Lipid Bodies Are Reservoirs of Cyclooxygenase-2 and Sites of Prostaglandin-E_2 Synthesis in Colon Cancer Cells

Maria T. Accioly,[1] Patricia Pacheco,[2] Clarissa M. Maya-Monteiro,[2] Nina Carrossini,[1] Bruno K. Robbs,[1] Silvia S. Oliveira,[1] Cristiane Kaufmann,[1] José A. Morgado-Diaz,[1] Patricia T. Bozza,[2] and João P.B. Viola[1]

[1]Division of Cellular Biology, National Cancer Institute; and [2]Laboratory of Immunopharmacology, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil

Abstract

Lipid bodies (lipid droplets) are emerging as dynamic organelles involved in lipid metabolism and inflammation. Increased lipid body numbers have been described in tumor cells; however, its functional significance in cancer has never been addressed. Here, we showed increased number of lipid bodies in tumor tissues from patients with adenocarcinoma of colon submitted to surgical resection when compared with an adjacent normal tissue. Accordingly, increased numbers of lipid bodies were observed in human colon adenocarcinoma cell lines and in a H-*ras*V12–transformed intestinal epithelial cell line (IEC-6 H-*ras*V12) compared with nontransformed IEC-6 cells. The functions of lipid bodies in eicosanoid synthesis in cancer cells were investigated. CACO-2 cells have increased expression of cyclooxygenase-2 (COX-2) when compared with IEC-6 cells. We showed by immunolocalization that, in addition to perinuclear stain, COX-2 and prostaglandin E (PGE) synthase present punctate cytoplasmic localizations that were concordant with adipose differentiation–related protein–labeled lipid bodies. The colocalization of COX-2 at lipid bodies was confirmed by immunoblot of subcellular fractionated cells. Direct localization of PGE_2 at its synthesis locale showed that lipid bodies are sources of eicosanoids in the transformed colon cancer cells. Treatment with either aspirin or the fatty acid synthase inhibitor C75 significantly reduced the number of lipid bodies and PGE_2 production in CACO-2 and in IEC-6 H-*ras*V12 cells with effects in cell proliferation. Together, our results showed that lipid bodies in colon cancer cells are dynamic and functional active organelles centrally involved in PGE_2 synthesis and may potentially have implications in the pathogenesis of adenocarcinoma of colon. [Cancer Res 2008;68(6):1732–40]

Introduction

Increased lipogenesis has emerged as a common phenotype to numerous human carcinomas and has been associated to poor prognosis in breast, prostate, and colon cancer (1, 2). Altered lipid metabolism in cancer cells involves modulation of multiple lipogenic enzymes (1, 2). In fact, enzymes involved in fatty acid synthesis, as well as metabolic regulators, are potential targets for antineoplastic intervention and chemoprevention (1, 2). In virtually all organisms, including mammalian cells, cytoplasmic lipids are stored in hydrophobic organelles called lipid bodies or lipid droplets. Cytoplasmic lipid bodies are osmiophilic structures, surrounded by a phospholipid monolayer with a unique fatty acid composition, have a neutral lipid rich core, and contain a variable protein composition (3–5). Although in the past, the presence of lipid bodies in cells were largely associated with lipid storage and transport, it has become apparent that lipid bodies are dynamic and functionally active organelles (4, 5).

Increased numbers of lipid bodies are described pathologic observation in cancer cells both in experimental settings and in clinical conditions (4). However, mechanisms that regulate lipid body formation and their functional significance to the cellular biology of the tumor are not known. Recent studies based on lipid body content have shown that lipid bodies, beside their rich lipid content, contain several functionally diverse types of proteins, including adipose differentiation–related protein (ADRP) and caveolin (6–9), lipid metabolic enzymes and proteins of Rab family (10–12), eicosanoid-forming enzymes (13, 14), and protein kinases, such as phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase, and protein kinase C (PKC; refs. 15–17). Therefore, lipid bodies may function in lipid metabolism, membrane trafficking, and intracellular signaling and might have potential implications to mechanisms of cell proliferation and differentiation.

In inflammatory cells, lipid bodies have important roles in regulating arachidonic acid (AA; 20:4, n-6) metabolism. Indeed, AA, an essential polyunsaturated fatty acid with signaling functions and the precursor of prostaglandins and leukotrienes, is stored in its esterified form at lipid bodies (15, 18). Accumulating evidence indicates that AA metabolism plays an important role in colon carcinogenesis. Unesterified AA itself is a signal for apoptosis of colon cancer cells, and conversely, enzymatic conversion of AA or esterification of AA into lipid pools depletes unesterified AA, thereby removing a proapoptotic signal and promoting carcinogenesis (19, 20). Different products of AA metabolism are also implicated in carcinogenesis. As much as 80% to 90% of colon carcinomas show an enhanced cyclooxygenase-2 (COX-2; prostaglandin H synthase) expression compared with normal intestinal mucosa (21–23). COX-2 is the enzyme that catalyzes the rate-limiting step in eicosanoids synthesis, converting AA into prostaglandins (24). Accordingly, high levels of prostaglandins, particularly prostaglandin E_2 (PGE_2), were observed in colorectal cancers (25, 26). Moreover, compelling data from epidemiologic studies, clinical trials, and animal experiments indicate that aspirin and other nonsteroidal antiinflammatory drugs (NSAID) inhibit colorectal carcinogenesis (reviewed in refs. 27, 28).

Leukocyte lipid bodies associated with inflammatory responses were shown to compartmentalize enzymes involved in AA release ($cPLA_2$; ref. 15) and metabolic conversion into prostaglandins

Requests for reprints: João P.B. Viola, Divisão de Biologia Celular Instituto Nacional de Câncer, Rua André Cavalcanti, 37 Rio de Janeiro, 20231-050 RJ, Brazil. Phone: 55-21-3233-1322; Fax: 55-21-3233-1470; E-mail: jpviola@inca.gov.br.
©2008 American Association for Cancer Research.
doi:10.1158/0008-5472.CAN-07-1999

Downloaded from http://aacrjournals.org/cancerres/article-pdf/68/6/1732/2602354/1732.pdf by guest on 30 September 2022

(COX) and leukotrienes (5-lipoxygenase; LTC_4 synthase; refs. 13, 14). Moreover, quantitative correlations between an enhanced generation of eicosanoids and levels of lipid body formation have been established in different cell types, suggesting that lipid bodies might function as specific sites involved in eicosanoids synthesis by cells engaged in inflammation (14, 29, 30). Recently, direct assessment of newly synthesized eicosanoids into its locale of synthesis has placed lipid bodies as specific sites of LTC_4 and PGE_2 synthesis within inflammatory leukocytes (31, 32).

In the present study, the occurrence and functions of lipid bodies in colon cancer were investigated. Here, we showed that human colon adenocarcinoma cell lines and colon cancer biopsies from patients present a drastic increase in lipid body numbers. Lipid bodies in these cells contain COX-2 and are structurally distinct cytoplasmic sites for PGE_2 production in an adenocarcinoma cell line. Furthermore, treatment with either aspirin or fatty acid synthase (FAS) inhibitor inhibited PGE_2 production and cell proliferation that correlated to an inhibition of lipid body formation in cancer cells. Together, our results place lipid bodies as functional organelles involved in prostaglandin synthesis in cancer cells and might have roles to the pathogenesis of colon cancer.

Materials and Methods

Human subjects. Samples of colon cancer and adjacent grossly normal-appearing tissue were obtained from three patients at the time of surgery from patients undergoing colon surgical resection at Brazilian National Cancer Institute. Control specimens were collected from the accompanying normal mucosa distant at 5 to 10 cm from the carcinoma. Samples for electron microscopy were immediately fixed as described below. This study was carried out with approval of the Brazilian National Cancer Institute's Ethic Committee.

Cell culture. CACO-2, LOVO, HT-29, and HCT-116 cells were grown in DMEM containing 10% fetal bovine serum (FBS). IEC-6 cells were also grown in DMEM containing 10% FBS supplemented with 0.1 unit of insulin. All cells were grown at 37°C in a humidified 5% CO_2 incubator.

