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Review article

CFTR High Expresser Cells in cystic fibrosis and intestinal diseases

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ABSTRACT

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), the Cl^-/HCO_3^- channel implicated in Cystic Fibrosis, is critical to the pathophysiology of many gastrointestinal diseases. Defects in CFTR lead to intestinal dysfunction, malabsorption, obstruction, infection, inflammation, and cancer that increases morbidity and reduces quality of life. This review will focus on CFTR in the intestine and the implications of the subpopulation of CFTR High Expresser Cells (CHEs) in Cystic Fibrosis (CF), intestinal physiology and pathophysiology of intestinal diseases.

1. Introduction

Significant advances have been made in the development of pharmacotherapies to treat Cystic Fibrosis (CF), the genetic disease caused by loss of function mutations in the Cl^-/HCO_3^- anion channel Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR)*. Development of CFTR modulators have significantly improved CF lung disease and well-being, but all organs affected by CF have not equally benefitted from modulator therapies. The recent excitement around the pulmonary ionocyte, a rare lung epithelial cell type characterized by high CFTR expression, as a potential target in CF lung disease sparked our interest in revisiting the subpopulation of CFTR High Expresser Cells (CHEs) in the intestine and the potential importance of CHEs as a therapeutic target, especially given the challenges of reversing intestinal disease in CF. Recent attention has been directed at understanding the relative expression levels of CFTR in individual cell types of affected epithelial tissues, since this information is key for developing targeted therapies for CF. Although the pulmonary ionocyte was proposed as the likely therapeutic target for CF lung disease [1–3], a recent study of CFTR expressing cell types in the human lung concluded that while ionocytes express the highest CFTR per cell, secretory cells are the predominant source of functional CFTR and the therapeutic target [4]. Heterogeneous CFTR expression at the tissue- and cell-specific regulatory mechanisms have limited the ability to translate conclusions from studies of overexpression models to human disease. Moreover, epigenetic changes and differences in organ disease in CF animal models, further confound our ability to elucidate CF pathogenesis in humans. Despite intense efforts by the research community to overcome these challenges, CFTR protein remains one of the most elusive and challenging to study.

The mammalian small intestine can be divided into three functional regions: duodenum, jejunum, and ileum. Within each region, the epithelium is compartmentalized into the stem cell compartment, called the crypt, and the differentiated compartment, the villus

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[5]. Intestinal stem cells at the base of crypts generate a continuous stream of new cells that differentiate and migrate up the villus. The villus is comprised of several differentiated cell types: enterocytes, Goblet cells, Paneth cells, Tuft cells, Microfold (M) cells, and enteroendocrine cells (EECs) [6] (Fig. 1A). The expression levels of CFTR mRNA and protein within the intestine is heterogenous, varying along the length of the gut and across cell types. CFTR is expressed in the Brunner glands of mouse, rat and human duodenum and within crypts in a decreasing proximal-distal gradient along the gut length. In addition, in rat and human (but not mouse), *CFTR* is highly expressed in a subpopulation of cells predominantly within duodenum and jejunal villi (Fig. 1A–C) [7–9], termed CFTR High Expresser Cells (CHEs). Like all CFTR-expressing cells in the native intestine, second messenger-activation stimulates CFTR trafficking in CHEs from subapical vesicles to the apical membrane, confirming the importance of acute regulated CFTR trafficking in intestinal fluid secretion [9–12]. In the human CF intestine, lack of bicarbonate secretion is a major contributor to disease development. However, the specific CFTR-expressing cell types that regulate bicarbonate secretion and their role in CF intestinal disease remains poorly defined. Recent sc-RNA-seq studies confirmed that CHEs comprise a distinct cell fate and defined their gene expression profile [13–15]. This cutting-edge approach will facilitate new studies elucidating the functions and contributions of distinct cell types in disease pathophysiology. The study of their cell fate specification mechanisms has the potential to alter current paradigms of intestinal stem cell biology. In this review, we discuss features of CHEs and ionocytes and the importance of these cells to intestinal physiology and the pathophysiology of CF and intestinal diseases.

2. Mitochondria rich (MR) cells/ionocytes

MR cells, ionocytes, and proton-rich (PR)/H(+)-ATPase-rich (HR) cells are all terms used to describe a similar class of cells that possess overlapping characteristics. MR cells have been identified in fish, amphibians, sea turtles and higher mammalian species where they play a central role in maintaining body fluid homeostasis by actively secreting or absorbing ions (Table 1) [16]. They were first characterized in eel gills with roles in chloride secretion [16] and have been described as "*mitochondria rich*" cells in several organisms and tissues, including teleost brachial epithelium [17]. Because they have significant ion transport activity, they require an abundance of mitochondria to produce ATP to support active transport. MR cells are more commonly referred to as ionocytes in fish and aquatic species, emphasizing their specialized roles in Cl^- and ion transport [18]. Ionocytes are sites of active Cl^- secretion and increased ionic permeability, and there is a positive correlation between the density of Cl^- secreting cells and the rate of Cl^- transport [19]. Due to

