Published in final edited form as:

Drug News Perspect. 2009 ; 22(6): 313–318. doi:10.1358/dnp.2009.22.6.1395254.

GCC signaling in colorectal cancer: Is colorectal cancer a paracrine deficiency syndrome?

P. Li^{1,*}, J.E. Lin^{1,*}, G.P. Marszlowicz², M.A. Valentino¹, C. Chang², S. Schulz¹, G.M. Pitari¹, and S.A. Waldman¹

¹Department of Pharmacology and Experimental Therapeutics, Thomas Jefferson University, Philadelphia, PA 19107

²School of Biomedical Engineering, Science & Health Systems, Drexel University, Philadelphia, PA 19104

Summary

Guanylyl cyclase C (GCC) is the receptor expressed by intestinal cells for the paracrine hormones guanylin and uroguanylin that coordinate mucosal homeostasis and its silencing contributes to intestinal transformation. It orchestrates proliferative and metabolic circuits by limiting the cell cycle and programming metabolic transitions central to regeneration along the crypt-villus axis. Mice deficient in GCC are more susceptible to colon cancer induced by germline mutations or carcinogens. Moreover, guanylin and uroguanylin are the most commonly lost gene products in colon cancer. The role of GCC as a tumor suppressor and the universal loss of its hormones in transformation suggest a paradigm in which colorectal cancer is a disease of paracrine hormone insufficiency. Indeed, GCC signaling reverses the tumorigenic phenotype of human colon cancer cells by regulating proliferation and metabolism. These data suggest a pathophysiological hypothesis in which GCC is a tumor suppressor coordinating proliferative homeostasis whose silencing through hormone loss initiates transformation. The correlative therapeutic hypothesis suggests that colorectal cancer is a disease of hormone insufficiency that can be prevented or treated by oral hormone replacement therapy employing GCC ligands.

I. Introduction

Colorectal cancer is the 4th most common tumor and the second leading cause of cancer-related mortality, producing 10% of cancer-related deaths, worldwide [1–3]. While surgery is the mainstay of treatment, metastases produce disease recurrence in ~50% of patients [2, 4, 5] and adjuvant chemotherapy is only marginally effective [4, 6]. The poor prognosis and absence of highly effective therapies highlight the unmet clinical need for earlier prevention and treatment. The current paradigm suggests that colon cancer is a genetic disease, secondary to sequential accumulation of mutations in oncogenes and tumor suppressors [7], including sporadic or inherited alterations in the adenomatous polyposis coli (APC) gene, β -catenin, and the DNA mismatch repair genes, K-ras and p53 [8, 9]. Beyond this oncogenomic view of cancer, there is a novel paradigm suggesting that colorectal cancer initiates as a state of paracrine hormone insufficiency [10–12]. This hypothesis provides a novel opportunity for colorectal cancer prevention by hormone supplementation to re-engage disrupted signaling pathways opposing tumorigenesis [10, 13, 14].

Corresponding Author: Scott A. Waldman M.D., Ph.D., Department of Pharmacology and Experimental Therapeutics, Thomas Jefferson University, 132 South 10th Street, 1170 Main, Philadelphia, PA 19107; Tel: (215) 955-6086; Fax: (215) 955-5681; scott.waldman@jefferson.edu.

*Contribute equally.

II. Gut Homeostasis

The surface of the intestine is composed of a single layer of epithelial cells structured as vertical units essential for normal gut functions. This epithelium undergoes continuous renewal mediated by repetitive rounds of proliferation, migration, differentiation, apoptosis and shedding important for digestion, absorption, secretion, and barrier function (Fig. 1) [13–18]. Transit cells arise from stem cells near the crypt base and undergo multiple cycles of division. Both rapidly proliferating transit cells and slowly replicating stem cells encompass the proliferating zone, crypts in small intestine and the bottom of crypts in colon, providing for continuous epithelial renewal. Transit cells migrate up the vertical axis to the differentiated compartment where they undergo nuclear and cytoplasmic reprogramming which coordinates cytotaxis and differentiation. Multiple signaling pathways including NOTCH 1, MATH 1 or HES 1 program the transition from proliferation to differentiation [19–22]. Enterocytes comprise the principle lineage of intestinal epithelial cells and develop well-organized apical microvillus membranes, in which reside proteins mediating digestion and absorption. Goblet cells secrete mucin and establish the mucus layer protecting the epithelium and potentiating digestion and absorption [23]. Enteroendocrine cells are part of the neuro-endocrine system, secreting local paracrine hormones supporting neuromuscular activity, secretion, and central nervous system regulation of calorie consumption [23]. Paneth cells, produced only in small intestine, secrete antimicrobial peptides and growth factors [24] contributing to the barrier defending against microbial invasion and tumorigenesis [24]. As cells transition from proliferation to differentiation, they undergo metabolic reprogramming, shifting from glycolysis to mitochondrial oxidation-phosphorylation. The crypt-villus gradient of metabolism, with glycolysis prevailing in crypts and mitochondrial metabolism predominating in villi, is associated with the proliferative gradient along the vertical axis. Metabolic programming reflects the specific unique energy demands in different compartments, subserving the balance of proliferation and differentiation. In the crypt, cell division requires rapidly available energy from glycolysis, while in the differentiated compartment, mitochondria exploit the efficiency of oxidative phosphorylation to support catabolic demands in cells which have undergone lineage commitment [25, 26].

