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Translating Colorectal Cancer Prevention through the Guanylyl Cyclase C Signaling Axis

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Summary

Colorectal cancer is a major public health concern, ranking among the leading causes of cancer death in both men and women. Because of this continued burden there is a clear need for improved treatment, and more importantly prevention, of this disease. In recent years there is significant evidence to support the hypothesis that guanylyl cyclase C (GUCY2C) is a tumor suppressor in the intestine, and that the loss of hormone ligands for this receptor is an important step in the disease process. Thus, ligand replacement therapy has been proposed as a strategy to prevent colorectal cancer. Until recently this strategy was not clinically plausible; however, the recent regulatory approval of Linaclotide (LINZESS™, Forest Laboratories and Ironwood Pharmaceuticals, Inc.), an oral GUCY2C ligand, has raised the possibility of utilizing this strategy clinically to prevent colorectal cancer.

Keywords

Colorectal Cancer; Linaclotide; GUCY2C; Guanylin; Preventative Medicine

The Unmet Clinical Need for Colorectal Cancer Prevention

Colorectal cancer (CRC) is the fourth-most incident and second-most lethal cancer in the United States [1]. It has been estimated that in 2013, there will be ~143,000 new CRC diagnoses and ~51,000 patient deaths from CRC metastases [1]. Consequently, CRC-related healthcare expenditures are estimated to exceed 14 billion dollars annually [2]. Thus, the societal burden of CRC remains significant, highlighting the unmet need for improved preventative and therapeutic interventions for this disease.

Colorectal cancer was one of the first cancers for which a rigorous screening initiative was undertaken, and the CRC screening paradigm is widely considered the gold standard of such

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Disclosure

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cancer screening initiatives [3]. Colonoscopy is the mainstay for preventative CRC screening, allowing early detection and removal of precancerous polyps [4]. It is cost-effective, reliably detects precancerous and cancerous lesions, and decreases incidence and mortality of CRC [5]. However, polyps can be missed with this method and patient compliance remains an issue [5–7]. Fecal occult blood screening is an alternative to colonoscopy [8]; however, this strategy is inferior to colonoscopy, and the American College of Gastroenterology recommends its use only in populations with limited access to colonoscopy [9].

Prevention is recognized as an ideal in medicine, improving both outcomes and cost-effectiveness of care. Despite this ideal, cancer care has generally lagged behind other areas of medicine, notably cardiovascular diseases, in terms of prevention [10]. Although colonoscopy has enjoyed significant clinical success, the continued societal burden of CRC demands improved strategies to prevent the disease [11]. Just as cancer is a multi-factorial disease, so too a multi-pronged strategy must be utilized for prevention, combining this highly effective screening paradigm for CRC with other preventative interventions. This strategy has been described as a P4 paradigm: predictive, personalized, preventive and participatory [12].

Currently, there is no FDA-approved, generally accepted strategy for such chemoprevention of CRC, despite robust epidemiological evidence surrounding risk factors for CRC. Known risk factors of CRC include the umbrella of obesity (body weight, diet and physical activity level), intestinal inflammation, cigarette smoking and alcohol abuse [13]. However, there remains a significant knowledge gap surrounding the pathophysiologic mechanisms behind these risk factors, limiting the development preventative strategies.

There is an emerging link between body mass and intestinal malignancies, and obese patients have ~20–60% greater risk of, and ~2× greater mortality from, colorectal cancer [14–16]. Maintaining a healthy weight and keeping red meat intake to a minimum have been correlated to decreased CRC risk [13,17]. This relationship between diet and cancer has been an area of extensive research. Dietary intake of certain vitamins such as calcium and folic acid, fiber, antioxidants, and phytochemicals, including curcumin and resveratrol, has been implicated in protection against CRC [17,18]. Despite this work, the exact mechanisms by which obesity predisposes to CRC, and interventions to counteract these risks, remain elusive.

Inflammation is also widely considered to be a risk factor for CRC [19]. Notably, patients with inflammatory bowel disease (IBD) exhibit a marked increase in CRC incidence [20]. Therefore, the use of non-steroidal anti-inflammatory drugs (NSAIDs) has been proposed as a means of cancer prevention. A recent 10-year prospective study found that NSAID treatment, which inhibits the proinflammatory enzyme cyclooxygenase-2 (COX-2), reduced CRC risk by 35% in normal patients and by as much as 60% in a high-risk population when taken daily [21]. A number of drugs, including sulindac and celecoxib, have also been successfully used to prevent the growth of new polyps and cause regression of existing polyps in familial adenomatous polyposis (FAP) patients [22].