Production of recombinant retroviruses and infection of IEC-6 cells. The retroviral expression vector pBabe-H-*ras*V12 has been previously described and was kindly donated by Dr. Lowe (Cold Spring Harbor Laboratory; ref. [33]). BD EcoPack2 ecotropic packing cell line (BD Biosciences) was transiently transfected with retroviral vector by calcium phosphate precipitation for 24 h. Cell-free virus–containing supernatant was collected 48 h after transfection and concentrated using Amicon Ultracel 50k (Millipore). The supernatant was supplemented with 8 μg/mL polybrene (FLUKA Chemie) and immediately used for spin infection (2 × 45 min at 380 × g) of 5 × 10^4 IEC-6 cells. Infected cells were incubated at 37°C for further 24 h and selected for puromycin (Sigma-Aldrich) resistance (7.5 μg/mL) for 14 d.

Lipid body staining and enumeration. Cells (5 × 10^6 per well) were left unstimulated and untreated or treated with aspirin (500 μg/mL) or C75 (50 μg/mL) for 24 h. Lipid bodies were stained as previously described in each triplicate well ([29]). In brief, cells (10^5 cells/mL) were fixed in 3.7% formaldehyde in calcium/magnesium-free HBSS (pH 7.4), rinsed in 0.1 mol/L cacodylate buffer, stained in 1.5% OsO_4 (30 min), rinsed in distilled water (dH_2O), immersed in 1.0% thiocarbohydrazide (5 min), rinsed in 0.1 mol/L cacodylate buffer, restained in 1.5% OsO_4 (3 min), rinsed, and then dried and mounted. The morphology of fixed cells was observed, and lipid bodies were enumerated by light microscopy with 100× objective lens in 50 consecutively scanned cells.

Electron microscopy. Lipid bodies were analyzed as previously described ([34]). Briefly, samples were fixed for 1 h in 1% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.2). Then, samples were washed with 0.1 mol/L cacodylate buffer (pH 7.2) followed by a second wash with 0.1 mol/L imidazole buffer (pH 7.5). Samples were then postfixed for 30 min with 2% OsO_4 diluted in 0.1 mol/L imidazole buffer, washed in the 0.1 mol/L imidazole, dehydrated in a graded series of acetone and embedded in PolyBed 812 resin. Ultrathin sections were stained for 2 min with lead citrate and examined by transmission electron microscopy. Lipid bodies were enumerated by electron microscopy in 100 consecutively scanned cells.

Isolation of lipid bodies by subcellular fractionation. Lipid bodies were isolated by subcellular fractionation based on the buoyancy property of these lipid-rich organelles in sucrose gradients as described ([15, 16]). CACO-2 cells (10^8/mL) in 20 mmol/L Tris, 1 mmol/L EDTA, 1 mmol/L EGTA, 100 mmol/L KCl buffer (pH 7.4) containing 10 μg/mL leupeptin, 10 μg/mL benzamidin, 0.7 μg/mL pepstatin, and 0.1 mmol/L phenylmethylsulfonylfluoride were disrupted by nitrogen cavitation at 700ψ for 5 min at 4°C. The cavitate was collected dropwise and mixed with an equal volume of disruption buffer containing 1.08 mol/L sucrose. The homogenates were centrifuged at 1800 rpm for 5 min to remove the nuclei. The supernatants were overlaid sequentially with 2.0 mL each of 0.27 mol/L sucrose buffer, 0.135 mol/L sucrose buffer, and Top solution [25 mmol/L Tris-HCl, 1 mmol/L EDTA, and 1 mmol/L EGTA (pH 7.4)] and centrifuged at 35,000 rpm at 4°C for 1 h. Seven discrete fractions were collected sequentially from top to bottom: a top layer of lipid body (fraction 1), the mid-zone between lipid bodies and cytosol (fractions 2–3), the cytosol (fractions 4–5), and the microsomal and pellet (fractions 6–7). Proteins from subcellular fractions were concentrated by precipitation with 20% trichloroacetic acid overnight at 4°C. The precipitates were then washed twice with cold acetone. The protein contents in the fractions were analyzed by DC Protein Assay (Bio-Rad). The activity of lactate dehydrogenase was measured as cytosolic marker using the CytoTox 96 kit (Promega).

Western blotting. Proteins from cellular fractions or total cell lysates prepared in reducing and denaturing conditions were separated by SDS-PAGE on polyacrylamide gel. After transfer onto nitrocellulose membranes, nonspecific binding sites were blocked with 5% nonfat milk in TBS-Tween [TBST; 50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 0.05% Tween 20]. Membranes were probed with anti-ADRP monoclonal antibody (mAb; AP125, RDI), anti–COX-2 mAb (C-20; Santa Cruz Biotechnology), anti-H-Ras mAb (F132; Santa Cruz Biotechnology), and anti–β-actin mAb (AC-40; Sigma-Aldrich) in TBST with 1% nonfat dry milk. Proteins of interest were then identified by incubating the membrane with horseradish peroxidase–conjugated secondary antibodies (Amersham Biosciences) in TBST with 1% milk. The membrane was developed with enhanced chemiluminescence (Amersham Biosciences) reagent, and the luminescence was captured by exposure to an autoradiography film.

Immunolocalization. CACO-2 cells (2 × 10^4/mL) were fixed in 3% formaldehyde at room temperature for 10 min and permeabilized with 0.05% saponin/HBSS solution for 10 min. The unspecific sites were blocked with 1% normal human serum for 10 min. The cells were incubated for 1 h simultaneously with goat polyclonal anti–COX-2 (C-20; Santa Cruz Biotechnology) or goat polyclonal anti–PGE synthase (PGES; S-16; Santa Cruz Biotechnology) and mouse monoclonal anti-ADRP (AP125; RDI) diluted in 0.05% saponin/HBSS solution. Then, cells were blocked with 1% donkey serum for 10 min before simultaneous incubation with Cy3-conjugated affiniPure $F(ab')$ fragment donkey anti-mouse and Cy2-conjugated affiniPure $F(ab')$ fragment donkey anti-goat (Jackson ImmunoResearch Laboratories) for 45 min. Then, cells were washed several times with HBSS, mounted in an aqueous mounting medium, and visualized with 100× objective by both phase contrast and fluorescence microscopy.

Immunodetection of PGE_2 at its sites of production. Immunodetection of PGE_2 was performed as previously described ([32]). In brief, cells (2 × 10^4/mL) were fixed and permeabilized during 1 h at 37°C with water-soluble 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDAC; 0.5% in HBSS). Then, cells were washed with HBSS and blocked with 2% donkey serum for 15 min before incubation with anti-PGE_2 mAb (Cayman Chemicals) for 45 min. The cells were washed with HBSS and incubated with Cy3-conjugated affiniPure $F(ab')$ fragment donkey anti-mouse for 45 min. Lipid bodies were fluorescent labeled with Bodipy 493/503 (Molecular Probes), added together with the secondary antibodies. The

Downloaded from http://aacrjournals.org/cancerres/article-pdf/68/6/1732/2602354/1732.pdf by guest on 30 September 2022

