**Guanylyl Cyclase C Prevents Colon Cancer Metastasis by Regulating Tumor Epithelial Cell Matrix Metalloproteinase-9**

Wilhelm J. Lubbe,1 David S. Zuzga,1 Zengyi Zhou,2 Weili Fu,2 Joshua Pelta-Heller,1 Ruth J. Muschel,2 Scott A. Waldman,1 and Giovanni M. Pitari1

1Department of Pharmacology and Experimental Therapeutics, Thomas Jefferson University; 2Department of Pathology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

**Abstract**

Matrix metalloproteinase-9 (MMP-9) produced by colorectal cancer cells is a critical determinant of metastatic disease progression and an attractive target for antimetastatic strategies to reduce colon cancer mortality. Cellular signaling by cyclic GMP (cGMP) regulates MMP-9 dynamics in various cell systems, and the bacterial enterotoxin receptor guanylyl cyclase C (GCC), the principle source of cGMP in colonocytes, which is overexpressed in colorectal cancers, inhibits tumor initiation and progression in the intestine. Here, we show that ligand-dependent GCC signaling through cGMP induces functional remodeling of cancer cell MMP-9 reflected by a compartmental redistribution of this gelatinase, in which intracellular retention resulted in reciprocal extracellular depletion. Functional remodeling of MMP-9 by GCC signaling reduced the ability of colon cancer cells to degrade matrix components, organize the actin cytoskeleton to form locomotory organelles and spread, and hematogenously seed distant organs. Of significance, GCC effects on cancer cell MMP-9 prevented establishment of metastatic colonies by colorectal cancer cells in the mouse peritoneum in vivo. Because endogenous hormones for GCC are uniformly deficient in intestinal tumors, reactivation of dormant GCC signaling with exogenous administration of GCC agonists may represent a specific intervention to target MMP-9 functions in colon cancer cells. The notion that GCC-mediated regulation of cancer cell MMP-9 disrupts metastasis, in turn, underscores the unexplored utility of GCC hormone replacement therapy in the chemoprevention of colorectal cancer progression.

[ Cancer Res 2009;69(8):OF1–8 ]

**Introduction**

The majority of patients (~60%) with colorectal cancer, the third most common neoplasm in men and women in developed nations, exhibit metastatic disease at the time of diagnosis (1). Progress in postsurgical adjuvant chemotherapy is effectively improving clinical management by prolonging disease-free intervals in patients with metastatic spread confined at regional sites (2, 3). However, advanced metastatic disease with tumor colonization of distant organs remains untreatable (3), reflected by high disease-specific mortality rate (~50%; ref. 4). In that regard, colorectal cancer cell dissemination is the result of a complex progression regulated by universal metastatic mediators encompassing extracellular matrix (ECM) remodeling, invasion through tissue boundaries, and seeding of target organs (5). The vulnerability of the metastatic cascade, including a coordinated series of rate-limiting steps (5), predicts that disruption of one or more of these underlying mechanisms may greatly improve the prognosis of patients with colon cancer by interrupting disease progression.

Matrix metalloproteinase-9 (MMP-9) promotes key processes underlying tumor cell propagation, from migration and invasion to angiogenesis and tumor growth (6, 7). A zinc-dependent metalloendopeptidase, MMP-9, cleaves specific factors constituting or present in the ECM, including collagen IV in basement membranes and angiogenic molecules (7), thereby creating a physical and biochemical path for tumor invasion and neovascularization (8). MMP-9 confers invasive abilities to cancer cells by regulating the function of dynamic membrane regions (9, 10). In turn, actin cytoskeleton remodeling associated with membrane protrusion (lamellipodia and invadopodia) formation induces polarized MMP-9 release (9, 11). In colorectal cancer, expression of tumor epithelial cell MMP-9 positively correlates with disease progression (12), suggesting that cancer cell MMP-9 controls essential functions mediating metastasis. Indeed, colorectal cancer cells secrete MMP-9, which promotes ECM degradation and tumor cell seeding (13). Thus, targeting tumor epithelial cell MMP-9 may represent a novel therapeutic strategy for colorectal cancer by disrupting fundamental processes underlying metastasis. However, an obstacle to this approach is the lack of selective therapeutics that inhibit MMP-9 mediating metastasis in cancer cells, without perturbing the physiologic activity of MMP-9 in normal tissues (6, 14, 15).