Disruption of these regenerative cycles, associated with dysregulation of proliferative and metabolic programs, particularly the transition from cell division to lineage commitment, produces crypt over-population and a maturational shift to the left (more immature), associated with a Warburg-type metabolic phenotype, contributing to the development of colon cancer [15–18]. Epithelial renewal is regulated by a variety of antiproliferative mechanisms. Their corruption produces perpetual cycles of DNA replication, proliferation and crypt hyperplasia, potentiating the accumulation of mutations which, in turn, drive unrestricted cell division, failure of lineage commitment, and failure of apoptosis, establishing invasive cancer. The cell cycle is primarily controlled by regulating entry and progression through the G1/S interface. Accelerated transit through G₁ and premature entry into S amplifies genetic instability [27–30]. Moreover, transformed cells fail to differentiate and, rather, undergo metabolic reprogramming to enhance glycolysis to meet energy requirements for rapid proliferation. Hyperproliferation produces a microenvironment that amplifies genetic instability, potentiating neoplasia resulting in colorectal cancer initiation, progression, invasion and metastasis [31–33].

III. Guanylyl Cyclase C

Guanylyl cyclase C (GCC), functioning as a dimer or trimer (Fig. 2A) [34–36], is the intestinal epithelial cell receptor [37–41] for diarrheagenic bacterial heat-stable enterotoxins (STs) and the endogenous paracrine hormones guanylin and uroguanylin (Fig. 2A). STs are

structurally and functionally homologous to guanylin and uroguanylin, with 10-fold greater potency than those paracrine hormones [42, 43]. Hormone-receptor interaction activates the intracellular catalytic domain which converts GTP to cyclic GMP (cGMP; Fig. 2B). This cyclic nucleotide activates its downstream effectors [43], including cGMP-dependent protein kinase (PKG), which phosphorylates the cystic fibrosis transmembrane conductance regulator, producing secretion of salt and water and, in the case of ST, diarrhea [44].

Guanylin and uroguanylin are organized in a tail-to-tail configuration on chromosome 1p in humans [45, 46]. Preliminary studies suggested that these hormones are the most commonly lost gene products in colorectal cancer in animals and humans [47–51]. Their loss occurs at the earliest stages along the neoplastic continuum, with hormone deficiency detected as early as dysplastic crypts [52] and hyperplastic polyps and adenomas [10, 47–51]. More recently, >500 normal adjacent tissue (NAT) and tumors from >300 colon cancer patients, including 125 matched NAT and tumor specimens were analyzed [53]. Guanylin (~20-fold) and uroguanylin (~20-fold) expression was coordinately reduced in tumors, compared to NAT ($p < 0.0001$), in 81% and 76% of patients, respectively, a frequency comparable to APC mutation rates in colon cancer [54, 55]. These observations suggest that GCC hormone loss generally is associated with the earliest stages of sporadic colorectal cancer [10–12, 56, 57].

GCC is the only defined receptor for guanylin and uroguanylin, although some studies suggest there might be other receptors, since GCC deficient mice exhibit uroguanylin-induced natriuresis and kaliuresis in response to uroguanylin administration [58]. In that context, GCC is expressed primarily in apical membranes of intestinal epithelial cells from the duodenum to the rectum. In humans, the specificity of GCC expression has been demonstrated by RT-PCR and confirmed with GCC-specific [32 P]-labeled riboprobe hybridization to PCR products [38] as well as functional assay by competitive binding with 125I ST [38, 40]. Some studies suggest that, in addition to intestine, GCC is expressed and functions in epithelia from lung [59, 60], kidney [61, 62], gallbladder [63], pancreas [64, 65], salivary glands [66, 67] and reproductive epithelium [68] in human and rats by RT-PCR, western blot or immunohistochemistry. However, these observations are inconsistent with studies using more sensitive and specific probes to quantify RT-PCR products and functional binding analysis [38, 69–71]. Apparent discrepancies in the specificity of GCC expression in different studies may reflect the design of primers and probes for RT-PCR analyses and non-specific cross-reactivity of antibodies used for immunohistochemistry. The family of guanylyl cyclases share >50% sequence homology in their intracellular catalytic domains while, in contrast, the extracellular domains exhibit <10% homology between members of the family [43]. Primers and probes corresponding to the catalytic domain may be of relatively low stringency [60] while primers corresponding to GCC introns may detect DNA, rather than mRNA [67].