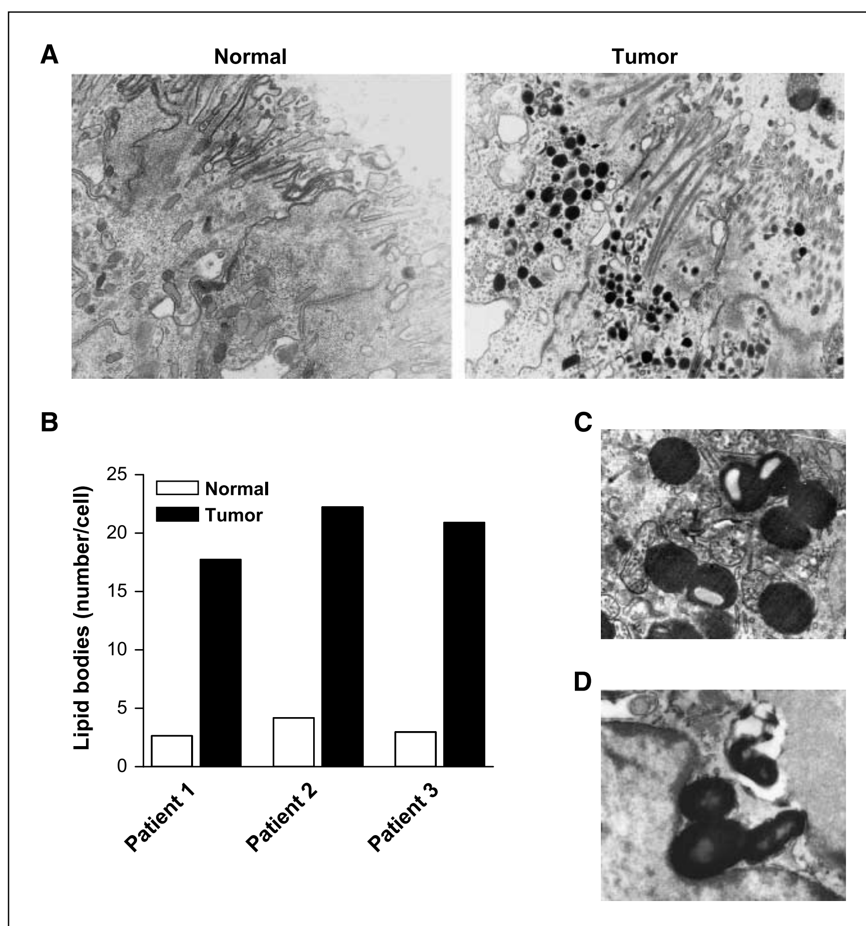

Figure 1. Analysis of lipid bodies of paired samples of human colon cancer and normal tissue. Pairs of samples of colon cancer and adjacent nonneoplastic tissue obtained at the time of surgery from patients undergoing colon surgical resection as described. *A,* analysis of thin sections of the adjacent normal tissue (*Normal*) and colon cancer (*Tumor*) examined by transmission electron microscopy. *B,* quantitative analyses of the lipid body numbers of the adjacent normal tissue and colon cancer. Lipid bodies were enumerated by electron microscopy and presented as mean from 100 consecutively scanned cells in tissues from three independent patients. *C* and *D,* electron microscopy analysis of the lipid bodies of the colon cancer sample. The presented images are representative from three independent patients.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/68/6/1732/2602354/1732.pdf by guest on 30 September 2022

cells were washed with HBSS, then mounted in an aqueous mounting medium, and visualized with 100× objective by both phase contrast and fluorescence microscopy. The specificity of the PGE_2 immunolabeling was ascertained by the incubation (10 min before EDAC) with the COX inhibitor indomethacin (10 μg/mL) to avoid the synthesis of PGE_2 and by the use of an isotype control (MOPC 31c; BD Pharmigen).

Cell proliferation assay. Cells (5×10^4 per well) were left unstimulated and untreated or treated with aspirin (500 μg/mL) or C75 (50 μg/mL) for 24, 48, 72, and 96 h. After treatment, cell proliferation was assayed in each triplicate well. Briefly, cells were fixed with ethanol for 10 min, stained with 0.05% violet crystal in 20% ethanol for 10 min, solubilized with methanol, and read by spectrometer at 595 nm.

EIA. Cells (2×10^6 per well) were left unstimulated and untreated or treated with aspirin (500 μg/mL) or C75 (50 μg/mL) for 24 h. After the treatment, PGE_2 was assayed in each triplicate well supernatant. Determination of PGE_2 levels by enzyme immunoassay was accomplished using PGE_2 monoclonal enzyme immunoassay kit (Cayman Chemical).

Statistical analysis. Statistical analysis of values from control and treated groups was determined using an unpaired Student's *t* test for single comparison or an ANOVA followed by the Student-Newman-Keuls for multiple comparisons. $P < 0.05$ was considered to be statistically significant.

Results

Lipid bodies are increased in human colon cancer. Ultrastructural evaluation of lipid bodies were performed by electron microscopy of paired samples of human colon cancer and normal tissue distant from the tumor. Patients' pairs of samples of cancer and nonneoplastic tissues were processed as described in material and methods. Analysis of adenocarcinoma of colon from patients showed round, nonmembrane-bound lipid bodies exhibiting variable osmiophilia, ranging from light to strong electron dense organelles that were prominently increased in number and size in tumor tissues when compared with a normal tissue from the same patient submitted to surgical resection (Fig. 1*A*). Electron microscopy quantitative analyzes showed a dramatic increase in lipid body numbers in all studied patients (Fig. 1*B*). Moreover, lipid bodies in tumor tissues were imaged as heterogeneous organelles. They frequently displayed lucent areas and granular and/or membranous internal structures or showed irregular surfaces (Fig. 1). Images suggesting budding, coalescence, or fusions of lipid bodies were identified (Fig. 1). Remarkably, lipid bodies from tumor tissues showed clear interaction with nucleus, ER, and mitochondria (Fig. 1*C* and *D*).

Adenocarcinoma cell line CACO-2 presents an increased number of lipid bodies, COX-2 overexpression, and high levels of PGE_2 production. To evaluate the possible involvement of lipid bodies in the pathogenesis of colon cancer, the presence of lipid bodies in a human colon adenocarcinoma cell line (CACO-2 cells) was compared with a nontransformed rat intestinal epithelial cell line (IEC-6 cells). Both cell lines were maintained in culture medium by 24 h and stained with osmium tetroxide. As shown in Fig. 2*A*, CACO-2 cells present enhanced lipid body formation when compared with IEC-6 cells, which have few or absence of these cytoplasmic structures. Electron microscopy analysis showed cytoplasmic organelles that were identified by their shape,

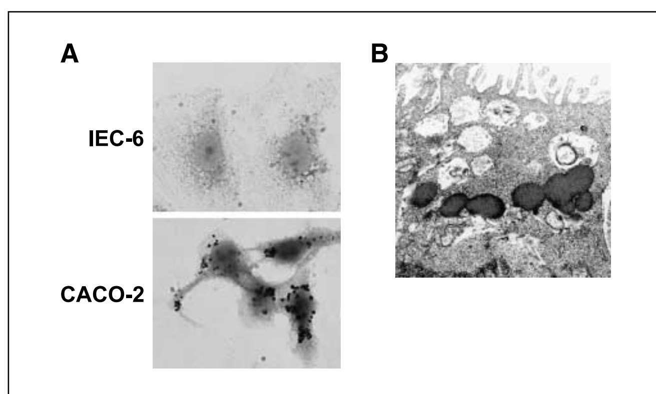

Figure 2. Analysis of lipid bodies in IEC-6 and CACO-2 cell lines. *A,* IEC-6 and CACO-2 cells were grown without stimulation for 24 h and stained for lipid bodies as described. Then, cells were analyzed by phase contrast microscopy with 100× objective lens. *B,* analysis of lipid bodies by electron microscopy in unstimulated CACO-2 cells as described in *A*. These results are representative from three independent experiments.

osmiophilia, and lack of a limiting membrane as lipid bodies (Fig. 2*B*). Images suggesting fusion of lipid bodies with each other were frequently observed, as well as, interactions with other organelles including ER, nuclei, and cytoplasmic vacuoles (Fig. 2*B* and data not shown).

Quantitative analysis of lipid bodies shows a 4-fold to 5-fold increase of lipid bodies numbers in CACO-2 cells when compared with IEC-6 cells (Fig. 3*A*). To evaluate if increased lipid body numbers were a unique feature of CACO-2 cells or if it was a common aspect to other human colon adenocarcinoma cell lines, LOVO, HT-29, and HCT-116 cells were analyzed (Fig. 3*A*). Increased lipid body numbers in all these cancer cell lines were observed when compared with IEC-6 cells (Fig. 3*A*). Several evidences suggest that the inflammatory process and COX-2 have a central role in colorectal carcinogenesis. COX-2 expression was evaluated in CACO-2 and IEC-6 cell lines. Western blot analysis showed increased expression of COX-2 protein in CACO-2 cells when compared with IEC-6 cells (Fig. 3*B*). Furthermore, CACO-2 cells also presented an enhanced production of PGE_2 when compared with IEC-6 cells (Fig. 3*C*).

