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Review

The multiple and enigmatic roles of guanylyl cyclase C in intestinal homeostasis

Najla Arshad, Sandhya S. Visweswariah*

Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore 560012, India

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ABSTRACT

Guanylyl cyclase C (GC-C) is predominantly expressed in intestinal epithelial cells and serves as the receptor for the gastrointestinal hormones guanylin and uroguanylin, and the heat-stable enterotoxin, the causative agent for Travellers' Diarrhea. Activation of GC-C results in an increase in intracellular levels of cGMP, which can regulate fluid and ion secretion, colon cell proliferation, and the gut immune system. This review highlights recent findings arising from studies in the GC-C knockout mouse, along with enigmatic results obtained from the first descriptions of human disease caused by mutations in the GC-C gene. We provide some insight into these new findings and comment on areas of future study, which may enhance our knowledge of this evolutionarily conserved receptor and signaling system.

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1. Introduction

The importance of intestinal health has been recognized for over 2000 years, most famously by Hippocrates who said, "All disease begins in the gut". Although not every disease may initiate in the intestine, overall human health and well-being is strongly dependent on the integrity of the gastrointestinal (GI) tract. The GI tract is the largest organ-system in the body, and is responsible for the ingestion, digestion and assimilation of food for the survival of the organism. In this process the digestive system also has to adapt to a large number of microbes that are ingested with the food and colonize the intestine [1]. There are several defence strategies employed by the GI tract to achieve this, which include the secretion of antibodies in the mouth [2], the acidic pH in the stomach, and cellular defence mechanisms that operate within the intestine [3].

The intestine is the major site of digestion and absorption of nutrients. Therefore, to enhance the surface area and thus the absorptive capacity, intestinal villi form finger-like projections into the lumen of the intestine. The villi are separated from each other by crypts [4]. Epithelial cells that line the intestine are primarily comprised of enterocytes which are absorptive in function [5]. The enterocytes are polarized whereby the cell membrane is distinctly sectioned into an apical and basolateral region, separated by tight junctions. Other specialized cells present in the GI tract in-

clude goblet cells which secrete mucous and other glycoproteins, the enteroendocrine cells which secrete hormones, the dendritic cells which are antigen presenting cells, the M-cells or microfold cells which are responsible for antigen sampling, and the Paneth cells, which are found in the crypts and produce antibacterial peptides [3]. Located at the base of the crypt are the intestinal stem cells that provide daughter transit amplifying cells that ultimately form all the differentiated cells of the intestine [6]. The epithelial layer of cells is protected from the contents of the lumen by a thick mucosal layer made up of glycoproteins and mucins, which can contain antibodies and antimicrobial peptides.

The human gut is a rich source of nutrition and plays host to a variety of microbes that co-exist with a mutual dependence called commensalism. The microbiome is comprised mainly of bacteria [3], some protozoans [7] and even fungi [8]. They contribute to host nutrition by producing enzymes that breakdown complex polysaccharides for efficient digestion of ingested food, and by producing metabolites such as vitamins [9]. These microbes do not disrupt the normal physiology of the body and their numbers are kept in check by basal immune activity, which is stimulated by factors such as peptidoglycans from the bacterial cell wall [10]. Genetic variation in humans give rise to differences in their immune system, which then reflects in the composition of the microbiota, and these differences are being correlated to the susceptibility of the individual to immune-related diseases such as asthma and intestinal inflammation [3].

The balance and composition of the gut microflora can be altered by the use of antibiotics or by colonization of the intestine by pathogens. This change may also induce, or be induced by, modifications in the body's immune response, ultimately leading

* Corresponding author. Address: Biology Building-GA09, Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore 560012, India. Fax: +91 80 23600999.

E-mail address: sandhya@mrdg.iisc.ernet.in (S.S. Visweswariah).

to disease. Thus, when intestinal homeostasis is compromised, it can frequently lead to inflammation and invasion of the epithelial layer, diarrhea and poor nutrient absorption [11,12].

This review will focus on a member of the receptor guanylyl cyclase family of proteins, GC-C, in regulating intestinal function. While it was realized many years ago that GC-C was the target of a family of bacterial heat-stable enterotoxins that caused watery diarrhea in humans and animals [13], recent information has highlighted the role of GC-C and cGMP in maintaining intestinal homeostasis [14,15]. Aspects of GC-C structure have been described in a comprehensive review that has been published recently [16], and we will therefore not dwell on this in the current review. Instead, we will highlight recent findings arising from genetic approaches using mice, as well as evidence from human populations, that indicate the important roles that GC-C and cGMP have to play in intestinal physiology.

2. Guanylyl cyclase C, intestinal fluid secretion and feeding behavior

Diarrhea was initially considered a primitive mechanism by which the host attempts to 'flush out' infectious agents, but it is now appreciated that microbes disrupt the host's secretory and absorptive machinery in an attempt to be successfully transmitted. There are various mechanisms by which microbes and their products cause diarrhea, and include direct lysis of epithelial cells, production of pore forming toxins, or by manipulation of host signaling pathways via second messengers such as cyclic AMP, cyclic GMP and calcium ions [17].