Of significance, unlike its paracrine hormones, GCC continues to be produced after epithelial cells undergo transformation and become colorectal cancer cells [38, 69–71]. Further, GCC expression has been identified in all primary and metastatic colorectal tumors obtained from patients [38, 69–71]. Moreover, GCC is over-expressed in colon cancers compared to normal adjacent tissues, which might reflect receptor supersensitization in the context of hormone loss [70, 71]. Selective expression of GCC in intestine and colorectal tumors fulfills the criteria for a specific therapeutic target for cancer prevention and therapy [70, 71]. The novel paradigm in which colon cancer starts as a disease of hormone insufficiency in the face of over-expression of receptors suggests a previously unrecognized cancer prevention strategy involving hormone replacement therapy employing guanylin and uroguanylin.

IV. GCC Signaling Opposes Carcinogenesis

Beyond fluid regulation [72, 73], GCC and its ligands regulate intestinal regenerative homeostasis by restricting proliferative dynamics and coordinating cell cycle and metabolic circuits [12, 26, 74, 75]. Moreover, interruption of GCC signaling potentiates tumorigenesis [12, 26, 74, 75] in mice reflecting disrupted proliferative and metastatic processes. Eliminating expression of GCC ($GCC^{-/-}$) [12, 26] or guanylin [75] in mice increases the number of crypts, which exhibit higher proliferative indices, associated with an accelerated cell cycle [12]. Hyperproliferation in the context of silenced GCC is associated with over-expression of cell cycle drivers, including cyclin D and pRb, which regulate transition through the G1-S interface, but decreased cell cycle suppressors, including p27 [12, 26, 74]. Hyperplasia of the proliferating compartment is associated with bioenergetic remodeling with the induction of glycolysis and the reciprocal suppression of mitochondrial biogenesis, required for energy independence and anabolic biosynthesis supporting hyperproliferation [26, 76, 77]. Indeed, the gradient of mitochondria along the crypt-villus axis reflecting the transition from proliferation to differentiation is eliminated in $GCC^{-/-}$ mice [26]. These data suggest a role for GCC in coordinating proliferation and metabolism important in physiological programs organizing the crypt-villus axis. Normally, GCC and its paracrine hormones drive cGMP production in an ascending gradient along the crypt-villus axis (Fig. 1) [43, 78] restricting proliferation and maintaining glycolytic ATP production in crypts. Corruption of those programs, reflecting loss of paracrine hormones, induces hyperproliferation marked by cell cycle acceleration and glycolytic reprogramming universally characterizing tumorigenesis [76, 77].

$GCC^{-/-}$ mice are hypersensitive to $Apc^{Min/+}$ and azoxymethane (AOM)-induced tumorigenesis, exhibiting increased tumor incidence, multiplicity, and burden [12]. GCC-associated tumorigenesis reflects corrupted proliferation, with increases in cell cycle drivers [12]. Indeed, human colorectal tumors exhibit alterations in proteins driving the cell cycle and glycolytic programming which are identical to those induced by disruption of GCC in mice [26]. Hyperproliferation and glycolytic reprogramming in $GCC^{-/-}$ mice reinforce DNA damage and chromosomal instability producing neoplastic transformation [26, 74]. Thus, GCC is a tumor suppressor that regulates proliferative and metabolic homeostasis in intestine. Loss of GCC paracrine hormones at the earliest stages of tumorigenesis suggests a mechanistic basis for hormone replacement to prevent and treat colorectal cancer [13, 26, 79].

Together, these data suggest that GCC suppresses tumorigenesis by coordinating proliferative and metabolic programming. Thus, disruption of GCC signaling due to early loss of guanylin and uroguanylin might be one central initiating event in human colorectal cancer by corrupting cell cycle and metabolic regulation and compromising genomic stability, producing accumulation of mutations in tumor suppressors and oncogenes promoting disease progression [26, 74].

V. Targeted Prevention For Colorectal Cancer

The role of GCC in proliferative and metabolic regulation opposing tumorigenesis, and the loss of ligands but over-expression of receptors in human colorectal carcinogenesis, highlight a new therapeutic paradigm for targeted colon cancer prevention and treatment involving oral hormone replacement therapy. This presumes that in the pathophysiological state, GCC is a dormant tumor-suppressing receptor whose re-engagement by hormones coordinately rescues cell cycle regulation and reprograms glycolytic metabolism to restrict proliferation.

GCC signaling inhibits the cell cycle of normal human intestinal cells and human colon carcinoma cells *in vitro* and *ex vivo* [11, 12, 26, 56, 74]. Activation of GCC induces a G1-S delay that restricts progression through the cell cycle, without apoptosis. Cytostasis is specifically mediated by GCC, associated with accumulation of cGMP, mimicked by the cell-permeant analog 8-Br-cGMP, and reproduced and potentiated by the cGMP-specific phosphodiesterase inhibitor zaprinast, but not an inactive ST analog [11, 56, 80]. Cytostasis induced by GCC downstream signaling was associated with reduced expression of cell cycle drivers like cyclin D, pRb, and increased expression of cell cycle inhibitors including p27, restricting the transition through the G1/S interface [11, 12, 56].

