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Emergent Concepts from the Intestinal Guanylyl Cyclase C Pathway

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1. Introduction

Cancer is one of the world's top killers accounting for 7.4 million deaths, or 13% of all deaths (World Health Organization [WHO], 2011). Colorectal cancer is the third most common and deadly cancer worldwide (Jemal et al., 2011). An unequal geographic distribution of the disease burden exists, with less developed areas of the world exhibiting the lowest incidence and mortality (Pitari et al., 2003). In contrast, populations dwelling in western countries are at increased risk to develop and die of colorectal cancer. In the US, 141,210 new cases are estimated to be diagnosed in 2011 and 49,380 patients are expected to die for this disease, representing an intolerable socio-economical toll (Siegel et al., 2011).

Promises derive from substantial advancements in early detection and prevention strategies, which have contributed to reduce colorectal cancer incidence and mortality rates in recent years (Siegel et al., 2011). However, new chemotherapeutic approaches have not emerged and terminal clinical stages of the disease remain incurable. Specifically, invasion and metastatic disease progression, traditionally unnameable to surgical resection, are largely refractory to pharmacological therapy. About 90% of patients with distant metastasis die of the disease within 5 years from diagnosis (Siegel et al., 2011). Moreover, racial and educational health-disparities exist in which minorities and less educated individuals of the affected population exhibit the worst clinical prognosis and the highest mortality, in part reflecting their more advanced stages at diagnosis compared to other patient segments (Siegel et al., 2011). Together, these considerations underscore the enormous impact that therapeutic target discovery might have on western societies, especially if they would translate into innovative, curative pharmacological approaches that will prolong the survival of patients with colorectal cancer.

Crucial systems regulating the intestinal crypt-villus axis are also important determinants of the carcinogenetic process (Aoki et al., 2003; Fodde et al., 2001; Korinek et al., 1998). Among these, the signalling pathway orchestrated by the surface receptor guanylyl cyclase C (GCC) has recently emerged as both an integral component of intestinal mucosa homeostasis and a negative regulator of the malignant cell phenotype. GCC, expressed in the epithelial layer of the gastrointestinal wall, and its endogenous ligands guanylin and uroguanylin control fluid balance and renewal crypt dynamics by operating sophisticated biochemical circuits in both the small and large intestine. Intriguingly, a bacterial mimicry of endogenous

hormones exists, the E. coli heat-stable enterotoxin (ST), which may confer both harmful (watery diarrhea) and beneficial (colorectal cancer resistance) effects to exposed individuals (Lucas et al., 2000; Pitari et al., 2001). In this model, the uneven epidemiological distribution of colon cancer incidence across different geographic areas of the world reflect, in part, inverse differences in the prevalence of enterotoxigenic E. coli infections (Pitari et al., 2003). Moreover, an unexplained mutation early in colorectal tumorigenesis leads to the loss of guanylin and uroguanylin expression, producing a dormant GCC pathway in neoplastic cells (Fig. 1) (Pitari et al., 2007).

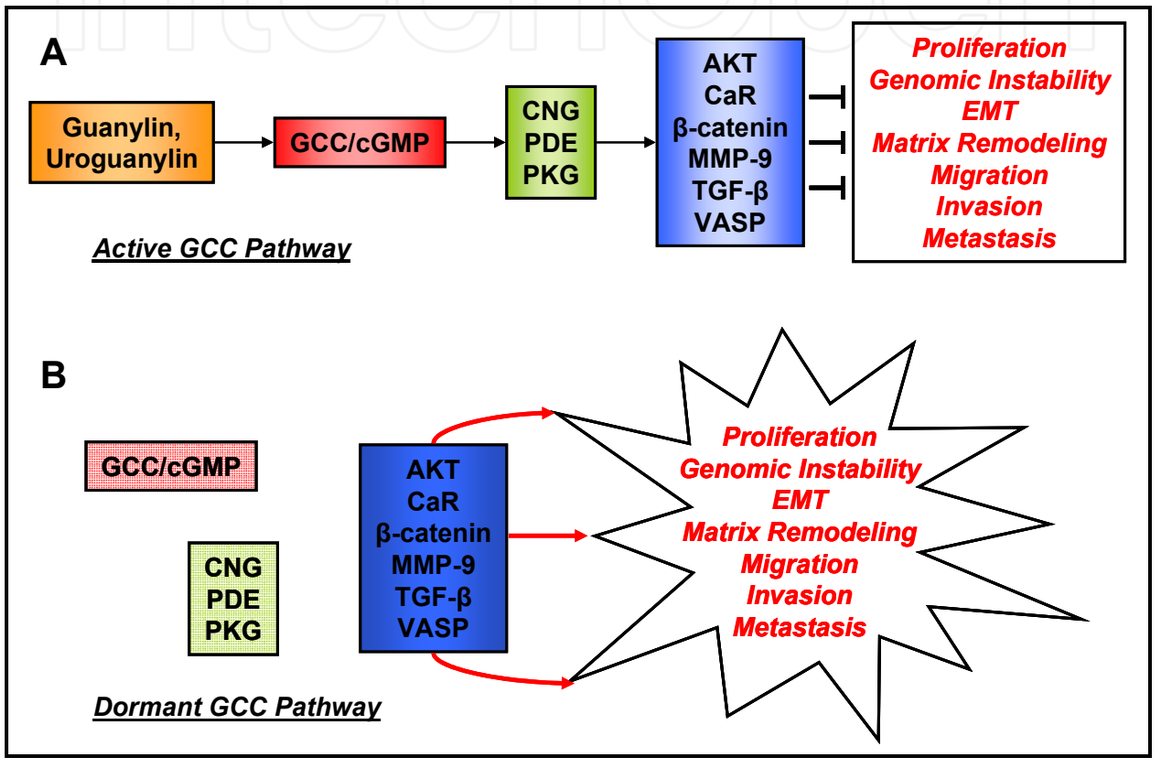

Fig. 1. Significance of the dormant guanylyl cyclase C (GCC) pathway for colorectal carcinogenesis.
Selected GCC signalling components with reported impact on tumorigenesis are depicted. A) In normal intestinal physiology, the GCC pathway is constitutively activated by paracrine hormonal regulation with the endogenous GCC agonists guanylin and uroguanylin. The active GCC pathway promotes signalling by proximal cGMP effectors cyclic nucleotide-gated channel (CNG), phosphodiesterases (PDE) and protein kinase G (PKG) that, in turn, affect the function of key distal effectors, including the v-akt murine thymoma viral oncogene homolog (AKT), Ca^{2+}-sensing receptor (CaR), β-catenin, matrix metalloproteinase 9 (MMP-9), transforming growth factor β (TGF-β), and vasodilator-stimulated phosphoprotein (VASP). As a result, tumorigenic forces are restrained and normal intestinal mucosa homeostasis is maintained. B) During neoplastic transformation the GCC pathway becomes dormant, principally because of the loss of endogenous hormone expression. Loss of signalling between GCC and the proximal cGMP effectors deregulates the distal components of the pathway, thereby producing an oncogenic system favouring colorectal cancer progression and metastasis. EMT, epithelial-mesenchymal transition.

This chapter will details the consequences at the functional and cellular level of the silenced GCC signalling for colorectal tumor formation and progression. Key molecular effectors comprising the GCC pathway with high clinical translational significance will be presented and their potential impacts for both diagnostic and therapeutic advances discussed.

