Cytoplasmic Phospholipase A2 Deletion Enhances Colon Tumorigenesis

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Abstract
Cellular pools of free arachidonic acid are tightly controlled through enzymatic release of the fatty acid and subsequent utilization by downstream enzymes including the cyclooxygenases. Arachidonic acid cleavage from membrane phospholipids is accomplished by the actions of phospholipase A2 (PLA2). Upon release, free arachidonic acid provides substrate for the synthesis of eicosanoids. However, under certain conditions, arachidonic acid may participate in ceramide-mediated apoptosis. Disruption of arachidonic acid homeostasis can shift the balance of cell turnover in favor of tumorigenesis, via overproduction of tumor-promoting eicosanoids or alternatively by limiting proapoptotic signals. In the following study, we evaluated the influence of genetic deletion of a key intracellular phospholipase, cytoplasmic PLA2 (cPLA2), on azoxymethane-induced colon tumorigenesis. Heterozygous and null mice, upon treatment with the organotropic colon carcinogen, azoxymethane, developed a significant increase in colon tumor multiplicity (7.2-fold and 5.5-fold, respectively) relative to their wild-type littermates. This enhanced tumor sensitivity may be explained, in part, by the attenuated levels of apoptosis observed by terminal deoxynucleotidyl transferase–mediated nick end labeling staining within the colonic epithelium of heterozygous and null mice (850% of wild type). The lower frequency of apoptotic cells corresponded with reduced ceramide levels and increased tumorigenesis resulting from cPLA2 deletion occurred despite a significant reduction in prostaglandin E2 production, even in cyclooxygenase-2–overexpressing tumors. These data contribute new information that supports a fundamental role of cPLA2 in the control of arachidonic acid homeostasis and cell turnover. Our findings indicate that the proapoptotic role of cPLA2 in the colon may supercede its contribution to eicosanoid production in tumor development. (Cancer Res 2005; 65(7): 2636–43)

Introduction
Cellular fatty acid metabolism and downstream signaling events play a critical role in colon carcinogenesis. For example, cyclooxygenase-2 (COX-2) levels are elevated in colorectal cancers (1, 2), resulting in increased production of prostaglandins (PG) that contribute to proliferation, angiogenesis, and inflammation (3–8). Modulation of arachidonic acid metabolism with COX inhibitors can provide an effective strategy for suppressing colon cancer risk or preventing recurrence of polyps (9–13). Regression of established adenomatous polyps in patients with familial adenomatous polyposis has been shown in case reports and randomized controlled studies (14, 15); more recently, COX-2–selective inhibitors have shown chemopreventive efficacy in animal studies as well as in clinical trials (10, 16).

Upstream of the COXs, phospholipase A2 (PLA2) play an important role in generating bioactive lipid mediators, including prostaglandin E2 (PE2 refs. 17, 18). PLA2s represent a family of enzymes that hydrolyze glycerophospholipids at the sn-2 position, liberating free fatty acids, including arachidonic acid (6). The secretory PLA2s (sPLA2) are low molecular mass (14 kDa) enzymes that require mmol/L Ca2+ for enzymatic activity and are induced by proinflammatory cytokines (7, 19). Cytoplasmic phospholipase A2 (cPLA2) are ubiquitously distributed 85 kDa enzymes that preferentially cleave arachidonic acid (20, 21). There are at least three cPLA2 isoforms, called cPLA2α (or IVA), cPLA2β (or IVC), and cPLA2γ (or IVC; ref. 22). cPLA2 is the most well characterized isoform and the only one that is widely expressed in the colon (20). cPLA2 is activated both by Ca2+ binding and mitogen-activated protein kinase phosphorylation (23, 24). In resting cells, cPLA2 is present in the cytosol and upon stimulation by Ca2+ binding, translocates to membranes where it is positioned adjacent to the COXs within the nuclear envelope and endoplasmic reticular membrane, thus serving as a major source of arachidonic acid substrate for PG production (24).