Similarly, GCC signaling reverts the tumorigenic metabolic phenotype in human colon cancer cells. GCC signaling inhibits glycolysis by reducing the expression of key rate-limiting enzymes, associated with a decrease in glucose uptake and lactate production [26]. Further, GCC signaling in human colon cancer cells induces expression of critical transcription factors required for mitochondrial biogenesis, including PGC1 alpha, mtTFA, and NRF1 [26]. Additionally, GCC signaling promotes mitochondrial biogenesis by increasing mitochondria content associated with enhanced mitochondrial oxygen consumption, dehydrogenase activity and ATP production [26].

In summary, restoration of GCC signaling in human colorectal cancer cells inhibits proliferation through cGMP-dependent mechanisms by coordinate regulation of the cell cycle and metabolism. In the context of universal hormone loss early in neoplasia, reconstitution of dormant receptor signaling by oral ligand supplementation may prevent the initiation and progression of colon cancer by suppressing proliferation and the associated metabolic reprogramming restricting premature entry into S phase and, therefore, maintaining genomic integrity [26, 74].

VI. The Paracrine Hormone Hypothesis of Colorectal Cancer

The emerging paradigm in which colorectal cancer is initiated by hormone insufficiency suggests that, at inception intestinal neoplasia could be prevented by oral hormone replacement to reconstitute tumor suppressing signaling pathways [13, 14]. Specifically, GCC signaling, compromised early in intestinal tumorigenesis reflecting hormone loss, maintains homeostasis by coordinating proliferation, metabolism, and genomic integrity along the crypt-villus axis. Corruption of this paracrine hormone axis compromises epithelial dynamics, producing crypt hyperplasia, maturational failure, and metabolic reprogramming. Disruption of these fundamental processes produces genetic mutations ultimately providing an evolutionary cellular advantage permitting emergence of progressive neoplastic transformation. Beyond this model of pathophysiology, these observations suggests a therapeutic hypothesis in which colorectal cancer can be prevented employing the well-established paradigm originating in endocrinology in which diseases of hormone insufficiency are treated by hormone replacement therapy. In that context, the near-universal loss of GCC ligands early along the neoplastic continuum [10, 47, 49–51] and the specificity and compensatory over-expression of GCC in colorectal cancer cells [38, 40, 70, 71] suggest the utility of oral GCC ligand replacement to prevent colorectal cancer. Oral supplementation of GCC ligands should restore intestinal paracrine hormone functions and defend proliferation, differentiation, metabolism and genomic stability that oppose colorectal carcinogenesis.

Abbreviations

AOM

azoxymethane

APC	adenomatous polyposis coli
BrdU	5'-bromo-2'-deoxyuridine
CFTR	cystic fibrosis transmembrane conductance regulator
cGMP	cyclic GMP
CNG	cyclic nucleotide-gated channel
GCC	guanylyl cyclase C
IHC	immunohistochemistry
PKA	cyclic AMP-dependent protein kinase
PKG	cyclic GMP-dependent protein kinase
pRb	phosphorylated retinoblastoma
RT-PCR	reverse transcriptase-polymerase chain reaction
ST	bacterial heat-stable enterotoxin
PGC1 alpha	peroxisome proliferator-activated receptor gamma coactivator 1alpha
mtTFA	mitochondria transcription factor A
NRF1	nuclear respiratory factors 1

Acknowledgments

These studies were supported by grants from NIH (CA75123, CA95026) and Targeted Diagnostic and Therapeutics Inc. to SAW and the Pennsylvania Department of Health and the Prevent Cancer Foundation to GMP. The Pennsylvania Department of Health specifically disclaims responsibility for any analyses, interpretations or conclusions. SAW is the Samuel M.V. Hamilton Endowed Professor.

References

1. Cresanta JL. Epidemiology of cancer in the United States. *Prim Care*. 1992; 19:419–441. [PubMed: 1410056]
2. Greenwald P. Colon cancer overview. *Cancer*. 1992; 70:1206–1215. [PubMed: 1511368]
3. Jessup JM, Gallick GE. The biology of colorectal carcinoma. *Curr Probl Cancer*. 1992; 16:261–328. [PubMed: 1337732]
4. Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. *N Engl J Med*. 2005; 352:476–487. [PubMed: 15689586]
5. Pihl E, Hughes ES, McDermott FT, Milne BJ, Price AB. Disease-free survival and recurrence after resection of colorectal carcinoma. *J Surg Oncol*. 1981; 16:333–341. [PubMed: 7253653]
6. Sobrero A, Kerr D, Glimelius B, Van Cutsem E, Milano G, Pritchard DM, Rougier P, Aapro M. New directions in the treatment of colorectal cancer: a look to the future. *Eur J Cancer*. 2000; 36:559–566. [PubMed: 10738119]
7. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med*. 1988; 319:525–532. [PubMed: 2841597]
8. Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, et al. Clues to the pathogenesis of familial colorectal cancer. *Science*. 1993; 260:812–816. [PubMed: 8484121]
9. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med*. 2003; 348:919–932. [PubMed: 12621137]
10. Shailubhai K, Yu HH, Karunanandaa K, Wang JY, Eber SL, Wang Y, Joo NS, Kim HD, et al. Uroguanylin treatment suppresses polyp formation in the Apc(Min/+) mouse and induces