2. GCC and the intestinal crypt-villus axis

GCC is a member of membrane-bound guanylyl cyclases (GCA to GCG), enzymes which catalyze the formation of cyclic guanosine monophosphate (cGMP) from GTP. Although they exhibit unique physicochemical and antigenic properties, particulate guanylyl cyclases are homodimeric transmembrane domain proteins sharing conserved cytoplasmic portions with tyrosine kinase-like and cyclase catalytic domains (Lucas et al., 2000). The amino acid sequence of GCC considerably diverges from the other isoforms in the extracellular domain, which represents the ligand binding domain for the E. coli heat-stable enterotoxin ST and the endogenous peptides guanylin and uroguanylin (Lucas et al., 2000). Beyond selected dopaminergic neurons in the central nervous system (Gong et al., 2011), in mammals GCC expression is principally restricted to brush-border membranes of epithelial cells lining the intestinal inner surface from the duodenum to the rectum, uniformly distributed along the crypt-villus axis (Lucas et al., 2000). This unique anatomical distribution subserves the functional role of GCC as a critical regulator of the intestinal mucosa homeostasis. In particular, the signalling pathway regulated by GCC and its second messenger cGMP contributes to the control of epithelial self-renewal and maturation dynamics underlying the integrity of the crypt-villus axis (Pitari et al., 2007).

2.1 The GCC pathway

Modulation of intracellular cGMP concentrations represents the fundamental event of a variety of signal transduction circuits shaping cellular behaviour. Synthesis (by guanylyl cyclases) and breakdown (by phosphodiesterases) are recognized as the major mechanisms defining cGMP levels in tissues. In intestinal epithelial cells, GCC is the principle source of cGMP (Lucas et al., 2000). GCC activity defines the type, intensity and duration of biological responses mediated by cGMP through unique physical, spatial and temporal dynamics at intestinal mucosal surfaces. The most important modality to regulate GCC activity is by ligand binding to its extracellular domain, which induces an intramolecular conformational change that is transmitted down to the cytoplasmic C-terminus catalytic domain. In this way, cellular cGMP levels can be raised numerous folds over basal states (Lucas et al., 2000; Schulz et al., 1989). Furthermore, the three ligand peptides know to induce GCC activation in mammalian cells exhibit different affinities and potencies for GCC, resulting in different patterns of cGMP concentrations/effects. The exogenous ligand ST, produced by E. coli and responsible for life-threatening diarrhoeagenic syndromes, is the most potent GCC agonist and consists of 18 amino acids with three intrachain disulfide bonds (Guarino et al., 1989). In contrast the endogenous paracrine hormones, guanylin and uroguanylin, are 15-16 amino acid long with two intrachain disulfide bonds and uneven tissue distributions and physico-chemical characteristics. Thus, while uroguanylin is a more potent (~100 fold) and abundant GCC agonist at acidic pH of proximal intestinal tracts, guanylin is more potent (~4 fold) as a GCC agonist at basic pH and is highly expressed in the colon and rectum (Forte, 1999; Hamra et al., 1997). Finally, elegant spatio-temporal constrains along the crypt-villus axis

represent additional determinants of cGMP signalling by GCC. At the tissue level, maximal GCC activity and the dependant cGMP functions are imposed at the epithelial crypt/villus interface by increased GCC ligand expression (Cohen et al., 1995; Whitaker et al., 1997). In addition at the cellular level, compartmentalization of GCC-ligand interactions at luminal membrane borders establishes an increasing baso-apical cGMP gradient, wherein highest nucleotide concentrations are ensured at microvillus cell domains (Lucas et al., 2000).

Beyond GCC activation, the functional consequences of cGMP rises in intestinal epithelial cells reflect the specific expression and compartmentalization of downstream target molecules. Two evolutionarily distinct allosteric binding sites for cGMP exist in eukaryotic cells: one is present in cGMP- (PKG) and cAMP- (PKA) dependent protein kinases and in the cyclic nucleotide gated (CNG) cation channels, while the other is expressed in cGMP-regulated phoshodiesterases (PDEs). These proteins represent the intracellular receptors for cGMP and permit the selective transmission of information in a cell-(and subcellular-) specific manner. PKGs are Ser/Thr protein kinases comprising the soluble type I, widely distributed across tissues and including the isoforms Iα and Iβ, and the particulate type II, mainly expressed in the intestine (Pfeifer et al., 1999). PKA is a tetrameric kinase preferentially activated by cAMP (Chao et al., 1994). CNG channels are heterotetrameric proteins of α- and β-subunits, which mediate membrane Na^+ and Ca^{2+} influx by cGMP in intestine as well as different other tissues (Bielet al., 1999). Further, cGMP-regulated PDEs (eg, PDE2, PDE5) are hydrolytic enzymes specialized in cleaving the cyclic nucleotide phosphodiester bond, thereby terminating correspondent biological activities (Corbin & Francis, 1999; Francis et al., 2011). Cyclic GMP binding to the consensus site of these intracellular targets results in regulation of important downstream effectors which control specific biochemical networks and cellular functions. Molecules distal to cGMP with paramount significance for intestinal cell biology include ions, ion channels, cytoskeleton regulators and enzymes. For instance, cGMP binding to two allosteric sites present at the amino-terminal region of PKG II fully activates the enzyme and induces phosphorylation and opening of the cystic fibrosis transmembrane conductance regulator (CFTR), a pivotal mechanism underlying control of intestinal fluid homeostasis (Pfeifer et al., 1999). For an in depth discussion on the regulation of the various biochemical cGMP-dependent targets, such as the CFTR channel, the reader is referred to other comprehensive reviews (Browning et al., 2010; Lucas et al., 2000; Steinbrecher & Cohen, 2011). Here, the focus will be on those molecular elements of the GCC and cGMP pathway that affect the epithelial cell phenotype, including its proliferative, morphogenetic and migratory attributes that greatly influence the crypt-villus homeostasis and the process of neoplastic transformation.

2.2 Regulation of the intestinal epithelial cell phenotype

The human intestinal mucosa is characterized by minute tubular invaginations called crypts, of maximal length in large intestinal tracts. In addition, the small intestinal mucosa exhibits lumenal protrusions of multi- (villi) and sub-cellular (microvilli) dimensions devoted to digestive activities. As a result, the inner intestinal surface is enormously expanded to optimally serve fundamental processes, from food processing and absorption to pathogen protection and immune system control (Montgomery et al., 1999). In this context, the complexity of those processes, constantly exposing the organism to potentially harmful external factors, is reflected by the sophisticated functional organization adopted by the columnar monolayer of epithelial cells lining the intestine. First, a self-renewal epithelial

ability is conferred by multipotent stem cells located at crypt bottoms, ensuring weekly cycles of cellular replacement to eliminate damaged or aging cells worn out by the demanding intestinal functions (Potten & Loeffler, 1990). Also, intriguing maturation dynamics are operating that turn proliferating progenitor cells into differentiated, cell cycle-arrested epithelial cells with specialized functions, mostly populating the upper crypt and villus areas. They include a) the enterocytes, absorptive cells with food digestive functions (Montgomery et al., 1999), b) goblet cells, mucus-producing cells with detergent activities (Koldovsky et al., 1995), c) enteroendocrine cells, hormone-secreting cells comprising an intestinal endocrine system (Koldovsky et al., 1995), and d) Paneth cells, which secrete antimicrobial peptides and growth factors and are uniquely located at crypt bases (Bry et al., 1994). Further, incompletely understood check-up mechanisms constantly detect genetic or epigenetic defects and direct epithelial cells to the appropriate maintenance (e.g., cell resistance), self-repair (e.g., DNA excision repair) or death (e.g., apoptosis, autophagy) program (Potten & Loeffler, 1990). Finally, the spatio-temporal coordination of this variety of processes is ensured by the migratory nature of the epithelial monolayer that physically maps the proliferation-differentiation transition at crypt/villus (small intestine) or lower/upper crypt (colon) interfaces, and drives the shedding of senescent cells at mucosal tips (Montgomery et al., 1999).

