Robles EF, Rabal O, de Miguel I, Panizo C, *et al*: Targeting the anion exchanger 2 with specific peptides as a new therapeutic approach in B lymphoid neoplasms. Haematologica 103: 1065-1072, 2018.
13. Vigliarolo T, Zocchi E, Fresia C, Booz V and Guida L: Abscisic acid influx into human nucleated cells occurs through the anion exchanger AE2. Int J Biochem Cell Biol 75: 99-103, 2016.
14. Wu C, Liu X, Sun R, Sun R, Qin Y, Liu Z, Yang S, Tang T, Zhu Z, Yu D and Liu F: Targeting anion exchange of osteoclast, a new strategy for preventing wear particles induced-osteolysis. Front Pharmacol 9: 1291, 2018.
15. Gawenis LR, Ledoussal C, Judd LM, Prasad V, Alper SL, Stuart-Tilley A, Woo AL, Grisham C, Sanford LP, Doetschman T, *et al*: Mice with a targeted disruption of the AE2 Cl^-/HCO_3^- exchanger are achlorhydric. J Biol Chem 279: 30531-30539, 2004.
16. Yang Y, Wu PP, Wu J, Shen WW, Wu YL, Fu AF, Zheng L, Jin XL and Fu GH: Expression of anion exchanger 2 in human gastric cancer. Exp Oncol 30: 81-87, 2008.
17. Sasaki M, Sato Y and Nakanuma Y: An impaired biliary bicarbonate umbrella may be involved in dysregulated autophagy in primary biliary cholangitis. Lab Invest 98: 745-754, 2018.
18. Alper SL: Molecular physiology and genetics of Na^+-independent SLC4 anion exchangers. J Exp Biol 212: 1672-1683, 2009.
19. Romero MF: Molecular pathophysiology of SLC4 bicarbonate transporters. Curr Opin Nephrol Hypertens 14: 495-501, 2005.
20. Cordat E and Reithmeier RA: Structure, function, and trafficking of SLC4 and SLC26 anion transporters. Curr Top Membr 73: 1-67, 2014.
21. Liu Y, Yang J and Chen LM: Structure and function of SLC4 Family [Formula: See text] Transporters. Front Physiol 6: 355, 2015.
22. Stewart AK, Kurschat CE, Vaughan-Jones RD and Alper SL: Putative re-entrant loop 1 of AE2 transmembrane domain has a major role in acute regulation of anion exchange by pH. J Biol Chem 284: 6126-6139, 2009.
23. Stewart AK, Kerr N, Chernova MN, Alper SL and Vaughan-Jones RD: Acute pH-dependent regulation of AE2-mediated anion exchange involves discrete local surfaces of the NH2-terminal cytoplasmic domain. J Biol Chem 279: 52664-52676, 2004.
24. Layden TJ, Schmidt L, Agnone L, Lisitza P, Brewer J and Goldstein JL: Rabbit esophageal cell cytoplasmic pH regulation: Role of Na^+-H^+ antiport and H^+-dependent HCO_3^- transport systems. Am J Physiol 263: G407-G413, 1992.
25. Tobey NA, Reddy SP, Khalbuss WE, Silvers SM, Cragoe EJ Jr and Orlando RC: Na(+)-dependent and -independent Cl^-/HCO_3^- exchangers in cultured rabbit esophageal epithelial cells. Gastroenterology 104: 185-195, 1993.
26. Laczkó D, Rosztóczy A, Birkás K, Katona M, Rakonczay Z Jr, Tiszlavicz L, Róka R, Wittmann T, Hegyi P and Venglovecz V: Role of ion transporters in the bile acid-induced esophageal injury. Am J Physiol Gastrointest Liver Physiol 311: G16-G31, 2016.
27. Abdulnour-Nakhoul S, Nakhoul HN, Kalliny MI, Kalliny MI, Gyftopoulos A, Rabon E, Doetjes R, Brown K and Nakhoul NL: Ion transport mechanisms linked to bicarbonate secretion in the esophageal submucosal glands. Am J Physiol Regul Integr Comp Physiol 301: R83-R96, 2011.
