Prostaglandin E₂ Regulates the Complement Inhibitor CD55/Decay-accelerating Factor in Colorectal Cancer*

Received for publication, July 2, 2004 and in revised form, September 14, 2004 Published, JBC Papers in Press, November 1, 2004, DOI 10.1074/jbc.M407403200

Vijaykumar R. Holla‡, Dingzhi Wang‡, Joanne R. Brown‡, Jason R. Mann‡, Sharada Katkuri‡, and Raymond N. DuBois¶\

From the ‡Department of Medicine, Vanderbilt University Medical Center and the ¶Department of Cancer Biology, Vanderbilt-Ingram Cancer Center, Nashville, Tennessee 37232-2279

Cyclooxygenase-derived prostaglandin E₂ (PGE₂) stimulates tumor progression by modulating several proneoplastic pathways. The mechanisms by which PGE₂ promotes tumor growth and metastasis through stimulation of cell migration, invasion, and angiogenesis have been fairly well characterized. Much less is known, however, about the molecular mechanisms responsible for the immunosuppressive effects of PGE₂. We identified PGE₂ target genes and subsequently studied their biologic role in colorectal cancer cells. The complement regulatory protein decay-accelerating factor (DAF or CD55) was induced following PGE₂ treatment of LS174T colon cancer cells. Analysis of PGE₂-mediated activation of the DAF promoter employing 5′-deletion luciferase constructs suggests that regulation occurs at the transcriptional level via a cyclic AMP/protein kinase A-dependent pathway. Nonsteroidal anti-inflammatory drugs blocked DAF expression in HCA-7 colon cancer cells, which could be restored by the addition of exogenous PGE₂. Finally, we observed an increase in DAF expression in the intestinal mucosa of ApcMin+/− mice treated with PGE₂ in vivo. In summary, these results indicate a novel immunosuppressive role for PGE₂ in the development of colorectal carcinomas.

Immune evasion represents an important mechanism by which cancer cells survive in a hostile environment. This complex process involves the modulation of several multifaceted complex pathways that regulate host immunity. These include targeted disruption of T-cell function through production of molecules that inhibit effector T-cells (1), suppression of T-cell cytotoxic death receptors (2), and induction of proapoptotic molecules targeted at tumor infiltrating lymphocytes (3, 4). Other methods of immune evasion include inhibition of B-cell and dendritic cell function (5) as well as secretion of the immunosuppressive mediators, such as transforming growth factor-β, interleukin-10 (IL-10), and cyclooxygenase-2 (COX-2)-derived PGE₂ (6, 7). Finally, malignant cells evade host immunity through induction of complement regulatory proteins (CRPs) that function to inhibit complement-mediated cell death (8–10).

Complement protects the host against microbial invasion through opsonization, and its activation triggers humoral and cellular inflammatory responses (11, 12). Normal cells anchor CRPs in the plasma membrane to defend host tissues from autologous complement attack. These include membrane cofactor protein (CD46), protectin (CD59), and decay-accelerating factor (also known as CD55). Stimulation of C3 convertase is the most important step in complement activation. Under physiologic and pathologic conditions, DAF decreases mis-targeted complement attack by preventing C3 convertase from forming and triggering rapid inactivation of the enzyme (13). Previous reports suggest that tumor cells induce CRPs as an effective mechanism to evade immune surveillance and complement-mediated cytotoxicity. Increased expression of CRPs has been described in several different malignancies, including colorectal (14), gastric (15), lung (16), renal (17), and breast (18) cancers. Specifically, increased DAF expression has been observed in 75% of colorectal cancers compared with matched normal tissue (19), and increased DAF expression has been associated with poor prognosis in patients with colorectal cancer (14). In addition to the angiogenic growth factors such as vascular endothelial growth factor and basic fibroblast growth factor (20), DAF expression is regulated by proinflammatory mediators such as lipopolysaccharide (21), tumor necrosis factor-α (22), and IL-1β (23).