The enterotoxigenic *Escherichia coli* or ETEC adhere to the intestine and produce two types of toxins, the heat-labile (LT) and the heat-stable enterotoxins (ST). The LT shares 82% amino acid homology to cholera toxin, binds to the GM1 ganglioside and mediates the onset of diarrhea in a manner similar to that of cholera toxin, via elevation of intracellular cAMP [18]. ST is an 18-amino acid long peptide with three disulfide bridges, and causes an increase in chloride ion secretion, leading to secretory diarrhea [13]. The ST receptor is guanylyl cyclase C [19]. GC-C is a multi-domain protein, and a member of the receptor guanylyl cyclase family. GC-C is primarily expressed in intestinal epithelial cells, and binds to the endogenous gastrointestinal hormones guanylin and uroguanylin [20–22]. Binding of guanylin or uroguanylin to GC-C results in receptor activation, catalyzing the production of cGMP (Fig. 1). Cyclic GMP can activate cGMP-dependent protein kinase II (PKGII), or inhibit the activity of a cAMP-specific phosphodiesterase, PDE3, thereby cross-activating cAMP-dependent protein kinase (PKA). PKGII and PKA phosphorylate the cystic fibrosis transmembrane conductance regulator or CFTR, increasing its chloride-secreting activity [23].

A marked reduction in Na⁺ absorption, and consequently decreased fluid uptake by the intestinal cell, is also brought about by ST and the guanylin family peptides [24]. This is mediated by inhibition of the sodium-hydrogen exchanger, NHE3, following its interaction with PKGII and the scaffolding protein NHERF2 [25]. In addition, cGMP enhances duodenal bicarbonate secretion through an unknown channel [26]. Cyclic GMP can also directly activate cyclic nucleotide gated channels (CNGs), leading to Ca²⁺ influx [27] (Fig. 1).

GC-C signaling is terminated by hydrolysis of cGMP to GMP by a cGMP-dependent phosphodiesterase, PDE5 [16]. Secretion of water into the intestinal lumen is necessary for the lubrication and breakdown of the bolus of food, and this fluid secretion is regulated by the endogenous ligands of GC-C, guanylin and uroguanylin [21,28]. ST is a super-agonist of GC-C, and induces abnormally high levels of intracellular cGMP which causes aberrant fluid-ion efflux, leading

to diarrhea. This disease is usually self-limiting in adults and symptoms resolve within a few days, therefore no specific treatment is required except oral rehydration. However, the incidence of mortality in children can be very high [17].

Given the role that activation of GC-C has in stimulating fluid secretion in the intestine, attempts have been made to exploit this pathway to relieve symptoms of chronic constipation. Currently, an orally administered synthetic ST mimic, linaclotide [29], has been successfully shown to alleviate constipation [30], and this drug is currently awaiting FDA approval.

The mechanism of ST-induced diarrhea is unique in that it is the only example where the primary target of a diarrheal disease-causing agent is a receptor directly controlling intestinal fluid-ion homeostasis. GC-C is well conserved from Pisces to Mammalia and given its function and exploitation by microbes to cause a disease, it was anticipated that the receptor was conserved because of its importance in intestinal physiology [16]. Two independent groups generated knock-out mice for *GUCY2C*, the gene encoding GC-C. Surprisingly, the GC-C knock-out mouse showed no abnormalities in the gut that could be associated with luminal dehydration, but were found to be resistant to ST-induced diarrhea [31,32]. This appeared to be paradoxical for a receptor so well conserved evolutionarily, suggesting that the critical role(s) of GC-C would become apparent when animals were subjected to stresses not normally encountered in the restricted environment in which these knock-out mice were bred.

It is worth mentioning at this stage that extra-intestinal roles for GC-C, specifically in the nervous system, emerged on studies with the knock-out mouse, and warrant a brief summary. Hyperphagia, obesity and metabolic syndrome were observed in GC-C knock-out mice, and these functions appeared to be controlled by circulating levels of uroguanylin, and neuronally-expressed GC-C [33]. This finding was the first report of specific extra-intestinal roles for both GC-C and uroguanylin, highlighting the endocrine function of uroguanylin, which is primarily synthesized in the intestine and acts on intestinal GC-C in a paracrine manner [34].

A second example for the role of GC-C in the nervous system was reported by Gong et al. [35]. Midbrain dopamine neurons express GC-C, and on activation enhance excitatory responses mediated by glutamate and acetylcholine in a cGMP and PKG-dependent manner. GC-C knock-out mice demonstrated attention deficiency and hyperactive behavior, which was reversed on administration of a PKG activator.

GC-C, guanylin and uroguanylin are expressed in reproductive tissues of the rat [36], though no role for this signaling pathway has been described in these tissues. Perhaps subtle roles for this signaling axis, in controlling fertility and reproduction may become evident in the future through studies on the GC-C knock-out mouse.