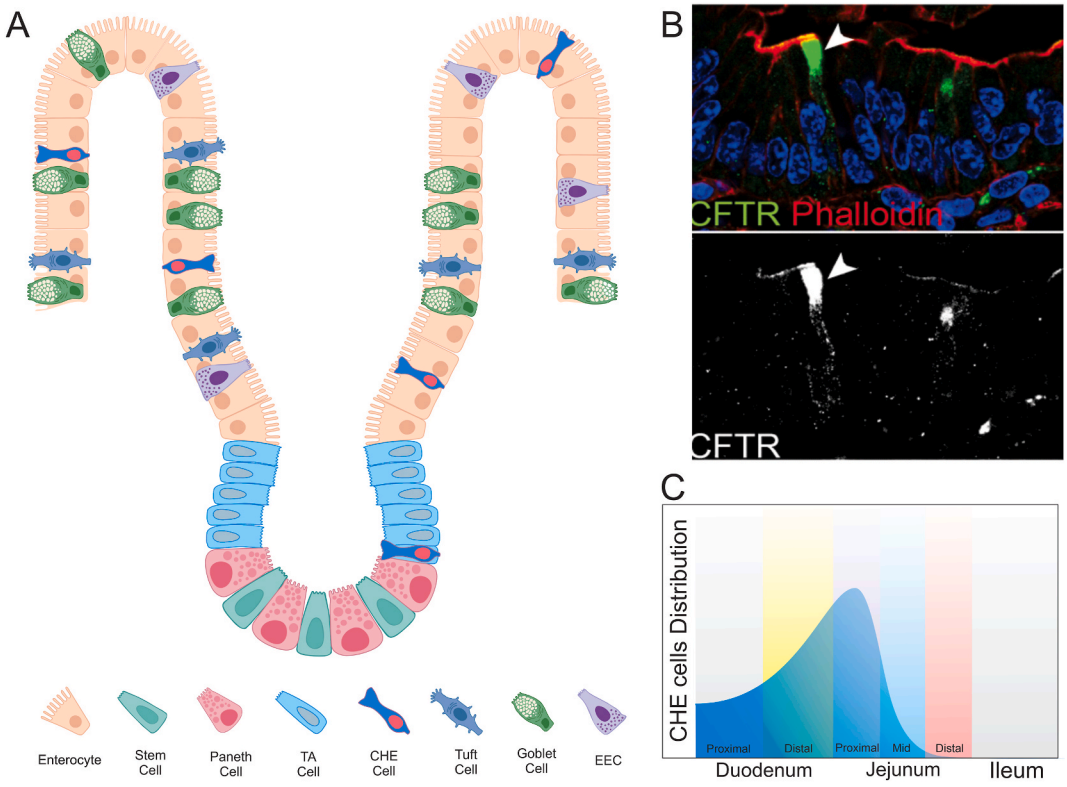

Fig. 1. CHE cells in the proximal small intestine. A: Schematic diagram of the distribution of cell types along the crypt-villus axis. Intestinal stem cells, Transit-Amplifying (TA) Cells, and differentiated cells are located in spatially defined regions along the crypt-villus axis. In the crypt, stem cells, TA cells, Paneth Cells and occasional CHE cells are present. CHE cells are more abundant along the villus, frequently located adjacent to Goblet Cells. EEC, enteroendocrine cells. B: CFTR label identifies very high concentration of CFTR in the apical domain of a villus CHE cell. C: Schematic diagram of CHE cell distribution along the proximal-distal axis of rat small intestine.

Table 1
Comparison of ion channels and other proteins expressed in MR cells/ionocytes and CHEs across different species.

	MR cells/Ionocytes		Mammalian				CHEs	
	Fish	Amphibian	Inner ear	Intercalated cells	Epididymal clear cells	Pulmonary ionocytes	Rat	Human
Ion channels								
BEST4	–	–	–	–	–	–	–	Yes
CFTR	–	–	Yes	Yes	–	Yes	Yes	Yes
NBCE1	Yes	–	–	Yes	–	–	Yes	–
NHE	Yes[a]	–	Yes	Yes	–	–	No	–
NKCC	–	Yes	Yes	Yes	Yes	–	Yes	–
Slc26a3/DRA	–	–	–	–	–	–	No	–
SLC26A4/Pendrin	Yes	Yes	Yes	Yes	Yes	–	–	–
SLC36A1/PAT1	Yes	–	–	–	–	–	No	–
SLC4A1/AE1		Yes	–	–	Yes	–	–	–
SLC4A11/AE11	–	–	–	Yes	–	–	–	–
SLC4A9/AE4	–	–	–	Yes	–	–	–	–
v-ATPase	Yes	Yes	Yes	Yes	Yes	Yes	Yes	–
Miscellaneous								
CA	–	Yes	–	–	Yes	Yes	–	Yes
GN	–	–	–	–	–	–	–	Yes
UGN	–	–	–	–	–	–	–	Yes
References	[29,31,38,39]	[28,30]	[21,23]	[25,32,35,36,41]	[33,36]	[1,2,4]	[9,45]	[14]