Prostaglandins modulate immune function through a variety of mechanisms (24, 25). PGE₂ in particular has some known roles in the regulation of humoral and cellular immunity. PGE₂ is generated from arachidonic acid by the enzymes COX-1 and COX-2. The inducible isoform, COX-2, is highly expressed at sites of inflammation (26, 27), and COX-2-derived PGE₂ can signal via four distinct G-protein-coupled cell surface receptors (EP1–EP4) (28). Activation of EP2 and EP4 receptors leads to increased intracellular cyclic AMP (cAMP) levels through activation of Gaα, coupled protein. High levels of cAMP stimulate the cAMP-dependent kinase, protein kinase A, which ultimately increases expression of cAMP-responsive genes. In contrast, the activation of EP3 leads to decreased cAMP levels, whereas EP1 does not directly regulate intracellular levels of cAMP but increases free cytosolic Ca²⁺ levels through activation of Goq.

Numerous reports have demonstrated increased expression of COX-2 in a variety of human malignancies (29, 30), and high nonsteroidal anti-inflammatory drugs; CRP, complement regulatory protein; EP, E prostaglandin; Th, T (cell) helper.

* This work was supported in part by United States Public Health Services Grants RO-DK-62112 and PO-CA-77839. 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.

¶ Hortense B. Ingram Professor of Molecular Oncology and the recipient of a National Institutes of Health MERIT award (R37-DR47297). To whom correspondence should be addressed: Vanderbilt-Ingram Cancer Center, 691 Preston Bldg., 2300 Pierce Ave., Nashville, TN 37232-6838. Tel.: 615-343-0527; Fax: 615-936-6865; E-mail: raymond.dubois@vanderbilt.edu.

§ The abbreviations used are: IL, interleukin; COX, cyclooxygenase; PG, prostaglandin; DAF, decay-accelerating factor; CRE, cAMP response element; MOPS, 4-morpholinepropanesulfonic acid; PBS, phosphate-buffered saline; PI3K, phosphatidylinositol 3'-kinase; NSAID, nonsteroidal anti-inflammatory drugs; CRP, complement regulatory protein; EP, E prostaglandin; Th, T (cell) helper.
COX-2 expression correlates with a poor clinical outcome (31). High levels of COX-2-derived PGE₂ is associated with resistance to programmed cell death (32) as well as increased cell migration, proliferation, and angiogenesis (33). Immunosuppressive roles of PGE₂ reported previously include suppression of T- and B-cell proliferation (34) as well as modulation of professional antigen-presenting cell activity (5). Finally, elevated PGE₂ contributes to malignancy through immunosuppression of natural killer cell cytotoxicity (35, 36) and modulation of T-cell-derived cytokine production through inhibition of Th1 cytokines (interferon-γ and IL-2) (37, 38) and stimulation of Th2 cytokines (IL-4, IL-5, and IL-10) (39).

We sought to identify PGE₂-regulated genes downstream of elevated COX-2 activity in colon cancer. Using a variety of approaches, we present data in this study suggesting that decays-accelerating factor, a complement regulatory protein, is a direct target of PGE₂ in LS174T colon carcinoma cells. This is the first indication that prostaglandins regulate components of the complement cascade, which may allow malignant cells to evade complement-mediated cytotoxicity and contribute to carcinogenesis. Ultimately, this novel observation may shed light on the adverse clinical outcomes of patients with high levels of tumor-derived cyclooxygenase-2.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Prostaglandins (PGE₂, PGF₂, PGE₂, PGF₂, thromboxane B₂, and PGD₂) and antibody to COX-2 (catalogue number 160106) were obtained from Cayman Chemical (Ann Arbor, MI). LY294002, H-89, and chola toxin were purchased from Calbiochem. Antibodies to DAF (catalogue number SC-9156) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and monoclonal β-actin antibody and 3,3-diaminobenzidine were obtained from Sigma.

**Cell Culture**—LS174T, HCT-15, and OVCA-3 cells were purchased from the ATCC (Manassas, VA), and HCA-7 cells were a generous gift from Susan Kirkland. LS174T, HCT-15, and HCA-7 cells were maintained in McCoy's 5A medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin in a 5% CO₂ atmosphere. OVCA-3 cells were maintained in RPMI 1640 media containing 20% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin in a 5% CO₂ atmosphere.