3. Role of GC-C and cGMP in colon cancer cell cytostasis

The intestinal epithelium undergoes homeostatic cycles of proliferation, migration, differentiation and apoptosis, driven by multipotent stem cells. An imbalance between cell proliferation and apoptosis can therefore lead to the formation of tumours within the gastrointestinal tract. Some years ago, it was noted that there appeared to be an inverse correlation between the incidence of colorectal cancer and secretory diarrhea caused by ETEC [37], leading to the hypothesis that activated GC-C could act as an anti-proliferative agent in the intestine. Studies on both colorectal cell lines [38] as well as GC-C knock-out mice [39] indicated that GC-C exerts a cytostatic effect on epithelial cells following ligand-mediated activation. This induction of colon cell cytostasis was mediated by the regulation of calcium ion influx via a cyclic nucleotide gated channel, and was mimicked by analogs of cGMP [27,40,41].

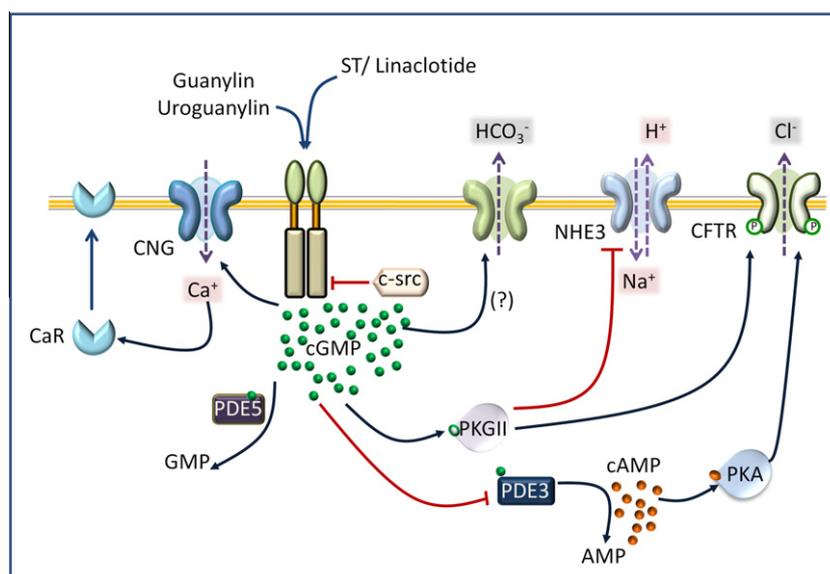


Fig. 1. Signalling mechanisms mediated by GC-C. GC-C expressed on the surface of enterocytes serves as the receptor for the endogenous ligands uroguanylin and guanylin, or ST produced by enterotoxigenic *E. coli*. Linaclotide, an ST peptide mimic, also binds to GC-C. Ligand binding increases intracellular levels of cGMP. Cyclic GMP activates cGMP-dependent protein kinase II (PKGII) and inhibits the activity of the cAMP-phosphodiesterase PDE3, thereby cross-activating cAMP-dependent protein kinase (PKA). PKGII and PKA phosphorylate the cystic fibrosis transmembrane conductance regulator (CFTR), increasing its chloride-secreting activity. Cyclic GMP also enhances duodenal bicarbonate secretion in a CFTR-dependent manner, and inhibits NHE3. These processes maintain fluid and ion homeostasis. Cyclic GMP also directly activates cyclic nucleotide gated channels (CNG) leading to Ca^{2+} influx. Elevated intracellular Ca^{2+} levels bind to calcium sensing receptors (CaRs), resulting in cell differentiation and migration. GC-C signaling is terminated by hydrolysis of cGMP-GMP by a cGMP-dependent phosphodiesterase, PDE5. Cyclic GMP production by GC-C is inhibited by c-src mediated phosphorylation of a specific tyrosine residue in the guanylyl cyclase domain of GC-C.

Additional signaling pathways in the intestinal cell also appear to be modulated by GC-C activity. Thus, in GC-C knock-out mice, an increased activity of Akt (protein kinase B) was observed, resulting in accelerated cell proliferation, which could be reversed by the administration of cGMP [39]. In human colonic cell lines, activation of GC-C resulted in changes in the expression of genes that are involved in the Akt signaling pathway, suggesting that GC-C could prevent intestinal tumour formation by inhibiting Akt signaling [39]. It is not readily apparent at this time whether calcium influx and/or lower Akt activity are principally responsible for mediating the cytostatic effects of activated GC-C. It is equally likely that the main driving force for inducing colon cell cytostasis may involve other signaling molecules that are operative under a particular intestinal milieu, and such studies are awaited.

It is important to note that the GC-C expression is retained in colon tumours, and therefore GC-C expression can be used as a marker to diagnose metastatic colon cancer [42]. However, expression of uroguanylin and guanylin is often lost in colorectal cancer progression [43]. Therefore, the anti-proliferative activity of GC-C could perhaps be restored by administration of ST peptide analogs such as linaclotide, or by the elevation of intracellular cGMP following inhibition of cyclic nucleotide phosphodiesterases such as PDE5 [44].