Yes = positive expression, No = negative expression, - = not known.
BEST4 = bestrophin 4, CFTR = cystic fibrosis transmembrane conductance regulator, NBCE1 = Electrogenic sodium bicarbonate cotransporter 1, NHE = sodium-hydrogen exchanger, NKCC = sodium-potassium-chloride cotransporter, DRA = down-regulated in adenoma, SLC26A3 = solute carrier family 26 member 3, SLC36A1 = solute carrier family 36 member 1, PAT1 = proton-assisted amino acid transporter 1, SLC4A1 = solute carrier family 4 member 1, AE1 = anion exchanger 1, SLC4A11 = solute carrier family 4 member 11, AE11 = anion exchanger 11, SLC4A9 = solute carrier family 4 member 9, AE4 = anion exchanger 4, CA = carbonic anhydrase, GN = guanylin, UGN = uroguanylin.
[a] Only type III and IV ionocytes express NHE protein.

their highly polar nature, ionocytes maintain a specific combination of channels or transporters on the apical and basolateral membranes, allowing for bidirectional movement of ions and acid base molecules. A comprehensive description of ionocyte classification, subtypes in aquatic species, and specific polarized distribution of ion transporters and their physiological relevance to ion transport was recently documented [20].

2.1. Mammalian MR cells/Ionocytes

Ionocytes have been identified in several mammalian tissues, particularly in those in which fluid regulation is important (Table 1). For example, MR cells in the inner ear express high levels of the Cl^-/HCO_3^- exchanger Slc26a4/pendrin, and loss of Slc26a4 results in defective NaCl reabsorption and hearing loss during embryonic mouse development [21]. In humans, Pendred Syndrome is caused by loss-of-function mutations in SLC26A4 and results in early hearing loss [22–24]. In the mammalian kidney, ionocytes are called intercalated cells, and three distinct subtypes express various combinations of v-ATPase and SLC26A4. They have important roles in regulating acid-base homeostasis and water and ion reabsorption (reviewed in Ref. [25]). In males, ionocytes in the epididymis are called clear cells. Epididymal ionocytes express v-ATPase and CFTR and play a critical role in luminal acidification, which is important for sperm development [26]. Taken together, it is clear that mammalian ionocytes are critical regulators of fluid homeostasis, acid/base levels and ion transport. Interestingly, however, the unique functions of each tissue likely require distinct gene/protein expression profiles in ionocytes to fulfill their role. Therefore, how ionocytes are specified and how their activity is regulated must be studied in tissue-specific contexts.

2.2. Pulmonary ionocytes

While the presence of a rare CFTR-enriched cell was identified in human airway submucosal glands over thirty years ago [27], recent sc-RNA-seq analyses in the mouse and human airway transcriptionally defined this same rare cell type enriched for CFTR and mitochondrial gene expression [1,2]. These cells were named pulmonary ionocytes in reference to ionocytes in *Xenopus laevis* [28]. Pulmonary ionocytes account for less than 1% of the epithelial population in the mouse airway, but they express more than half of all detected *Cftr* [2]. Based on the high level of *Cftr* expression, ionocytes were proposed to be the primary site of ion secretion and the major cellular target in CF [1,2]. However, studies of human airways demonstrated that CFTR-mediated Cl^- secretion was associated with secretory cell types and not ionocytes [4]. In addition to its roles in Cl^- secretion, CFTR is also a bicarbonate transporter, whose secretion is important in regulating mucus viscosity, luminal pH and bacterial killing. Whether ionocytes are the primary source of bicarbonate secretion or whether pulmonary ionocytes play roles in stimulus response in the airway has not been addressed.

2.3. Regulation of MR cells/ionocytes

MR cell specification is regulated by both intrinsic and extrinsic mechanisms. An important factor that determines the presence, number, and function of MR cells is environmental pH. In zebrafish embryos, acidic water was sufficient to increase both the relative proportion of MR cells and the acid secretion function of each cell [29]. At the transcriptional level, a single transcription factor has important roles in the specification of many ionocytes/MR cells across species and tissues. Foxi1 is a member of the Fox (Forkhead box) family of transcription factors and it is essential for development of ionocytes in *Xenopus* [30] and fish [31]. In mammals, Foxi1 is required for ionocyte fate specification in the kidney, epididymis, and the inner ear [32–34]. In addition, it is sufficient to drive pulmonary ionocyte fate in human airway cultures [1]. Foxi1 likely regulates ionocyte fate in part by directly promoting the expression of v-ATPase subunit genes and other important ion exchangers (Table 1) [33,35–37]. Foxi1 regulation of v-ATPase gene expression is evolutionarily conserved, as Foxi1-driven expression of v-ATPase in MR cells of several aquatic species is critical for ion transport adaptation from seawater to freshwater [31,38,39]. In zebrafish embryonic skin, ionocyte fate is regulated by the Foxi1-related transcription factors Foxi3a and Foxi3b [40]. Foxi3 induces expression of the Notch ligand Jagged2a. Notch signaling works through a mechanism of contact inhibition; therefore, interaction of membrane-bound Jagged2a with its Notch receptor on adjacent cells represses ionocyte fate in neighbors. The role for Notch signaling in regulating fate is also a conserved feature of ionocyte specification, though the effects of Notch seem to be tissue/organism-specific. Similar to the zebrafish epidermal ionocyte, Notch inhibition in *Xenopus* epidermis and mammalian kidney increased ionocyte fate specification [28,41]. In contrast, mammalian pulmonary ionocytes are enriched for Notch target genes and inhibition of Notch signaling decreased ionocyte abundance, suggesting that Notch is an important upstream positive regulator of the ionocyte fate program [1]. Taken together, these data suggest that while Notch signaling is an important regulator of ionocyte fate, its downstream effects are context-dependent.