Independent of its role in regulating eicosanoid production, cPLA2 has also been implicated in regulating apoptosis in response to extracellular signaling molecules, such as proinflammatory cytokines (25). We recently reported that tumor necrosis factor (TNF)-α–induced apoptosis was attenuated in cultured mouse colonocytes in which cPLA2 expression was reduced by treatment with antisense oligos (26). Several other studies have shown that when TNF-α induces cPLA2, arachidonic acid is released, triggering activation of sphingomyelinases to generate ceramide, which in turn signals G0–G1 cell cycle arrest and apoptosis (24, 27–30). The direct dose-dependent stimulation of sphingomyelinate activity by arachidonic acid has been previously established in a cell-free system (28). Furthermore, Cao et al. (29) have suggested that accumulation of arachidonic acid may in turn initiate a set of changes that lead to apoptosis and ultimately to suppression of tumor cell growth. It has been shown that arachidonic acid release occurs concomitantly with activation of caspases and DNA

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fragmentation (26, 31), thus leading to cell shrinkage, compaction, and phagocytosis-facilitated membrane breakdown (20, 21, 25, 32). In fact, it has been proposed that the tumor-suppressive properties of nonsteroidal anti-inflammatory drugs may not be entirely related to reduced PG production, but may actually result from elevated levels of arachidonic acid associated with pharmacologic inhibition of the COXs (33–35). This mechanism is supported by the effects of nonsteroidal anti-inflammatory drug treatment of colon tumor cells (HCT116 and SW480), in which elevated levels of arachidonic acid were found to stimulate conversion of sphingomyelin to ceramide (35). Consistent with this reasoning, Tsuji and Dubois (36) reported that overexpression of COX-2 in rat intestinal epithelial cells inhibited apoptosis by reducing the pools of unesterified arachidonic acid available to signal apoptosis.

In the following study, we have focused on the role of cPLA2 in colon carcinogenesis. We report that the absence or reduction of cPLA2 dramatically enhances the tumorigenic effects of the organotrophic colon carcinogen, azoxymethane. We further evaluated the mechanism of this enhanced tumorigenic response and found significantly reduced numbers of apoptotic cells within the colonic epithelium of cPLA2-deficient mice, an effect that may reflect reduced ceramide production in this tissue. These findings raise fundamental questions regarding the role of cPLA2 in colonocyte turnover and provide a potential mechanism whereby cPLA2 directly impacts the progression of colon cancer.

Materials and Methods

Characterization of cytoplasmic phospholipase A2 mice. Generation of the cPLA2 null line has been previously described (37). Briefly, targeted inactivation of the cPLA2 (Pla2g4) gene was achieved by introduction of a neomycin resistance gene into exon 10, beginning with codon 187. The original knockout line was generated on a C57Bl/6j-129/Sv (B6-129) chimeric background. Mice were subsequently backcrossed >10 generations onto a pure BALB/c background. BALB/c cPLA2 heterozygous mice were then crossed to generate mice of all three genotypes for the following study. Wild-type mice used in this study were littermates of the heterozygous and homozygous null mice. Mice were randomized and placed into the Wild-type mice used in this study were littermates of the heterozygous and then crossed to generate mice of all three genotypes for the following study.

Determination of prostaglandin E2 levels. Colon tissues were homogenized in 1 mL final volume containing 12.5 μL 2× TaqMan Universal Master Mix (Applied Biosystems), 1.25 μL 20× Target Assay Mix (Applied Biosystems), and 2.5 μL cDNA. Standard curves were generated for relative comparisons of targets by assessing 5-fold serial dilutions of control cDNA for each gene. Results were normalized to HPRT and reported as fold changes relative to the gene of interest for the cPLA2 wild-type saline-treated mice.

Measurement of apoptosis. Levels of apoptosis in distal colon tissue from saline-treated mice were determined by the terminal deoxynucleotidyl transferase–mediated nick end labeling (TUNEL) method as previously described (44). Five micron frozen tissue sections were stained using the ApopTag peroxidase method (Chemicon International, Temecula, CA). Hepatocytic guanine phosphoribosyltransferase (HPRT) was used as a loading control. Primers and probes for all genes (with the exception of Pla2g4f) were designed to span intron junctions in these genes (Assays-on-Demand, Applied Biosystems). The primers and probe for cPLA2 were designed using Primer Express software to specifically span the region that was disrupted by the neomycin gene within the cPLA2-null allele. qRT-PCR was done in a 25 μL final volume containing 12.5 μL 2× TaqMan Universal Master Mix (Applied Biosystems), 1.25 μL 20× Target Assay Mix (Applied Biosystems), and 2.5 μL cDNA. Standard curves were generated for relative comparisons of targets by assessing 5-fold serial dilutions of control cDNA for each gene. Results were normalized to HPRT and reported as fold changes relative to the gene of interest for the cPLA2 wild-type saline-treated mice.