A characteristic feature of most colorectal cancers is the high activity of c-src tyrosine kinase seen in colon carcinoma cells [45]. We have demonstrated that GC-C is regulated by c-src mediated inhibitory phosphorylation of a specific tyrosine residue in GC-C. Consequently, the addition of ligands of GC-C to colonic cancer cells that contain elevated levels of active c-src does not result in an increase in intracellular cGMP [46], and therefore would prevent anti-proliferative signaling mediated by GC-C. Thus, a combinatorial therapy of c-src inhibitors, such as dasatinib [47], which is in phase-2 clinical trials for the treatment of colorectal cancer, along with activators of GC-C, such as linaclotide, may be an effective regime in the treatment of colon cancers.

4. Roles of GC-C and cGMP in intestinal inflammation

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder and two main clinical presentations are Crohn's disease and ulcerative colitis [48]. Genetic factors, the host immune system and the gut microbiome contribute to IBD, and disturbances in the interaction between the intestinal epithelial cells and the immune cells in the intestine appear to be responsible for these syndromes. Therefore, any malfunctioning of the epithelial cells could alter the responses of the immune cells in the gut, resulting in inflammation.

Heightened inflammation, as a result of intestinal electrolyte imbalance, is seen in infectious diarrheal disease and in patients suffering from congenital chloride diarrhea [49]. Since GC-C regulates ion secretion, it may be a mitigating factor in causing the inflammation. Intestinal biopsies from patients suffering from inflammatory bowel disease (IBD) showed down regulation of the sodium-hydrogen ion exchanger 3 (NHE3) [50], and NHE3 knock-out mice overproduce inflammatory cytokines, and are more susceptible to dextran sodium sulfate (DSS)-induced epithelial injury [51]. Thus, the inhibition of NHE3 activity that is seen following GC-C activation may contribute to the inflammation seen in diarrheal disease. Suggestive of this is the observation that GC-C knock-out mice, and to a lesser extent, guanylin knock-outs, show reduced TNF β production and diminished apoptosis in the distal colon following acute administration of DSS [52]. This may be correlated with the lower levels of cGMP in the intestinal epithelia of GC-C and guanylin knock-out mice, which may have resulted in hyper activation of NHE3, and therefore reduced inflammation.

Paradoxically, a disruption of the integrity of the intestinal barrier is also seen in GC-C knock-out mice [53]. It is known that a disruption of epithelial barrier function can result in pathological inflammatory responses as is seen in IBD. Moreover, down regulation of guanylin and uroguanylin mRNA is seen in Crohn's disease

and ulcerative colitis [54] implying that reduced cGMP levels may actually aggravate inflammation. It is therefore apparent that the molecular bases, if any, of GC-C and cGMP-mediated regulation of inflammation in the intestine, either via tight junction breakdown or altered pro-inflammatory cytokine expression in the gut, are far from clear.

An interaction between intestinal microflora and host factors is necessary for the development of intestinal inflammation, since in the background of a genetic mutation in the host, specific viruses or bacteria need to be present, along with environmental factors, in the intestine in order to manifest disease [55]. We speculate that perhaps fluid and ion secretion regulated by GC-C could affect the composition of the microbiome. Therefore, in GC-C knock-out mice, the microbiota may differ from wild-type mice, and this could contribute to the reduced inflammation that is seen, in spite of tight junction disassembly.

5. GC-C and cGMP: of mice and men

As is usually the case for many genes, most of the studies that relate to GC-C have to date been carried out in mouse models, or in cell lines derived from human colorectal cancer tissue. What appeared to be a lacuna was any report of a human disorder that resulted from a mutation in the GC-C gene, *GUCY2C*. However, this year, two studies appeared where point mutations in *GUCY2C* were associated with human disease. The importance of these two findings in emphasizing the role of GC-C in human intestinal physiology cannot be overstated.

Familial diarrheas are severe and generally caused by recessive mutations. Irritable bowel syndrome and Crohn's disease are well documented examples that show a genetic predisposition, and are generally believed to be multifactorial, with a number of genetic susceptible loci contributing to their occurrence in families. Recently, a large Norwegian family was identified which demonstrated a dominantly inherited, fully penetrant syndrome of frequent diarrhea. Whole genome SNP-linkage based analysis and exome sequencing identified a heterozygous mutation in *GUCY2C* [14]. The effect of the Ser840 mutation to an Ile in GC-C was characterized by heterologous expression of the mutant receptor. The mutation interestingly resulted in a hyper-responsive form of GC-C, whereby levels of cGMP produced by the mutant receptor were 6–10-fold higher than observed for the wild type receptor, at equivalent concentrations of ST peptide, uroguanylin or guanylin. What was intriguing was that while the affinity of mutant GC-C for ST or uroguanylin did not change, the EC₅₀ for activation by uroguanylin was significantly lower in the mutant receptor in comparison with the wild type receptor [14]. We therefore argued that patients harboring this mutation would have elicited higher intracellular levels of cGMP in the intestine in response to the endogenous levels of uroguanylin or guanylin present in their gastrointestinal tract. Thus, this mutant GC-C was a gain-of-function mutation, which would have resulted in over-activation of signaling pathways that emanate from GC-C. One such pathway may regulate fluid and ion secretion from the intestine, and involve activation of CFTR and inhibition of NHE3, thus resulting in the chronic and frequent episodes of diarrhea that these patients experience.