Lipid bodies are sites for PGES and COX-2 localization and PGE_2 production in adenocarcinoma cell line. Lipid bodies in inflammatory cells have been shown to function as main sites involved in the oxidative metabolism of AA and eicosanoid synthesis (reviewed in ref. 5). To address the relationship between COX-2 and lipid bodies in cancer cells, the cellular compartment for COX-2 localization was evaluated. Initially, CACO-2 cells were subject to subcellular fractionation, and then cellular fractions were immunoblotted with anti-COX-2. ADRP was used as a marker of lipid bodies for colocalization purpose. ADRP is ubiquitously expressed in many cells and tissues as a major component of lipid droplets (6, 7). As shown in Fig. 4*A*, COX-2 protein localize in fractions where lipid bodies and nuclei are isolated. On the other hand, ADRP, a protein that is associated to lipid bodies in different cell types, only localize in the lipid body fraction (Fig. 4*A*).

We then used indirect immunofluorescence microscopy to confirm the localization of COX-2 in intact cells with specific conditions for fixation and permeabilization, which prevent dissolution of lipid bodies. In CACO-2 cells, lipid bodies are readily visualized by a strong ring shape staining for ADRP, indicating a staining pattern localized to the peripheral surface of lipid bodies similar to the staining pattern for ADRP-associated lipid body in adipocytes and other cells (6, 7). CACO-2 cells stained with anti–COX-2 polyclonal antibody, in addition to perinuclear membrane, showed focal punctate cytoplasmic staining (Fig. 4*B, top*). The presence of COX-2 within lipid body was confirmed by ADRP and COX-2 colocalization within CACO-2 cells (Fig. 4*B, top*). Interestingly, COX-2 lipid body staining was not restricted to lipid body surface, suggestive that COX-2 pervade lipid body core (Fig. 4*B insert*). PGE_2 is formed from AA by cyclooxygenase-catalyzed formation of PGH_2 and further transformation by downstream terminal enzyme PGES (35). Next, we evaluated the localization of the inducible isoform of PGES, mPGES, in CACO-2 cells. As shown in Fig. 4*B* (*bottom*), mPGES shows a similar localization pattern as the observed for COX-2 with colocalization with ADRP-labeled lipid bodies in addition to perinuclear staining.

Because CACO-2 cells have increased COX-2–enriched and PGES-enriched lipid bodies and enhanced PGE_2 formation, we hypothesized that lipid bodies were domains for compartmentalized PGE_2 synthesis. To investigate intracellular sites of newly formed PGE_2 in colon cancer cells, a recently developed strategy for direct *in situ* immunolocalization of PGE_2 synthesis were used (32). EDAC was used to cross-link eicosanoid carboxyl groups to amines in adjacent proteins, and immobilized PGE_2 was immunoflorescently detected. CACO-2 cells showed intense and punctate immunofluorescent staining for PGE_2 (Fig. 4*C, top*). As shown in Fig. 4*C* (*top right*), PGE_2 intracellular site of production match

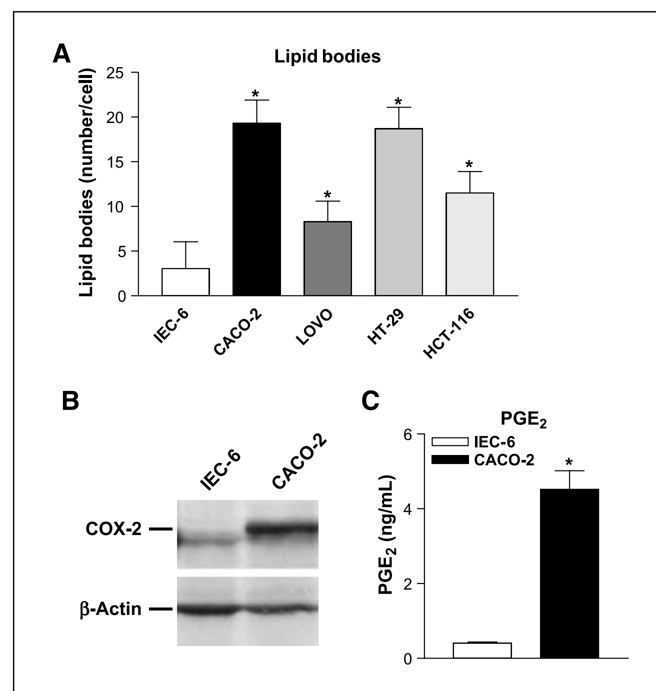

Figure 3. Quantitative analysis of lipid bodies, COX-2, and PGE_2 in colon cancer cell lines. *A,* quantitative analyses of the lipid body numbers in unstimulated IEC-6 and CACO-2, LOVO, HT-29, and HCT-116 cells. The lipid bodies were enumerated in 50 consecutively scanned cells. *Columns,* mean; *bars,* SD. *, significantly different values relative to IEC-6 cells ($P < 0.05$). *B,* analysis of COX-2 (*top*) and β-actin (*bottom*) expression in total cell lysates of unstimulated IEC-6 and CACO-2 cells by Western blot. β-Actin levels were used for control of protein loading. *C,* analysis of PGE_2 production in unstimulated IEC-6 and CACO-2 cells. In each supernatant, PGE_2 was assayed in triplicate by EIA. *Columns,* mean; *bars,* SD. *, significantly different values relative to IEC-6 cells ($P < 0.05$). These results are representative from three independent experiments.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/68/6/1732/2602354/1732.pdf by guest on 30 September 2022