**Northern Blotting**—Total cellular RNA was isolated from cells by TRIZol (Molecular Research Center, Cincinnati, OH) following the manufacturer’s protocol. Five micrograms of total RNA were fractionated with a MOPS-formaldehyde-agarose gel and transferred to Hybond N1 membrane (Amersham Biosciences). Following UV cross-linking, the blots were prehybridized for 30 min at 42 °C in Hybriol is-Benzol Company, Purchase, NY), hybridized using 32P-labeled cDNA in the same buffer at 42 °C, and subjected to autoradiography. The 0.5-kb DAF (NM_000674) probe was amplified by reverse transcription-PCR using primers 5'-CTGAGGGGCTCTGTGCGGAG-3' (sense, 683–702) and 5'-TAAGTCGAAACCCCATGTT-3' (antisense, 1191–1210). The 0.4-kb actin (BC004251) probe was amplified by reverse transcription-PCR using primers 5'-GGGATACCACTCTCTATTCAACACCCAGT-3' (sense, 317–336) and 5'-CTCATCTGTCTGAATGTCACAAG-3' (antisense, 723–742).

**Western Blotting**—Cells were washed with PBS and lysed with radiimmune precipitation assay buffer (50 mM Tris-Cl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% sodium deoxycholate, 0.1% SDS, and protease inhibitors from Roche Diagnostics). Protein concentrations were measured using Bio-Rad reagent (Bio-Rad). Proteins were then separated on precast SDS-polyacrylamide gels and electrotransferred onto nitrocellulose membranes. Membranes were blocked in 5% milk in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 1% sodium deoxycholate, 0.1% Tween 20) and incubated with primary antibody overnight at 4 °C. The membranes were then treated with horseradish peroxidase-conjugated secondary antibody and developed using an ECL kit (Amersham Biosciences).

**Promoter Constructs, Transient Transfection, and Luciferase Assay—** Various lengths of the human DAF promoter (40) spanning −724 to +80 bp with respect to the transcription initiation site were amplified using human genomic DNA (Promega, Madison, WI). For cloning purposes, the primer pairs containing BglII (forward) and HindIII (reverse) restriction sites at the 5' site were amplified by PCR, digested with BglII/HindIII enzymes, and cloned into pGL3-Basic firefly luciferase reporter vector (Promega). Substitution mutations at the CAMP response element (CRE) binding site were generated by PCR using forward 5'-TTCGGGACCTCTGCACTG and (reverse) 5'-GACCCCATCATGTCAGG-3' and (reverse) 5'-GAGTGGCAGCAGGAGCCCGAGG-3' and (reverse) 5'-GACCCCATCATGTCAGG-3' and (reverse) 5'-GAGTGAGCCACAGGAGCCCGAGG-3' and (reverse) 5'-GACCCCATCATGTCAGG-3'. Following amplification of the −383/+80 promoter construct using Pfu turbo DNA polymerase (Stratagene), the PCR product was digested with DpnI enzyme and transformed into DH5 incompetent cells. Small cultures from individual colonies were used for Miniprep DNA preparations (Qiagen). Each construct was sequence-verified before further evaluation. For luciferase assays, 1.3 × 10⁵ LS174T cells were cultured in a 12-well plate 24 h before transfection. The transfection was carried out using Lipofectamine (Invitrogen) in serum-free media containing 100 ng of reporter plasmid and 5 ng of Renilla luciferase reporter plasmid pRL-SV40 as an internal control following the manufacturer’s protocol. This transfection mixture was added to cells, and the plates were incubated at...
37 °C for 24 h. Prostaglandins and the other experimental reagents were added after 24 h and incubated for a further 24 h. Firefly and Renilla luciferase activities were measured using a dual luciferase assay kit (Promega) and a luminometer. Firefly luciferase values were normalized to Renilla values.

Immunohistochemistry—Six-week-old male ApcMin/H11001/H11002 mice were given 300 μg/kg PGE2 or sterile PBS vehicle control per os twice a day for 7 weeks. Pilot experiments consistently demonstrated that 300 μg/kg PGE2 achieved 2–3-fold higher levels of circulating and intestinal-derived PGE2 when compared with vehicle-treated control mice (data not shown). After 7 weeks, the mice were sacrificed by CO2 asphyxiation, and the entire intestine was dissected, washed in PBS, and immediately fixed in 10% neutral buffered formalin overnight at room temperature for paraffin embedding. Sections (5 μm) were dehydrated with xylene and rehydrated; the epitopes were revealed by microwave. Once endogenous peroxidase activity was quenched, nonspecific immunoglobulins were blocked with normal goat serum (Vector Laboratories, Burlingame, CA), and samples were incubated overnight at 4 °C with rabbit anti-human DAF primary antibody (1:50 dilution), which cross-reacts with mouse DAF protein. Negative controls received no primary antibody. The Vectastain ABC peroxidase system (Vector Laboratories) was used for immunodetection following the manufacturer’s instructions, and immunolocalization was visualized with the peroxidase substrate 3,3-diaminobenzidine. Samples were counterstained with hematoxylin and mounted. All results were verified by a blind independent second observer.