28. Shiozaki A, Hikami S, Ichikawa D, Kosuga T, Shimizu H, Kudou M, Yamazato Y, Kobayashi T, Shoda K, Arita T, *et al*: Anion exchanger 2 suppresses cellular movement and has prognostic significance in esophageal squamous cell carcinoma. Oncotarget 9: 25993-26006, 2018.
29. Persi E, Duran-Frigola M, Damaghi M, Roush WR, Aloy P, Cleveland JL, Gillies RJ and Ruppin E: Systems analysis of intracellular pH vulnerabilities for cancer therapy. Nat Commun 9: 2997, 2018.
30. Seidler U, Song P, Xiao F, Riederer B, Bachmann O and Chen M: Recent advances in the molecular and functional characterization of acid/base and electrolyte transporters in the basolateral membranes of gastric and duodenal epithelial cells. Acta Physiol (Oxf) 201: 3-20, 2011.
31. Humphreys BD, Jiang L, Chernova MN and Alper SL: Hypertonic activation of AE2 anion exchanger in Xenopus oocytes via NHE-mediated intracellular alkalinization. Am J Physiol 268: C201-C209, 1995.

32. Rossmann H, Bachmann O, Wang Z, Shull GE, Obermaier B, Stuart-Tilley A, Alper SL and Seidler U: Differential expression and regulation of AE2 anion exchanger subtypes in rabbit parietal and mucous cells. J Physiol 534: 837-848, 2001.
33. Alper SL: Molecular physiology of SLC4 anion exchangers. Exp Physiol 91: 153-161, 2006.
34. Recalde S, Muruzábal F, Looije N, Kunne C, Burrell MA, Sáez E, Martínez-Ansó E, Salas JT, Mardones P, Prieto J, *et al*: Inefficient chronic activation of parietal cells in Ae2a,b(-/-) mice. Am J Pathol 169: 165-176, 2006.
35. Kurschat CE, Shmukler BE, Jiang L, Wihelm S, Kim EH, Chernova MN, Kinne RK, Stewart AK and Alper SL: Alkaline-shifted pHo sensitivity of AE2c1-mediated anion exchange reveals novel regulatory determinants in the AE2 N-terminal cytoplasmic domain. J Biol Chem 281: 1885-1896, 2006.
36. Huang QR, Li Q, Chen YH, Li L, Liu LL, Lei SH, Chen HP, Peng WJ and He M: Involvement of anion exchanger-2 in apoptosis of endothelial cells induced by high glucose through an mPTP-ROS-Caspase-3 dependent pathway. Apoptosis 15: 693-704, 2010.
37. Wang T, Fei HJ, Yang Y, Jiang XS, Yan M, Zeng Z, Wu J, Song LJ, Tian H and Fu GH: Expression of AE1/p16 promoted degradation of AE2 in gastric cancer cells. BMC Cancer 16: 716, 2016.
38. Wang T, Zhao L, Yang Y, Tian H, Suo WH, Yan M and Fu GH: EGR1 is critical for gastrin-dependent upregulation of anion exchanger 2 in gastric cancer cells. FEBS J 280: 174-183, 2013.
39. Cui Y, Li SB, Peng XC, Wu J and Fu GH: Trastuzumab inhibits growth of HER2-negative gastric cancer cells through gastrin-initialized CCKBR signaling. Dig Dis Sci 60: 3631-3641, 2015.
40. Walker NM, Flagella M, Gawenis LR, Shull GE and Clarke LL: An alternate pathway of cAMP-stimulated Cl secretion across the NKCC1-null murine duodenum. Gastroenterology 123: 531-541, 2002.
41. Murek M, Kopic S and Geibel J: Evidence for intestinal chloride secretion. Exp Physiol 95: 471-478, 2010.
42. Zhu JX, Xue H, Ji T and Xing Y: Cellular localization of NKCC2 and its possible role in the Cl- absorption in the rat and human distal colonic epithelia. Transl Res 158: 146-154, 2011.