Determination of ceramide levels. Colon tissues were homogenized in PBS containing protease inhibitors (1 mol/L DTT and 100 mM/L phenylmethylsulfonyl fluoride) before lipid extraction by the method of Bligh and Dyer (45). Levels of ceramide in colon tissues were quantified using the diacylglycerol (DAG) kinase assay as previously described (46). Total ceramide levels were normalized to the actual wet tissue weight used in the assay and reported as picomoles of ceramide per milligram of tissue.

Determination of prostaglandin E2 levels. Normal colon and tumor tissues were homogenized in ice-cold, 0.1 mol/L phosphate buffer containing 1 mol/L EDTA and 10 μmol/L indomethacin (Sigma). The homogenate was

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mixed with two volumes of ethanol, incubated at 4°C for 5 minutes, and centrifuged at 3,000 × g for 10 minutes. The ethanol was evaporated and the supernatant was acidified (pH 4) by addition of acetate buffer. The sample was then applied to a methanol-activated C-18 reverse phase column (Cayman Chemicals, Ann Arbor, MI). The column was washed with water and hexanes before elution of the PGE2 with ethyl acetate containing 1% methanol, and samples were evaporated to dryness. PGE2 levels were determined by a competitive ELISA assay according to manufacturer’s instructions (Prostaglandin E2 EIA-Monoclonal kit, Cayman Chemicals). Results were normalized to the actual wet tissue weight used in the assay.

Western blot analysis. Protein was extracted from colon tissues using TRIzol reagent. Purified protein was quantified by the Bio-Rad DC Protein Assay (Bio-Rad Laboratories, Hercules, CA). Thirty-five micrograms of protein from each mouse colon were separated on a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane. The membrane was probed with primary antibodies against COX-2 (polyclonal rabbit anti-muCOX-2, Cayman Chemicals, 1:1,000 dilution) and β-actin (monoclonal mouse anti-β-actin antibody, Sigma, 1:3,000). For COX-2, the membrane was incubated with goat anti-mouse horseradish peroxidase–conjugated secondary antibody (1:1,000 dilution, Upstate Biotechnology, Inc., Charlottesville, VA). For β-actin, the membrane was incubated in donkey anti-rabbit horseradish peroxidase–conjugated secondary antibody (1:1,000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA). The enhanced chemiluminescence Western blot analysis system (Santa Cruz Biotechnology) was used for detection.

Statistical analysis. A two-tailed, unpaired Student’s t test was done to determine statistical significance by the probability of difference between the means. P < 0.05 is considered statistically significant. Values in the graphs are expressed as means ± SE.

Results

Tumor multiplicity, incidence, and load. To evaluate the influence of cPLA2 status on colon cancer, mice of each cPLA2 genotype (+/+), (+/-), and (-/-) were examined for colon tumor formation 24 weeks after the last of six weekly injections with azoxymethane (or saline). As shown in Fig. 1A, there was a significant increase in colon tumor multiplicity in cPLA2 heterozygous (6.5 ± 2.1 tumors/mouse, P = 0.004) and null (5.0 ± 0.87 tumors/mouse, P = 0.0006) mice compared with their wild-type littermates (0.9 ± 0.43 tumors/mouse). There was also a significant increase in tumor load (volume) in cPLA2 heterozygous (106.8 mm³ ± 44.6, P = 0.02) and null (68.3 mm³ ± 9.7, P = 0.0003) mice compared with the wild-type controls (14.3 mm³ ± 7.2; Fig. 1B). Whereas the trend in average tumor multiplicity and tumor load was higher in heterozygotes compared with the null animals, the differences were not statistically significant (P = 0.44 and P = 0.28, respectively). cPLA2 status also affected colon tumor incidence. Whereas only 40% of wild-type mice receiving azoxymethane developed colon tumors, tumor incidence was elevated in the azoxymethane-treated heterozygous and null mice (83% and 100%, respectively).