The GCC signalling pathway represents one of the elaborate homeostatic mechanisms evolved to direct the integration of each component supporting the intestinal epithelial cell phenotype. Indeed, targeted deletion of guanylin expression induces expansion of the proliferating crypt compartment and accelerated cell migration in mouse colon, presumably as a consequence of reduced GCC activation and cGMP-dependent signalling (Steinbrecher et al., 2002). In agreement with this notion, elimination of GCC in mice produces hyperplastic colonic crypts, populated by a higher number of fast-cycling and fast-migrating progenitor cells, associated with impaired cell maturation and death dynamics, with fewer Paneth and goblet cells but increased apoptotic events (Li et al., 2007a). Moreover, compound mice in which the expression of uroguanylin or GCC has been disrupted exhibit similar structural alterations in the crypt-villus axis, with loss of tight junction-mediated intestinal barrier function and increased mucosal permeability (Han et al., 2011). Of relevance, intestine-specific expression of GCC is under the control of the caudal homeobox gene Cdx2, a transcriptional factor regulating development and cell fate specification of intestinal epithelial cells (Park et al., 2000). The Cdx2 gene product binds a consensus site present in the GCC proximal promoter and stimulates GCC transcription in an intestine-specific fashion (Di Guglielmo et al., 2001). Thus, it is tempted to speculate that GCC expression and the dependant cGMP signalling are part of the universal developmental program supporting the integrity of the intestinal crypt-villus axis.

Molecular mechanisms underlying effects of the GCC pathway on the intestinal cell phenotype have only recently been investigated. In one paradigm, lumenal Ca^{2+} is the key distal mediator of GCC activity (Pitari et al., 2003, 2008). The role of dietary Ca^{2+} as antiproliferative agent and promoter of differentiation and cell death along the epithelial crypt-villus axis is well defined (Lipkin & Newmark, 1995; Whitfield, 1992), and Ca^{2+}-deficient diets induce larger proliferative compartments in mouse colonic crypts (Rozen et al., 1989). One key molecular target for antiproliferative effects by dietary Ca^{2+} is the Ca^{2+}-sensing receptor (CaR), a G protein-coupled receptor present at apical membranes of intestinal epithelial cells (Sheinin et al., 2000). Binding of Ca^{2+} to the N-terminal extracellular

domain of CaR initiates discreet intracellular events mediated by the second messengers inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG), which result in mobilization of intracellular Ca^{2+} and protein kinase C (PKC) activation, respectively (Berridge et al., 2000; Gama et al., 1997; Rhee, 2001). Intriguingly, elimination of GCC expression in mice is associated with loss of CaR from enterocyte brush borders (Pitari et al., 2008). Moreover, expression of CaR and GCC ligands is maximal at upper-crypt areas (Chakrabarty et al., 2005; Cohen et al., 1995; Whitaker et al., 1997), where epithelial cells stop proliferation and enter the maturation program, suggesting that CaR activity may be subordinated to GCC signalling along the crypt–villus axis (Pitari et al., 2008). Further, lumenal Ca^{2+} (1-3 mM) triggering cell cycle arrest at colonic mid-crypts (Whitfield et al., 1995) opposes pro-proliferative β–catenin activity and favour p21- and p27-mediated differentiation (Chakrabarty et al., 2003, 2005), molecular effectors also regulated by cGMP signalling in the intestine (Lin et al., 2010; Liu et al., 2001). Thus, GCC signalling may promote the proliferation-differentiation transition of intestinal epithelial cells through CaR regulation (Pitari et al., 2008). This is important as CaR is also the receptor for other polyvalent cations (i.e., Gd^{3+}, Mg^{2+}, Ni^{2+}) and polyamines (i.e., spermine, spermidine, putrescine) produced by commensal colonic bacteria (Hofer & Brown, 2003), pointing to a crucial role of the GCC-CaR pathway as regulator of a variety of antiproliferative signals in intestine (Pitari et al., 2008). In addition, lumenal Ca^{2+} may mediate intestinal GCC actions through CaR-independent mechanisms, including ionic currents by CNG channels (Biel et al., 1999; Pitari et al., 2003, 2008). Cyclic GMP-gated Ca^{2+} current through CNG is a major regulator of signal generation and transmission in excitable cells (Ames et al., 1999; Zufall et al., 1997). Of relevance, in colon cancer cells GCC signalling slows cell cycle progression, in part, by inducing cGMP-dependent CNG channel activation, intracellular Ca^{2+} influx and cytosolic Ca^{2+} rises (Pitari et al., 2003).

Another model proposes the v-akt murine thymoma viral oncogene homolog (AKT) as the master biological effector of the GCC pathway (Lin et al., 2010). AKT regulates survival and metabolic circuits in proliferating intestinal cells, and AKT over activation promotes crypt hyperplasia and tumorigenesis in mouse intestine (Sakatani et al., 2005). Importantly, elimination of GCC expression in mice results in hyperactivation of AKT signalling pathways, associated with expanded crypt compartments populated by glycolytic cells with accelerated G_1-S cell cycle transition (Lin et al., 2010). Conversely, loss of GCC and cGMP signalling restricts the differentiated villus compartment and diminishes mitochondria-dependent oxidative metabolism in intestinal epithelial cells (Lin et al., 2010). Investigations employing genetic and pharmacologic manipulation of AKT confirmed that GCC signalling through cGMP control the proliferative cell metabolism by decreasing the function of AKT (Lin et al., 2010). Thus, AKT-dependent regulation of cell survival and glycolytic metabolism along the crypt-villus axis, at the basis of intestinal mucosa development and homeostasis, may be conditionally regulated by the activity of the GCC pathway, whose increasing crypt-villus gradient directly correlates with differentiation and oxidative phosphorylation.

2.3 Regulation of epithelium-stroma interactions

Beyond the epithelium, the intestinal wall also encompasses mesenchymal and a smooth muscle layers. The intestinal mesenchymal compartment comprises mucosal and submucosal layers of connective tissues, composed of both acellular (e.g., glycoproteins,

hyaluronic acid, proteoglycans, collagen I and III) and cellular components (e.g., fibroblasts, myofibroblasts, leukocytes, endothelial cells). The basement membrane, a balanced mix of matrix components (e.g., nidogen, laminin, collagen IV, perlecan), physically separates the enterocytes from the underlying mesenchyme. The basement membrane and all the other mesenchymal components significantly contribute to the dynamic renewal of intestinal epithelial cells. Indeed, intestinal epithelium-stroma interactions contribute to maintain the crypt-villus homeostasis through direct cell-matrix and cell-cell contacts or paracrine signalling (Montgomery et al., 1999; Pinchuk et al., 2010). In contrast, corrupted epithelium-stroma interactions promote the initiation and progression of an array of intestinal pathologies (Kraus & Arber, 2009; Suzuki et al., 2011).

Studies with targeted deletion of GCC in mice revealed striking morphogenetic alterations affecting the extra-epithelial layers of the intestine (Gibbons et al., 2009). Indeed, the intestinal wall of these mice is significantly enlarged compared to mice with normal GCC expression. The mesenchymal compartment exhibits hypertrophy as a result of both exaggerated activation of its cellular elements and increased deposition of its interstitial matrix components (Gibbons et al., 2009). In particular, an increased ratio of activated myofibroblasts over quiescent fibroblasts is present in mice with loss of GCC signalling, an alteration which contributes to the establishment of a reactive stromal environment characterized by overexpression of collagen I, tenascin C and matrix metalloproteinase 9 (MMP-9) (Gibbons et al., 2009). In part, these alterations appear to be the consequence of an increased interstitial activity of the profibrinogenic transforming growth factor β (TGF-β), as GCC signalling through cGMP inhibits TGF-β secretion and function in intestinal epithelial cells and opposes stromal remodelling underlying inflammatory processes (Gibbons et al., 2009). Further, intestinal smooth muscle layers of mice lacking GCC signalling exhibit hyperplasia and hypertrophy, which represent important contributors of the transmural gut enlargement in these animals (Gibbons et al., 2009). Thus, the GCC pathway operating in intestinal mucosal cells exerts strong developmental and functional influences on the underlying stroma, presumably by regulating discreet hormonal circuits supporting epithelial-mesenchymal crosstalk (Pitari et al., 2007). Given the established role of the intestinal mesenchyme in inflammation and tumorigenesis (Kraus & Arber, 2009; Pinchuk et al., 2010; Suzuki et al., 2011), it is possible to speculate that dysregulation of GCC signalling in intestinal epithelial cells may favour the emergence of a reactive stromal environment promoting pathological processes.