Human Colorectal Tissue Samples—Human colorectal tumor specimens were obtained from surgical resections with Vanderbilt University internal review board approval. For each tumor sample, matched adjacent normal mucosa was collected for comparison. All samples were snap frozen and stored in liquid nitrogen until use. RNA preparation from tissues was performed using TRI reagent as described above.

RESULTS

PGE2 Induces DAF mRNA and Protein in LS174T Cells—To evaluate the temporal profile of PGE2-mediated DAF expression, we conducted a time course following PGE2 treatment (1 μM). Northern blot analysis revealed that PGE2 rapidly induces DAF expression, beginning at 1.5 h (5-fold) and reaching a maximum by 4 h (20-fold) (Fig. 1A). PGE2 treatment increased DAF protein levels by 4 h (2-fold), and DAF expression remained elevated for 24 h (Fig. 1B). Furthermore, adding PGE2 induced DAF expression in a dose-dependent manner (Fig. 1C). mRNA levels were maximal at 2 μM (8-fold), whereas protein levels peaked at 10 μM (11-fold) PGE2 (Fig. 1D).

PGE2 also increased human DAF promoter activity in LS174T cells. Based on the identified transcription initiation start site (41), a series of 5′ DAF promoter deletion constructs were generated to evaluate the promoter activity of DAF in response to PGE2 treatment using reporter assays. The activity of these promoter constructs, which was assessed by their ability to drive luciferase expression, is shown in Fig. 2A. Transfection of the DAF −383/+80 construct resulted in max-
imal (~15-fold) PGE₂-mediated luciferase activity compared with the other constructs. Significantly, deletion of the CRE dramatically reduced luciferase activity, suggesting that PGE₂-dependent DAF induction is mediated by cAMP. This was further verified by substitution mutation of the CRE element from TGACACAG to TGATTCAG in the −383/+80 construct. This two-base mutation in the CRE decreased PGE₂ induction of DAF by 90% relative to the wild-type construct (Fig. 2B).

PGE₂ also induced DAF promoter activity in a dose-dependent manner with maximal activity at a concentration of 0.5 μM (Fig. 2C).

**PGE₂ Stimulates DAF in LS174T Cells in a cAMP/protein kinase A-dependent mechanism**—Four G-protein-coupled receptors are known to mediate PGE₂ signal transduction via distinct second-messenger pathways. To elucidate the specific mechanism by which PGE₂ induces DAF expression, we utilized a compound known to elevate intracellular cAMP levels. Incubation with 1 μg/ml cholera toxin, which activates adenylyl cyclase and increases cAMP levels, significantly induced DAF expression (25-fold) (Fig. 3A). Induction of DAF by PGE₂ via cAMP signaling was further evaluated using H-89, a selective inhibitor of the cAMP-dependent protein kinase A. H-89 blocked the induction of DAF by PGE₂ (Fig. 3). These findings are consistent with the hypothesis that PGE₂ induces DAF expression in a cAMP/protein kinase A-dependent manner.

We further evaluated PGE₂ stimulation of DAF by other signaling pathways known to affect cAMP levels. We have shown previously that PGE₂ has a positive effect on the phosphatidylinositol 3’-kinase (PI3K) and protein kinase B (AKT) pathways (42). Others have demonstrated that activation of the PI3K/AKT pathway reduces cAMP levels through increasing the activity of cyclic nucleotide phosphodiesterase 3B (43). Consistent with a model in which inhibiting the PI3K/AKT/phosphodiesterase 3B pathway increases cAMP levels (44), pretreatment with the PI3K inhibitor LY294002 prior to adding PGE₂ induced a robust increase in DAF expression (40-fold) in LS174T cells (Fig. 3A).