All gastrointestinal tumors were restricted to the distal colon of azoxymethane-treated mice, consistent with the organotropism of this carcinogen (40). Representative photomicrographs of the distal colon from the various treatment groups are shown in Fig. 1C. Histologically, tumors displayed loss of goblet cells, anisokaryosis, and elongation of the nuclei with occasional pseudostratification (Fig. 1C). Azoxymethane-treated cPLA2 wild-type mice developed lesions that ranged from dysplastic aberrant crypt foci and adenomas to a single superficial adenocarcinoma in situ. In the heterozygous and null animals, a more pronounced effect was observed. As shown in Fig. 1, multiple adenomas and in situ adenocarcinomas, including an invasive adenocarcinoma, were produced.

The effect of cPLA2 deletion on tumorigenesis was not limited to the colon. Interestingly, a total of five adenocarcinomas were found within the lung parenchyma of two cPLA2 null mice treated with azoxymethane (Fig. 2). Whereas BALB/c mice are known to develop azoxymethane (AOM) or saline, colons were harvested and examined macroscopically for evidence of tumors. Colon tumor multiplicity (average number of tumors per mouse; A) and tumor load (total tumor volume in mm³; B) was compared in mice from all three genotypes. Columns, mean number or volume of tumors per mouse; bars, SE. *Significant difference (P < 0.05) in the number or volume of tumors per mouse compared with wild-type mice in the same treatment group. N.D., not detected. C, photomicrographs of distal colon visualized under a dissecting microscope at ×7 magnification (right). H&E-stained sections of colon tumors (>200; left). Superficial exophytic adenoma from a cPLA2 wild-type mouse (top left); superficial exophytic adenoma from a cPLA2 null mouse (middle left); large exophytic, infiltrative adenocarcinoma from a cPLA2 heterozygous mouse, displaying loss of goblet cells and marked anisokaryosis with several large, deeply basophilic to vesicular nuclei and several moderately elongated, stratified nuclei (bottom left). Note the tumor cell invasion into the muscularis mucosa and the infiltration of lymphocytes into the submucosa.
lung tumors throughout their life span (47, 48), no lung tumors were observed in the 36-week-old wild-type or heterozygous mice receiving azoxymethane, or any of the mice receiving saline. A combination of histopathologic examination and positive staining with an antibody against thyroid transcription factor 1 revealed that these lung lesions were consistent with primary bronchial lung tumors rather than colon metastases (data not shown).

**Phospholipase A2 expression.** cPLA2 status was confirmed by expression analysis using qRT-PCR. As shown in Fig. 3A, cPLA2 mRNA levels in the normal colon were correlated with the cPLA2 genotype. In the small intestine, however, cPLA2 mRNA was barely detectable in the heterozygous and wild-type mice (Fig. 3A). Because there may be some functional redundancy between PLA2 isofoms (19), we also evaluated the expression levels of sPLA2 IIa, sPLA2 V, and sPLA2 X, each of which have been shown to release arachidonic acid in the colon (19). As shown in Fig. 3B-D, there were no significant differences in the expression levels of sPLA2 IIa, sPLA2 V, or sPLA2 X among the cPLA2 genotypes. In the case of sPLA2 IIa, however, there was a marked elevation (up to 100-fold) in tumors compared with untreated and azoxymethane-treated adjacent normal tissue.

**Levels of apoptosis.** In various cell culture systems, cPLA2 activity has been directly associated with apoptosis (25, 49, 50). To determine whether such an association occurs in vivo, we evaluated the frequency of apoptotic cells within the colonic epithelium of each genotype, using the TUNEL assay. As shown in Fig. 4A, the number of apoptotic cells per field was significantly reduced in the heterozygous and null groups (2.62 ± 0.52, P < 0.05 and 2.56 ± 0.38, P < 0.05, respectively) compared with the wild type (4.74 ± 0.54). Representative photomicrographs depicting TUNEL-stained colonocytes are shown in Fig. 4B. Because arachidonic acid release has been associated with ceramide production, we also measured ceramide levels in the colon using the DAG kinase assay. As shown in Fig. 4C, ceramide levels were reduced in parallel with the frequency of apoptotic cells. In azoxymethane-treated mice, the levels of ceramide were reduced by 31% and 54% relative to wild type, in heterozygous and null groups, respectively.