43. Hashemian F, Ghorbanian MA, Hashemian F, Mortazavi SA, Sheikhi M, Jahanshahi J and Poorolajal J: Effect of topical furosemide on rhinosinusal polyposis relapse after endoscopic sinus surgery: A randomized clinical trial. JAMA Otolaryngol Head Neck Surg 142: 1045-1049, 2016.
44. Walker NM, Liu J, Stein SR, Stefanski CD, Strubberg AM and Clarke LL: Cellular chloride and bicarbonate retention alters intracellular pH regulation in Cftr KO crypt epithelium. Am J Physiol Gastrointest Liver Physiol 310: G70-G80, 2016.
45. Peña-Münzenmayer G, Catalán MA, Kondo Y, Jaramillo Y, Liu F, Shull GE and Melvin JE: Ae4 (Slc4a9) anion exchanger drives Cl- uptake-dependent fluid secretion by mouse submandibular gland acinar cells. J Biol Chem 290: 10677-10688, 2015.
46. Kaji I and Kaunitz JD: Luminal chemosensing in the gastroduodenal mucosa. Curr Opin Gastroenterol 33: 439-445, 2017.
47. Takeuchi K, Kita K, Hayashi S and Aihara E: Regulatory mechanism of duodenal bicarbonate secretion: Roles of endogenous prostaglandins and nitric oxide. Pharmacol Ther 130: 59-70, 2011.
48. Tuo B, Wen G, Wei J, Liu X, Wang X, Zhang Y, Wu H, Dong X, Chow JY, Vallon V and Dong H: Estrogen regulation of duodenal bicarbonate secretion and sex-specific protection of human duodenum. Gastroenterology 141: 854-863, 2011.
49. Alrefai WA, Tyagi S, Nazir TM, Barakat J, Anwar SS, Hadjiagapiou C, Bavishi D, Sahi J, Malik P, Goldstein J, *et al*: Human intestinal anion exchanger isoforms: Expression, distribution, and membrane localization. Biochim Biophys Acta 1511: 17-27, 2001.
50. Alper SL, Rossmann H, Wilhelm S, Stuart-Tilley AK, Shmukler BE and Seidler U: Expression of AE2 anion exchanger in mouse intestine. Am J Physiol 277: G321-G332, 1999.
51. Bachmann O and Seidler U: News from the end of the gut-how the highly segmental pattern of colonic HCO_3^- transport relates to absorptive function and mucosal integrity. Biol Pharm Bull 34: 794-802, 2011.
52. Ikuma M, Geibel J, Binder HJ and Rajendran VM: Characterization of Cl-HCO_3 exchange in basolateral membrane of rat distal colon. Am J Physiol Cell Physiol 285: C912-C921, 2003.
53. Gawenis LR, Bradford EM, Alper SL, Prasad V and Shull GE: AE2 Cl^-/HCO_3^- exchanger is required for normal cAMP-stimulated anion secretion in murine proximal colon. Am J Physiol Gastrointest Liver Physiol 298: G493-G503, 2010.
54. Jeong YS and Hong JH: Governing effect of regulatory proteins for Cl(-)/HCO_3(-) exchanger 2 activity. Channels (Austin) 10: 214-224, 2016.
55. O'Mahony F and Harvey BJ: Sex and estrous cycle-dependent rapid protein kinase signaling actions of estrogen in distal colonic cells. Steroids 73: 889-894, 2008.
56. Song LJ, Liu RJ, Zeng Z, Alper SL, Cui HJ, Lu Y, Zheng L, Yan ZW and Fu GH: Gastrin inhibits a novel, pathological colon cancer signaling pathway involving EGR1, AE2, and P-ERK. J Mol Med (Berl) 90: 707-718, 2012.