3. GCC and intestinal transformation

Colorectal carcinogenesis comprises a pathological continuum turning pre-cancerous lesions into invasive malignant tumors. The process begins with single (epi)genetic mutations driven by carcinogenic insults that disrupt the physiological epithelial cell phenotype (Gryfe et al., 1997; van Engeland et al., 2011). As a result, the balance of migration, proliferation, differentiation and cell death along the colonic crypt-surface axis is perturbed and neoplastic cells with limitless replicative potential emerge. Remodelling of the surrounding stroma also participates to the promotion and progression of transformation, imposing cell non-autonomous drivers of tumorigenesis such us angiogenesis and inflammation (Kraus & Arber, 2009; Suzuki et al., 2011). Ultimately, malignant cells lose their epithelial characteristics and acquire a mesenchymal phenotype that enables them to translocate and

establish new colonies at distant sites, such as the liver, lung and peritoneum (Nicolson, 1988; Polyak & Weinberg 2009; Suzuki et al., 2011).

The paracrine hormone hypothesis of colorectal cancer suggests that sporadic intestinal tumorigenesis is a process initiated by loss of endogenous GCC ligand expression, which induces a state of guanylinopenia and uroguanylinopenia (Pitari et al., 2007). Indeed, extensive studies have demonstrate that early in transformation intestinal epithelial cells acquire a mysterious mutation that renders pre-cancerous adenomatous lesions devoid of guanylin and uroguanylin (Birkenkamp-Demtroder et al., 2002; Cohen et al., 1998; Notterman et al., 2001; Steinbrecher et al., 2000). Those reports suggest that guanylin and uroguanylin, organized in a tail-to-tail configuration on human chromosome 1p, are the most commonly lost gene products in colorectal cancer in both animals and humans, exhibiting mutational frequency rates comparable to that of APC. Conversely, GCC is retained in colorectal cancer cells, which often exhibit higher GCC expression levels compared to normal epithelial tissues (Schulz et al., 2006; Witek et al., 2005). Increased GCC in the context of reduced guanylin and uroguanylin expression probably reflects the common pharmacological paradigm of receptor upregulation following specific ligand deprivation. More importantly, dysregulation of GCC signalling with an intact, but silent (for failure of ligand-dependent activation), intracellular molecular pathway produces a dormant cGMP-regulated system, which might be pathognomonically associated with neoplastic disease progression (Pitari et al., 2007). In this model, colorectal carcinogenesis following paracrine GCC ligand insufficiency reflects the central role of GCC in coordinating processes maintaining epithelial cell homeostasis and the crypt-villus axis, including the proliferation-differentiation balance, migration, metabolic programming and mesenchymal development (Li et al., 2007a; Pitari et al., 2007).

3.1 Regulation of the colon cancer cell phenotype

Neoplastic cell transformation ensues from the stepwise accumulation of mutations that produce hyper functioning oncogenes and silenced tumor suppressors (Bishop & Weinberg, 1996). Universally, the final combination of all the mutations and signalling deregulations occurring in cancers has similar functional consequences, the promotion of tumor cell growth and dissemination, and the evasion of host mechanisms of elimination (e.g., immuno-surveillance) (Hanahan & Weinberg, 2000). In intestinal tumorigenesis, acquisition of these malignant traits resembles a pathological amplification of the crypt stem cell phenotype, which self-perpetuates through relentless rounds of cell proliferation and migration (Montgomery et al., 1999; Potten & Loeffler, 1990). Conversely, invasive cancer cells progressively lose the morphology and metabolism of the differentiated epithelium, acquiring the ancestral functional plasticity of pluripotent stem cells. Indeed, overexpression of molecules (Wnt, β-catenin, Tcf) that support the crypt cell compartment (Gregorieff & Clevers, 2005; Korinek et al., 1998), or disruption of gene products (the adenomatous polyposis coli gene APC, Smad, CDX-2) restricting it (Aoki et al., 2003; Fodde et al., 2001; Tang et al., 2005) promotes intestinal tumorigenesis in animal models. In close agreement with these observations, the majority of sporadic human colorectal cancers exhibits a perturbed APC signalling as the initial mutational event, which crystallizes crypt-like nuclear proliferative programs driven by the β-catenin/Tcf complex (Fodde et al., 2001).

Since it regulates crypt compartments and the proliferation-differentiation balance along the crypt-villus axis (Li et al., 2007a; Pitari et al., 2007), dormancy of the GCC signalling pathway contributes to neoplastic transformation in the intestine (Li et al., 2007a; Pitari et al., 2007). Indeed, the increased migration and proliferation induced by loss of GCC signalling in mucosal colonocytes (Li et al., 2007a) represents a significant oncogenic stress (Aoki et al., 2003; Spruck et al., 1999) that creates the pre-neoplastic intestinal crypt (Pitari et al., 2007). Accordingly, cell cycle progression and growth of human colon cancer cells, experimental mimicry of the GCC dormancy characterizing the human disease because they express GCC but not the endogenous ligands (Lucas et al., 2000; Pitari et al., 2001), are greatly impaired upon reactivation of GCC signalling with exogenous supplementation of its specific agonists (Pitari et al., 2001, 2003, 2005, 2008). Ligand-dependent GCC activation restores lost cGMP-regulated circuits and imposes cancer cytostasis by reducing nuclear DNA synthesis and the G_1/S transition (Lin et al., 2010; Pitari et al., 2001). Antiproliferation by GCC, in part, is mediated by extracellular Ca^{2+} actions at cancer cell membrane surfaces, through its dependant effects on CaR activation and CNG channel-mediated Ca^{2+} influx (Pitari et al., 2003; Pitari et al., 2008). In addition, reactivation of GCC signalling through cGMP opposes the Wnt/β-catenin/Tcf4 signalling axis, the regulator of the proliferative crypt phenotype and tumor promoter in intestine (Pinto & Clevers 2005; Reya & Clevers 2005; van Es et al., 2005), by directly inhibiting β-catenin stability (Liu et al., 2001; Thompson et al., 2000). Underscoring the significance of the dormant GCC pathway in colon cancer, elimination of GCC in mice significantly enhances intestinal tumor initiation and progression (Li et al., 2007b). Mice deficient of GCC signalling exhibit enhanced sensitivity to tumorigenesis induced by $Apc^{Min/+}$ and the carcinogen azoxymethane, reflected by increased tumor incidence, multiplicity, and burden (Li et al., 2007b). A principal mechanism by which GCC promotes colorectal tumorigenesis is the perturbation of regulators of G_1/S cell cycle transition, including increased expression of oncogenes cyclin D_1 and pRb, and decreased activity of tumor suppressor p27 (Li et al., 2007b). Beyond hyperproliferation, GCC-deficient mice also exhibit increased genomic instability in their intestinal mucosa cells. In particular, an increased incidence of DNA breaks, loss of heterozygosity and point mutations in genes central to tumorigenesis, including APC and β-catenin, are observed along the crypt-villus axis (Li et al., 2007b). Although it remains unclear, the molecular mechanism mediating maintenance of the genome by GCC, including damage detection or mutational repair, appears to be distinct from that regulating proliferation (Li et al., 2007b). Rather, proliferative restriction and genomic quality control reflect two reinforcing systems by which the GCC pathway opposes intestinal carcinogenesis (Li et al., 2007b; Pitari et al., 2007). While accelerated G_1/S cell cycle transition favours inheritance and amplification of genetic mutations (Aoki et al., 2003; Spruck et al., 1999), instability involving tumor suppressors or oncogenes further deregulates the cancer cell cycle (Spruck et al., 1999).