What remained enigmatic, however, were the additional severe symptoms reported by some patients [14]. Some individuals had undergone laparotomy for suspected bowel obstruction, and ileal inflammation, volvulus or adhesional bands, was confirmed. Some showed anatomical variants in the ileocaecal region, and needed to undergo resection of the terminal ileum or caecum. In addition, Crohn's disease was observed in four of such patients. No emotional disturbances, which could be a consequence of hyperactive

GC-C in neurons [35], were observed in any patients, and no one showed any signs of leanness [33]. Clearly, the presence of over active GC-C, and presumably elevated levels of cGMP, in these patients led to phenotypes that could not be predicted from results seen in the GC-C knock-out mice.

The increased risk of inflammatory bowel disease in these patients could have been a consequence of enhanced inflammatory cytokine production, as has been observed in NHE3 null mice [50,56], and patients suffering from chloride diarrhea [49]. It could be speculated that, in contrast to the GC-C knock-out mouse, hyperactive GC-C could have led to a breakdown of intestinal barrier function, resulting in the inflammation that is seen in these patients. Alternatively, as discussed above, the microbiota of these patients may differ, consequent to the life-long incidence of diarrhea, resulting in an increased susceptibility to inflammation.

Following this first report of a human disease correlated with a gain-of-function mutation in the *GUCY2C* gene, inactivating mutations of GC-C in a small Bedouin population were reported [15]. These patients also reported multiple disturbances, which led to ileus, and blockage of the small intestine caused by thick mucoid stools, presumably as a result of the lack of adequate fluid being secreted via the GC-C signaling pathway. Here again, these results contrast with mice data, where the GC-C knock-out mouse was normal in terms of regular gastrointestinal function. Two inactivating mutations were reported in the Bedouin population, one which was a substitution of Asp387 by a Gly residue, which reduced cGMP production by ~50% in the mutant receptor in contrast with the wild type receptor. The second was an insertion of a single base following Asn757, which would have truncated the receptor protein before the guanylyl cyclase domain, causing the mutant protein to be non-responsive to ligands of GC-C. The authors speculate that these mutations would have rendered the carriers resistant to ST-induced diarrhea, which could have been an advantage to the desert dwelling Bedouins [15].

These to date remain the only two reports of mutations in GC-C found in humans. Perhaps this once again emphasized the critical requirement of the presence of an optimally functional GC-C in the intestine, since neither its loss, nor its hyperactivity, can be tolerated in humans without severe consequences. In addition, in recognition of the differences that are known between the mouse and the human immune system [57], it becomes important to realize that perhaps not all of the reported roles of GC-C in the mouse may be applicable in human physiology.

6. Future perspectives

Very important findings related to the roles of GC-C, and consequently cGMP, have emerged over the past few years. Nevertheless, there are still a number of questions that remain. What are the precise molecular mechanisms by which GC-C is able to aid in colon cell cytotaxis? Are there new players that may emerge from a greater understanding of downstream effects of elevated cGMP levels in the cell? What would be the consequences of a knock-in hyperactivating mutation in GC-C in the mouse? Would the phenotypes in mice mimic those seen in the Norwegian family? If they did, this would provide a very good model to dissect the underlying molecular mechanisms that contribute to the disturbances seen in patients, thereby aiding in the development of therapeutics for this disorder.

Cyclic GMP has always been considered the 'poor cousin' of cAMP. Nevertheless, its signaling machinery is no less sophisticated. A knock out of PKGII, the isoform predominantly expressed in the intestine, resulted in mice being resistant to ST-mediated fluid accumulation, as might be expected [58]. However, an unexpected finding was that these mice showed skeletal abnormalities

and dwarfism, indicating that cGMP and PKG II were involved in bone development [58,59]. PKGII knock-out mice were also defective in resetting the circadian clock, since PKG II activity was required for the correct modulation of Per1 and Per2 levels [60]. Intriguingly, guanylin and uroguanylin show circadian regulation in the rat intestine [61], thus suggesting a tantalizing link between the intestine, behavior and circadian rhythm.

The multiple abnormalities seen in patients with altered GC-C activity or expression reveal an underlying complexity in the precise roles of GC-C and its ligands in mediating intestinal homeostasis. We anticipate that additional signaling outputs from GC-C may depend on its apical or basolateral localization in the epithelial cell, which could allow its association with specific signaling partners, perhaps via direct protein–protein interaction. The multi-domain nature of GC-C, and perhaps novel post-translational modifications mediated via phosphorylation, may make GC-C amenable for such interactions, as has been reported for c-src [16]. A large fraction of GC-C is localized to the endoplasmic reticulum (ER), and glycosylated differently to the form that is found on the plasma membrane [62,63]. The precise role of this 'intracellular' pool of GC-C is enigmatic. Is it a fraction that is poised to be secreted to the plasma membrane in a regulated manner? Or are there specific roles for ER-localized GC-C, in that it may respond to stimuli other than its known ligands, in a cGMP-independent manner? These are some of the questions that we wish to address in the coming years. Our motivation stems primarily from recent observations indicating the somewhat serious consequences of abnormal GC-C signaling in the intestine, that suggest that much remains unknown about this receptor, its ligands and cGMP signaling in the intestinal epithelial cell.