- apoptosis in human colon adenocarcinoma cells via cyclic GMP. *Cancer Res.* 2000; 60:5151–5157. [PubMed: 11016642]
11. Pitari GM, Zingman LV, Hodgson DM, Alekseev AE, Kazerounian S, Bienengraeber M, Hajnoczky G, Terzic A, et al. Bacterial enterotoxins are associated with resistance to colon cancer. *Proc Natl Acad Sci U S A.* 2003; 100:2695–2699. [PubMed: 12594332]
 12. Li P, Lin JE, Chervoneva I, Schulz S, Waldman SA, Pitari GM. Homeostatic control of the crypt-villus axis by the bacterial enterotoxin receptor guanylyl cyclase C restricts the proliferating compartment in intestine. *Am J Pathol.* 2007; 171:1847–1858. [PubMed: 17974601]
 13. Li P, Lin JE, Snook AE, Gibbons A, Zuzga D, Schulz S, Pitari GM, Waldman SA. Colorectal cancer as a paracrine deficiency syndrome amenable to oral hormone replacement therapy. *Clinical Translational Science.* 2008; 1:163–167.
 14. Pitari GM, Li P, Lin JE, Zuzga D, Gibbons AV, Snook AE, Schulz S, Waldman SA. The paracrine hormone hypothesis of colorectal cancer. *Clin Pharmacol Ther.* 2007; 82:441–447. [PubMed: 17687268]
 15. Pinto D, Clevers H. Wnt, stem cells and cancer in the intestine. *Biology of the Cell.* 2005; 97:185–196. [PubMed: 15715524]
 16. Potten CS, Loeffler M. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development.* 1990; 110:1001–1020. [PubMed: 2100251]
 17. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature.* 2005; 434:843–850. [PubMed: 15829953]
 18. Sancho E, Batlle E, Clevers H. Signaling pathways in intestinal development and cancer. *Annu Rev Cell Dev Biol.* 2004; 20:695–723. [PubMed: 15473857]
 19. Yang Q, Bermingham NA, Finegold MJ, Zoghbi HY. Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. *Science.* 2001; 294:2155–2158. [PubMed: 11739954]
 20. Jensen J, Pedersen EE, Galante P, Hald J, Heller RS, Ishibashi M, Kageyama R, Guillemot F, et al. Control of endodermal endocrine development by Hes-1. *Nat Genet.* 2000; 24:36–44. [PubMed: 10615124]
 21. Crosnier C, Stamatakis D, Lewis J. Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. *Nat Rev Genet.* 2006; 7:349–359. [PubMed: 16619050]
 22. Vincent Zecchini RD. Doug Winton and Phil Jones, Notch signaling regulates the differentiation of post-mitotic intestinal epithelial cells. *Genes & Dev.* 2005; 19:1686–1691. [PubMed: 16024658]
 23. Koldovsky O, Dobiasova M, Hahn P, Kolinska J, Kraml J, Pacha J. Development of gastrointestinal functions. *Physiol Res.* 1995; 44:341–348. [PubMed: 8798267]
 24. Bry L, Falk P, Huttner K, Ouellette A, Midtvedt T, Gordon JI. Paneth cell differentiation in the developing intestine of normal and transgenic mice. *Proc Natl Acad Sci U S A.* 1994; 91:10335–10339. [PubMed: 7937951]
 25. Gassler N, Newrzella D, Bohm C, Lyer S, Li L, Sorgenfrei O, van Laer L, Sido B, et al. Molecular characterisation of non-absorptive and absorptive enterocytes in human small intestine. *Gut.* 2006; 55:1084–1089. [PubMed: 16556670]
 26. Lin JE, Snook AE, Li P, Schulz S, Dasgupta A, Hyslop TM, Pitari GM, Waldman SA. GUCY2C establishes lineage dependence in intestinal tumorigenesis through AKT. 2009 Submitted.
 27. Aoki K, Tamai Y, Horiike S, Oshima M, Taketo MM. Colonic polyposis caused by mTOR-mediated chromosomal instability in Apc+/Delta716 Cdx2+/- compound mutant mice. *Nat Genet.* 2003; 35:323–330. [PubMed: 14625550]
 28. Spruck CH, Won KA, Reed SI. Deregulated cyclin E induces chromosome instability. *Nature.* 1999; 401:297–300. [PubMed: 10499591]
 29. Morgan DO. Cyclin-dependent kinases: engines, clocks, and microprocessors. *Annu Rev Cell Dev Biol.* 1997; 13:261–291. [PubMed: 9442875]
 30. Murray AW. Recycling the cell cycle: cyclins revisited. *Cell.* 2004; 116:221–234. [PubMed: 14744433]
 31. Oving IM, Clevers HC. Molecular causes of colon cancer. *European Journal of Clinical Investigation.* 2002; 32:448–457. [PubMed: 12059991]
 32. Clevers H. Wnt breakers in colon cancer. *Cancer Cell.* 2004; 5:5–6. [PubMed: 14749120]