Despite these encouraging anticancer effects, there are still concerns about the efficacy and safety of long-term use of NSAIDS, most notably the risk of gastrointestinal bleeding [23]. To mitigate these risks, a class of drugs known as NO-NSAIDs is being evaluated clinically. These NSAIDs release nitric oxide (NO) for prolonged periods of time, and there is evidence that this NO release is protective against many of the toxicities associated with NSAIDs [24].

A number of other studies suggest that the cytostatic and apoptotic properties of NSAIDS and NO-NSAIDs may be due to COX-independent targets, such as cyclic GMP phosphodiesterases (cGMP PDEs), c-MYC, nuclear factor- κ B (NF- κ B), p38, and NSAID-activated gene-1 (NAG-1), in part because antitumorigenic effects still occur in cell models that lack COX expression [21,22,25]. The lack of clear safety and efficacy data and mechanism of action have clouded the potential use of NSAIDs and NO-NSAIDs as a strategy for chemoprevention.

Despite this significant body of work on CRC prevention, no easy answer has arisen for disease prevention. Thus, there remains a substantial need for novel targets and strategies in CRC prevention. Present and future work should focus on uncovering these novel targets, perhaps by utilizing strategies which focus on common molecular defects in the disease itself. Fortunately recent advances in high throughput technology have greatly improved our understanding of many diseases [26,27]. In that context, our knowledge of the molecular signature of CRC has grown exponentially over the last decade, providing opportunities for identifying such targets.

Molecular Mechanisms of Colorectal Cancer

CRC development usually takes decades, reflecting the requirement for multiple independent mutations to accumulate, mediating the progression from normal mucosa to adenomatous polyps and eventually to carcinomas [11]. There are a few molecular factors that are commonly mutated in most CRC patients. Moreover, while hereditary forms of CRC are not as frequent as sporadic cases, much has been learned about the genetics of sporadic CRC by examining the driving germ-line mutations in hereditary syndromes. The inherited FAP syndrome is caused by mutations in the *adenomatous polyposis coli* (APC) gene. APC also plays a principle role in sporadic CRC, with 70–80% of adenomas and carcinomas harboring inactivating mutations in the gene [28]. The APC protein is an essential negative control element in the canonical Wnt signaling pathway, participating in the degradation of β -catenin to minimize proliferative gene expression in the absence of Wnt ligand [29]. Inactivation of APC thus enables hyperplasia and increased transcription of β -catenin gene targets, many of which are proto-oncogenes such as *C-MYC* and *CCND1* (cyclin D1) [30,31]. Another inherited syndrome is hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome, which arises from mutations in mismatch repair (MMR) genes such as *MSH2* and *MLH1* [32]. Deficient MMR leads to genomic instability resulting in accumulation of DNA sequence repeats called microsatellites, imparting an MSI-H phenotype. Although mutations in MMR genes are infrequent in sporadic CRC, MSI-H still occurs in approximately 15% of cases, indicating that genomic instability and deficient

repair exists in sporadic CRC as well [28,33]. Other genes which frequently undergo somatic mutation include *p53*, *KRAS*, *TGF β IR*, and *PIK3CA* [28,34–37].

Despite the recurrence of certain gene mutations in sporadic CRC, the initiating transformative event in the disease remains elusive. The paracrine hormone hypothesis of colorectal cancer suggests a possible mechanism of initiation, whereby silencing of the intestinal paracrine hormone ligands for the receptor guanylyl cyclase C (GUCY2C) renders the intestine vulnerable to transforming mutations [38]. GUCY2C is encoded by the *GUCY2C* gene, and is the intestinal epithelial cell receptor for diarrheagenic *E. coli* heat-stable enterotoxins (STa) [39,40]. These toxins are structurally and functionally homologous to the endogenous GUCY2C paracrine hormone ligands guanylin and uroguanylin, but with 10-fold greater potency [39,41,42]. Upon ligand-receptor interaction, the intracellular catalytic domain of GUCY2C is activated and intracellular GTP is converted to cyclic (c)GMP. Cyclic GMP then activates its downstream effectors, including cGMP-dependent protein kinase (PKG) II, which phosphorylates the cystic fibrosis transmembrane conductance regulator (CFTR) inducing secretion of salt, and therefore water, leading to diarrhea [39]. Despite the health burden that STa creates worldwide, an inverse correlation exists between regions where enterotoxigenic bacterial infections are common and CRC incidence [43,44]. This suggests a possible role for GUCY2C ligands, in this case STa, in preventing CRC. Indeed, there is a higher incidence of CRC in more developed countries with low levels of ETEC, suggesting that ETEC-mediated activation of GUCY2C signaling may prevent CRC [44,45].