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References

- Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W. and Pettersson, S. (2012) Host-gut microbiota metabolic interactions. *Science* 336, 1262–1267.
- Mandel, I.D. (1989) The role of saliva in maintaining oral homeostasis. *J. Am. Dent. Assoc.* 119, 298–304.
- Goto, Y. and Kiyono, H. (2012) Epithelial barrier: an interface for the cross-communication between gut flora and immune system. *Immunol. Rev.* 245, 147–163.
- Lloyd, D.A.J. and Gabe, S.M. (2009) Intestinal Morphology, Intestinal Regeneration and the Promise of Tissue Engineering in: *Intestinal Failure: Diagnosis, Management and Transplantation* (Langnas, A.N., Quigley, E.M.M. and Tappenden, K.A., Eds.), pp. 11–19, Blackwell Publishing Ltd, Oxford, UK.
- Barrett, K.E. (2008) New ways of thinking about (and teaching about) intestinal epithelial function. *Adv. Physiol. Educ.* 32, 25–34.
- Snippert, H.J. et al. (2010) Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* 143, 134–144.
- Mbuhi, J.V., Ntonifor, H.N. and Ojong, J.T. (2010) The incidence, intensity and host morbidity of human parasitic protozoan infections in gastrointestinal disorder outpatients in Buea sub division, cameroon. *J. Infect. Dev. Ctries.* 4, 38–43.
- Schulze, J. and Sonnenborn, U. (2009) Yeasts in the gut: from commensals to infectious agents. *Dtsch. Arztebl. Int.* 106, 837–842.
- Kau, A.L., Ahern, P.P., Griffin, N.W., Goodman, A.L. and Gordon, J.I. (2011) Human nutrition, the gut microbiome and the immune system. *Nature* 474, 327–336.
- Sekirov, I., Russell, S.L., Antunes, L.C. and Finlay, B.B. (2010) Gut microbiota in health and disease. *Physiol. Rev.* 90, 859–904.
- Hodges, K. and Gill, R. (2010) Infectious diarrhea: cellular and molecular mechanisms. *Gut. Microbes* 1, 4–21.
- Brown, K.H. (2003) Diarrhea and malnutrition. *J. Nutr.* 133, 328S–332S.
- Field, M., Graf Jr., L.H., Laird, W.J. and Smith, P.L. (1978) Heat-stable enterotoxin of *Escherichia coli*: in vitro effects on guanylate cyclase activity, cyclic GMP concentration, and ion transport in small intestine. *Proc. Natl. Acad. Sci. U.S.A.* 75, 2800–2804.
- Fiskerstrand, T. et al. (2012) Familial diarrhea syndrome caused by an activating *GUCY2C* mutation. *N. Engl. J. Med.* 366, 1586–1595.
- Romi, H. et al. (2012) Meconium ileus caused by mutations in *GUCY2C*, encoding the CFTR-activating guanylate cyclase 2C. *Am. J. Hum. Genet.* 90, 893–899.
- Basu, N., Arshad, N. and Visweswariah, S.S. (2010) Receptor guanylyl cyclase C (GC-C): regulation and signal transduction. *Mol. Cell. Biochem.* 334, 67–80.
- Kopic, S. and Geibel, J.P. (2010) Toxin mediated diarrhea in the 21 century: the pathophysiology of intestinal ion transport in the course of ETEC, *V. cholerae* and rotavirus infection. *Toxins (Basel)* 2, 2132–2157.
- Vanden Broeck, D., Horvath, C. and De Wolf, M.J. (2007) *Vibrio cholerae*: cholera toxin. *Int. J. Biochem. Cell Biol.* 39, 1771–1775.
- Schulz, S., Green, C.K., Yuen, P.S. and Garbers, D.L. (1990) Guanylyl cyclase is a heat-stable enterotoxin receptor. *Cell* 63, 941–948.
- Basu, N. and Visweswariah, S.S. (2011) Defying the stereotype: non-canonical roles of the Peptide hormones guanylin and uroguanylin. *Front. Endocrinol. (Lausanne)* 2, 14.
- Hamra, F.K. et al. (1993) Uroguanylin: structure and activity of a second endogenous peptide that stimulates intestinal guanylate cyclase. *Proc. Natl. Acad. Sci. U.S.A.* 90, 10464–10468.
- Forte, L.R. and Currie, M.G. (1995) Guanylin: a peptide regulator of epithelial transport. *FASEB J.* 9, 643–650.
- Vaandrager, A.B. et al. (1998) Membrane targeting of cGMP-dependent protein kinase is required for cystic fibrosis transmembrane conductance regulator Cl-channel activation. *Proc. Natl. Acad. Sci. U.S.A.* 95, 1466–1471.