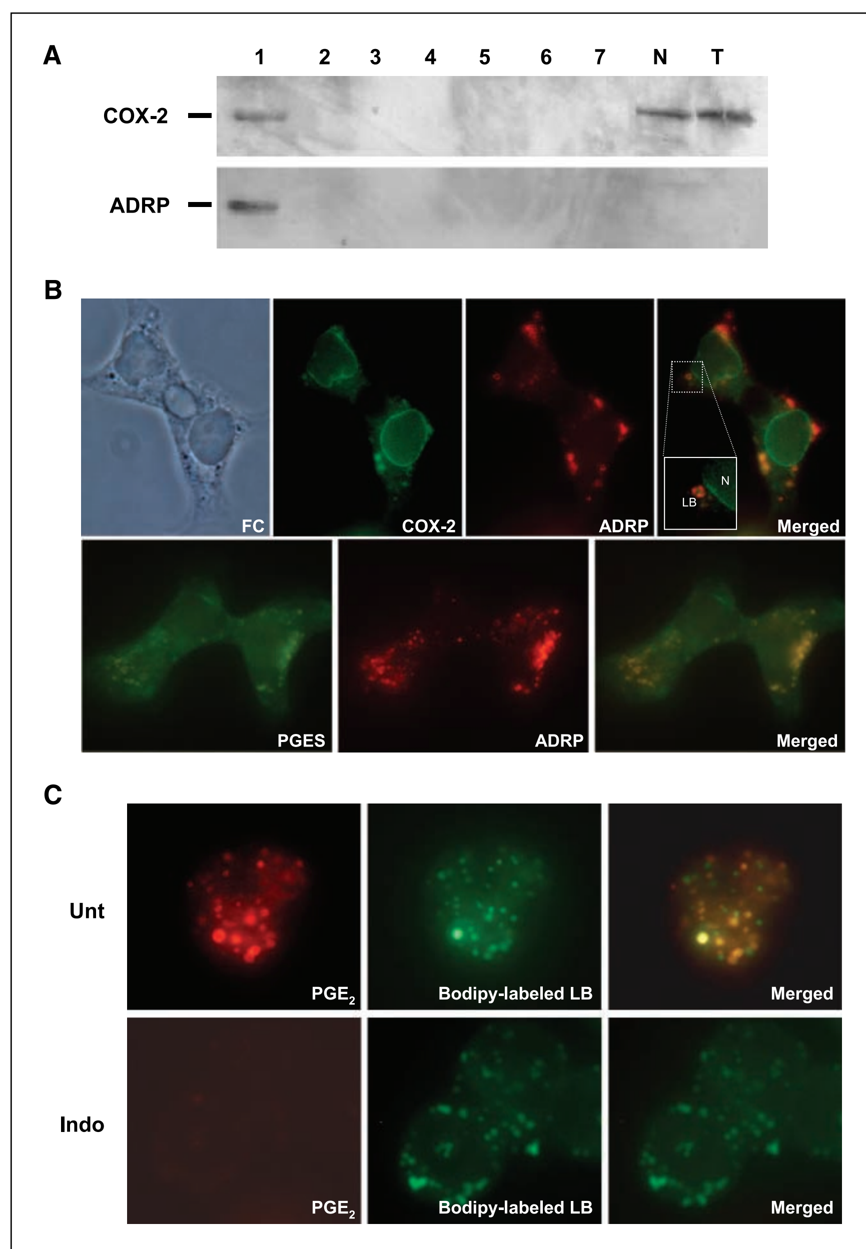

Figure 4. Analysis of COX-2, PGES, and PGE_2 localization in CACO-2 cells. *A,* unstimulated cells were subjected to subcellular fractionation, and fractions were recovered separately. *Fractions 1 and 2,* lipid bodies; *fractions 3 and 4,* cytosol; *fractions 5, 6, and 7,* microsome and membrane; *N,* nuclear; *T,* total lysate. Then, fractions were immunoblotted with anti–COX-2 (*top*) or anti-ADRP (*bottom*) antibodies. *B,* unstimulated cells were stained with anti–COX-2, anti-PGES, or anti-ADRP antibodies. *Top,* phase contrast (*FC*), anti-COX-2 (*COX-2*), anti-ADRP (*ADRP*), and merge of COX-2/ADRP (*Merged*); *bottom,* anti-PGES (*PGES*), anti-ADRP (*ADRP*), and merge of PGES/ADRP (*Merged*). *C,* unstimulated cells were left untreated (*Unt*) or treated with indomethacin (*Indo*) and then stained with anti-PGE_2 antibody and fluorescent Bodipy 493/503. The sequence shows anti-PGE_2 (*PGE_2*), fluorescent Bodipy 493/503 (*Bodipy*), and merge of PGE_2/Bodipy (*Merged*). All results are representative from two independent experiments.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/68/6/1732/2602354/1732.pdf by guest on 30 September 2022

bodipy-stained lipid bodies, placing lipid bodies as sites of PGE_2 synthesis within colon cancer cells. Of note, perinuclear PGE_2-positive staining could also be observed. Specificity of the immunofluorescence for PGE_2 was ascertained by the lack of PGE_2 immunolabeling within indomethacin pretreated CACO-2 cells (Fig. 4*C, bottom*) and also supported by the absence of immunostaining when an isotype control antibody replaced the anti-PGE_2 mAb (data not shown). These results showed that lipid bodies are intracellular sites for COX-2 and mPGES protein localization and locales of newly formed PGE_2 production in colon cancer cells.

Aspirin and FAS inhibitor C75 inhibited lipid body formation, PGE_2 production, and proliferation in adenocarcinoma cell line. NSAIDs, which inhibit COX, are used as chemopreventive agents for carcinogenesis of colon and cause apoptosis of cancer cells. The mechanism for this response is not fully understood, but it might result from an absence of a prostaglandin product, an accumulation of AA substrate, or shunting of substrate into a different pathway (28, 36). In addition, NSAIDs, including aspirin and NS398, have been previously shown to inhibit lipid body formation induced by cys-unsaturated fatty acids through COX-independent mechanisms (29, 37). The capacity of aspirin to inhibit lipid body formation in CACO-2 cells was investigated. CACO-2 cells were left untreated or treated with aspirin (500 μg/mL) for 24 hours, and lipid bodies and PGE_2 were evaluated as described above. As shown in Fig. 5*A* and *B*, aspirin inhibited lipid body formation and PGE_2 generation in CACO-2 cells. To gain further insights on the functions of lipid bodies in eicosanoid metabolism and roles in cell growth, we analyzed the effect of a noncyclooxygenase inhibitor of lipid body biogenesis.

To this end, CACO-2 cells were treated with the FAS inhibitor C75. FAS is the key biosynthetic enzyme in the fatty acid synthesis.

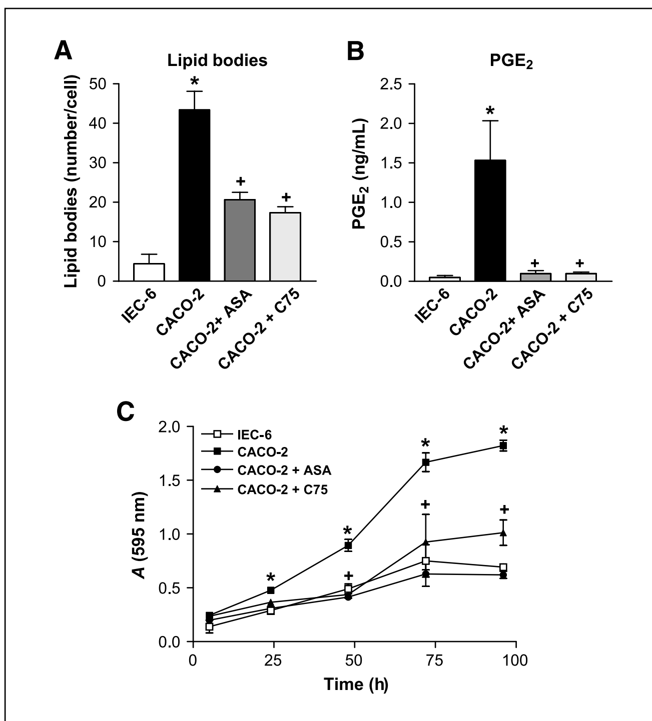

Figure 5. Analysis of lipid bodies, PGE_2 production, and cell growth in CACO-2 cells treated with aspirin and C75. *A,* cells were left unstimulated, and CACO-2 cells were left untreated or treated with aspirin (500 μg/mL) or C75 (50 μg/mL) for 24 h. Then, the lipid bodies were enumerated as described in Fig. 3*A*. *B,* cells were left unstimulated and CACO-2 cells were left untreated or treated with aspirin (500 μg/mL) or C75 (50 μg/mL) for 24 h. Then, PGE_2 was assayed by EIA as described in Fig. 3*C*. *C,* cells were left unstimulated and CACO-2 cells were left untreated or treated with aspirin (500 μg/mL) or C75 (50 μg/mL) for 24, 48, 72, and 96 h. Then, cells were stained with violet crystal and plates were analyzed by spectrometer at 595 nm as described. *Points,* mean; *bars,* SD. *, significantly different values relative to IEC-6 cells ($P < 0.05$); +, significantly different values relative to untreated CACO-2 cells ($P < 0.05$). All results are representative from two independent experiments.

Increased expression of FAS has emerged as a common phenotype in most human cancer, including colon cancer (38, 39) and its increased expression and activity associated to poor prognosis in cancer (reviewed in ref. 2). Moreover, it has been well established that FAS pharmacologic inhibition or knockdown by RNA interference leads to selective cytotoxic to human cancer cells (40–42). The mechanisms involved in FAS-induced killing of cancer cells remain an area of intense investigation. The effect of C75 in lipid body formation and PGE_2 production was analyzed. As shown in Fig. 5*A*, C75 significantly inhibited lipid body biogenesis in CACO-2 cells. Accordingly, C75 was shown to significantly inhibit lipid body formation in preadipocytes (43). Strikingly, although C75 is not an enzymatic inhibitor of COX-2 it significantly inhibited PGE_2 production by CACO-2 cells (Fig. 5*B*), thus suggesting that COX-2 compartmentalization within lipid bodies play roles in the enhanced PGE_2 synthetic capacity by colon cancer cells. It should be noted that cell viability 24 hours after treatment with either C75 or aspirin was greater then 95% as assessed by trypan blue exclusion (not shown).

