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Overexpression of the Nonpancreatic Secretory Group II PLA₂ Messenger RNA and Protein in Colorectal Adenomas from Familial Adenomatous Polyposis Patients¹

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ABSTRACT

The synovial fluid or group II secretory phospholipase A₂ (sPLA₂) has been implicated in various inflammatory processes and has been shown to release arachidonic acid for prostaglandin biosynthesis. In human colorectal cancer, both arachidonic acid and eicosanoid levels are elevated. Recently, sPLA₂ has been identified as a candidate gene that modifies the *Apc* gene in the *Min* mouse, a murine model for familial adenomatous polyposis (FAP). Loss of sPLA₂ gene function results in susceptibility to the *Min* phenotype and the formation of multiple intestinal polyps, whereas mice expressing an active sPLA₂ gene are resistant to polyp formation. Therefore, there are two potentially contrasting roles for sPLA₂ in colon cancer; one is protection against polyp formation, and the other, the release of arachidonic acid for prostaglandin production and subsequent tumor promotion. To investigate these contrasting dual roles of sPLA₂, we have examined the expression and sequence of the sPLA₂ mRNA in normal mucosa and duodenal and colorectal polyps from FAP patients. In 11 of 14 patients, there was a significant increase in sPLA₂ mRNA levels in the adenoma over the normal tissue. In some cases, there was over 100-fold increase in mRNA levels in the adenoma compared with normal tissue. Analysis of multiple adenomatous polyps from individual patients revealed that not all polyps contained elevated levels of sPLA₂ mRNA. Immunoblot analysis also showed that sPLA₂ protein expression was elevated in adenoma over normal tissue in five of six FAP patients analyzed. Furthermore, sequence analysis of sPLA₂ mRNA present in these samples did not reveal mutations in the coding region. The implications of the up-regulation of sPLA₂ in FAP is not clear, but unlike the *Min* mouse model, it does not seem to have a significant effect on polyp formation. In contrast, the high level of sPLA₂ expression is more likely contributing to the elevated levels of arachidonic acid found in colorectal cancer and, in conjunction with the elevated expression of cyclooxygenase-2, could be another factor in tumor formation.

INTRODUCTION

sPLA₂³ is a lipolytic enzyme that hydrolyzes the release of the fatty acid from the *sn*-2 position of glycerophospholipids (reviewed in Refs. 1-3). When this fatty acid is arachidonic acid, which is usually esterified at this position in phospholipids, it can lead to the production of the various prostaglandin and leukotriene proinflammatory mediators. sPLA₂ has been shown to be involved in the release of arachidonic acid for prostaglandin production (1, 2, 4), and like Cox-2, sPLA₂ expression is induced by various inflammatory stimuli such as lipopolysaccharide, interleukin 1 β , and tumor necrosis

factor- α (1, 2). Recently, a new role has been proposed for sPLA₂ as a modifier of the *Apc* gene in the *Min* mouse (5). The *Min* mouse, which carries a nonsense mutation in the *Apc* gene, develops multiple intestinal adenomas and is, therefore, a murine model for FAP, an inherited human disorder that predisposes to colorectal adenomas and cancer (6-9). It was found that mouse strains with a disrupted sPLA₂ gene (5, 10) were susceptible to the *Min* phenotype and develop numerous intestinal polyps, whereas mouse strains with an active sPLA₂ enzyme were resistant and developed only a few polyps (5). The mechanism by which sPLA₂ promotes resistance to the *Apc* mutation in the mouse is unclear, but because the *Min* mouse is a model for FAP, one might predict that some FAP patients with a more severe phenotype may also have mutations in the human sPLA₂ gene. Contrary to this is the observation that PLA₂ activity (11) and arachidonic acid levels (12) are increased in human colorectal cancer. In addition, nonsteroidal anti-inflammatory drugs, which inhibit Cox and thus prevent prostaglandin synthesis, can reduce the number and size of colorectal adenomas (13). Finally, recent evidence indicates that increased Cox-2 levels promote colorectal tumorigenesis in the mouse model for FAP (14). Therefore, there are two contrasting activities for sPLA₂ in FAP: a protective role, supposedly by reducing polyp number; and a potentially detrimental role, by contributing to elevated prostaglandin production through Cox-2, thus leading to increased cell proliferation and tumor growth. These apparently contrasting dual roles of sPLA₂ in FAP were investigated by examining the expression and sequence of the sPLA₂ mRNA in normal mucosa and adenomatous polyps from FAP patients. In agreement with recent reports (15-17), we did not find mutations in the sPLA₂ mRNA, but in nearly all cases, there was a substantial increase in sPLA₂ mRNA and protein levels in colorectal adenomas compared with normal tissue.