2.4. CFTR localization in CHEs

As enterocytes move up the crypt-villus axis and undergo a progressive differentiation program [42], they downregulate *CFTR* mRNA and protein levels to create a gradient of *CFTR* expression with levels highest at the crypt base and lowest at the villus tips [7,8,43]. Therefore, downregulation of *CFTR* is an intrinsic property of enterocytes undergoing terminal differentiation. This decreasing

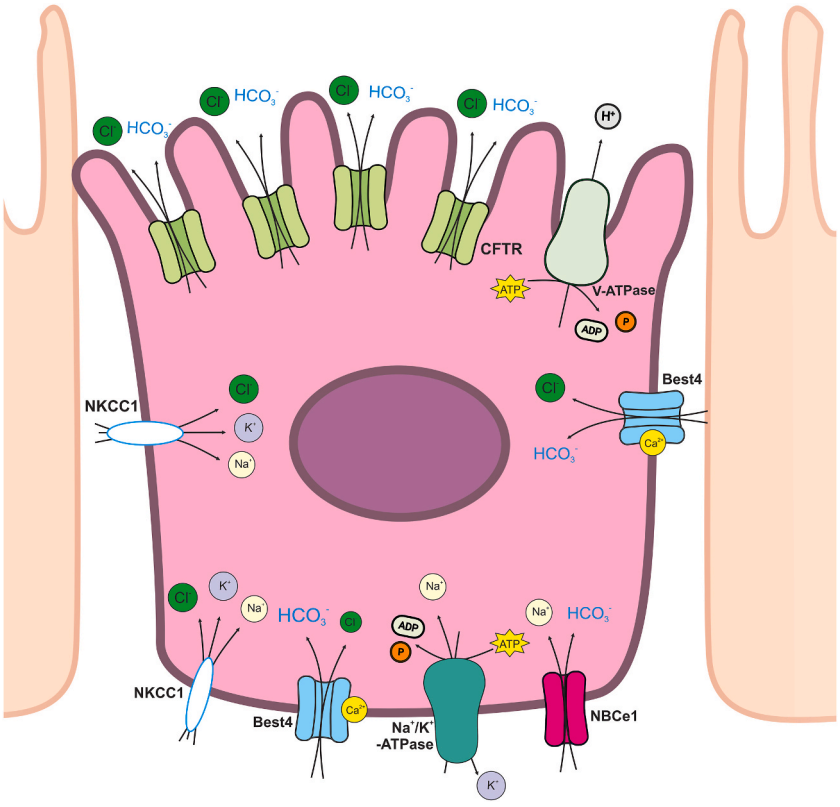

Fig. 2. Distribution of ion channels and transporters in a villus CHE cell. The Cl-and HCO3- levels can be controlled by CFTR in the apical membrane. In the apical membrane, V-ATPase (Vacuolar ATPase) pump participates in H^+ transport. Best4 (bestrophin 4) and NKCC1 (Na–K–Cl cotransporter 1) are on the basolateral membrane, along with NBCe1 (Na^+/HCO_3^- cotransporter 1) and Na/K^+-ATPase. Co-ordinated activities of basolateral transporters control Cl^-, Na^+, K+, HCO_3^- entry and exit to maintain homeostasis.

expression gradient aligns well with the anion secretory function of *CFTR* in the crypt, the major recognized site for fluid secretion in the intestine. The existence of CHEs in rat and human duodenum and jejunum, with much higher *CFTR* expression compared to progenitor cells, is in direct contrast to the accepted paradigm for intestinal *CFTR* expression and function [43]. In fact, as CHEs migrate up the villus, they continue to increase their CFTR mRNA and protein expression. Immuno-electron microscopy confirmed that CHEs localize within villi and in the upper crypt but are excluded from the stem cell zone at the crypt base. Morphologically, they resemble absorptive enterocytes [11]. Prior to ultrastructural analysis, it was thought that CHEs could possibly be EECs, but they do not express the EEC marker Chromogranin A. At the time CHEs were identified and initially described, nothing was known regarding their link to MR cells/ionocytes, transcriptional regulation, or gene expression outside of their high CFTR expression. CHEs represent about 1–2% of intestinal epithelial cells, are more abundant on the villus, sporadically localized in the duodenum and jejunum in rat and human intestine but are distinctly absent in mice [44,45]. They are most abundant in the jejunum [9] (Fig. 1C).