57. Hulikova A, Black N, Hsia LT, Wilding J, Bodmer WF and Swietach P: Stromal uptake and transmission of acid is a pathway for venting cancer cell-generated acid. Proc Natl Acad Sci USA 113: E5344-E5353, 2016.
58. Roussa E, Alper SL and Thévenod F: Immunolocalization of anion exchanger AE2, Na(+)/H(+) exchangers NHE1 and NHE4, and vacuolar type H(+)-ATPase in rat pancreas. J Histochem Cytochem 49: 463-474, 2001.
59. Rakonczay Z Jr, Fearn A, Hegyi P, Boros I, Gray MA and Argent BE: Characterization of H^+ and HCO_3^- transporters in CFPAC-1 human pancreatic duct cells. World J Gastroenterol 12: 885-895, 2006.
60. Rakonczay Z Jr, Hegyi P, Hasegawa M, Inoue M, You J, Lida A, Lgnáth I, Alton EWFWA, Griesenbach U, Ovári G, *et al*: CFTR gene transfer to human cystic fibrosis pancreatic duct cells using a Sendai virus vector. J Cell Physiol 214: 442-455, 2008.
61. Park HW, Nam JH, Kim JY, Namkung W, Yoon JS, Lee JS, Kim KS, Venglovecz V, Gray MA, Kim KH and Lee MG: Dynamic regulation of CFTR bicarbonate permeability by [Cl-]i and its role in pancreatic bicarbonate secretion. Gastroenterology 139: 620-631, 2010.
62. Yamaguchi M, Steward MC, Smallbone K, Sohma Y, Yamamoto A, Ko SB, Kondo T and Ishiguro H: Bicarbonate-rich fluid secretion predicted by a computational model of guinea-pig pancreatic duct epithelium. J Physiol 595: 1947-1972, 2017.
63. Hong JH, Muhammad E, Zheng C, Hershkovitz E, Alkrinawi S, Loewenthal N, Parvari R and Muallem S: Essential role of carbonic anhydrase XII in secretory gland fluid and HCO_3(-) secretion revealed by disease causing human mutation. J Physiol 593: 5299-5312, 2015.
64. Venter J, Francis H, Meng F, DeMorrow S, Kennedy L, Standeford H, Hargrove L, Wu N, Wan Y, Frampton G, *et al*: Development and functional characterization of extrahepatic cholangiocyte lines from normal rats. Dig Liver Dis 47: 964-972, 2015.
65. Tietz PS, Marinelli RA, Chen XM, Huang B, Cohn J, Kole J, McNiven MA, Alper S and LaRusso NF: Agonist-induced coordinated trafficking of functionally related transport proteins for water and ions in cholangiocytes. J Biol Chem 278: 20413-20419, 2003.
66. Aranda V, Martínez I, Melero S, Lecanda J, Banales JM, Prieto J and Medina JF: Shared apical sorting of anion exchanger isoforms AE2a, AE2b1, and AE2b2 in primary hepatocytes. Biochem Biophys Res Commun 319: 1040-1046, 2004.
67. García C, Montuenga LM, Medina JF and Prieto J: In situ detection of AE2 anion-exchanger mRNA in the human liver. Cell Tissue Res 291: 481-488, 1998.
68. Beuers U, Maroni L and Elferink RO: The biliary HCO(3)(-) umbrella: Experimental evidence revisited. Curr Opin Gastroenterol 28: 253-257, 2012.
69. Rodrigues PM, Perugorria MJ, Santos-Laso A, Bujanda L, Beuers U and Banales JM: Primary biliary cholangitis: A tale of epigenetically-induced secretory failure? J Hepatol 69: 1371-1383, 2018.
70. Hohenester S, Wenniger LM, Paulusma CC, van Vliet SJ, Jefferson DM, Elferink RP and Beuers U: A biliary HCO_3-umbrella constitutes a protective mechanism against bile acid-induced injury in human cholangiocytes. Hepatology 55: 173-183, 2012.