**Cyclooxygenase-2 and prostaglandin E2 levels.** Because COX-2 is a key enzyme in the production of prostaglandins (51), COX-2 expression was measured in tumors and normal tissue using both qRT-PCR and Western blot analysis. As shown in Fig. 5A-B, COX-2 expression was markedly elevated (~7-fold) in tumor tissue, an effect that was independent of cPLA2 genotype. PGE2 levels were also determined using a competitive ELISA-based assay. As shown

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**Figure 2.** Total number of primary lung tumors and lung tumor pathology in cPLA2+/− mice. A, total primary lung tumors were compared in mice of all three genotypes. All five lesions observed were restricted to two azoxymethane-treated cPLA2−/− mice. Columns, total number of tumors; bars, SE. *Significant difference (P < 0.05) in the number of tumors compared with wild-type mice in the same treatment group. B, H&E-stained section of a representative lung adenocarcinoma with masses and ribbons of cuboidal to columnar cells and enlarged hyperchromatic nuclei (>200).

**Figure 3.** Expression analysis of a panel of PLA2s in cPLA2+/+, +/-, and −/− mice. RNA was isolated from the colon and small intestine of mice from each genotype 24 weeks after treatment with azoxymethane (or saline). qRT-PCR was done as described in Materials and Methods. A, relative expression of cPLA2 in the colon and small intestine from saline-treated normal, azoxymethane-treated adjacent normal, and azoxymethane-treated tumor tissues. B-D, relative expression of sPLA2 IIa, sPLA2 V, and sPLA2 X in colon tissues from saline- and azoxymethane-treated mice. Columns, relative expression compared with wild-type saline-treated normal colon; bars, SE. Data are normalized to expression of HPRT. *Significant difference (P < 0.05) in expression compared with wild-type mice.
in Fig. 5C, PGE2 levels were significantly elevated (~15-fold, 
P <0.05) within colon tumors harvested from wild-type mice. However, the fold increase in PGE2 was significantly attenuated in the cPLA2 heterozygous and null mice, providing indirect evidence that eicosanoid production may be limited by the diminished availability of arachidonic acid substrate within tumors. PGE2 levels in normal tissues, however, were largely unaffected by cPLA2 genotype.

Discussion

The growth-regulatory role of cPLA2 within the colon epithelium is complex. On the one hand, the enzymatic actions of cPLA2 provide arachidonic acid for the synthesis of eicosanoids, many of which can promote tumor cell growth, proliferation, and angiogenesis. However, there is considerable evidence that intracellular pools of arachidonic acid released by cPLA2 play a key role in ceramide-mediated signal transduction that culminates in apoptosis (24, 25, 28, 35). During tumorigenesis, arachidonic acid homeostasis is often altered, and the tightly controlled balance between apoptotic and proliferative signals is potentially disrupted. In one such scenario, COX-2 overexpression may deplete pools of free arachidonic acid, thereby limiting apoptotic signaling mediated by the downstream actions of the ceramide pathway (25, 26, 29, 35). In the following study, we created a colonic environment in which the generation of arachidonic acid is limited by genetic deletion of the cPLA2 gene. Under the stress of repeated carcinogen exposures, this model predicts that an important tumor surveillance mechanism may be compromised in the cPLA2 null animals, thereby facilitating the initiation and promotion of colon tumors.