2.5. CHE morphology and ultrastructure

CFTR is distributed throughout the cytoplasm of CHEs with enrichment in the apical region and prominent vesicular staining in the subapical compartment (Fig. 1B). Cryo-immunogold electron microscopy identified CFTR protein on microvilli membranes, in coated pits on the apical membrane, on clathrin-coated vesicles, and endosomes within the subapical cytoplasm of CHEs [11]. Ultrastructural studies of villus CHEs in rat jejunum revealed that the cells are elongated and pear-shaped, are more electron dense than neighboring enterocytes, and have a shorter somewhat disordered brush border [11,45]. They have prominent desmosomes, abundant subapical vesicles, and long actin fibers and motor filaments that traverse the cells, and abundant mitochondria [11,45]. Taken together, the prominence of mitochondria, apical microvilli, and CHE morphology bear similarities to MR cells.

2.6. CHE protein expression and function

Rat intestinal CHEs express high levels of v-ATPase on the brush border but lack the usual absorptive hydrolases and ion transporters found on absorptive enterocytes, including Slc9a3/NHE3 (Na^+/H^+ exchanger 3), Slc36a1/PAT1 (Putative Anion Exchanger 1), and Slc26a3/DRA (Down-Regulated in Adenoma) (Fig. 2) [9]. Furthermore, they express low levels of myosin 1a, the villus enterocyte-specific motor protein critical for brush border development and retention of sucrase-isomaltase [9,46,47]. CFTR trafficking from subapical vesicles to the brush border membrane of enterocytes requires myosin 1a [48], but the specific motor responsible for apical CFTR trafficking in CHEs has yet to be identified.

NKCC1, a critical basolateral co-transporter involved in intestinal Cl^- secretion, is expressed at similar levels in rat CHEs and

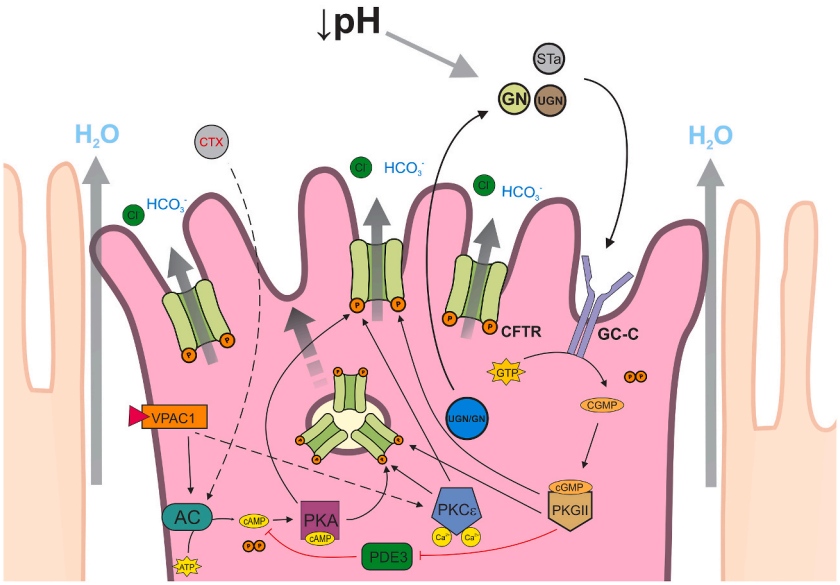

Fig. 3. Signaling in CHE cells. Stimuli including low pH, high salinity, or STa (Heat-Stable Enterotoxin) activate the Guanylate cyclase-C (GC-C) receptor. cGMP (cyclic GMP) generation by GC-C activates PKGII (protein kinase G II) to phosphorylate CFTR in vesicles and induce traffic and activation in the membrane. CTX (cholera toxin) activates adenylate cyclase (AC) to signal cAMP (cyclic AMP) and PKA (protein kinase A) activation. PKA phosphorylates endosomal and membrane CFTR, translocating more activated CFTR to the membrane. PKGII can also increase the PKA activation, inhibiting PDE3 (phosphodiesterase 3) responsible for cAMP degradation. Activation of VPAC1 (Vasoactive intestinal peptide receptor type 1) by ligand is responsible for PKA and PKCε (Protein kinase C ε) activation. The principal effect of its phosphorylation is the endosomal trafficking and activation of CFTR on the apical membrane. Increased activated CFTR on the apical membrane results in secretion of Cl^- and $HCO3^-$ into the lumen, that drives water flow, resulting in fluid secretion and diarrhea.

neighboring enterocytes at steady state (Fig. 2). However, NKCC1 robustly increased at CHE basolateral membranes upon cyclic AMP (cAMP) or acetylcholine stimulation. cAMP and acetylcholine stimulation also increased CFTR trafficking to CHE brush border membranes, supporting a role for CHEs in intestinal Cl^-/HCO_3^- secretion [9]. Furthermore, rat CHEs responded to a decrease in lumen pH by rapidly recruiting CFTR and NBCe1 to their specific domains, supporting a role for CHEs in pH sensing and regulation [12].