Beyond inhibition of catalytic activity (6), regulation of transcription, translation, or intracellular trafficking through tumor-specific signal transduction mechanisms represent attractive modalities to decrease the prometastatic function of MMP-9 in the pericellular space (16, 17). In that context, the guanylyl cyclase/cyclic GMP (cGMP) transduction system reduces MMP-9 mRNA levels in rat renal mesangial cells (18) and inhibits the secretion of MMP-9 in vascular smooth muscle cells (19). Intriguingly, intestinal epithelial cells selectively express the transmembrane receptor guanylyl cyclase C (GCC), a particulate guanylyl cyclase promoting GTP to cGMP conversion on binding to endogenous ligands guanylin and uroguanylin and diarrheagenic bacterial heat-stable enterotoxins (ST; refs. 20–22). Normally confined to apical membranes of intestinal mucosa cells (23), GCC is uniformly overexpressed in human colon tumors including those at extraintestinal sites (24–26), where it behaves as a selective cell surface receptor for targeted drug delivery (27).

The present study showed that GCC and cGMP induce a compartmental redistribution of cancer cell MMP-9, which resulted...
in suppression of the proteolytic activity of that gelatinase in the pericellular space and inhibition of tumor seeding and metastasis. These observations underscore the potential of GCC-targeted strategies inhibiting cancer cell MMP-9 for metastatic colorectal cancer chemoprevention.

Materials and Methods

Reagents. ST, the inactive analogue TJU 1-103 (28), and human recombiant proMMP-9 (29) were prepared as described. Uroguanylin and guanylin were from Peninsula Laboratories, whereas purified human recombinant active MMP-9 (67 kDa form) was from Calbiochem. Antibodies to human MMP-9 and MMP-2 were from Biomol. Antibody to human GAPDH was from Santa Cruz Biotechnology. Alexa Fluor 555 anti-rabbit IgG, Oregon Green 488 phalloidin, and Slowfade Gold with 4',6-diamidino-2-phenylindole were obtained from Molecular Probes. cGMP analogues (8-br-cGMP and 8-pCPT-cGMP) and all other chemicals were from Sigma.

Cell culture. T84 and Caco-2 human colon carcinoma cells (American Type Culture Collection) were cultured (37°C, 5% CO2) with 10% fetal bovine serum in DMEM/F-12 or DMEM, respectively. Unless otherwise indicated, treatments were conducted for 24 h in serum-free medium.

Cell transductions. Full-length MMP-9 cDNA (Invitrogen) was subcloned into XhoI-EcoRI multiple cloning site of mouse stem cell vector (MSCV)-puro retroviral vector employing forward (5'-ATCTCGAGATGAGGCTCTGGGCAGCCC-3') and reverse (5'-ATGAAATTCTAGTCTCAGGGGACCTGC-3') primers. HEK 293T cells were transfected with 1 μg of either purified MSCV-puro or MSCV-puro-MMP-9 plasmid plus 1 μg of the packaging vector pCM-Ampho employing FuGene (Roche). Viral supernatants supplemented with 4 μg/ml polybrene were used to transduce T84 (for 72 h). Cells transduced with MMP-9 or empty vector were selected with 5 μg/ml puromycin (for 1 week) and maintained in 1 μg/ml puromycin.

Immunofluorescence. Cancer cells were fixed (15 min) with ice-cold paraformaldehyde (4% in PBS) and permeabilized (10 min) with ice-cold acetone. Following overnight incubations (4°C; in PBS containing 2% bovine serum albumin) with rabbit anti-MMP-9 antibody (1:100) and 25 μl/m. Oregon Green 488 phalloidin, cells were incubated (60 min at room temperature) with Alexa Fluor 555 anti-rabbit IgG. Specificity of antigen/antibody reactions was assessed employing the secondary antibody alone. To identify cell nuclei, preparations were counterstained with SlowFade Gold anti-fade reagent with 4',6-diamidino-2-phenylindole. Images were acquired with a confocal laser scanning microscope (LSM 510; Carl Zeiss).