Our results clearly show that the absence (or reduction) of cPLA2 enhances azoxymethane-induced colon carcinogenesis. Homozygous and heterozygous deletion of cPLA2 resulted in a significant increase in tumor multiplicity and tumor load, providing indirect evidence that disruption of arachidonic acid availability may have profound effects on both tumor initiation and promotion. These data further support a role for cPLA2 in tumor progression, as shown by the increased frequency of colon tumors displaying more advanced dysplasia when cPLA2 levels were reduced or absent. Underscoring this effect was the formation of a large, exophytic, invasive adenocarcinoma harvested from the colon of a cPLA2 null animal. This finding was surprising because mice are largely resistant to azoxymethane-induced tumor cell infiltration (43). We further conclude that cPLA2 heterozygotes display a haploinsufficiency phenotype because deletion of only one allele resulted in a tumorigenic response that was at least comparable with the effect observed in the homozygous null mice. The enhanced tumorigenic response in the heterozygotes suggests that diminished pools of arachidonic acid are sufficient to maintain the production of proliferative eicosanoids (e.g., PGE2), yet are inadequate in affording protection to the colonic epithelium via activation of the sphingomyelinase-ceramide pathway.

In two earlier studies, the influence of cPLA2 on intestinal tumorigenesis was examined in mice harboring germ line mutations in both cPLA2 and the Apc tumor suppressor gene (38, 52). In contrast to our findings, these studies reported that the deletion of cPLA2 protected against tumor formation in the small intestine of Apc<sup>min</sup> and Apc<sup>1716</sup> mice (38, 52). The impact of cPLA2 deletion in the colons of Apc mutant mice is somewhat difficult to assess, however, because the effects of tumor multiplicity did not reach statistical significance in either study. However, the trend in tumor numbers was consistent with our findings using azoxymethane as an initiating agent, suggesting that Apc status alone is not responsible for the divergent effects of cPLA2 gene deletion in the small and large intestine. This conclusion is further strengthened by the observation that Apc mutations also occur to some extent in colon tumors harvested from azoxymethane-treated mice (53–55). The tissue-specific phenotypes conferred by genetic deletion of cPLA2 should also be considered within the context of its basal expression. cPLA2 is normally expressed at very low levels within the small intestinal mucosa, and thus its physiologic role in regulating apoptosis in this organ may be minimal. On the other hand, colonocytes that normally express high endogenous levels of disease.
cPLA2 may be particularly sensitive to the apoptotic signals derived from arachidonic acid release.

The effects of cPLA2 on intestinal tumorigenesis extends to other organs, including the lung. In a recent report (56), cPLA2 deletion afforded partial protection against urethane-induced lung cancer. These findings contrast with the results in which cPLA2 deletion may have accelerated spontaneous lung tumorigenesis. It is difficult to directly compare the results of these two studies, however, because we found only a limited number of lung tumors (total of five) that were restricted to 17% of mice in the cPLA2-null azoxymethane treatment group. A consideration of genetic background that was used in the earlier study further complicates a direct comparison. BALB/c mice were used in our study, whereas the urethane study was conducted on a mixed genetic background consisting of lung tumor sensitive (129/Sv) and resistant (C57Bl/6) backgrounds (56). Furthermore, Meyer et al. (56) argued that impaired PGE2 production in the lung underlies the phenotype observed in the small intestine (38, 52). Our data, however, indicate that reduced PGE2 production in the colon does not protect against tumorigenesis. Kisley et al. (57) recently reported that in BALB/c mice, the tumorigenic effects of cPLA2 deletion resulted in significantly reduced PGE2 levels at a level that is sufficient to attenuate apoptotic signaling via the inhibition of either FACL4 or COX-2, thereby diminishing pools of free arachidonic acid and eliminating a potential stimulus for apoptosis. In addition, the inhibition of either FACL4 or COX-2 in HT-29 colon cancer cells replenished arachidonic acid levels, and as a consequence, restored apoptosis (25). Our results provide additional evidence that disruption in arachidonic acid balance through the deletion of cPLA2 limits the extent of apoptosis in the colon. To gain insight into potential mechanisms by which cPLA2 may elicit this effect, we evaluated ceramide levels directly within the colon. The rationale for this analysis is based on previous studies that have shown that arachidonic acid released by cPLA2 activates sphingomyelinase, which in turn converts sphingomyelin to ceramide, a bioactive lipid molecule that is linked to cell death pathways (28, 29, 49, 50, 58). We found that deletion of cPLA2 resulted in significantly reduced ceramide levels, suggesting that free pools of arachidonic acid are sufficiently limited to attenuate apoptotic signaling via the sphingomyelinase-ceramide pathway. Our ceramide data, thus, provide a potential mechanism whereby the absence of cPLA2 may enable tumorigenic cells to evade apoptotic death.