We next examined the effect of aspirin or C75-induced inhibition of lipid body formation in CACO-2 cellular proliferation. CACO-2 cells were left untreated or treated with aspirin (500 μg/mL) or C75 (50 μg/mL) for 24, 48, and 72 hours. As shown in Fig. 5*C*, CACO-2 cells presented an enhanced proliferation when compared with IEC-6 cells. However, the treatment with either aspirin or C75 significantly decreased the CACO-2 cells proliferation at levels of IEC-6 cells (Fig. 5*C*).

Aspirin and FAS inhibitor C75 inhibited lipid body formation, PGE_2 production, and proliferation in H-Ras transformed IEC-6. To gain further insight into the association of cell transformation and lipid body biogenesis, we transformed IEC-6 cells with a retrovirus construct of H-*ras*V12. Consistent with published data (44), oncogenic Ras-mediated transformation of intestinal epithelial cells (IEC-6 H-*ras*V12) shows COX-2 overexpression (Fig. 6*A*) and is associated with an increased cell proliferation phenotype (Fig. 6*B*). As shown in Fig. 6, H-Ras transformed IEC-6 exhibits highly increased lipid body biogenesis (Fig. 6*C*) and enhanced PGE_2 production (Fig. 6*D*) when compared with IEC-6 cells. In addition, treatment with phorbol 12-myristate 13-acetate (PMA; 10 nmol/L for 1 h) increased lipid body formation in IEC-6 cells (4.8 ± 2.5 in untreated to 23.6 ± 3.5 lipid bodies per cell in IEC-6 cells treated with PMA; $P < 0.05$).

As shown in Fig. 5*C* and *D*, pretreatment with either aspirin (500 μg/mL) or C75 (50 μg/mL) significantly inhibited lipid body biogenesis and PGE_2 in IEC-6 H-*ras*V12 cells. Furthermore, the treatment with either aspirin or C75 in parallel to the inhibition of lipid body formation significantly decreased the IEC-6 H-*ras*V12 cell proliferation to the levels of IEC-6 cells (Fig. 6*B*). Of note, pretreatment with aspirin or C75 failed to inhibit basal lipid body numbers in wild-type IEC-6 (Fig. 6*C*) and inhibited IEC-6 cell growth in <30% within 72 hours (not shown).

Discussion

Lipid bodies are increasingly recognized as dynamic and multifunctional organelles in different cell systems and organisms. The occurrence and functions of lipid bodies in cancer cells are largely unknown. Here, we showed that lipid bodies are increased in neoplasic cells. Furthermore, lipid bodies in colon cancer cells are functionally active organelles that compartmentalize eicosanoid-forming enzymes and are sites for newly formed lipid mediator (PGE_2) production. Together, these findings yielded insights into the functions of lipid bodies in AA metabolism in cancer cells and place lipid bodies as potential targets for therapeutic interventions.

Inflammation is considered a well-established cancer risk factor, and a number of inflammatory conditions predispose to colon cancer, including ulcerative colitis and Crohn's disease (45, 46). Increased biogenesis of lipid bodies has been extensively characterized in cells associated with several human inflammatory diseases (reviewed in ref. 5), including infiltrating leukocytes and intestinal epithelium of patients with Crohn's disease (47). We showed drastic increased numbers of lipid bodies in the tumor tissues from patients with adenocarcinoma of colon submitted to surgical resection when compared with paired normal tissues. Lipid bodies within adenocarcinoma cells appeared as round amorphous osmiophilic inclusions showing variable electron densities and frequently showed an electron-dense rim, devoid of a bilayer membrane, typical features of lipid bodies in other cells. Although our study is limited by its small sample size, it gives support to future studies with large numbers of patients where the relationship between lipid bodies and the different aspects of colon cancer, including the stage, invasiveness, and the outcome could be addressed.

The surface of lipid bodies in cancer cell was shown to be enriched with the protein ADRP, which is in agreement with

Downloaded from http://aacrjournals.org/cancerres/article-pdf/68/6/1732/2602354/1732.pdf by guest on 30 September 2022

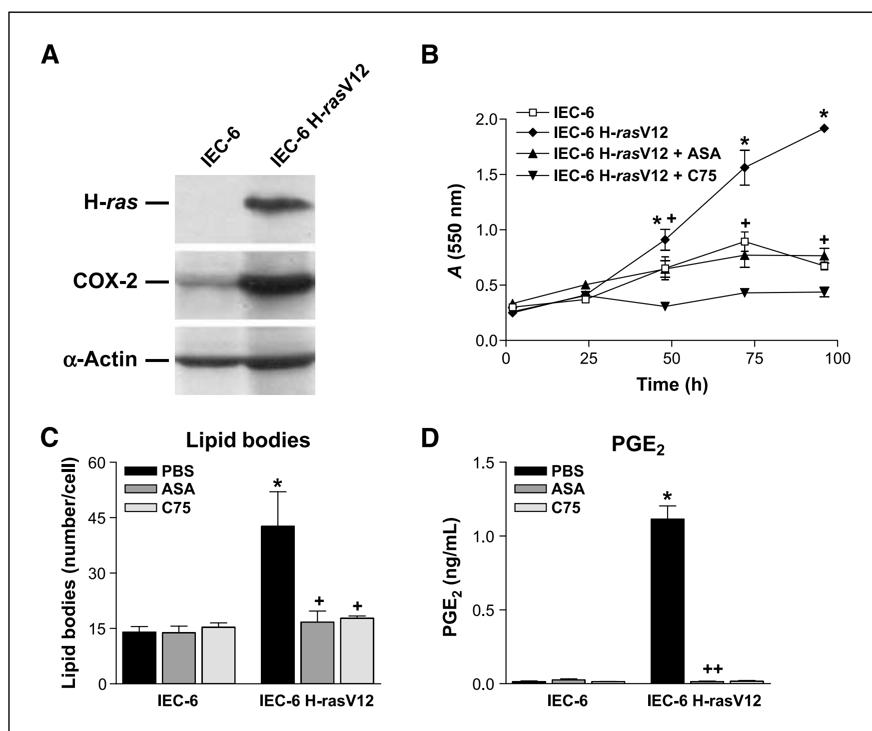

Figure 6. Analysis of lipid bodies, PGE_2 production, and cell growth in H-*ras* transformed IEC-6 cells treated with aspirin and C75. *A,* analysis of H-Ras (*top*), COX-2 (*middle*), and β-actin (*bottom*) expression in total cell lysates of unstimulated IEC-6 and IEC-6 H-*ras*V12 cells by Western blot. β-Actin levels were used for control of protein loading. *B,* cells were left unstimulated and untreated or treated with aspirin (500 μg/mL) or C75 (50 μg/mL) for 24, 48, 72, and 96 h. Then, cells were stained with violet crystal and plates were analyzed by spectrometer at 595 nm as described. *C,* cells were left unstimulated and untreated or treated with aspirin (500 μg/mL) or C75 (50 μg/mL) for 24 h. Then, the lipid bodies were enumerated as described in Fig. 3*A*. *D,* cells were left unstimulated and untreated or treated with aspirin (500 μg/mL) or C75 (50 μg/mL) for 24 h. Then, PGE_2 was assayed by EIA as described in Fig. 3*C*. *Columns,* mean; *bars,* SD. *, significantly different values relative to IEC-6 cells ($P < 0.05$); +, significantly different values relative to untreated IEC-6 H-*ras*V12 cells ($P < 0.05$). All results are representative from two independent experiments.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/68/6/1732/2602354/1732.pdf by guest on 30 September 2022