Another consequence of a dormant GCC pathway in colorectal transformation is the promotion of the cancer cell metabolism (Lin et al., 2010). As discussed above, intestinal crypt stem cells principally rely on glycolysis to produce ATP and support their metabolism. Activation of GCC signalling restricts the glycolytic crypt compartment and favours the acquisition of mitochondria-mediated oxidative phosphorylation by differentiated epithelial cells in villi (Lin et al., 2010). Importantly, neoplastic cells utilize glycolytic ATP as their source of energy, even in the context of optimal environmental oxygen levels (Capuano et

al., 1997; Kroemer, 2006; Pelicano et al., 2006). Dr. Otto Warburg first described this malignant paradox suggesting that cancer cells undergo metabolic reprogramming, wherein they switch from oxidative phosphorylation to aerobic glycolysis to produce ATP (Warburg, 1956). This malignant transition provides a competitive advantage to cancer cells that have a readily accessible supply of energy and substrates to support proliferation, adapt to the hypoxic tumor microenvironment, and promote invasion. Of relevance, restoration of GCC signalling by exogenous ligand administration increases the number, size and function of mitochondria in human colon cancer cells (Lin et al., 2010). Tumor reversion to mitochondria-dependent oxidative metabolism is associated with concurrent reduction in rate-limiting glycolytic enzymes, and reflects modulation of AKT and its downstream effectors (e.g., mTOR) by GCC signalling reactivation (Lin et al., 2010). Thus, while GCC signalling in human colon cancer cells induces expression of critical transcription factors required for mitochondrial biogenesis (PGC1α, mtTFA, NRF1), inhibition of glycolysis by GCC results in a reduced ability of tumors to uptake glucose and produce lactate (Lin et al., 2010). Importantly, elimination of AKT rescues the tumorigenic intestinal phenotype of mice deficient in GCC signalling (Lin et al., 2010), underscoring the central role of metabolic circuits in mediating inhibition of colorectal carcinogenesis by GCC. Together, these observations suggest that the dormant GCC pathway, produced by hormone deprivation early in transformation (Birkenkamp-Demtroder et al., 2002; Cohen et al., 1998; Notterman et al., 2001; Steinbrecher et al., 2000), can be envisioned as a loss-of-function mutation of a tumor suppressor system, which promotes crypt stem-like proliferation and metabolism and favours genomic instability and the development of the colon cancer cell phenotype.

3.2 Regulation of the colon tumor microenvironment

The tumor microenvironment is recognized as a major determinant of cancer formation, growth and dissemination (Fidler, 2001; Kraus & Arber, 2009; Suzuki et al., 2011). Both cellular and acellular components comprising the tumor stroma contribute to intestinal transformation, reflecting the intimate crosstalk between tumor epithelial cells and the underneath mesenchyme (Kraus & Arber, 2009; Suzuki et al., 2011; Witz & Levy-Nissenbaum, 2006). Thus, interstitial matrix remodelling, secretion of paracrine factors by stromal cells, lymphocyte-mediated immunoresponses, and neo-angiogenesis significantly influence cancer development (Fidler, 2001; Kraus & Arber, 2009; Suzuki et al., 2011). Among the molecular mediators of cancer-mesenchyme interactions, the matrix metalloproteinases (MMPs) play an essential role (Zucker & Vacirca, 2004). MMPs are a family of zinc-dependent metalloendopeptidases that cleave interstitial matrix components, growth factors, chemokines and cell surface receptors creating a nurturing niche for cancer growth and invasion (Cox & O'Byrne, 2001; Curran & Murray, 2000; McCawley & Matrisian, 2001). Depending on their substrate specificities, MMPs are divided into collagenases, gelatinases, stromelysins, and matrilysins (Stamenkovic, 2003).

The soluble collagenase MMP-9 has been conclusively linked with colorectal carcinogenesis (Chu et al., 2011; Lubbe et al., 2006; Nascimento et al., 2010; Zucker & Vacirca 2004; Zuzga et al., 2008). Structurally, MMP-9 (92-kDa protein) consists of a pro-peptide sequence, a catalytic domain containing the zinc binding site and fibronectin type II-like repeats, which promote MMP-9 binding to gelatin and elastin (Fridman et al., 2003; Shipley et al., 1996). Although enzymatic-independent signalling also has been reported (Bjorklund et al., 2004; Librach et al., 1991), the catalytic activity of MMP-9 is the principal mediator of tumor

matrix remodelling (Fridman et al., 2003; Lubbe et al., 2006). In this way, MMP-9 degrades basement membrane collagen type IV, allowing intestinal tumor epithelial cells to invade the adjacent stromal compartment (Fridman et al., 2003). Moreover, MMP-9 promotes tumor angiogenesis by specifically processing and releasing TGF-β and VEGF from cancer cell surfaces and the interstitial matrix, respectively (Bergers et al., 2000; Qian et al., 1997; Yu & Stamenkovic, 2000). Given its crucial role in those pathological processes, MMP-9-dependent proteolytic activity is considered a driving force conferring the migratory and invasive phenotype to cancer cells and favouring tumor progression (Bergers et al., 2000; Fridman et al., 2003; Lubbe et al., 2006; Yu & Stamenkovic, 2000). Consequently, MMP-9 activity needs to be tightly controlled in biological tissues. Indeed, normally MMP-9 is a silent protease, secreted by cancer cells as a pro-zymogen that is activated only upon cleavage of its 10-kDa N-terminal pro-peptide by various proteases (e.g., MMP-2, MMP-3, MMP-13, plasmin, thrombin) (Ahmed et al., 2003; Fridman et al., 2003; Ramos-DeSimone et al., 1999). Endogenous inhibitors of MMP-9 also exists (e.g., the tissue inhibitor of matrix metalloproteinase 1) which bind to both the pro- and the active-form of MMP-9 and neutralize its proteolytic activity (Goldberg et al., 1992; Stamenkovic, 2003).

Beyond inhibition of catalytic activity, regulation of zymogen expression and secretion represents additional effective modalities to contain tumorigenic MMP-9 functions (St-Pierre et al., 2003; Zhang et al., 2006). Cyclic GMP inhibits the synthesis and secretion of MMP-9 in various cell systems (Akool el et al., 2003; Gurjar et al., 1999). Accordingly, restoration of ligand-dependent GCC signalling though cGMP induces a compartmental redistribution of colon cancer cell MMP-9, in which intracellular retention results in reciprocal extracellular depletion of that collagenase (Lubbe et al., 2009). As a consequence, MMP-9 proteolytic activities at the pericellular tumor space are suppressed, with abrogation of MMP-9-dependent interstitial matrix remodelling and cell spreading (Lubbe et al., 2009). Conversely, mutational dormancy of the GCC pathway early in transformation (Birkenkamp-Demtroder et al., 2002; Cohen et al. 1998; Notterman et al., 2001; Steinbrecher et al., 2000) may permit the emergence of a pro-tumorigenic stromal environment characterized by increased MMP-9 secretion, break-down of epithelial basement membranes by MMP-9 catalytic activity and disruption of homeostatic epithelial-mesenchymal interactions. It has been proposed that GCC effect on spatiotemporal MMP-9 dynamics in colon cancer cells has a profound impact on the overall tumor phenotype, because by disrupting its surface localization, membrane anchoring and focal catalytic activity it suppresses oncogenic MMP-9 functions (Lubbe et al., 2009).