Two recent studies performed sc-RNA-seq on the human gastrointestinal tract and demonstrated that duodenal CHEs are enriched for *CFTR*, bestrophin 4 (*BEST4*), guanylin (*GUCA2A*), and uroguanylin (*GUCA2B*) (Fig. 2) [14,49]. Bestrophins are ion channels that can function as Cl^-, HCO_3^-, or voltage-gated Ca^{2+} channels [50]. Best4$^+$ epithelial cells are present in both the human small and large intestine [51], though Best4$^+$ cells in the large intestine do not express CFTR, suggesting that these cells may function differently across the small and large intestine [14]. Studies of isolated human Best4+ colonocytes demonstrated that their intracellular pH decreases upon incubation in acidic conditions [15], further supporting a role for these cells in pH sensing and regulation, in part by importing protons.

Guanylin (GN) and uroguanylin (UGN) are low molecular-weight endogenous hormone peptides that are mainly synthesized in the intestine as pre-hormones (Fig. 3). They bind to guanylate cyclase C receptor (GC-C) and generate cyclic GMP (cGMP) [52–55]. Increased cGMP targets several kinases, phosphodiesterases, and ion channels, including CFTR [56]. GC-C is expressed on the apical brush border of epithelial cells from duodenum to the rectum [57,58]. GN and UGN are both secreted peptides involved in regulating intestinal fluid homeostasis. During a salt-rich meal, GN and UGN secretions increase, resulting in Na^+ absorption inhibition by NHE3 and increased Cl^- and HCO_3^- secretion by CFTR to drive water secretion [59].

In the intestine, UGN expression is restricted to the proximal small intestine [60], while guanylin is most prominent in the distal small intestine and colon [61–67]. Co-localization studies of UGN and chromogranin A indicated that EECs are not the main source of UGN in rat and human tissues. Instead, UGN is highly expressed by scattered enterocytes [66], which interestingly bear similar morphology and localization patterns to our previous reports of CHEs. The transcriptomic results that *Guca2b* is enriched in CHEs and the striking resemblance of UGN-rich cells to CHEs suggest that CHEs may be the major source of UGN in the rat and human proximal small intestine. Future studies will be necessary to better define the cellular source of UGN in the intestine. Gut organoids and genetic manipulation will be useful to elucidate the role of UGN in CHEs.

2.7. CHEs and cell fate

While CHEs have now been preliminarily characterized by gene expression patterns based on sc-RNA-seq data in humans [14,49], factors, pathways and mechanisms driving their specification have not been elucidated. In pulmonary ionocytes, Foxi1 was shown to be necessary for their fate [2] and loss of Foxi1 resulted in decreased CFTR expression and other ionocyte-enriched transcription factors like Ascl3 (Achaete-Scute Family BHLH Transcription Factor 3). As mentioned above, Foxi1 is a strong regulator of v-ATPase subunit gene expression [28,36], consistent with its role in promoting ionocyte fate. Therefore, an outstanding question remains regarding which transcription factors are enriched in or specific to CHEs that drive their fate specification program. A recent study demonstrated the presence of CHEs in human duodenum by sc-RNA-seq and suggested that CHEs arise from secretory progenitors [49].

Table 2
Potential role of CHEs in diseases.

Disease		Mechanism	References
1. Non-congenital secretory diarrhea	*Cholera*	Cholera toxin binds to monosialoganglioside (GM1) receptors on the luminal surface of epithelial cells which activates adenylate cyclase and increases cAMP. High levels of intracellular cAMP results in the activation of downstream targets that leads to CFTR activation and secretory diarrhea.	[77, 102]
	Traveler's diarrhea	*Escherichia coli* heat-stable enterotoxins (STa) binds to GC-C receptor in the membrane of enterocytes resulting in cGMP increase and consequent activation of downstream targets, including PKGII, that promotes CFTR activation and secretory diarrhea.	[91]
2. Congenital secretory diarrhea	*Microvillus Inclusion Disease (MVID)*	Mutation in myosin Vb motor protein results to the mislocalization of receptors and ion channels to the apical and basolateral membranes of enterocytes. Depolarization of CFTR, DRA, and NHE3, and reduced levels of SGLT1 and GLUT2 are associated with malabsorption and secretory diarrhea in MVID.	[81, 82, 83, 84, 85]
3. Cystic Fibrosis (CF)		Mutations in the CFTR gene reduce or impair CFTR protein expression in the brush border of enterocytes, leading to an impairment of HCO3-/Cl- secretion which contributes to meconium ileus in CF newborns, acidic pH in the proximal duodenum, dysbiosis, nutrient malabsorption, and cancer. In CF, activation of the GC-C pathway by UGN/GN analogs ameliorate constipation and reduces obstruction episodes by inhibition of sodium absorption through NHE3.	[86, 87, 95, 96, 114, 115]
4. Obesity		UGN binding to GC-C in the hypothalamus results in the activation of the downstream anorexigenic pathways and potentiates leptin's ability to decrease appetite and food intake.	[98, 100, 101]
5. Colorectal cancer (CRC)		GC-C and cGMP signaling activation by UGN suppresses CRC development by maintaining genomic integrity, controlling proliferation, and increasing apoptosis of enterocytes.	[117, 118]

cAMP = adenosine 3',5'-cyclic monophosphate, CFTR = cystic fibrosis transmembrane conductance regulator receptor, cGMP = guanosine 3',5'-cyclic monophosphate, DRA = down-regulated in adenoma, GLUT2 = glucose transporter 2GN = guanylin, GC-C = guanylyl cyclase C, NHE3 = Sodium–hydrogen antiporter 3, PKGII = type II cGMP-dependent protein kinase, SGLT1 = Sodium-glucose cotransport 1, UGN = uroguanylin.