previous demonstrations that ADRP is the main surface protein of lipid bodies in different mammalian cells, including epithelial cells. Accumulating data points to an important role of ADRP in fatty acid uptake, cholesterol, and fatty acid binding and neutral lipid storage (48) and although the biological function of ADRP is not completely known, this protein is a specific probe for lipid bodies enabling its subcellular localization within intact cells and subcellular gradients (6, 7). To assess discrete sites involved in eicosanoid synthesis, eicosanoid-forming enzymes may be immunolocalized at specific intracellular sites. Based on immunolocalization studies of eicosanoid-forming enzymes in many cells, major sites of synthesis of cyclooxygenase pathway-derived eicosanoids are believed to be the ER and perinuclear membranes (24). In addition, extranuclear lipid bodies have been described as sites to where different enzymes involved in prostaglandin synthesis, including cPLA2 and COX-2, translocate upon leukocyte stimulation in inflammatory conditions (13–15, 30). Different evidences support the hypothesis that lipid bodies in neoplasic cells function as distinct intracellular domain for regulated eicosanoid production. First, lipid bodies were sites of COX-2 and PGES compartmentalization. Second, in this study we observed a significant correlation between lipid body numbers and PGE_2 generation in cancer colonic epithelial cells and in H-*ras*V12 transformed epithelial cells. Moreover, PGE_2 production was shown to occur at lipid bodies. Direct assessment of intracellular sites for prostaglandin generation has been elusive, as prostanoids and other eicosanoids are newly formed, nonstorable, and rapidly released upon cell stimulation. Recently, a new strategy to cross-link newly formed eicosanoids at its sites of synthesis has been described, enabling the immunofluorescent localization of newly formed eicosanoids at their intracellular formation locale and demonstrating that intracellular compartments involved in both leukotriene and prostaglandin synthesis differ according with the cell type studied and the stimulatory conditions used (31, 32). By investigating intracellular domains involved in prostaglandin synthesis in colon carcinoma cells, we showed that lipid bodies are intracellular domains for PGE_2 synthesis, placing lipid bodies as important sites in the cellular processing and metabolism of AA, in addition to nuclear membrane in colon cancer cells.

Increased lipid body biogenesis may contribute in different ways to cancer progression. As mentioned above, lipid body arachidonyl-phospholipids might provide a source of substrate AA leading to increased prostaglandin synthesis without requiring the perturbation of the integrity of membranes and directly coupled to cocompartmentalized PGES and COX-2. Lipid bodies could also function as a draining compartment to rapidly take up and reacylate free AA within lipid body lipids, with potentially detrimental outcomes for the host cell as AA has important functions in inducing apoptosis of tumor cells (19, 20).

Together, our results showed that adenocarcinoma of colon beside overexpression of COX-2, have increased numbers of lipid bodies equipped with active PGE_2-synthesizing machinery. These results prompt us to address if lipid body inhibition would have an effect on neoplastic cell proliferation. Different classes of drugs have been described with the capacity to inhibit lipid body formation, although no specific lipid body inhibitor has been identified thus far (reviewed in ref. 5). Thus, to address the hypothesis of lipid body involvement in colon cancer proliferation, parallel assessment of lipid bodies, PGE_2 production, and CACO-2 cell proliferation was obtained with two different classes of drugs that inhibit lipid body formation.

Epidemiologic studies showed that individuals who have taken NSAIDs have a markedly reduced risk of developing colon cancer. In mice, COX-2 inhibitors or traditional NSAIDs lower the incidence of polyps and cancer. It was suggested that inhibition of tumors by NSAIDs involves the common property of COX suppression and the resultant reduction in levels of prostaglandins in tissues (reviewed in refs. 28, 36). Our data show that treatment

with aspirin significantly reduced the number of lipid bodies and PGE_2 levels in CACO-2 and IEC-6 H-*ras*V12 cells (Figs. 5 and 6). The mechanisms involved in aspirin inhibition of lipid body biogenesis are not completely understood. Indeed, previous findings showed that lipid body biogenesis can be induced in COX-deficient cells and aspirin are able to inhibit lipid body formation even in COX-deficient cells, thus suggesting that aspirin inhibitory effect on lipid body biogenesis involves COX-independent mechanisms (29, 37). Clinical and experimental evidence strongly suggest that NSAIDs are anticarcinogenic, antiproliferative, and antineoplastic. In fact, the oncogenic potentials of the prostaglandins were related to its properties to promote cell proliferation and inhibit apoptosis in intestinal epithelial cells (49–51). We observed that CACO-2 cells present a progressive growth when compared with nontransformed epithelial intestinal cells. Inhibition of lipid body formation by either aspirin or FAS inhibitor, correlated to inhibition of PGE_2 generation and cell proliferation (Figs. 5 and 6). Thus, our data provides one more possible mechanism by which NSAIDs and FAS inhibitors exert their effects as anticarcinogenic drug. The inhibition of lipid body generation may affect the subcellular compartmentalization of COX-2 and in consequence inhibit the enhanced prostaglandin synthesis that is related to the pathogenesis of colon cancer.

Besides of its function as organelles involved in the generation of eicosanoids, lipid bodies constitute sites of compartmentalization of several signaling-relevant proteins, which may have functions beyond AA metabolism. Indeed, proteins with well-established roles in oncogenic cell transformation, tumorigenesis, and metastasis, including PI3K, extracellular signal-regulated kinase 1 (ERK1), ERK2, p38, PKC, and caveolin, were shown to localize in lipid bodies in a variety of cell types (8, 9, 15–17, 52). In addition, lipid body–specific protein ADRP has been recently described as a potential diagnostic and prognostic biomarker for renal cell and hepatocellular carcinoma (53, 54). In conclusion, our findings place lipid bodies as dynamic and functional active organelles centrally involved in PGE_2 synthesis in colon cancer cells that might potentially have implications with the pathogenesis of adenocarcinoma of colon. Thus, the evaluation of lipid bodies in human tissues could potentially be used as diagnostic and/or prognostic index in colon cancer or even as potential target to generate new drugs for cancer treatment.

Acknowledgments

Received 5/29/2007; revised 11/27/2007; accepted 1/9/2008.

Grant support: Brazilian Ministry of Health, FURNAS, CNPq (J.P.B. Viola), Brazilian Ministry of Health-DECIT/CNPq, and FAPERJ (P.T. Bozza). M.T. Accioly and S.S. Oliveira were supported by a fellowship from the Brazilian Ministry of Health, P. Pacheco and C.M. Maya-Monteiro by a fellowship from FAPERJ, N. Carrossini by a fellowship from CAPES, and B.K. Robbs by a fellowship from CNPq.

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.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/68/6/1732/2602354/1732.pdf by guest on 30 September 2022

References

1. Swinnen JV, Brusselmans K, Verhoeven G. Increased lipogenesis in cancer cells: new players, novel targets. Curr Opin Clin Nutr Metab Care 2006;9:358–65.

2. Kuhajda FP. Fatty acid synthase and cancer: new application of an old pathway. Cancer Res 2006;66: 5977–80.

3. Tauchi-Sato K, Ozeki S, Houjou T, et al. The surface of lipid droplets is a phospholipid monolayer with a unique fatty acid composition. J Biol Chem 2002;277: 44507–12.

4. Murphy DJ. The biogenesis and functions of lipid bodies in animals, plants and microorganisms. Prog Lipid Res 2001;40:325–438.

5. Bozza PT, Melo RC, Bandeira-Melo C. Leukocyte lipid bodies regulation and function: contribution to allergy and host defense. Pharmacol Ther 2007;113:30–49.

6. Brasaemle DL, Barber T, Wolins NE, et al. Adipose differentiation-related protein is an ubiquitously expressed lipid storage droplet-associated protein. J Lipid Res 1997;38:2249–63.

7. Heid HW, Moll R, Schwetlick I, et al.. Adipophilin is a specific marker of lipid accumulation in diverse cell types and diseases. Cell Tissue Res 1998;294:309–21.

8. Fujimoto T, Kogo H, Ishiguro K, et al. Caveolin-2 is targeted to lipid droplets, a new "membrane domain" in the cell. J Cell Biol 2001;152:1079–85.

9. Pol A, Luetterforst R, Lindsay M, et al. A caveolin dominant negative mutant associates with lipid bodies and induces intracellular cholesterol imbalance. J Cell Biol 2001;152:1057–70.

10. Liu P, Ying Y, Zhao Y, et al. Chinese hamster ovary K2 cell lipid droplets appear to be metabolic organelles involved in membrane traffic. J Biol Chem 2004;279: 3787–92.