**PGD₂ and PGJ₂ Also Stimulate DAF Expression**—The effect of PGE₂ prompted us to assess the effect of other prostaglandins on the regulation of DAF expression. We treated LS174T cells with a variety of prostaglandins (1 μM) and measured DAF expression. Although PGA₂, PGF₂α, and thromboxane B₂ had no effect, PGD₂ (38-fold) and its metabolic product, PGJ₂ (43-fold), stimulated DAF mRNA expression in a similar concentration as that of PGE₂ (24-fold) (Fig. 4A). Protein levels of DAF also increased with PGD₂ (1.8-fold) and PGJ₂ (2.2-fold) (Fig. 4B). These data complement the profile of LS174T prostaglandin receptor expression, which consists of receptors for PGE₂ (EP2 and -4) and PGD₂ (D prostaglandin) (data not shown). The Northern and Western blot results were further corroborated.
with DAF promoter studies (Fig. 4C)

**DAF Is Induced by PGE₂ in Other Cancer Cells**—To assess the ability of PGE₂ to induce DAF in other cancer cells, we evaluated HCA-7 (colon), HCT-15 (colon), and OVCAR-3 (ovary) cells. This panel of cell lines was selected because of their differential production of PGE₂ downstream from the two cyclooxygenase isoenzymes, COX-1 and COX-2. HCA-7 cells express high levels of COX-2 and endogenous PGE₂ (4 ng/10⁵ cells) (45), and concomitantly high levels of DAF are found in these cells as well (Fig. 5A). Consistent with this model, treatment with exogenous PGE₂ (1 μM) did not further induce DAF mRNA levels (Fig. 5A). On the other hand, OVCAR-3 cells express high levels of COX-2 and endogenous PGE₂ (4 ng/10⁵ cells) (45), and concomitantly high levels of DAF are found in these cells as well (Fig. 5A). Consistent with this model, treatment with exogenous PGE₂ (1 μM) did not further induce DAF mRNA levels (Fig. 5A). Consequently, adding exogenous PGE₂ induced DAF expression over basal levels (2-fold). Finally, HCT-15 cells do not express either COX-1 or COX-2, producing low prostaglandins levels (47). Thus, adding PGE₂ induced DAF expression (2-fold).

**In Vivo Studies of DAF Expression**—ApcMin/H11001/+H11002/+ mice are known to develop multiple intestinal polyps at 15 weeks of age (48). Although COX-2 and PGE₂ levels are relatively low initially, after 12 weeks of age these levels are elevated dramatically. We evaluated the correlation between COX-2 and DAF expression in intestinal polyps. Compared with adjacent normal tissue, expression of both COX-2 and DAF was greatly

---

**Fig. 4.** A variety of prostaglandins stimulate DAF expression. LS174T cells were cultured in serum-free media for 48 h prior to treatment with various prostaglandins (1 μM). Cells were harvested after 4 h. Total RNA was isolated, and equal amounts of RNA were loaded. DAF mRNA levels were determined by Northern blot analysis (A). Following the isolation of total cellular protein, equal amounts of protein were separated by SDS-PAGE and visualized with DAF antibody (B). LS174T cells were transfected with a −383/+80 construct and treated with different prostaglandins (1 μM). Luciferase activity was measured after 24 h as described above. The experiments were repeated three times, and representative results are shown (C). TXB₂, thromboxane B₂.

**Fig. 5.** DAF is expressed in multiple cancer cell lines and is inhibited in COX-2-expressing cells by an NSAID. HCA-7, OVCAR-3, and HCT-15 cells were cultured in serum-free media for 24 h prior to the addition of PGE₂ (1 μM). Cells were harvested after 24 h. Total RNA was isolated, and equal amounts of RNA were loaded. DAF mRNA levels were determined by Northern blot analysis (A). HCA-7 cells were grown in 6-well plates for 24 h prior to the addition of serum-free media containing indomethacin (Indo) (10 μM) and grown for 72 h. Following the isolation of total cellular protein, equal amounts of protein were separated by SDS-PAGE and visualized with DAF antibody (B). CTL, control.
increased in the polyps (Fig. 6, A and B).