Real-time reverse transcription-PCR. Total RNA was isolated from cancer cells with RNeasy kit (Qiagen) and from mouse diaphragms with TRIzol (Invitrogen). RNA was subjected to one-step reverse transcription-PCR (RT-PCR) on a 7000 Sequence Detection System for 45 cycles (95°C for 5 min, 94°C for 20 s, and 62°C for 1 min) using TaqMan EZ RT-PCR core reagents (Applied Biosystems). The mRNAs for human GAPDH, MMP-9, and β-actin and mRNA for mouse β-actin were quantified employing specific fluorescently labeled primer/probe sets (Assay-on-Demand; Applied Biosystems). Data were analyzed using Sequence Detection Software (Applied Biosystems) with thresholds set at 0.2. Template-negative controls were run on each PCR plate. MMP-9 or GCC mRNA levels were normalized to human or mouse β-actin mRNA, respectively.

Immunoblot analysis. Protein samples (in SDS loading buffer) were separated by electrophoresis on 10% acrylamide Tris-glycine gels, transferred to nitrocellulose membranes, and probed with rabbit polyclonal antibodies against MMP-9, MMP-2, or GAPDH (1:1,000 each) in TBS-Tween 20 (3.5% milk) overnight at 4°C. After washing the primary antibody, membranes were probed with goat anti-rabbit IgG antibody (1:2,000; Santa Cruz Biotechnology) for 1 h at room temperature. Immunostained bands were visualized by autoradiography using horseradish peroxidase and quantified by densitometry.

Gelatin zymography. Tumor cell conditioned medium (500 μL) was concentrated using Centricon-30 filters (Millipore) and resolved on 0.1% gelatin, 10% acrylamide electrophoretic gels under denaturing conditions. After renaturation (for 1 h), proteins were incubated (18 h; 37°C) in buffer containing 50 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl and 5 mmol/L CaCl2. Gelatinolytic activity was quantified by densitometry following Coomassie blue staining (30).
Flow cytometry. T84 cells were harvested with 0.05% trypsin-EDTA, pelleted by centrifugation, and fixed (20 min) in ice-cold 2% paraformaldehyde. After washing, cells (−1 × 10^5) were either permeabilized with 90% methanol or incubated with 1% bovine serum albumin in PBS (4°C for 20 min). Cells were washed again and incubated (4°C for 30 min) with rabbit anti-MMP-9 (1:100) in the presence or absence of a specific blocking peptide (Biomol). After additional washes, cells were incubated with Alexa Fluor 488 anti-rabbit secondary antibody (1:200) and analyzed employing a FACSCalibur flow cytometer (Becton Dickinson).

Cell spreading (13). Cancer cells (−5 × 10^4) were plated in T25 flasks. After 6 h, the number of cells that formed distinct protrusions was scored as the fraction of total cell number per microscopic field (≥100 cells) of an inverted microscope.

Metastatic lung seeding (31). Following treatments, cancer cells were fluorescently labeled (30 min for 37°C) with 0.2 μmol/L MitoTracker Red CMXRos (Molecular Probe), washed, and harvested with 0.05% trypsin-EDTA. Cells (−6 × 10^5/mL) were resuspended in serum-free medium and injected into the inferior vena cava of anesthetized (Nembutal) female nu/nu mice (4-6 weeks old; Taconic Farms), artificially ventilated by a pressure-controlled ventilator through a tracheotomy. After 5 min, the pulmonary vasculature was cleared of blood and lungs were excised, placed onto a slide chamber with ports for the tracheal cannula, and inflated with 5% CO² in air (31). Fluorescent tumor cells attached to pulmonary microvasculature in the left lobe were detected using inverted fluorescent microscopy and quantified by scanning 45 consecutive fields (1.1 mm² of field per picture) employing OpenLab (Improvision; ref. 31).

Peritoneal metastasis. Following treatments, cells (−1 × 10^6/mL) were injected into the lower right peritoneal cavity of lightly anesthetized male Cr:NIH- nu/nu-Xid mice (4-6 weeks old; National Cancer Institute). Two weeks after injections, mice were sacrificed by CO₂ inhalation and the diaphragmatic peritoneum was excised and either fixed with 3.7% formalin or placed in RNA-Later for RT-PCR analysis (see above). Paraffin-embedded sections (5 μm) of diaphragmatic peritoneal rolls were stained with H&E and examined with a computer attached to a light microscope. All animal procedures were approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University.