4. GCC and colorectal cancer metastasis

Cancer metastasis consists in the dissemination of tumor cells to distant locations (Fidler, 2003). Clinically, metastasis coincides with the most terminal disease stages, incurable conditions associated with poor prognosis and survival (Mehlen & Puisieux, 2006; Siegel et al., 2011). Pathogenetically, it comprises a sequence of distinct, individual processes including cancer cell invasion of the primary site, intravasation and distribution through blood or lymphatic vessels, and colonization of target organs (Fidler, 2003; Folkman, 1986; Nicolson, 1988). Following organ seeding, tumor cells have to migrate into and invade tissue parenchyma (Wanget al., 2004; Steeg, 2006), resist to local immune defences and establish a nurturing micro-environment to develop and growth (Fidler, 2003; Folkman, 1986). In colon cancer, preferred organs of metastatic colonization include the liver, lung and peritoneum.

Once colorectal cancer has spread to these organs, the risk of mortality increases dramatically, and ~90% of patients diagnosed with distant metastasis die within 5 years from diagnosis (Siegel et al., 2011). Indeed, the management of patients with colorectal cancer metastasis is characterized by the highest incidence of therapeutic failure, in which surgery is not practicable (Pihlet al., 1981; Shapiro, 1992) and adjuvant chemotherapy is ineffective (increasing median survival only few months) (Meyerhardt & Mayer, 2005).

The functional phenotype of metastatic cells is unique and very selective. It has been calculated that of intravasated tumor cells, only a minute fraction remains viable after 24 hour, and >99.99% are eliminated before reaching their target organ (Fidler, 1970). This metastatic inefficiency reflects the scarcity of cancer cell clones exhibiting the full molecular machinery to execute all the individual steps comprising the metastatic process (Fidler, 1970; Weiss, 1990). In that context, since its inception primary colorectal cancer consists of biologically heterogeneous cell subpopulations, among which are present those possessing the ability to migrate and spread to distant parenchyma (Fidler, 2003; Heppner, 1984). Intriguingly as demonstrated by extensive immune detection and mRNA analyses of clinical specimens, GCC is uniformly expressed in metastatic colon tumors regardless of anatomical location (Carrithers et al., 1994; Carrithers et al., 1996; Waldman et al., 1998). Moreover, the structural and functional integrity of GCC and its principal downstream effectors appears to be preserved in metastasis, as colorectal cancer cells at extra-intestinal sites exhibit identical binding characteristics to, and signalling activation by, the exogenous ligand ST to those of normal intestinal cells (Carrithers et al., 1994; Schulz et al., 2006; Witek et al., 2005). However away from its primary organ, GCC is a ligand-starved receptor with an intracellular dormant pathway, as normal mucosal cells in intestine are the principal producers of endogenous hormones guanylin and uroguanylin (Forte, 1999). Thus, the loss of GCC ligand expression early in transformation (Birkenkamp-Demtroder et al., 2002; Cohen et al., 1998; Notterman et al., 2001; Steinbrecher et al., 2000) may be part of the exclusive phenotypic mutations conferring a pro-metastatic evolutionary advantage to selected colon cancer clones (Lubbe et al., 2009; Pitari et al., 2007; Zuzga et al., 2011).

4.1 Control of invasive cell shape

To successfully execute the metastatic program, transformed cells require a dynamic actin cytoskeleton. Thus, a hallmark of metastasis is the abandon of the static epithelial cell polarity and the acquisition of plastic membrane borders with specialized actin-based organelles promoting locomotion and invasion (Fidler, 2003; Steeg, 2006). Similarly to lymphocytes or neutrophils at inflammatory sites, cancer cells constantly remodel their actin to assume atypical morphological architectures, a process often referred to as epithelial-mesenchymal transition (Polyak & Weinberg, 2009). Changes in cell shapes reflect profound molecular rearrangements at tumor surfaces, including loss of E-cadherin-dependent cell-cell contacts and transient assembly of integrin-driven cell-matrix adhesions (Avizienyte et al., 2004, 2005; Polyak & Weinberg, 2009). These processes permit *de novo* development of membrane protrusions, such as filopodia and lamellipodia for probing the matrix during spreading and migration, and invadopodia for focal proteolytic matrix degradation in invasion (Linder, 2007; Yamaguchi & Condeelis, 2007).

In general, common molecular regulators coordinate tumor cytoskeletal remodelling by transducing external signals into actin processes. In colon cancer cells, tyrosine kinase receptors (e.g., EGF receptor, Eph receptors, Met receptors), G protein-coupled receptors

(e.g., cholecystokinin receptors) and cytokine receptors (e.g., chemokine receptors, TGF-β receptor) have been established as important inducers of the metastatic cell morphology (Dienstmann & Tabernero 2010; Dong et al., 2009; Fulton, 2009; Kitamura et al., 2010; Larsen & Dashwood, 2010; Ongchin et al., 2009; Yuet al., 2006). They activate the intracellular signalling system controlling cytoskeletal actin (e.g., focal adhesion kinases, rho-GTPases, Arp2/3 complex), which assembles the membrane protrusive structures mediating invasion (Linder, 2007; Yamaguchi & Condeelis, 2007). Normally restricted at intestinal epithelial brush borders (Lucas et al., 2000), GCC is ideally positioned to affect those molecular networks and exert spatio-temporal control of actin remodelling. Indeed, ligand-dependent GCC signalling through cGMP appears to act as a suppressor of metastatic cell morphology in intestine (Lubbe et al., 2009; Zuzga et al., 2011). Thus, colon cancer cells assume a rounded shape upon GCC signalling activation, with elimination of F-actin rich filopodia and lamellipodia (Lubbe et al., 2009). The number and length of cancer cell invadopodia also significantly decreases after activation of the GCC pathway (Zuzga et al., 2011). Importantly, failure to form protrusive structures forces tumor cells to aggregate into compact colonies devoid of spreading and invading abilities (Lubbe et al., 2009; Zuzga et al., 2011). Together, these observations suggest that the GCC pathway is one of the intrinsic homeostatic systems that maintain the stable epithelial cell polarity, shape and tight junctions, which form the essential mucosal barrier between the intestine and the external environment (Han et al., 2011). This notion is further supported by the inhibitory role that GCC signalling exerts on known inducers of epithelial-mesenchymal transition (Polyak & Weinberg, 2009), including the reactive stromal environment (with enhanced TGF-β and MMP-9 activities) (Gibbons et al., 2009) and the stem cell-promoting PI3K/AKT system (Lin et al., 2010). Hence, dysregulation of GCC signalling in intestinal tumorigenesis may enable the epithelial-mesenchymal transition required for cancer cell dissemination (Lubbe et al., 2009).