- Vaandrager, A.B., Bot, A.G., Ruth, P., Pfeifer, A., Hofmann, F. and De Jonge, H.R. (2000) Differential role of cyclic GMP-dependent protein kinase II in ion transport in murine small intestine and colon. *Gastroenterology* 118, 108–114.
- Cha, B. et al. (2005) CGMP inhibition of Na⁺/H⁺ antiporter 3 (NHE3) requires PDZ domain adapter NHERF2, a broad specificity protein kinase G-anchoring protein. *J. Biol. Chem.* 280, 16642–16650.
- Sellers, Z.M. et al. (2005) Heat-stable enterotoxin of *Escherichia coli* stimulates a non-CFTR-mediated duodenal bicarbonate secretory pathway. *Am. J. Physiol. Gastrointest. Liver Physiol.* 288, G654–G663.
- Pitari, G.M., Lin, J.E., Shah, F.J., Lubbe, W.J., Zuzga, D.S., Li, P., Schulz, S. and Waldman, S.A. (2008) Enterotoxin preconditioning restores calcium-sensing receptor-mediated cytoskeleton in colon cancer cells. *Carcinogenesis* 29, 1601–1607.
- Forte, L.R., Eber, S.L., Turner, J.T., Freeman, R.H., Fok, K.F. and Currie, M.G. (1993) Guanylin stimulation of Cl⁻ secretion in human intestinal T84 cells via cyclic guanosine monophosphate. *J. Clin. Invest.* 91, 2423–2428.
- Busby, R.W. et al. (2010) Linaclotide, through activation of guanylate cyclase C, acts locally in the gastrointestinal tract to elicit enhanced intestinal secretion and transit. *Eur. J. Pharmacol.* 649, 328–335.
- Lembo, A.J. et al. (2011) Two randomized trials of linaclotide for chronic constipation. *N. Engl. J. Med.* 365, 527–536.
- Mann, E.A., Jump, M.L., Wu, J., Yee, E. and Giannella, R.A. (1997) Mice lacking the guanylyl cyclase C receptor are resistant to STA-induced intestinal secretion. *Biochem. Biophys. Res. Commun.* 239, 463–466.
- Schulz, S., Lopez, M.J., Kuhn, M. and Garbers, D.L. (1997) Disruption of the guanylyl cyclase-C gene leads to a paradoxical phenotype of viable but heat-stable enterotoxin-resistant mice. *J. Clin. Invest.* 100, 1590–1595.
- Valentino, M.A. et al. (2011) A uroguanylin-*GUCY2C* endocrine axis regulates feeding in mice. *J. Clin. Invest.* 121, 3578–3588.
- Forte Jr., L.R. (2004) Uroguanylin and guanylin peptides: pharmacology and experimental therapeutics. *Pharmacol. Ther.* 104, 137–162.
- Gong, R. et al. (2011) Role for the membrane receptor guanylyl cyclase-C in attention deficiency and hyperactive behavior. *Science* 333, 1642–1646.
- Jaleel, M., London, R.M., Eber, S.L., Forte, L.R. and Visweswariah, S.S. (2002) Expression of the receptor guanylyl cyclase C and its ligands in reproductive tissues of the rat: a potential role for a novel signaling pathway in the epididymis. *Biol. Reprod.* 67, 1975–1980.
- Pitari, G.M. et al. (2003) Bacterial enterotoxins are associated with resistance to colon cancer. *Proc. Natl. Acad. Sci. U.S.A.* 100, 2695–2699.
- Pitari, G.M., Di Guglielmo, M.D., Park, J., Schulz, S. and Waldman, S.A. (2001) Guanylyl cyclase C agonists regulate progression through the cell cycle of human colon carcinoma cells. *Proc. Natl. Acad. Sci. U.S.A.* 98, 7846–7851.
- Lin, J.E. et al. (2010) The hormone receptor *GUCY2C* suppresses intestinal tumor formation by inhibiting AKT signaling. *Gastroenterology* 138, 241–254.
- Kazerounian, S. et al. (2005) Proliferative signaling by store-operated calcium channels opposes colon cancer cell cytoskeleton induced by bacterial enterotoxins. *J. Pharmacol. Exp. Ther.* 314, 1013–1022.
- Pitari, G.M., Baksh, R.I., Harris, D.M., Li, P., Kazerounian, S. and Waldman, S.A. (2005) Interruption of homologous desensitization in cyclic guanosine 3',5'-monophosphate signaling restores colon cancer cytoskeleton by bacterial enterotoxins. *Cancer Res.* 65, 11129–11135.
- Carrithers, S.L., Barber, M.T., Biswas, S., Parkinson, S.J., Park, P.K., Goldstein, S.D. and Waldman, S.A. (1996) Guanylyl cyclase C is a selective marker for metastatic colorectal tumors in human extraintestinal tissues. *Proc. Natl. Acad. Sci. U.S.A.* 93, 14827–14832.