11. Brasaemle DL, Dolios G, Shapiro L, Wang R. Proteomic analysis of proteins associated with lipid droplets of basal and lipolytically stimulated 3T3-1 adipocytes. J Biol Chem 2004;279:46835–42.

12. Wan HC, Melo RC, Jin Z, et al. Roles and origins of leukocyte lipid bodies: proteomic and ultrastructural studies. FASEB J 2007;21:167–78.

13. Dvorak AM, Weller PF, Harvey VS, et al. Ultrastructural localization of prostaglandin endoperoxide synthase (cyclooxygenase) to isolated, purified fractions of guinea pig peritoneal macrophage and line 10 hepatocarcinoma cell lipid bodies. Int Arch Allergy Immunol 1993;101:136–42.

14. Bozza PT, Yu W, Penrose JF, et al. Eosinophil lipid bodies: specific, inducible intracellular sites for enhanced eicosanoid formation. J Exp Med 1997;186: 909–20.

15. Yu W, Bozza PT, Tzizik DM, et al. Co-compartmentalization of MAP kinases and cytosolic phospholipase A2 at cytoplasmic arachidonate-rich lipid bodies. Am J Pathol 1998;152:759–69.

16. Yu W, Cassara J, Weller PF. Phosphatidylinositide 3-kinase localizes to cytoplasmic lipid bodies in human polymorphonuclear leukocytes and other myeloid-derived cells. Blood 2000;95:1078–85.

17. Chen JS, Greenberg AS, Wang SM. Oleic acid-induced PKC isozyme translocation in RAW 264.7 macrophages. J Cell Biochem 2002;86:784–91.

18. Dvorak AM, Dvorak HF, Peters SP, et al. Lipid bodies: cytoplasmic organelles important to arachidonate metabolism in macrophages and mast cells. J Immunol 1983;131:2965–76.

19. Cao Y, Pearman AT, Zimmerman GA, et al. Intracellular unesterified arachidonic acid signals apoptosis. Proc Natl Acad Sci U S A 2000;97:11280–5.

20. Cao Y, Dave KB, Doan TP, et al. Fatty acid CoA ligase 4 is up-regulated in colon adenocarcinoma. Cancer Res 2001;61:8429–34.

21. Sano H, Kawahito Y, Wilder RL, et al. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. Cancer Res 1995;55:3785–9.

22. Eberhart CE, Coffey RJ, Radhika A, et al. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994;107:1183–8.

23. Kargman SL, O'Neill GP, Vickers PJ, et al. Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. Cancer Res 1995;55:2556–9.

24. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000;69:145–82.

25. Pugh S,Thomas GA. Patients with adenomatous polyps and carcinomas have increased colonic mucosal prostaglandin E_2. Gut 1994;35:675–8.

26. Oka M, Inaba A, Uchiyama T, et al. Prostaglandin E_2 levels and lymphocyte subsets in portal venous drainage of colorectal cancers. Am J Surg 1994;167:264–7.

27. Ulrich CM, Bigler J, Potter JD. Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. Nat Rev Cancer 2006;6: 130–40.

28. Cha YI, DuBois RN. NSAIDs and cancer prevention: targets downstream of COX-2. Annu Rev Med 2007;58: 239–52.

29. Bozza PT, Payne JL, Morham SG, et al. Leukocyte lipid body formation and eicosanoid generation: cyclooxygenase-independent inhibition by aspirin. Proc Natl Acad Sci U S A 1996;93:11091–6.

30. Pacheco P, Bozza FA, Gomes RN, et al. Lipopolysaccharide-induced leukocyte lipid body formation *in vivo*: innate immunity elicited intracellular Loci involved in eicosanoid metabolism. J Immunol 2002; 169:6498–506.

31. Bandeira-Melo C, Phoofolo M, Weller PF. Extranuclear lipid bodies, elicited by CCR3-mediated signaling pathways, are the sites of chemokine-enhanced leukotriene C4 production in eosinophils and basophils. J Biol Chem 2001;276:22779–87.

32. D'Avila H, Melo RC, Parreira GG, et al. Mycobacterium bovis bacillus Calmette-Guerin induces TLR2-mediated formation of lipid bodies: intracellular domains for eicosanoid synthesis *in vivo*. J Immunol 2006;176:3087–97.

33. Serrano M, Lin AW, McCurrach ME, et al. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 1997;88:593–602.

34. Angermuller S, Fahimi HD. Imidazole-buffered osmium tetroxide: an excellent stain for visualization of lipids in transmission electron microscopy. Histochem J 1982;14:823–35.

35. Samuelsson B, Morgenstern R, Jakobsson PJ. Membrane prostaglandin e synthase-1: a novel therapeutic target. Pharmacol Rev 2007;59:207–24.
36. Cao Y, Prescott SM. Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. J Cell Physiol 2002; 190:279–86.
37. Bozza PT, Pacheco P, Yu W, et al. NS-398: cyclooxygenase-2 independent inhibition of leukocyte priming for lipid body formation and enhanced leukotriene generation. Prostaglandins Leukot Essent Fatty Acids 2002;67:237–44.
38. Rashid A, Pizer ES, Moga M, et al. Elevated expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. Am J Pathol 1997;150: 201–8.
39. Visca P, Alo PL, Del Nonno F, et al. Immunohistochemical expression of fatty acid synthase, apoptotic-regulating genes, proliferating factors, and ras protein product in colorectal adenomas, carcinomas, and adjacent nonneoplastic mucosa. Clin Cancer Res 1999; 5:4111–8.
40. Kuhajda FP, Pizer ES, Li JN, et al. Synthesis and antitumor activity of an inhibitor of fatty acid synthase. Proc Natl Acad Sci U S A 2000;97:3450–4.
41. Zhou W, Simpson PJ, McFadden JM, et al. Fatty acid synthase inhibition triggers apoptosis during S phase in human cancer cells. Cancer Res 2003;63:7330–7.
42. De Schrijver E, Brusselmans K, Heyns W, et al. RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells. Cancer Res 2003;63:3799–804.
43. Schmid B, Rippmann JF, Tadayyon M, et al. Inhibition of fatty acid synthase prevents preadipocyte differentiation. Biochem Biophys Res Commun 2005;328:1073–82.
44. Sheng H, Shao J, Dixon DA, et al. Transforming growth factor-β1 enhances Ha-ras-induced expression of cyclooxygenase-2 in intestinal epithelial cells via stabilization of mRNA. J Biol Chem 2000;275:6628–35.
45. Jess T, Gamborg M, Matzen P, et al. Increased risk of intestinal cancer in Crohn's disease: a meta-analysis of population-based cohort studies. Am J Gastroenterol 2005;100:2724–9.
46. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–7.
47. Beil WJ, Weller PF, Peppercorn MA, et al. Ultrastructural immunogold localization of subcellular sites of TNF-α in colonic Crohn's disease. J Leukoc Biol 1995;58:284–98.
48. Brasaemle DL. The perilipin family of structural lipid droplet proteins: Stabilization of lipid droplets and control of lipolysis. J Lipid Res 2007;48:2547–59.
49. Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995;83:493–501.
50. Sheng H, Shao J, Morrow JD, et al. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res 1998;58:362–6.
51. Sheng H, Shao J, Washington MK, et al. Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. J Biol Chem 2001;276:18075–81.
52. Cohen AW, Razani B, Schubert W, et al. Role of caveolin-1 in the modulation of lipolysis and lipid droplet formation. Diabetes 2004;53:1261–70.
53. Yao M, Huang Y, Shioi K, et al. Expression of adipose differentiation-related protein: a predictor of cancer-specific survival in clear cell renal carcinoma. Clin Cancer Res 2007;13:152–60.
54. Yao M, Tabuchi H, Nagashima Y, et al. Gene expression analysis of renal carcinoma: adipose differentiation-related protein as a potential diagnostic and prognostic biomarker for clear-cell renal carcinoma. J Pathol 2005;205:377–87.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/68/6/1732/2602354/1732.pdf by guest on 30 September 2022