A key intracellular effector of the GCC pathway that regulates colon cancer cell shape is the vasodilator-stimulated phosphoprotein (VASP) (Zuzga et al., 2011). Ena/VASP family proteins control F-actin geometry supporting cell motility (Krause et al., 2003). VASP promotes filopodia and lamellipodia formation and extension by organizing molecular complexes comprising G-actin, F-actin and actin regulatory proteins (Krause et al., 2003). It functions by protecting actin barbed ends from binding to capping proteins, thereby permitting filament elongation (Bear et al., 2002; Mejillano et al., 2004). Three critical domains enable VASP to intimately interact with the actin cytoskeleton (Krause et al., 2003), including 1) the N-terminus Ena/VASP homology 1 (EVH1), which binds to focal adhesion proteins vinculin and zyxin, 2) the central prolin-rich region, which contains a consensus binding motif for the G-actin-binding protein profilin, and 3) the C-terminus EVH2, which binds to both G- and F-actin and mediates VASP oligomerization. Importantly, Ser239 within the EVH2 VASP domain is a preferred phosphorylation site for PKG, functioning as a biological marker for cGMP signalling in intestinal (Deguchi et al., 2002) and other cells (Krause et al., 2003; Yaroslavskiy et al., 2005). Cyclic GMP-dependent VASP phosphorylation inhibits membrane protrusion formation in normal cells (Krause et al., 2003; Lindsay et al., 2007). Accordingly, in colorectal cancer cells VASP Ser239 phosphorylation induced by ligand activation of GCC signalling through cGMP and PKG induces rapid disassembly (less than 10 minutes) of invasive and migratory membrane organelles (Zuzga et al., 2011). Herein, GCC promotes VASP removal from tumor membrane protrusions with subsequent collapse of the F-actin infrastructure supporting

filopodia and invadopodia (Zuzga et al., 2011). However, colorectal cancer cells expressing a mutant VASP construct not-phosphorylatable at Ser239 are resistant to GCC effects on VASP intracellular distribution and membrane protrusions (Zuzga et al., 2011). These findings are the most significant because they uncover the novel paradigm of a single intracellular biochemical reaction, VASP Ser239 phosphorylation, as an invasion suppressive mechanism for colon cancer (Zuzga et al., 2011). Hence, the loss of this mechanism during colorectal tumorigenesis, reflecting silencing of the GCC-cGMP-VASP system following hormonal deprivation (Birkenkamp-Demtroder et al., 2002; Cohen et al., 1998; Notterman et al., 2001; Steinbrecher et al., 2000), may favour the acquisition of the metastatic cell morphology, characterized by dissolution of normal cell-matrix and cell-cell contacts, increased actin polymerization dynamics, and enhanced formation of membrane protrusions (Zuzga et al., 2011).

4.2 Control of cancer cell dissemination

Relocation of cancer cells to distant sites requires acquisition of novel motor abilities, enabling them to spread through remodelled matrix surfaces at both primary and secondary tissues. In primary tumors, cancer cell spreading in the direction of blood vessels initiates the migratory journey of the intravasation process (Fidler et al., 1978; Fidler, 2003). In secondary organs, tumor cell adhesion and spreading onto vascular endothelial surfaces starts cancer invasion of target parenchyma (Im et al., 2004; Wang et al., 2004). In this context, polarized cell spreading drives cancer cell migration in the direction of invasion by permitting the establishment of specialized cell-matrix contacts at membrane protrusions, which mediates actin cytoskeleton-driven anchorage and traction of the cell body (Small et al., 1996). Thus, regulators of the cytoskeleton, adhesion receptors and extracellular proteases, which universally control spreading and migration in cells, are key players underlying cancer dissemination (Yamaguchi & Condeelis, 2007). Since its signalling through cGMP and VASP controls actin cytoskeletal dynamics and membrane protrusions in colon cancer cells (Zuzga et al., 2011), the GCC pathway may exert substantial impacts on those processes underlying formation of distant metastasis. Consistent with this hypothesis, elimination of GCC signalling in mice accelerates cell migration along the intestinal crypt-villus axis (Li et al., 2007a). Of relevance, basal GCC activity appears insufficient to restraining epithelial cell motility, as demonstrated by the increased migration of intestinal mucosa cells in mice with targeted ligand (guanylin) deletion (Steinbrecher et al., 2002). These observations suggest a model in which loss of hormone expression at the beginning of colorectal tumorigenesis (Birkenkamp-Demtroder et al., 2002; Cohen et al., 1998; Notterman et al., 2001; Steinbrecher et al., 2000) results in the acquisition of increased migratory abilities by transformed cells, driven by the accelerated formation of locomotory organelles mediating cell spreading and invasion (Zuzga et al., 2011).

A significant regulator of colorectal cancer cell migration and dissemination is the MMP-9 secreted by tumor epithelial cells (Lubbe et al., 2006). Beyond matrix degradation, this MMP-9 promotes the spreading and migration of colon cancer cells along two dimensional surfaces (Lubbe et al., 2006). Moreover, the catalytic activity of cancer cell MMP-9 is required for optimal colon tumor cell seeding of target mouse organs (Lubbe et al., 2006), an effect probably reflecting remodelling of the tumor pericellular microenvironment by MMP-9 (Fridman et al., 2003). Accordingly, inhibitors of MMP-9 suppress the formation of colorectal liver metastasis in an animal model (Aparicio et al., 1999). The significance of

MMP-9 for colon cancer metastasis is further underscored by its universal role in regulating migration and invasion across different cell types (Buisson et al., 1996; Leppert et al., 1995; Sanceau et al., 2003; Schultz et al., 1988; Yu & Stamenkovic, 1999). Importantly, ligand-dependent GCC signalling through cGMP suppresses the function of the MMP-9 produced by colorectal cancer cells (Lubbe et al., 2009). Activation of the GCC pathway suppresses tumor cell spreading, migration and dissemination by specifically inhibiting the secretion of cancer cell MMP-9 in the extracellular space (Lubbe et al., 2009). Further, colon tumor cells treated with GCC ligands fail to form metastatic colonies on mouse diaphragms following intraperitoneal injections (Lubbe et al., 2009). This effect also depends on the ability of GCC to inhibit cancer cell MMP-9, as demonstrated by the resistance of cells overexpressing MMP-9 to GCC-mediated inhibition of peritoneal metastasis (Lubbe et al., 2009). Conceivably, a silent GCC pathway in colorectal carcinogenesis (Birkenkamp-Demtroder et al., 2002; Cohen et al., 1998; Notterman et al., 2001; Steinbrecher et al., 2000) facilitates colon tumor invasion and metastatic dissemination by removing a key inhibitory mechanism restraining the oncogenic activity of cancer cell MMP-9.

5. The GCC pathway as a source of novel clinical targets

As discussed above, the loss of ligand-dependent GCC signalling produces a dormant GCC/cGMP pathway, which has significant impacts on the initiation, progression and metastasis of colorectal cancer. Conversely, deregulation of that pathway and its individual molecular components uncovers novel targets with unexploited clinical potential for improved diagnosis and therapy of patients. Thus, detection of hormone downregulation in colon biopsies could indicate presence of intestinal carcinogenesis and demand appropriate follow-up (Cohen et al., 1998; Notterman et al., 2001). The selective expression of GCC in colorectal tumor cells at metastatic sites (Carrithers et al., 1994, 1996; Waldman et al., 1998), suggests its utility as a diagnostic marker and specific target for delivering imaging and therapeutic agents *in vivo* (Gali et al., 2001; Wolfe et al., 2002). Indeed, clinical trials are confirming the value of GCC as a diagnostic marker for molecular staging of patients and prognostic indicator of colorectal cancer recurrence (Mejia et al., 2010; Waldman et al., 2009). Moreover, the structural preservation of GCC and its intracellular effectors offers the GCC hormone replacement therapy as a novel clinical paradigm for the prevention and treatment of colorectal cancer (Pitari et al., 2007). In this context, oral administration of uroguanylin prevents polyp formation in an animal model of intestinal tumorigenesis (Shailubhai et al., 2000). Further, the resistance to colon cancer initiation and progression exhibited by populations living in the developing world (Pitari et al., 2003; Shailubhai et al., 2000), where enterotoxigenic infections are highest, suggests that replacement therapy with the exogenous GCC ligand ST, the enterotoxin produced by E. coli, might be an effective treatment for colorectal cancer patients (Pitari et al., 2007). This latter consideration is supported by observations that ST is the most potent GCC agonist available (Lucas et al., 2000), and the only ligand successfully investigated to fully restore the tumor suppressor activities of the GCC pathway in colorectal cancer cells (Lubbe et al., 2009; Pitari et al., 2001, 2003, 2005, 2007; Zuzga et al.,2011).