In summary, our study establishes that heterozygous and homozygous deletion of cPLA2 markedly enhance tumorigenesis and a panel of lung carcinogens (including urethane) were not suppressed by co-administration of several COX-2 inhibitors (Celebrex, Sulindac), although PGE2 production was reduced significantly, reinforcing our conclusion that reduced PGE2 levels are not necessarily correlated with tumor suppression.

Our results raise additional questions regarding the source of arachidonic acid for eicosanoid production within the colon. A number of studies suggest functional redundancy between the secretory phospholipases and cPLA2 because both classes of enzymes are capable of releasing arachidonic acid (58–61). However, there is mounting evidence that sPLA2s do not compensate for the down-regulation or deletion of cPLA2 (26, 51, 59), and in fact may participate in functions other than providing substrate for eicosanoid synthesis (62–64). Our results in tumor tissue are consistent with the absence of functional redundancy. Despite the striking elevation (up to 100-fold) in the levels of sPLA2s in colon tumors, PGE2 production was correlated with cPLA2 status. Furthermore, in cPLA2-null mice, we saw no compensatory increase in the expression of the predominant sPLA2 in the colon (sPLA2A, sPLA2V, and sPLA2X; refs. 19, 51, 52). Interestingly, in normal tissue, PGE2 production was not altered by deletion of cPLA2, suggesting that low basal levels of PGE2 can be sustained from arachidonic acid generated by a source other than cPLA2. Taken together, our findings are consistent with functional coupling between sPLA2 with COX-1 in normal tissue and cPLA2 with COX-2 in tumor tissue proposed previously by Reddy and Herschman (65).

The influence of cPLA2 is clearly determined by its role in providing arachidonic acid substrate for eicosanoid synthesis. However, there is accumulating evidence that arachidonic acid released by cPLA2 contributes to apoptosis (reviewed in refs. 58, 66), providing a pathway through which cPLA2 may suppress colon tumorigenesis (28, 29, 50). Direct evidence for the involvement of cPLA2 in apoptosis was recently shown in vascular smooth muscle cells (67). Activation of cPLA2 with phospholipase activator peptide, resulted in increased arachidonic acid release with a concomitant increase in apoptosis, an effect that was reversed by treatment with a chemical inhibitor of cPLA2 (67).

Further evidence for the importance of arachidonic acid is shown by Cao et al. (25), in which apoptosis was blocked by overexpression of fatty acid-CoA ligase-4 (FACL4) and COX-2, thereby diminishing pools of free arachidonic acid and eliminating a potential stimulus for apoptosis. In addition, the inhibition of either FACL4 or COX-2 in HT-29 colon cancer cells replenished arachidonic acid levels, and as a consequence, restored apoptosis (25).
in the colon. The colonic epithelium is an organ that is under continuous regenerative stimulus that must maintain a critical balance between the tumor-suppressive and tumor-promoting properties of arachidonic acid and its downstream metabolites. However, under the toxicologic stress of carcinogen exposure, genetic disruption of cPLA2 may shift the balance in the colon toward cell growth and survival, an outcome that may ultimately favor tumorigenesis. Homozygous deletion of cPLA2 is predicted to limit the intracellular release of arachidonic acid, an effect that suppresses the production of prosurvival eicosanoids while disrupting proapoptotic signaling through the sphingomyelinase-ceramide pathway. In heterozygous animals, however, the reduced cPLA2 levels produce a tumor phenotype and frequency of apoptosis that is compatible with that of null mice, suggesting that the arachidonic acid supply, although limited, is adequate to maintain elevated PGE2 levels in tumor tissue at the expense of apoptosis. This pattern of enhanced tumorigenesis in mice with cPLA2 deletions indicates that the proapoptotic role of cPLA2 in the colon may predominate over its contribution to eicosanoid biosynthesis.

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