71. Chang JC, Go S, de Waart DR, Munoz-Garrido P, Beuers U, Paulusma CC and Oude Elferink R: Soluble adenylyl cyclase regulates bile salt-induced apoptosis in human cholangiocytes. Hepatology 64: 522-534, 2016.
72. Maillette de Buy Wenniger LJ, Hohenester S, Maroni L, van Vliet SJ, Oude Elferink RP and Beuers U: The cholangiocyte glycocalyx stabilizes the 'Biliary HCO_3 umbrella': An integrated line of defense against toxic bile acids. Dig Dis 33: 397-407, 2015.

73. Miszczuk GS, Banales JM, Zucchetti AE, Pisani GB, Boaglio AC, Saez E, Medina JF, Roma MG and Crocenzi FA: Adaptive downregulation of Cl^-/HCO_3^- exchange activity in rat hepatocytes under experimental obstructive cholestasis. PLoS One 14: e0212215, 2019.
74. Uriarte I, Banales JM, Sáez E, Arenas F, Oude Elferink RP, Prieto J and Medina JF: Bicarbonate secretion of mouse cholangiocytes involves Na(+)-HCO(3)(-) cotransport in addition to Na(+)-independent Cl(-)/HCO(3)(-) exchange. Hepatology 51: 891-902, 2010.
75. Erice O, Munoz-Garrido P, Vaquero J, Perugorria MJ, Fernandez-Barrena MG, Saez E, Santos-Laso A, Arbelaiz A, Jimenez-Agüero R, Fernandez-Irigoyen J, *et al*: MicroRNA-506 promotes primary biliary cholangitis-like features in cholangiocytes and immune activation. Hepatology 67: 1420-1440, 2018.
76. Banales JM, Prieto J and Medina JF: Cholangiocyte anion exchange and biliary bicarbonate excretion. World J Gastroenterol 12: 3496-3511, 2006.
77. Renzi A, DeMorrow S, Onori P, Carpino G, Mancinelli R, Meng F, Venter J, White M, Franchitto A, Francis H, *et al*: Modulation of the biliary expression of arylalkylamine N-acetyltransferase alters the autocrine proliferative responses of cholangiocytes in rats. Hepatology 57: 1130-1141, 2013.
78. Banales JM, Sáez E, Uriz M, Sarvide S, Urribarri AD, Splinter P, Tietz Bogert PS, Bujanda L, Prieto J, Medina JF and LaRusso NF: Up-regulation of microRNA 506 leads to decreased Cl^-/HCO_3^- anion exchanger 2 expression in biliary epithelium of patients with primary biliary cirrhosis. Hepatology 56: 687-697, 2012.
79. Brooks WH and Renaudineau Y: Epigenetics and autoimmune diseases: The X chromosome-nucleolus nexus. Front Genet 6: 22, 2015.
80. Wang W, Ren X, Cai Y, Chen L, Zhang W and Xu J: Rifampicin induces bicarbonate-rich choleresis in rats: Involvement of anion exchanger 2. Dig Dis Sci 61: 126-136, 2016.
81. Medina JF: Role of the anion exchanger 2 in the pathogenesis and treatment of primary biliary cirrhosis. Dig Dis 29: 103-112, 2011.
82. Tian L, Ye Z, Kafka K, Stewart D, Anders R, Schwarz KB and Jang YY: Biliary atresia relevant human induced pluripotent stem cells recapitulate key disease features in a dish. J Pediatr Gastroenterol Nutr 68: 56-63, 2019.
83. Arenas F, Hervías I, Sáez E, Melero S, Prieto J, Parés A and Medina JF: Promoter hypermethylation of the AE2/SLC4A2 gene in PBC. JHEP Rep 1: 145-153, 2019.
84. Melero S, Spirlì C, Zsembery A, Medina JF, Joplin RE, Duner E, Zuin M, Neuberger JM, Prieto J and Strazzabosco M: Defective regulation of cholangiocyte $Cl^-/HCO_3(-)$ and Na^+/H^+ exchanger activities in primary biliary cirrhosis. Hepatology 35: 1513-1521, 2002.