Another important mechanistic correlate implicating GUCY2C signaling in CRC prevention stems from the observation that the endogenous GUCY2C hormone ligands guanylin and uroguanylin are universally lost early in the temporal continuum of neoplastic transformation in colorectal tumors [46–48]. This suggests that silencing the expression of these hormones, and GUCY2C signaling, is necessary for tumor initiation and progression. Indeed, loss of GUCY2C signaling has been demonstrated to potentiate intestinal tumorigenesis and tumor progression [49–55]. There are several mechanisms by which GUCY2C exerts its protective effects, including inhibition of oncogenic Akt signaling, restriction of the proliferative compartment of the crypt, regulating the G1-S cell-cycle transition, maintaining genomic stability, coordinating metabolism, and defending the integrity of the epithelial barrier [49–55]. Additionally, GUCY2C receptor is frequently overexpressed in colorectal tumors, which could reflect a compensatory response to ligand loss and epithelial dysfunction [56,57]. The integration of these findings suggests that absence of GUCY2C signaling increases tumor susceptibility in hereditary and carcinogenic animal models of CRC [51].

Mechanistically, GUCY2C signaling inhibits the cell cycle of normal human intestinal cells and human colon carcinoma cells *in vitro* and *ex vivo* [50,52,58]. Activation of downstream GUCY2C signaling was associated with reduced expression of cell cycle drivers including β -catenin, cyclin D1, pRb, and increased expression of cell cycle inhibitors including p27. This reduced oncogenic milieu resulted in restriction of the transition through the G1/S interface, limiting progression through the cell cycle without inducing apoptosis. [50,52,58]. Together, these observations reveal GUCY2C as a key mediator of intestinal tumor

susceptibility and highlight GUCY2C ligands as promising preventative agents against CRC. However, until recently, therapeutic targeting of GUCY2C in humans was confined to the strictly theoretical. The recent regulatory approval of linaclotide (LINZESS™, Forest Laboratories and Ironwood Pharmaceuticals, Inc.), an oral GUCY2C ligand, creates the possibility for prevention of colorectal cancer in patients.

GUCY2C Ligands in Clinical Development

The role of GUCY2C in intestinal secretion and motility has been established for decades. Indeed, very early studies identified GUCY2C as the intestinal target for *E. Coli* heat stable enterotoxin (STa), mediating traveler's diarrhea and ETEC infection [59–61]. Binding of the toxin to the receptor causes increased fluid secretion, and thus diarrhea. Despite this early knowledge, therapeutic targeting of GUCY2C to utilize these pharmacologic effects is a relatively new development. Ultimately, the lack of effective treatment options for chronic constipation (CC) and irritable bowel syndrome with constipation (IBS-C) fostered the development of GUCY2C ligands designed to induce intestinal motility in these patients [62].

Three similar peptide agonists of GUCY2C have been approved by the FDA or entered clinical trials: linaclotide (LINZESS™, Forest Laboratories and Ironwood Pharmaceuticals, Inc.), and SP-304 (Plecanatide) and SP-333 (Synergy Pharmaceuticals, Inc.) [63–65]. Of these, only linaclotide is approved by the FDA. This compound shares over 60% structural homology with endogenous uroguanylin. Administration of this peptide to mice was shown to increase cGMP production and fluid secretion, consistent with GUCY2C activation [63]. Further, these pharmacologic effects were shown to be largely confined to the intestine, and the compound was minimally bioavailable [63].

Based on these promising preclinical data, linaclotide was evaluated in clinical trials in IBS-C patients. These trials supported the pharmacologic effects of linaclotide in treating IBS-C. Phase II and III trials of linaclotide demonstrated the ability of the compound to improve colonic transit, improve abdominal pain and bowel habits when compared to placebo [66–71]. Moreover, the side effect profile was generally favorable. The most common adverse event reported in approximately 20% of patients receiving linaclotide was diarrhea, which is not surprising given GUCY2C's role as a secretagogue. Other, less common events, included abdominal pain, flatulence, viral gastroenteritis, headache and abdominal distention [72]. However, none of these events were considered serious enough to discontinue development of the drug, and the FDA and international agencies approved its use in 2012 [66,72,101].