Distal components of the GCC pathway also could be exploited in original clinical applications against colon cancer. As expected for its significance in intestinal mucosa homeostasis, the intracellular GCC signalome comprises a complex molecular network

(Pitari et al., 2007), probably still incomplete and in which each of the molecular elements may deserve critical translational evaluations. For some of these, preclinical testing is currently ongoing that is revealing emerging features as promising colorectal cancer bio-targets (Table 1). One model is the CaR, whose surface expression in colon cancer cells is conditionally regulated by activation of GCC signalling (Pitari et al., 2008). The dormant GCC pathway probably contributes to the reduced CaR expression observed in colorectal tumors (Chakrabarty et al., 2003; Kallay et al., 2003), a mutational event with clinical potential as a diagnostic marker of disease progression (Pitari et al., 2008). Moreover, CaR activation by extracellular Ca^{2+} inhibits cell proliferation (Chakrabarty et al., 2003), and dietary Ca^{2+} supplementation has been proposed as a chemopreventive strategy for colon cancer (Cho et al., 2004). Since restoration of the GCC pathway with exogenous ST administration potentiates antitumorigenic CaR signalling in human colon carcinoma cells (Pitari et al., 2008), combinatorial therapies including dietary Ca^{2+} and GCC ligand replacement may represent promising clinical regimens for the prevention and treatment of colorectal cancer.

Protein	*Alteration in Colorectal Tumorigenesis*	*Diagnostic Target*	*Therapeutic Target*	*Ref.*
CaR	Reduced expression	Tumor formation	Inhibition of tumor growth	(Chakrabarty et al., 2003; Kallay et al., 2003; Pitari et al., 2008)
MMP-9	Increased cancer cell secretion	Distant metastasis	Metastasis prevention	(Lubbe et al., 2009; Zuzga et al., 2008)
VASP	Loss of Ser phosphorylation	Invasion, metastasis	Local invasion prevention	(Zuzga et al., 2011)

Table 1. Examples of emergent colon cancer molecular targets from the GCC pathway.

Another intriguing effector of the GCC pathway is MMP-9, whose cancer cell compartmentalization depends on intracellular cGMP signalling (Lubbe et al., 2009). A silent GCC network may favour increased release and proteolytic activity of MMP-9 at the tumor pericellular space (Lubbe et al., 2009), thereby promoting matrix remodelling and invasion (Curran & Murray, 1999). Importantly, colon cancer cell MMP-9 behaves as a selective prognostic and predictive biomarker for disease stage stratification and therapeutic regimen selection in patients (Bendardaf et al., 2010; Zuzga et al., 2008). Reactivation of the GCC pathway with ST, in turn, is one successful strategy to specifically inhibit MMP-9 in tumor epithelial cells, without collateral damage in normal tissue, that has been suggested for the chemoprevention of colorectal cancer metastasis (Lubbe et al., 2009). Further, recent studies are indicating VASP as yet another GCC target with attractive translational applications for patients with colon cancer (Zuzga et al., 2011). VASP is a crucial actin-binding protein controlling membrane protrusion geometry, cell adhesion and migration (Bear et al., 2002; Krause et al., 2003; Mejillano et al., 2004). Dormancy of the GCC pathway in tumorigenesis depletes colon cancer cells of the cGMP-dependent VASP Ser phosho-species, molecular regulators of VASP activity at dynamic membrane regions (Krause et al., 2003). Thus, loss of VASP Ser phosphorylation may represent a novel prognostic biomarker of colon cancer

progression (Zuzga et al., 2011). Conversely, reconstitution of VASP Ser phosphorylation could be exploited as an original paradigm for the chemoprevention of cancer migration and invasion, because the potent GCC ligand ST suppresses the malignant cell morphology and its pathological functions in colon cancer (Zuzga et al., 2011).

6. Conclusion

A novel paradigm is emerging in which colorectal cancer, one of the top cancer killers in the world, is pathogenetically conditioned by a dormant GCC pathway, developed early in tumorigenesis following specific ligand downregulation. Indeed, GCC and its paracrine hormones restrict the proliferative crypt phenotype and promote the normal epithelial cell morphology by orchestrating an articulated intracellular network comprising interconnected, but functionally distinct molecular effectors. Silencing of the pathway for loss of agonist-induced GCC/cGMP signalling alters the activity of those molecules with profound consequences for the initiation and progression of colorectal transformation (Fig. 1). Virtually all the key processes underlying carcinogenesis and metastasis are enhanced by dysregulation of the GCC pathway components, including proliferation, survival, genetic instability, migration, matrix remodelling and invasion. At the same time, the dormant pathway creates unexplored opportunities for novel diagnostic applications. This is because the biochemical deregulation that ensues from the silent cGMP-dependent machinery can be traced by analysis of the single pathway components at the molecular level. As a result, novel molecular fingerprints of colorectal carcinogenesis are emerging from the GCC pathway that can be exploited as clinical prognostic or predictive indicators of disease.
Restoration of the lost function by the GCC pathway in colorectal tumors also is proving its great translational value. Preclinical studies indicate that, though dormant, the pathway is largely intact and can be reconstituted simply by ligand replacement. Thus, administration of bacterial enterotoxin STs, potent GCC agonists, suppresses proliferation, migration, matrix degradation, invasion and metastasis by colorectal cancer cells. Altogether, these findings support the notion that oral replacement therapy with GCC ligands could represent a novel strategy for both the chemoprevention and cure of colorectal cancer. Additional therapies targeting the individual pathway components, either alone or in combination, also are being developed with the goal to improve clinical efficacy and selectivity. However, information from clinical testing is still missing and important questions remain to be addressed before this knowledge could be applied to the patient bed. In particular, general gastrointestinal toxicity worries need to be dissipated as GCC ligands such as ST are known for their potent diarrheagenic effects. Also, the temporal profile of GCC-targeted therapy will require complete characterization, including estimation of duration of treatments and effects. Finally, pharmacokinetics evaluation will need to be performed to accurately define dosing and timing regimens. In summary, the intestinal GCC pathway is an exciting potential source of novel diagnostic and therapeutic targets that could significantly affect the clinical management and disease outcome of patients with colorectal cancer.

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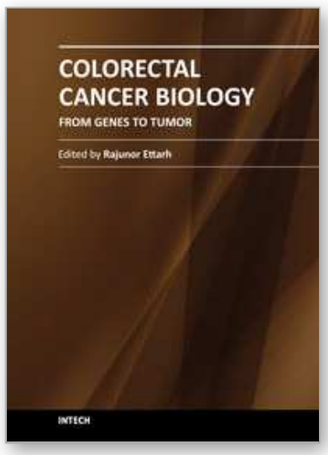

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Colorectal cancer is a common disease, affecting millions worldwide and represents a global health problem. Effective therapeutic solutions and control measures for the disease will come from the collective research efforts of clinicians and scientists worldwide. This book presents the current status of the strides being made to understand the fundamental scientific basis of colorectal cancer. It provides contributions from scientists, clinicians and investigators from 20 different countries. The four sections of this volume examine the evidence and data in relation to genes and various polymorphisms, tumor microenvironment and infections associated with colorectal cancer. An increasingly better appreciation of the complex inter-connected basic biology of colorectal cancer will translate into effective measures for management and treatment of the disease. Research scientists and investigators as well as clinicians searching for a good understanding of the disease will find this book useful.

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