85. Hisamoto S, Shimoda S, Harada K, Iwasaka S, Onohara S, Chong Y, Nakamura M, Bekki Y, Yoshizumi T, Ikegami T, *et al*: Hydrophobic bile acids suppress expression of AE2 in biliary epithelial cells and induce bile duct inflammation in primary biliary cholangitis. J Autoimmun 75: 150-160, 2016.
86. Prieto J, Qian C, García N, Díez J and Medina JF: Abnormal expression of anion exchanger genes in primary biliary cirrhosis. Gastroenterology 105: 572-578, 1993.
87. Concepcion AR, Salas JT, Sáez E, Sarvide S, Ferrer A, Portu A, Uriarte I, Hervás-Stubbs S, Oude Elferink RP, *et al*: CD8+ T cells undergo activation and programmed death-1 repression in the liver of aged $Ae2a,b^{-/-}$ mice favoring autoimmune cholangitis. Oncotarget 6: 28588-28606, 2015.
88. Salas JT, Banales JM, Sarvide S, Recalde S, Ferrer A, Uriarte I, Oude Elferink RP, Prieto J and Medina JF: Ae2a,b-deficient mice develop antimitochondrial antibodies and other features resembling primary biliary cirrhosis. Gastroenterology 134: 1482-1493, 2008.
89. Concepcion AR, Salas JT, Sarvide S, Sáez E, Ferrer A, López M, Portu A, Banales JM, Hervás-Stubbs S, Oude Elferink RP, *et al*: Anion exchanger 2 is critical for CD8(+) T cells to maintain pHi homeostasis and modulate immune responses. Eur J Immunol 44: 1341-1351, 2014.
90. Poupon R, Ping C, Chrétien Y, Corpechot C, Chazoillères O, Simon T, Heath SC, Matsuda F, Poupon RE, Housset C and Barbu V: Genetic factors of susceptibility and of severity in primary biliary cirrhosis. J Hepatol 49: 1038-1045, 2008.
91. Zhang L, Su H, Li Y, Fan YJ, Wang Q, Jiang J, Hu Y, Chen G, Tan B and Qiu F: Different effects of ursodeoxycholic acid on intrahepatic cholestasis in acute and recovery stages induced by alpha-naphthylisothiocyanate in mice. Toxicol Appl Pharmacol 342: 69-78, 2018.
92. Úriz M, Sáez E, Prieto J, Medina JF and Banales JM: Ursodeoxycholic acid is conjugated with taurine to promote secretin-stimulated biliary hydrocholeresis in the normal rat. PLoS One 6: e28717, 2011.
93. Wu TT, Hsieh YH, Wu CC, Tsai JH, Hsieh YS, Huang CY and Liu JY: Overexpression of anion exchanger 2 in human hepatocellular carcinoma. Chin J Physiol 49: 192-198, 2006.
94. Liu CJ, Hwang JM, Wu TT, Hsieh YH, Wu CC, Hsieh YS, Tsai CH, Wu HC, Huang CY and Liu JY: Anion exchanger inhibitor DIDS induces human poorly-differentiated malignant hepatocellular carcinoma HA22T cell apoptosis. Mol Cell Biochem 308: 117-125, 2008.
95. Hwang JM, Kao SH, Hsieh YH, Li KL, Wang PH, Hsu LS and Liu JY: Reduction of anion exchanger 2 expression induces apoptosis of human hepatocellular carcinoma cells. Mol Cell Biochem 327: 135-144, 2009.
96. Svastova E, Witarski W, Csaderova L, Kosik I, Skvarkova L, Hulikova A, Zatovicova M, Barathova M, Kopacek J, Pastorek J and Pastorekova S: Carbonic anhydrase IX interacts with bicarbonate transporters in lamellipodia and increases cell migration via its catalytic domain. J Biol Chem 287: 3392-3402, 2012.