This is consistent with their role as fluid secretors. However, unlike other secretory cells, including Goblet, Paneth, Tuft, and EEC, CHEs do not display a secretory cell morphology [9,45]. Instead, with an elongated columnar shape, they more closely resemble enterocytes (Fig. 1B).

The primary fate decision in the intestine is made as stem cells give rise to either absorptive or secretory progenitors. All absorptive progenitors become enterocytes, whereas secretory progenitors must then make secondary fate decisions to become one of the several secretory cell types, including EEC, Goblet, Tuft and Paneth cells. The initial choice between absorptive and secretory is controlled by Notch signaling. Notch regulates absorptive cell fate via Hes1, which directly represses the expression of the secretory cell transcription factor Atoh1 to inhibit secretory fates and promote absorptive fates [68–70]. Downstream of Atoh1, EEC, Goblet cells and Paneth cells are specified through distinct transcriptional programs [71–73]. Which signaling pathways regulate the secondary fate decision of secretory progenitors have not been elucidated. As discussed above, active Notch signaling promotes pulmonary ionocyte fate [1], though the Notch-dependent mechanism of ionocyte fate specification has not been identified. In contrast, ionocyte fate is induced in other organisms and tissues when Notch signaling is inhibited. Whether CHEs are regulated through Notch signaling at all, and whether Notch signaling promotes or represses CHE fate is an outstanding question. If they are indeed similar to pulmonary ionocytes in their fate decisions, then we would expect their specification to be Notch-dependent. However, because sc-RNA-seq analysis suggests they arise from secretory progenitors, CHE fate would require suppression of Notch signaling.

2.8. CHEs in intestinal homeostasis and disease

2.8.1. Diarrheal diseases

In the gastrointestinal (GI) tract, defects in secretion, absorption, motility, and sensation have been implicated in multiple disorders. Ion channels are involved in all these processes, which explains their ubiquitous expression along the GI tract [74]. CFTR plays a major role in intestinal and epithelial ion transport in other tissues [75]. CFTR dysfunction has been linked to life-threatening congenital and non-congenital secretory diarrheas, such as Microvillus Inclusion Disease (MVID) and cholera [76,77], respectively (Table 2).

MVID is caused by loss of function mutations in the myosin Vb (MYO5B) actin motor [78–81]. Loss of MYO5B causes mislocalization of several ion channels from the apical surface, but interestingly, some CFTR still traffics to the enterocyte apical membrane, suggesting redundant overlapping mechanisms for apical CFTR localization. Although a fraction of CFTR protein is mislocalized in MVID enterocytes, the apical CFTR protein is functional and contributes to the secretory component of diarrhea in MVID [82–84]. Notably, CHEs still exist in MVID human duodenum [82]. Unexpectedly, we found that CHEs also exist in the proximal small intestine of a tamoxifen-inducible *Myo5b*KO mouse model that displays all the features of human MVID [85]. However, as noted, CHEs are not present in mice under homeostatic conditions [44]. Whether these CHEs are transcriptionally similar to the CHEs found in rats and humans is unknown. Although neighboring villus enterocytes in the *Myo5b*KO mouse display basolateral redistribution of CFTR, it localizes normally in CHEs to the apical domain [85]. These observations raise new questions about cell type-specific CFTR trafficking mechanisms and the role of CHEs in MVID. The preservation of apical CFTR distribution in CHEs suggests that CHEs may contribute to electrogenic secretion in MVID diarrhea. Additionally, the expression of v-ATPase, Best4, and Otopetrin 2 (Otop2) in CHEs [14] reinforces their potential role in fluid secretion in MVID and other diarrheal diseases.

2.8.2. Cystic fibrosis

In CF, there is an impairment of HCO_3^-/Cl^- secretion, leading to an acidic duodenal pH, which contributes to intestinal obstruction, malabsorption, bacterial overgrown, gut inflammation, and cancer. Impaired fluid secretion in CF newborns contributes to meconium ileus, characterized by viscid and thick meconium that obstructs the terminal ileum and leads to small bowel dilation proximal to the obstruction [86,87]. CF patients experience chronic constipation and intestinal obstructive episodes, which are generally managed with laxatives [88].