Statistical analysis. Unless otherwise indicated, data are mean ± SE of three of more independent experiments. Statistical analyses were done employing the unpaired Student’s t test.

Results

GCC and cGMP signaling inhibits MMP-9 release into the extracellular compartment by colon cancer cells. Induction of GCC signaling with two membrane-permeant cGMP analogues or the bacterial enterotoxin ST, a potent GCC agonist inducing cytostasis by increasing intracellular cGMP (28), significantly diminished the accumulation of the MMP-9 proform (proMMP-9, 92 kDa) in the medium conditioned by human colon cancer cells (Fig. 1A). ProMMP-9 depletion in the extracellular space did not reflect increased cleavage of the inhibitory propeptide and proMMP-9 conversion into the active MMP-9 form (84 kDa; ref. 7), because accumulation of active MMP-9 was also reduced in the medium conditioned by tumor cells with induced cGMP signaling as assessed by gelatin zymography (Fig. 1B, top). Rather, cGMP-mediated MMP-9 depletion was associated with reduced MMP-9-dependent gelatinolytic activity in tumor cell conditioned medium (Fig. 1B, bottom). Moreover, cGMP effects on MMP-9 did not reflect changes in gene expression, as MMP-9 mRNA levels by RT-PCR were nearly identical in cancer cells treated with the vehicle PBS or agents inducing cGMP signaling (Fig. 1C). However, flow cytometry analyses revealed that ST induces a dramatic increase (≥10 fold) in intracellular MMP-9 compared with the vehicle control (Fig. 2A, top middle; cells were methanol-permeabilized). In contrast, the total amount of membrane-bound

![Figure 2](image-url)
MMP-9 protein was not altered by ST (Fig. 2A, bottom middle; cells were not permeabilized). The specificity of these observations was shown by omitting the primary antibody (Fig. 2A, left) or administering the specific blocking peptide (Fig. 2A, right), procedures that eliminated any detectable MMP-9 signal. Immunoblot analyses of total cell lysates (Fig. 2B) and confocal microscopy (Fig. 3A, MMP-9) with anti-MMP-9 polyclonal antibody confirmed that cancer cells exposed to ST exhibit intracellular accumulation of MMP-9. Importantly, intracellular MMP2 levels were not affected by ST (Fig. 2B). Thus, GCC activation induces a selective compartmental redistribution of MMP-9 in colorectal cancer cells, wherein MMP-9 retention in the intracellular compartment results in MMP-9 depletion in the extracellular space associated with reduced MMP-9-dependent proteolytic activity.

**GCC-mediated regulation of cancer cell MMP-9 prevents tumor cell spreading.** The catalytic activity of MMP-9 secreted by cancer cells promotes tumor cell locomotion through enhanced formation of membrane protrusions (filopodia and lamellipodia; ref. 13). Colon cancer cells extend membrane protrusions to spread and migrate along two-dimensional surfaces (Fig. 3A, top left, arrows). However, formation of these locomotory organelles was disrupted by ST treatment (Fig. 3A, bottom left). Indeed, compared with the vehicle control, tumor cells with ST-stimulated GCC activity displayed a rounded morphology in the absence of actin-rich filopodia and lamellipodia (Fig. 3A, β-actin). Moreover, rounded ST-treated cells exhibited intracellular MMP-9 accumulation (Fig. 3A, MMP-9), consistent with the hypothesis that, in colon cancer cells, GCC signaling inhibits membrane protrusion formation, in part, by inducing intracellular MMP-9 retention. Accordingly, ST significantly reduced membrane protrusion-driven cell spreading, an effect mimicked by the endogenous GCC ligands guanylin and uroguanylin but not an inactive ST analogue (Fig. 3B). GCC effects on cell spreading were cGMP- and MMP-9-dependent, because a membrane-permeant cGMP analogue mimicked ST actions, whereas administration of active MMP-9 blocked inhibition of cell spreading by ST or cGMP (Fig. 4A). To confirm that MMP-9 is the downstream molecular target of impaired tumor cell locomotion by GCC, a stable colon cancer cell line that overexpressed the endogenous GCC ligand uroguanylin or a membrane-permeant cGMP analogue mimicked ST effects on cell spreading (Fig. 4B) and oversecreted (Fig. 4C) MMP-9 was generated. As expected, MMP-9-overexpressing, but not empty vector control, cells were resistant to ST-induced inhibition of cell spreading (Fig. 4D).