33. Zauber NP, Wang C, Lee PS, Redondo TC, Bishop DT, Goel A. Ki-ras gene mutations, LOH of the APC and DCC genes, and microsatellite instability in primary colorectal carcinoma are not associated with micrometastases in pericolic lymph nodes or with patients' survival. *Journal of Clinical Pathology*. 2004; 57:938–942. [PubMed: 15333653]
34. Qiu Y, Ogawa H, Miyagi M, Misono KS. Constitutive activation and uncoupling of the atrial natriuretic peptide receptor by mutations at the dimer interface. Role of the dimer structure in signalling. *J Biol Chem*. 2004; 279:6115–6123. [PubMed: 14600147]
35. Vijayachandra K, Guruprasad M, Bhandari R, Manjunath UH, Somesh BP, Srinivasan N, Suguna K, Visweswariah SS. Biochemical characterization of the intracellular domain of the human guanylyl cyclase C receptor provides evidence for a catalytically active homotrimer. *Biochemistry*. 2000; 39:16075–16083. [PubMed: 11123935]
36. Vaandrager AB, van der Wiel E, Hom ML, Luthjens LH, de Jonge HR. Heat-stable enterotoxin receptor/guanylyl cyclase C is an oligomer consisting of functionally distinct subunits, which are non-covalently linked in the intestine. *J Biol Chem*. 1994; 269:16409–16415. [PubMed: 7911466]
37. Cagir B, Gelmann A, Park J, Fava T, Tankelevitch A, Bittner EW, Weaver EJ, Palazzo JP, et al. Guanylyl cyclase C messenger RNA is a biomarker for recurrent stage II colorectal cancer. *Ann Intern Med*. 1999; 131:805–812. [PubMed: 10610624]
38. Carrithers SL, Barber MT, Biswas S, Parkinson SJ, Park PK, Goldstein SD, Waldman SA. Guanylyl cyclase C is a selective marker for metastatic colorectal tumors in human extraintestinal tissues. *Proc Natl Acad Sci U S A*. 1996; 93:14827–14832. [PubMed: 8962140]
39. Carrithers SL, Ott CE, Hill MJ, Johnson BR, Cai W, Chang JJ, Shah RG, Sun C, et al. Guanylin and uroguanylin induce natriuresis in mice lacking guanylyl cyclase-C receptor. *Kidney Int*. 2004; 65:40–53. [PubMed: 14675035]
40. Carrithers SL, Parkinson SJ, Goldstein S, Park P, Robertson DC, Waldman SA. Escherichia coli heat-stable toxin receptors in human colonic tumors. *Gastroenterology*. 1994; 107:1653–1661. [PubMed: 7958675]
41. Frick GS, Pitari GM, Weinberg DS, Hyslop T, Schulz S, Waldman SA. Guanylyl cyclase C: a molecular marker for staging and postoperative surveillance of patients with colorectal cancer. *Expert Rev Mol Diagn*. 2005; 5:701–713. [PubMed: 16149873]
42. Carpick BW, Garipey J. The Escherichia coli heat-stable enterotoxin is a long-lived superagonist of guanylin. *Infect Immun*. 1993; 61:4710–4715. [PubMed: 8104900]
43. Lucas KA, Pitari GM, Kazerounian S, Ruiz-Stewart I, Park J, Schulz S, Chepenik KP, Waldman SA. Guanylyl cyclases and signaling by cyclic GMP. *Pharmacol Rev*. 2000; 52:375–414. [PubMed: 10977868]
44. Giannella RA. Escherichia coli heat-stable enterotoxins, guanylins, and their receptors: what are they and what do they do? *Journal Of Laboratory And Clinical Medicine*. 1995; 125:173–181. [PubMed: 7844467]
45. Sciaky D, Jenkins NA, Gilbert DJ, Copeland NG, Sonoda G, Testa JR, Cohen MB. Mapping of guanylin to murine chromosome 4 and human chromosome 1p34–p35. *Genomics*. 1995; 26:427–429. [PubMed: 7601480]
46. Sciaky D, Kosiba JL, Cohen MB. Genomic sequence of the murine guanylin gene. *Genomics*. 1994; 24:583–587. [PubMed: 7713512]
47. Birkenkamp-Demtroder K, Lotte Christensen L, Harder Olesen S, Frederiksen CM, Laiho P, Aaltonen LA, Laurberg S, Sorensen FB, et al. Gene expression in colorectal cancer. *Cancer Res*. 2002; 62:4352–4363. [PubMed: 12154040]
48. Cohen MB, Hawkins JA, Witte DP. Guanylin mRNA expression in human intestine and colorectal adenocarcinoma. *Laboratory Investigation*. 1998; 78:101–108. [PubMed: 9461126]
49. Notterman DA, Alon U, Sierk AJ, Levine AJ. Transcriptional gene expression profiles of colorectal adenoma, adenocarcinoma, and normal tissue examined by oligonucleotide arrays. *Cancer Res*. 2001; 61:3124–3130. [PubMed: 11306497]
50. Steinbrecher KA, Tuohy TM, Heppner Goss K, Scott MC, Witte DP, Groden J, Cohen MB. Expression of guanylin is downregulated in mouse and human intestinal adenomas. *Biochemical & Biophysical Research Communications*. 2000; 273:225–230. [PubMed: 10873591]

51. Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, Vogelstein B, Kinzler KW. Gene expression profiles in normal and cancer cells. *Science*. 1997; 276:1268–1272. [PubMed: 9157888]
52. Glebov OK, Rodriguez LM, Soballe P, DeNobile J, Cliatt J, Nakahara K, Kirsch IR. Gene expression patterns distinguish colonoscopically isolated human aberrant crypt foci from normal colonic mucosa. *Cancer Epidemiol Biomarkers Prev*. 2006; 15:2253–2262. [PubMed: 17119054]
53. Wilson C, Schulz S, Hyslop, Waldman SA. Silencing of guanylin and uroguanylin expression in colon cancer. 2009 Submitted.
54. Miyaki M, Iijima T, Kimura J, Yasuno M, Mori T, Hayashi Y, Koike M, Shitara N, et al. Frequent mutation of beta-catenin and APC genes in primary colorectal tumors from patients with hereditary nonpolyposis colorectal cancer. *Cancer Res*. 1999; 59:4506–4509. [PubMed: 10493496]
55. Sparks AB, Morin PJ, Vogelstein B, Kinzler KW. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. *Cancer Res*. 1998; 58:1130–1134. [PubMed: 9515795]
56. Pitari GM, Di Guglielmo MD, Park J, Schulz S, Waldman SA. Guanylyl cyclase C agonists regulate progression through the cell cycle of human colon carcinoma cells. *Proc Natl Acad Sci U S A*. 2001; 98:7846–7851. [PubMed: 11438734]
57. Li P, Lin JE, Snook AE, Schulz S, Pitari GM, Waldman SA. Colorectal cancer is a paracrine deficiency syndrome which can be prevented and treated by oral hormone replacement therapy. *Current Molecular Pharmacology*. 2008 In press.
58. Carrithers SL, Hill MJ, Johnson BR, O'Hara SM, Jackson BA, Ott CE, Lorenz J, Mann EA, et al. Renal effects of uroguanylin and guanylin in vivo. *Braz J Med Biol Res*. 1999; 32:1337–1344. [PubMed: 10559834]
59. Kulaksiz H, Schmid A, Honscheid M, Ramaswamy A, Cetin Y. Clara cell impact in air-side activation of CFTR in small pulmonary airways. *Proc Natl Acad Sci U S A*. 2002; 99:6796–6801. [PubMed: 12011439]
60. Schulz S, Chrisman TD, Garbers DL. Cloning and expression of guanylin. Its existence in various mammalian tissues. *J Biol Chem*. 1992; 267:16019–16021. [PubMed: 1379587]
61. Fonteles MC, Carrithers SL, Monteiro HS, Carvalho AF, Coelho GR, Greenberg RN, Forte LR. Renal effects of serine-7 analog of lymphoguanylin in ex vivo rat kidney. *Am J Physiol Renal Physiol*. 2001; 280:F207–F213. [PubMed: 11208595]
62. London RM, Eber SL, Visweswariah SS, Krause WJ, Forte LR. Structure and activity of OK-GC: a kidney receptor guanylate cyclase activated by guanylin peptides. *Am J Physiol*. 1999; 276:F882–F891. [PubMed: 10362777]
63. Kulaksiz H, Schlenker T, Rost D, Stiehl A, Volkmann M, Lehnert T, Cetin Y, Stremmel W. Guanylin regulates chloride secretion in the human gallbladder via the bile fluid. *Gastroenterology*. 2004; 126:732–740. [PubMed: 14988827]
64. Kulaksiz H, Cetin Y. Uroguanylin and guanylate cyclase C in the human pancreas: expression and mutuality of ligand/receptor localization as indicators of intercellular paracrine signaling pathways. *J Endocrinol*. 2001; 170:267–275. [PubMed: 11431160]
65. Kulaksiz H, Schmid A, Honscheid M, Eissele R, Klempnauer J, Cetin Y. Guanylin in the human pancreas: a novel luminocrine regulatory pathway of electrolyte secretion via cGMP and CFTR in the ductal system. *Histochem Cell Biol*. 2001; 115:131–145. [PubMed: 11444148]
66. Kulaksiz H, Rausch U, Vaccaro R, Renda TG, Cetin Y. Guanylin and uroguanylin in the parotid and submandibular glands: potential intrinsic regulators of electrolyte secretion in salivary glands. *Histochem Cell Biol*. 2001; 115:527–533. [PubMed: 11455453]
67. Kulaksiz H, Rehberg E, Stremmel W, Cetin Y. Guanylin and functional coupling proteins in the human salivary glands and gland tumors : expression, cellular localization, and target membrane domains. *Am J Pathol*. 2002; 161:655–664. [PubMed: 12163390]
68. Jaleel M, London RM, Eber SL, Forte LR, Visweswariah SS. 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*. 2002; 67:1975–1980. [PubMed: 12444076]
69. Waldman SA, Cagir B, Rakinic J, Fry RD, Goldstein SD, Isenberg G, Barber M, Biswas S, et al. Use of guanylyl cyclase C for detecting micrometastases in lymph nodes of patients with colon cancer. *Dis Colon Rectum*. 1998; 41:310–315. [PubMed: 9514425]