Among CHE-enriched transcripts, UGN represents a notable druggable candidate given its importance in gut homeostasis (Table 2). The duodenum protects epithelial cells against acid stomach secretions, and its low pH elicits increased UGN production, secretion, and function. UGN is more effective in acidic pHs, while GN has the highest activity in alkaline pH [89]. As mentioned earlier, UGN secretion increases cGMP levels in the duodenum, leading to PKG II activation and subsequent CFTR phosphorylation to induce HCO_3^-/Cl^- and water secretion into the lumen (Fig. 3) [90–92]. At the same time, Na^+ absorption is inhibited through NHE3, impeding fluid absorption from the lumen. As a consequence, duodenal pH increases to prevent epithelial damage and increase fluid secretion [93]. Neutralization of the duodenal pH is also important for preventing dysbiosis [94] and promoting fat metabolism, since pancreatic lipase is inactivated at low pH.

FDA-approved GC-C agonists, analogs of UGN and GN, are in clinical use for treatment of Irritable Bowel Syndrome with constipation (IBS–C) and have been used in CF mouse models to improve gastrointestinal transit and alkalinity [95,96]. Although CFTR function was impaired in these models due to mutations, GC-C agonist treatment inhibited NHE3 to decrease Na^+ and fluid absorption, improving gastrointestinal transit. GC-C agonism functionally rescued murine F508del and R117H *Cftr* and CFTR mutants in CF patient-derived ileal organoids [97]. Whether CHEs regulate pH and fluid secretion in CF through the GC-C/UGN pathway is unknown but should be examined. Indeed, although CHEs are present in the human small intestine, the localization and function of CHEs in the human CF intestine remains unknown. In addition to the *Myo5b* KO mouse model of MVID, we have observed CHEs in a humanized mouse model of DF508CFTR (unpublished data). These intriguing results suggest that in pathological states where there is a disturbance of CFTR physiology, mice can generate intestinal CHEs. Whether the number of CHEs changes in different diseases and the mechanisms that regulate these cells are critical to understand.

2.8.3. Metabolism and obesity

In addition to the established role for UGN in fluid secretion and pH regulation, recent discoveries have revealed a novel UGN function in the mouse brain and expanded our understanding of GC-C/UGN pathway-mediated homeostasis (Table 2). The presence of GC-C expression in the hypothalamus, the site of homeostatic coordination and regulation, has implicated a novel function of UGN in satiety and energy balance [98]. Indeed, loss of *Gucy2c*, the gene encoding the GN/UGN receptor GC-C, resulted in dysregulated energy homeostasis with an increased rate of weight gain over time compared to wild type mice. GC-C/UGN signaling in the hypothalamus regulates weight and satiety through cGMP-dependent activation of cyclic nucleotide-gated channels, causing depolarization or hyperpolarization of membrane potentials [99]. In diet-induced obesity models, pharmacological upregulation of UGN suppresses appetite and facilitates weight loss [100]. Conditional transgenic UGN expression in brain restored hormone insufficiency and reduced appetite, food consumption, and weight gain over months in diet-induced obesity mouse models [101], thereby reinforcing the role of GC-C/UGN signaling in controlling obesity. Given the elongated shape of CHEs, their responsiveness to neuronal stimuli [102], and their UGN expression, it is feasible that CHEs play an important role in metabolism homeostasis and obesity.

2.8.4. Obesity and cancer

Although malnutrition has long been associated with CF, overweight and obesity have increasingly become a concern in the CF population [103]. Several single center studies have reported that as much as 25% of adults with severe CF mutations were obese/overweight [104–106]. Obesity is associated with hypertension and cardiometabolic risk factors in CF patients [105,107].

Epidemiological data suggest that obesity is associated with an increased risk of colorectal cancer (CRC) [108,109]. CRC is the third most diagnosed GI malignancy and the second leading cause of cancer-related death worldwide [110,111]. The incidence of CRC in the CF population is 5–10 times higher than the general population, particularly in patients who received an organ transplant [112–115]. The causal mechanisms between obesity and CRC are not well established, but insulin resistance, metabolic syndrome, and changes in adipocytokine level are risk factors [116]. In addition, a link between GC-C silencing and intestinal tumorigenesis has recently been identified. Indeed, GC-C silencing increases tumorigenesis and tumor growth by disturbing genomic integrity and increasing proliferation in a colon cancer mouse model [117]. Corroborating these findings, oral UGN therapy reduces polyp formation in the $Apc^{Min/+}$ mouse for CRC and induces apoptosis in human colon adenocarcinoma cells via cGMP [118]. Future studies are necessary to reveal whether CHEs contribute to CRC pathogenesis.

There is a clear link between UGN dysregulation and disease. Although CHEs are the likely primary source of intestinal UGN, their role in each of these diseases has not been investigated.

3. Conclusion

CFTR is widely studied in the pulmonary context of cystic fibrosis. While CFTR modulators such as ivacaftor, lumacaftor, tezacaftor, and elexacaftor have improved symptoms in the airways and generally improved well-being, the benefits of these modulators are not equally observed across all organs, including the intestine.

CHEs in the intestine have a unique morphology and remarkable features, including unusually high CFTR expression. Some of their features resemble MR cells/ionocytes. Sc-RNA-seq analysis now provides a unique opportunity to expand molecular and functional studies to define the physiological and pathophysiological role of CHEs in the gastrointestinal tract that will facilitate therapeutic approaches to treat CF and other intestinal diseases.

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