**GCC-mediated regulation of cancer cell MMP-9 reduces hematogenous metastatic seeding.** Metastatic seeding of the mouse pulmonary microvasculature, an MMP-9-dependent process (13), can be visualized by fluorescence microscopy following intracaval injection of fluorescently labeled tumor cells (Fig. 5A; refs. 13, 31). Colon cancer cells treated in vitro with ST exhibit a reduced ability to attach to the lung microvasculature in vivo (Fig. 5A). The endogenous GCC ligand uroguanylin or a membrane-permeant cGMP analogue mimicked, whereas addition of purified human proMMP-9 blocked ST effects on metastatic seeding (Fig. 5B). Moreover, a broad inhibitor of MMP-dependent catalytic activity restored sensitivity to ST-induced inhibition of hematogenous seeding in tumor cells supplemented with proMMP-9 (Fig. 5B), confirming that the proteolytic activity of endogenous MMP-9 in the pericellular space confers seeding abilities (13). Finally, whereas it significantly reduced metastatic seeding by tumor cells stably expressing the empty vector, ST had no effect on
MMP-9-overexpressing tumor cells (Fig. 5a and B), showing that GCC inhibits metastatic cell dissemination by regulating MMP-9. 

Targeting GCC signaling suppresses peritoneal metastasis by colon cancer cells. Treatment with ST in vitro prevented the establishment of metastatic tumors in the mouse peritoneum in vivo by human colorectal cancer cells (Fig. 6). Whereas cancer cells treated with the vehicle control produced substantial diaphragmatic micrometastases 2 weeks after intraperitoneal injection (Fig. 6A, left, arrows), human cancer colonies from ST-treated cells could not be detected by H&E on mouse peritoneal rolls (Fig. 6A, right). Quantification of metastatic tumor burden by RT-PCR for GCC mRNA revealed that ST significantly reduced the amount of persistent colon cancer cells, which resided in the mouse peritoneum, compared with the vehicle controls (Fig. 6B). Importantly, ST effects on peritoneal metastasis were abolished in MMP-9-overexpressing cancer cells (Fig. 6B), indicating that MMP-9 mediates GCC-dependent inhibition of metastasis formation. Further, ST reduced (~30–40%) the incidence of peritoneal metastasis, defined as the percentage of mice with any detectable human GCC mRNA in peritoneal specimens, in animals injected with either wild-type colon cancer cells (PBS 60% versus ST 42.8%) or isogenic clones expressing the empty vector (PBS 62.5% versus ST 35%) but was ineffective in cells overexpressing MMP-9 (PBS 67% versus ST 66%). Together, these observations suggest that ligand-dependent GCC signaling suppresses the ability of colorectal cancer cells to invade and establish metastatic colonies in target organs by regulating the function of cancer cell MMP-9.

Discussion

GCC and cGMP have emerged as key physiologic regulators of intestinal crypt-villus homeostasis (27). Mice with loss of GCC signaling exhibit dysregulated intestinal proliferation and differentiation (32) and are susceptible to colorectal tumorigenesis (33). Protective effects by GCC depend on discreet cGMP-mediated signaling events, including protein kinase activation and ion channel currents (20, 32, 34). The present findings that ligand-induced GCC signaling negatively regulates MMP-9 and its dependent functions in colon cancer cells reveal a previously unappreciated arm of the GCC/cGMP signalome, probably contributing to antitumorigenesis (27). Importantly, GCC is uniformly overexpressed during transformation and may be exploited as a selective therapeutic target for metastatic colorectal cells in extraintestinal sites (24, 25, 35), including the use of GCC ligands to regulate cancer cell MMP-9.