- [43] Li, P., Lin, J.E., Marszlowicz, G.P., Valentino, M.A., Chang, C., Schulz, S., Pitari, G.M. and Waldman, S.A. (2009) GCC signaling in colorectal cancer: Is colorectal cancer a paracrine deficiency syndrome? *Drug News Perspect.* 22, 313–318.
- [44] Zhu, B. and Strada, S.J. (2007) The novel functions of cGMP-specific phosphodiesterase 5 and its inhibitors in carcinoma cells and pulmonary/cardiovascular vessels. *Curr. Top. Med. Chem.* 7, 437–454.
- [45] Cartwright, C.A., Kamps, M.P., Meisler, A.I., Pipas, J.M. and Eckhart, W. (1989) Pp 60c-src activation in human colon carcinoma. *J. Clin. Invest.* 83, 2025–2033.
- [46] Basu, N., Bhandari, R., Natarajan, V.T. and Visweswariah, S.S. (2009) Cross talk between receptor guanylyl cyclase C and c-src tyrosine kinase regulates colon cancer cell cytostasis. *Mol. Cell. Biol.* 29, 5277–5289.
- [47] Kopetz, S. et al. (2009) Synergistic activity of the SRC family kinase inhibitor dasatinib and oxaliplatin in colon carcinoma cells is mediated by oxidative stress. *Cancer Res.* 69, 3842–3849.
- [48] Abraham, C. and Cho, J.H. (2009) Inflammatory bowel disease. *N. Eng. J. Med.* 361, 2066–2078.
- [49] Wedenoja, S., Hoglund, P. and Holmberg, C. (2010) Review article: the clinical management of congenital chloride diarrhoea. *Aliment. Pharmacol. Ther.* 31, 477–485.
- [50] Sullivan, S. et al. (2009) Downregulation of sodium transporters and NHERF proteins in IBD patients and mouse colitis models: potential contributors to IBD-associated diarrhea. *Inflamm. Bowel Dis.* 15, 261–274.
- [51] Kiela, P.R. et al. (2009) Changes in mucosal homeostasis predispose NHE3 knockout mice to increased susceptibility to DSS-induced epithelial injury. *Gastroenterology* 137 (965–975), 975 e1–975 e 10.
- [52] Steinbrecher, K.A., Harmel-Laws, E., Garin-Laflam, M.P., Mann, E.A., Bezerra, L.D., Hogan, S.P. and Cohen, M.B. (2011) Murine guanylate cyclase C regulates colonic injury and inflammation. *J. Immunol.* 186, 7205–7214.
- [53] Han, X., Mann, E., Gilbert, S., Guan, Y., Steinbrecher, K.A., Montrose, M.H. and Cohen, M.B. (2011) Loss of guanylyl cyclase C (GCC) signaling leads to dysfunctional intestinal barrier. *PLoS One* 6, e16139.
- [54] Wu, F. et al. (2007) Genome-wide gene expression differences in Crohn's disease and ulcerative colitis from endoscopic pinch biopsies: insights into distinctive pathogenesis. *Inflamm. Bowel Dis.* 13, 807–821.
- [55] Maloy, K.J. and Powrie, F. (2011) Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature* 474, 298–306.
- [56] Laubitz, D., Larmonier, C.B., Bai, A., Midura-Kiela, M.T., Lipko, M.A., Thurston, R.D., Kiela, P.R. and Ghishan, F.K. (2008) Colonic gene expression profile in NHE3-deficient mice. evidence for spontaneous distal colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 295, G63–G77.
- [57] Mestas, J. and Hughes, C.C. (2004) Of mice and not men: differences between mouse and human immunology. *J. Immunol.* 172, 2731–2738.
- [58] Pfeifer, A., Aszodi, A., Seidler, U., Ruth, P., Hofmann, F. and Fassler, R. (1996) Intestinal secretory defects and dwarfism in mice lacking cGMP-dependent protein kinase II. *Science* 274, 2082–2086.
- [59] Rangaswami, H. et al. (2010). Cyclic GMP and protein kinase G control a Src-containing mechanosome in osteoblasts. *Sci. Signal* 3, ra91.
- [60] Oster, H., Werner, C., Magnone, M.C., Mayser, H., Feil, R., Seeliger, M.W., Hofmann, F. and Albrecht, U. (2003) CGMP-dependent protein kinase II modulates mPer1 and mPer2 gene induction and influences phase shifts of the circadian clock. *Curr. Biol.* 13, 725–733.
- [61] Scheving, L.A. and Jin, W.H. (1999) Circadian regulation of uroguanylin and guanylin in the rat intestine. *Am. J. Physiol.* 277, C1177–C1183.
- [62] Ghanekar, Y., Chandrasher, A., Tatu, U. and Visweswariah, S.S. (2004) Glycosylation of the receptor guanylate cyclase C: role in ligand binding and catalytic activity. *Biochem. J.* 379, 653–663.
- [63] Ghanekar, Y., Chandrasher, A. and Visweswariah, S.S. (2003) Cellular refractoriness to the heat-stable enterotoxin peptide is associated with alterations in levels of the differentially glycosylated forms of guanylyl cyclase C. *Eur. J. Biochem.* 270, 3848–3857.