70. Schulz S, Hyslop T, Haaf J, Bonaccorso C, Nielsen K, Witek ME, Birbe R, Palazzo J, et al. A validated quantitative assay to detect occult micrometastases by reverse transcriptase-polymerase chain reaction of guanylyl cyclase C in patients with colorectal cancer. *Clin Cancer Res.* 2006; 12:4545–4552. [PubMed: 16899600]
71. Birbe R, Palazzo JP, Walters R, Weinberg D, Schulz S, Waldman SA. Guanylyl cyclase C is a marker of intestinal metaplasia, dysplasia, and adenocarcinoma of the gastrointestinal tract. *Hum Pathol.* 2005; 36:170–179. [PubMed: 15754294]
72. Sellers ZM, Mann E, Smith A, Ko KH, Giannella R, Cohen MB, Barrett KE, Dong H. Heat-stable enterotoxin of *Escherichia coli* (STa) can stimulate duodenal HCO₃(-) secretion via a novel GC-C- and CFTR-independent pathway. *FASEB J.* 2008; 22:1306–1316. [PubMed: 18096816]
73. Giannella RA, Mann EA. *E. coli* heat-stable enterotoxin and guanylyl cyclase C: new functions and unsuspected actions. *Trans Am Clin Climatol Assoc.* 2003; 114:67–85. discussion 85-66. [PubMed: 12813912]
74. Li P, Schulz S, Bombonati A, Palazzo JP, Hyslop TM, Xu Y, Baran AA, Siracusa LD, et al. Guanylyl cyclase C suppresses intestinal tumorigenesis by restricting proliferation and maintaining genomic integrity. *Gastroenterology.* 2007; 133:599–607. [PubMed: 17681179]
75. Steinbrecher KA, Wowk SA, Rudolph JA, Witte DP, Cohen MB. Targeted inactivation of the mouse guanylin gene results in altered dynamics of colonic epithelial proliferation. *Am J Pathol.* 2002; 161:2169–2178. [PubMed: 12466132]
76. Bui T, Thompson CB. Cancer's sweet tooth. *Cancer Cell.* 2006; 9:419–420. [PubMed: 16766260]
77. Deberardinis RJ, Sayed N, Ditsworth D, Thompson CB. Brick by brick: metabolism and tumor cell growth. *Curr Opin Genet Dev.* 2008; 18:54–61. [PubMed: 18387799]
78. Forte LR. Guanylin regulatory peptides: structures, biological activities mediated by cyclic GMP and pathobiology. *Regulatory Peptides.* 1999; 81:25–39. [PubMed: 10395405]
79. Lin EJ, Li P, Snook AE, Schulz S, Pitari GM, Waldman SA. Guanylyl cyclase C in colorectal cancer: susceptibility gene and potential therapeutic target. *Future Oncology.* 2009 In press.
80. Pitari GM, Baksh RI, Harris DM, Li P, Kazerounian S, Waldman SA. Interruption of homologous desensitization in cyclic guanosine 3',5'-monophosphate signaling restores colon cancer cytostasis by bacterial enterotoxins. *Cancer Res.* 2005; 65:11129–11135. [PubMed: 16322263]

Figure 1. Intestinal crypt-villus axis

Intestinal epithelium is a rapidly regenerating tissue which undergoes cycles of proliferation, migration, differentiation and apoptosis that maintain the integrity of the crypt-surface axis. Stem cells give rise to rapidly proliferating transit cells, located in the proliferating crypt compartment, which migrate up to the villus, the differentiated compartment, or down to the bottom of the crypt to become terminally differentiated cells. Transit cells give rise to four principal differentiated cell lines supporting digestive, absorptive and defense functions: absorptive enterocytes, goblet cells, enteroendocrine cells and Paneth. There is a gradient of metabolism, with glycolysis predominating in crypts and mitochondrial oxidative phosphorylation dominant in villi. Similarly, there is a gradient of cGMP production, lowest in crypts and greatest in villi. Adapted from [14]

Figure 2. Guanylyl cyclase C and its paracrine hormones

GCC is the intestinal epithelial cell receptor for the (A) diarrheagenic bacterial heat-stable enterotoxins (ST) and the endogenous paracrine hormones guanylin and uroguanylin. (B) Ligand binding to the GCC extracellular domain activates the intracellular catalytic domain which converts GTP to cyclic GMP and the cyclic nucleotide, as the second messenger, activates its downstream effectors.