Although significant, the role of MMP-9 in metastasis is controversial. MMP-9 is a fundamental component of invadopodia, dynamic membrane structures directing ECM degradation and tumor cell invasion (11, 36). Increased expression and secretion of MMP-9 at invadopodia tips conferred metastatic behavior to CT26 murine colon adenocarcinoma cells (37). Moreover, MMP-9 promotes the angiogenetic switch supporting tumor progression by activating ECM-embedded vascular endothelial growth factor (38). Because it regulates most of the rate-limiting steps underlying metastasis (39), MMP-9 could represent an ideal target for effective disruption of the metastatic cascade. However, MMP-9 also mediates wound healing and inflammation (14, 15) and opposes tumor growth by mobilizing angiogenetic inhibitors angiostatin and endostatin (40). The dual role in pathologic and physiologic processes may underlie the disappointing results obtained in cancer clinical trials with MMP inhibitors (6). Of significance, mechanisms of tissue repair and tumor suppression primarily depend on the MMP-9 contributed by stromal cells, including cancer-associated fibroblasts, which are principle sources of MMPs within tumors (41). Conversely, MMP-9 contributed by colorectal cancer cells specifically mediates pathologic processes promoting...
metastasis (13). Further, relative overexpression of MMP-9 in tumor epithelial, but not stromal, cells compared with matched normal adjacent tissues correlates with lymph node metastasis and disease progression in colon cancer (12). Confinement of GCC expression and signaling exclusively to epithelial cell populations within both normal and transformed intestinal tissues (24, 25, 35), in the context of the GCC-mediated inhibition of MMP-9 functions described here, offers the unrecognized therapeutic opportunity to selectively target the pathologic MMP-9 species mediating metastasis in colon cancer employing specific GCC ligands.

Beyond physicochemical differences, unique prometastatic activities by colon cancer cell MMP-9 probably reflect stringent spatiotemporal requirements of its functional regulation. Indeed, successful lamellipodia- or invadopodia-driven migration and invasion relies on the polarized release of MMP-9 at critical membrane microdomains, an obligatory step toward productive MMP-9 activation at the pericellular space (9, 11). Here, GCC signaling in cancer cells induced a compartmental shift of MMP-9, reflected by intracellular MMP-9 retention and decreased enzyme activity in the extracellular compartment. Arguably, GCC effects on MMP-9 are intimately linked to spatiotemporal remodeling of MMP-9 functional dynamics imposed by changes in the cellular/pericellular MMP-9 balance affecting surface localization, productive membrane binding, and catalytic activity (7). Accordingly, GCC-mediated compartmental redistribution of cancer cell MMP-9 was associated with reduced MMP-9-dependent membrane protrusion formation, motility, and metastatic seeding.

Functional remodeling of cancer cell MMP-9 by GCC and cGMP signaling is reminiscent of other tumor-based mechanisms, which inhibit the release and prometastatic activity of MMP-9 in the extracellular environment. In that context, integrins induce MMP-9 expression and secretion in various cell types (42, 43), and inhibition of selected integrin-dependent signaling at adhesion complexes has been suggested to disrupt MMP-9-dependent metastasis in colon cancer (44). Moreover, caveolins (45) and gangliosides (17) regulate the appropriate delivery and intracellular localization of MMP-9 to specialized tumor cell surface domains mediating MMP-9 secretion and activation, and potentiation of their dependent intracellular signaling suppresses metastasis in various cancer models. Thus, functional remodeling of MMP-9 by altering its intracellular trafficking, secretion, and compartmental distribution represents a general strategy for metastasis prevention. Specifically in colorectal cancer, tumor epithelial cell MMP-9 is the key promoter of discreet processes defining metastatic disease progression (12, 13), and GCC-mediated functional MMP-9 remodeling critically compromises those pathologic processes impeding invasion, seeding, and establishment of ectopic colonies (present results). These observations suggest a model (Fig. 6C) in which the catalytic activity of secreted MMP-9 promotes colorectal cancer cell metastasis by driving both invadopodia-mediated ECM