We next evaluated the ability of PGE\textsubscript{2} to effect DAF expression as part of the polyp formation process. For these experiments, we treated 5-week-old Apc\textsuperscript{Min}/H11001/H11002 mice with PGE\textsubscript{2} for 7 weeks and then examined DAF expression by immunohistochemical analysis of intestinal sections. Compared with vehicle-treated control mice (Fig. 6C, III and IV), 300 \( \mu \)g/kg PGE\textsubscript{2} treatment (Fig. 6C, I and II) induced DAF expression exclusively on the luminal surface of the small intestine. DAF expression was heterogeneous, revealing apical surface immunolocalization in differentiated villus epithelial cells. Sections from PBS-treated mice stained negatively for DAF, as did sections from PGE\textsubscript{2}-treated mice incubated without primary antibody as a negative control (data not shown).

Finally, assessment of DAF in human colon cancers and matched normal tissues revealed increased expression levels in malignant tissues. Northern blot analysis shows increased DAF expression in the large majority (14 of 16) of colon carcinoma samples as compared with adjacent normal mucosa (Fig. 7).

**DISCUSSION**

Studies exploring the relationship between host immune status and susceptibility to cancer represents an important field with tremendous potential to improve human health (49). The signaling networks involved are complex, and the molecular mechanisms modulating inflammation, immune surveillance, and tumorigenesis remain poorly understood. The present study sought to examine the hypothesis that two established mediators of immunosuppression in cancer, prostaglandin E\textsubscript{2} and decay-accelerating factor, are connected in a causal and directional relationship.

The inducible cyclooxygenase isoenzyme, COX-2, is significantly over-expressed at sites of inflammation and in various malignant tissues, with concomitant overproduction of the major arachidonate metabolite, PGE\textsubscript{2}. A large body of evidence has revealed a 40–50\% reduction in colorectal cancer in individuals taking NSAIDs regularly. These effects are due, at least in part, to the inhibition of the cyclooxygenase enzymes and decreased production of PGE\textsubscript{2}. Although increased levels of COX-2-derived PGE\textsubscript{2} are found in several different solid tumors, all of the effector genes downstream of this bioactive lipid are not well understood. We sought to carefully examine the role of COX-2 and prostaglandins in epithelial biology and carcinogenesis by identifying PGE\textsubscript{2}-regulated genes that mediate the effects of elevated COX-2 expression in colorectal cancer.

The well studied roles of COX-2 in malignant and metastatic disease have been shown to involve inhibition of apoptosis, stimulation of angiogenesis, and promotion of tumor invasion. However, the role of PGE\textsubscript{2} in subversion of the immune system has been less well characterized. Using a variety of methods, we describe for the first time the ability of PGE\textsubscript{2} to induce a major complement regulatory protein, DAF, through a cAMP/protein kinase A-dependent mechanism in human colon cancer cells. Polyps from Apc\textsuperscript{Min}/mice showed increased DAF expression compared with adjacent normal tissue that correlated...
PGE\(_2\) Regulates Decay-accelerating Factor

The novel findings presented in this study suggest that adjunct treatment of colorectal cancer with NSAIDs in combination with immunotherapy may increase the overall efficacy of colorectal cancer treatment. Because of increased potency and reduced side effects, immunotherapy continues to gain momentum for the treatment of a variety of human cancers (64). Future studies will yield greater insight into which effector genes mediate tumorigenicity downstream of COX-2-derived PGE\(_2\). Future work in this area will reveal the mechanisms involved in the complex progression from chronic inflammation and immunosuppression to overt tumor formation in the intestine.

Acknowledgments—We thank the T. J. Martell Foundation and the National Colorectal Cancer Research Alliance for generous support. We also thank Dr. S. K. Dey for valuable input throughout this study.

REFERENCES


PGE\textsubscript{2} Regulates Decay-accelerating Factor

483
Prostaglandin E$_2$ Regulates the Complement Inhibitor CD55/Decay-accelerating Factor in Colorectal Cancer

Vijaykumar R. Holla, Dingzhi Wang, Joanne R. Brown, Jason R. Mann, Sharada Katkuri and Raymond N. DuBois

doi: 10.1074/jbc.M407403200 originally published online November 1, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M407403200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 64 references, 19 of which can be accessed free at http://www.jbc.org/content/280/1/476.full.html#ref-list-1