Additionally there are other peptides in development. SP-304 is an analog of uroguanylin, an endogenous ligand of GUCY2C [73]. SP-304 was designed to exert its effect on the small intestine with minimal systemic exposure. In early phase IIa clinical trials, patients with chronic constipation exhibited a marked improvement in bowel habits compared with placebo [74]. SP-333 is structurally similar to SP-304 with the exception of several amino acid substitutions which render it resistant to proteolysis, thus giving it potentially greater utility in treating colonic disorders [75]. This important feature gives this drug potentially

wider therapeutic applications because it can elicit signaling throughout the gastrointestinal tract. SP-333 is therefore also currently in clinical trials for the treatment of inflammatory bowel disease (IBD) [76,102].

There is an established clinical need for improved screening, treatment and prevention strategies for colorectal cancer. Given the well-established role for disruption of the GUCY2C axis in intestinal tumorigenesis, linaclotide and similar compounds may provide a unique opportunity for study as cancer preventative agents [49–55]. This strategy is particularly feasible given that linaclotide is already FDA approved, and the others are in clinical development. These compounds are also well-tolerated, and possess pharmacologic activity exclusively in the target tissue for disease (intestinal lumen).

Exploiting the GUCY2C Signaling Axis for Cancer Prevention

In the healthy state, GUCY2C plays a key regulatory role regulating homeostasis of intestinal epithelial cells [50,52], and disruption of these processes by paracrine hormone loss initiates intestinal tumorigenesis [77]. These observations highlight a potential prevention strategy for colorectal cancer involving oral hormone replacement therapy. This strategy presumes that during colorectal carcinogenesis, GUCY2C persists as a dormant tumor-suppressing receptor following ligand loss, and that its re-activation by exogenous ligand rescues cell cycle regulation and restricts aberrant growth.

Applying these findings, several pivotal animal studies provide proof-of-concept for the cancer prevention effects of exogenous ligand supplementation. One study investigated the use of oral administration of uroguanylin which induced apoptosis and opposed tumorigenesis in a mouse model of CRC [45]. Further studies utilized a genetic approach, generating transgenic mice to overexpress guanylin. These mice serve as a model for chronic exposure to excess GUCY2C ligand, and were resistant to DSS-induced colitis [53]. Remarkably, the effects of GUCY2C signaling persist over the lifetime of the animal, without obvious attenuation or desensitization. Importantly, in addition to these protective effects, mice in both of these models were healthy and had no adverse effects inside or outside of the GI tract.

Taken together, these findings strongly support using linaclotide as a safe, effective and novel approach to decrease the socioeconomic burden posed by colorectal cancer. Although studies have confirmed the cancer-preventative effects of some GUCY2C ligands, the specific ligands that are in clinical use (linaclotide and similar compounds) may need to be evaluated in preclinical models of CRC before such a paradigm is investigated in humans.

Interestingly, linaclotide may have a role in preventing other tumor types outside the intestine. This potential application stems from the role for GUCY2C in regulating intestinal barrier. Indeed, the aforementioned preclinical models of GUCY2C activation also promote intestinal barrier integrity [53,54]. This, in turn, may prevent leakage of environmental toxins or carcinogens and thereby protects against DNA damage in other organs outside the intestine [53]. Thus, oral linaclotide supplementation in high-risk populations may markedly decrease the incidence of other forms of cancer in addition to CRC.

Expert Commentary

Recently, there has been a paradigm shift in cancer research from treatment to prevention. This evolution in thinking can be attributed to a number of factors. On the one hand, improvement in clinical outcomes for most cancers has been modest in recent decades, despite billions of dollars in research spending, suggesting that a change in strategy may be necessary [78]. This failure is accentuated by the use of small molecule inhibitors for cancer treatment, which often has been plagued by the problem of acquired resistance, yielding mere months of survival despite billions of dollars in development costs [79–81]. On the other hand, this research, largely through high-throughput data analysis, has yielded an unprecedented quantity of new information that can be applied to disease prevention. Thus, this change in focus towards prevention has evolved somewhat naturally, and has the potential to change the course of cancer care.