Figure 5. GCC signaling reduces hematogenous seeding of mouse lung. A, representative inverted fluorescence microscope fields of MitoTracker-stained wild-type T84 cells and isogenic clones stably expressing the MSCV-empty vector (T84-V) or the MSCV-MMP-9 vector (T84-MMP-9). Cells were treated in vitro (24 h in serum-free medium) with PBS (top) or ST (1 μM; bottom). Bar, 200 μm. B, tumor cell seeding of mouse lung in vivo was quantified as described in Materials and Methods. Percentages of respective vehicle-treated controls. Uroguanylin, 1 μM; 8-pCPT-cGMP, 1 mmol/L; MMP-9, 500 ng/mL purified human proMMP-9; BB94, a broad MMP inhibitor (60 nmol/L). MMP-9 and BB94 were added to cell cultures during the last 2 h of incubations. With the exception of uroguanylin (n = 2), at least three animals per condition were examined.

* P < 0.05; ***, P < 0.005 versus respective control.
degradation, migration, and invasion at the primary tumor site and attachment of intravasated tumor cells to exposed basement membrane components in the microvasculature of target organs (31). MMP-9 effects on metastasis can be independently regulated, contributing to the ordered sequence of distinct pathologic processes, or mutually reinforcing, as each individual process may promote the proper execution of other processes comprising the metastatic sequence (Fig. 6C). Indeed, MMP-9 independently regulates ECM degradation, cell spreading, and tumor seeding (13), but ECM remodeling by MMP-9 may be a prerequisite for proper cytoskeleton-driven membrane extension, cell migration, and cell attachment to the host microvasculature (5, 31). In turn, GCC signaling through cGMP disrupts cancer cell MMP-9 functions at the primary tumor, decreasing local tissue invasion, and in intravasated metastatic cells, preventing productive seeding (Fig. 6C). As a result, primary tumors are contained within tissue boundaries, tumor cells are cleared from the circulation, and colon cancer metastatic progression is interrupted by inhibition of underlying mutually reinforcing mechanisms (Fig. 6C).

In the context of the data presented here, loss of GCC signaling at the onset of colorectal carcinogenesis may be permissive to the emergence of the metastatic phenotype imposed by tumor epithelial cell MMP-9. Importantly, reactivation of the silent, but conserved, GCC signalome in cancer cells with exogenous administration of specific GCC ligands may oppose tumorigenesis and metastasis (27). Antimetastatic therapy with GCC ligands is tumor-specific, because it targets only metastatic colon cancer cells uniquely overexpressing GCC at extraintestinal sites (24, 35), and mechanism-based, because it inhibits pathologic functions of colon cancer cell MMP-9 by activating cGMP-dependent signaling. Thus, GCC hormone replacement therapy represents an unexplored approach to reduce colon cancer mortality by disrupting metastasis and its component processes in patients.

Disclosure of Potential Conflicts of Interest
S.A. Waldman is a paid consultant to Merck; is the Chair of the Scientific Advisory Board of Targeted Diagnostics & Therapeutics Inc. (uncompensated); receives research funding from Merck and Targeted Diagnostics & Therapeutics Inc.; and is an inventor on patents that include information contained in this article. Targeted Diagnostics & Therapeutics Inc. holds the license to commercialize intellectual property which includes information contained in this article. G.M. Pitari receives research salary support from Merck and is an inventor on patents that include information contained in this article. The other authors disclosed no potential conflicts of interest.
Acknowledgments

Received 1/8/09; revised 2/4/09; accepted 2/9/09; published OnlineFirst 3/31/09.

Grant support: Pennsylvania Department of Health and Prevent Cancer Foundation (GLM, Pitar), NIH grants CA75123 and CA59526 and Targeted Diagnostic and Therapeutics (A. W. Meiyer), and NIH Institutional Training Award ST32 CA09692 and Meaney Foundation M1D/PLDL fellowship (W. J. Lubbe). The Pennsylvania Department of Health specifically disclaims responsibility for any analyses, interpretations, or conclusions.

S. A. Waldman is the Samuel M. V. Hamilton Professor of Medicine.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Dr. Rafael Frildman for critical reading of the article.

References


Guanylyl Cyclase C Prevents Colon Cancer Metastasis by Regulating Tumor Epithelial Cell Matrix Metalloproteinase-9


Cancer Res  Published OnlineFirst March 31, 2009.

Updated version  Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-09-0067