The potential use of GUCY2C-targeted therapeutics to prevent/treat CRC was first suggested by early observations of an inverse relationship between rates of colorectal cancer and exposure to ETEC bacteria in the developing world [43,44]. Further, there is a large body of literature supporting GUCY2C's role as a tumor suppressor, the function of which is dysregulated early in the disease by loss of endogenous GUCY2C ligands [49–55]. In all of these studies, preventative ligand supplementation appears to be the obvious logical clinical translation of the work. However, until recently, using this strategy in patients was not feasible. Thus, the recent regulatory approval of linaclotide, and further clinical development of similar compounds, provides investigators with a unique opportunity to apply this robust body of preclinical literature to benefit patients.

Linaclotide is a safe, well-tolerated drug, which is minimally absorbed systemically and thus has selective pharmacologic activity at the site of disease (the intestine). The obvious candidates for initial studies of preventative treatment with linaclotide would be those patients most at-risk for the disease: those with an APC mutation, those with a family history of CRC, or those with a positive colonoscopy. Additionally, the drug profile is sufficiently favorable that this preventative strategy might be extended to other average-risk populations as well. A large chemopreventative trial, similar to the NSAID trials, must be carried out to balance the benefits with the risks, since little is known at this time about the effects of widespread and long-term treatment with linaclotide. Moreover, activation of the GUCY2C signaling axis may even prevent extra-intestinal malignancies, by activating intestinal barrier function.

Five-Year View

In the past several years evidence contributing to our understanding of the molecular defects underlying cancer has increased exponentially. These studies will continue to yield strategies aimed at both treatment and prevention of cancer. This body of work supports the hypothesis that the loss of GUCY2C function increases susceptibility to CRC, and this loss of function is mediated by loss of GUCY2C ligands. Further work has demonstrated the potential efficacy of ligand supplementation to reduce the incidence of cancer. Thus, the recent approval of linaclotide, a GUCY2C ligand, creates the opportunity to advance this

hypothesis into patient care. Because linaclotide is approved for clinical use, a low regulatory burden exists for adopting this use. Additionally, other compounds with the same mechanism of action are in development clinically. Coupling these developments with the large amount of supporting preclinical data, it is likely that this application will be explored clinically in the near future, with the potential to influence patient outcomes in CRC.

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Abbreviations

APC	<i>adenomatous polyposis coli</i>
CRC	Colorectal Cancer
ETEC	Enterotoxigenic Escherichia coli
FAP	familial adenomatous polyposis
GUCY2C	Guanylyl Cyclase C
GUCA2A	Guanylin
IBD	Inflammatory Bowel Disease
IBS-C	Irritable Bowel Syndrome- Constipation Prevalent
MMR	Mismatch Repair
NSAID	Non-steroidal anti-inflammatory drugs
STa	E. Coli heat stable enterotoxin

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Key Issues

- Colorectal cancer is a major cause of morbidity and mortality in the developed world.
- Although preventative strategies are presently in clinical use for this disease in the form of screening tests, there remains a clear need for improved methods of disease prevention.
- Although the molecular defects in colorectal cancer are heterogeneous, one of the most common events in sporadic and genetic disease is the loss of expression of guanylin, the endogenous hormone ligand for the guanylyl cyclase C receptor. There is substantial evidence to support a role for GUCY2C as a tumor suppressor in intestine, and that loss of signaling by silencing of guanylin expression is permissive for tumorigenesis.
- Regulatory agencies recently approved the oral GUCY2C ligand linaclotide (LINZESS™, Forest Laboratories and Ironwood Pharmaceuticals, Inc.) for clinical use in treating patients with chronic constipation. The drug is well-tolerated and possesses selective pharmacologic activity in intestine.
- Given the body of literature that GUCY2C silencing promotes tumorigenesis, linaclotide possesses substantial potential for use as hormone replacement therapy to prevent cancer by replacing lost endogenous ligands.

Figure 1. Colorectal Cancer Prevention by GUCY2C Ligands

In normal colonic mucosa, the endogenous hormone guanylin binds GUCY2C regulating intestinal homeostasis. Guanylin loss promotes epithelial dysfunction via silencing of GUCY2C signaling. This dysfunction subsequently promotes adenoma and tumor formation via oncogenic transformation. Linaclotide and similar compounds mimic the endogenous effect of guanylin and restore epithelial homeostasis, preventing colorectal cancer. Importantly, the persistence of the GUCY2C receptor in both tumors and adenomas suggests that linaclotide and similar compounds may also be effective in reversing these precancerous lesions and preventing colorectal cancer.