MATERIALS AND METHODS

FAP patients were identified from an ongoing clinical surveillance program offered to affected polyposis kindreds through the Familial GI Cancer Registry at Mount Sinai Hospital, Toronto, Canada. Patient data and specimen collection were carried out according to a protocol approved by the Ethics Committee, University of Toronto. Samples were obtained from a panel of 25 FAP patients, consisting of histopathologically confirmed normal mucosa and matched colorectal ($n = 16$) or duodenal adenomas ($n = 9$).

Northern blot analysis was performed on total RNA prepared from 12 matched normal mucosa and colorectal adenomatous polyps and from two unmatched colorectal adenomas. RNA was prepared using Trizol (BRL) reagent as described by the manufacturer. Total RNA (8 μ g) was separated on 1% agarose/formaldehyde gels and transferred to nitrocellulose (18). Prehybridization and hybridization were carried out at 42°C in 50% formamide, 5 \times SSPE (1 \times SSPE = 0.15 M NaCl, 0.01 M NaH₂PO₄ · H₂O, 0.001 M Na₂EDTA), 10 \times Denhardt's, 2% SDS, and 100 μ g/ml sheared and denatured herring sperm DNA. The probes used were human cDNAs for sPLA₂ (19), Cox-2 (20), and glyceraldehyde-3-phosphate dehydrogenase (Clontech). The cDNAs were labeled by random priming (Pharmacia), and hybridization was done using a probe concentration of 1-3 \times 10⁶ cpm/ml. The blots were washed in 0.5 \times SSC and 0.5% SDS at 60°C and exposed to film at -70°C. Typically for sPLA₂, signals could be detected within 3-6 h, whereas for Cox-2, the film

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³ The abbreviations used are: sPLA₂, secretory group II phospholipase; Cox, cyclooxygenase; FAP, familial adenomatous polyposis; RT-PCR, reverse transcription-PCR; FP, forward primer; RP, reverse primer; SSCP, single-strand conformation polymorphism.

needed at least a 1-week exposure. Quantitation of hybridization signal was performed on a Molecular Dynamics laser densitometer.

RT-PCR was used to amplify the sPLA₂ mRNA sequence present in matched normal and colorectal adenomatous polyp tissue samples from nine FAP patients. The primers used for amplification were as follows: FP, 5'-CCGAATTCATGAAGACCCTCTACTGTTGGCA-3'; and RP, 5'-CCA-GATCTCAGCAACGAGGGGTGCTCCCTCTG-3' (19). The RT-PCR (Perkin-Elmer) reactions were carried out on approximately 1 µg of total RNA using the following cycling conditions: 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s for 35 cycles. The PCR products were electrophoresed on a 1.5% low-melt agarose gel. The sPLA₂ cDNA fragment was cut out and recovered using a QIAquick gel extraction kit (Qiagen). The recovered PCR products were directly sequenced on both strands using Dye Terminator cycle sequencing (Perkin-Elmer) and analyzed on an ABI 373A automated DNA sequencer.

SSCP analysis was used to screen the sPLA₂ mRNA sequence in matched normal mucosa and duodenal adenomas from nine FAP patients. RNA was extracted and reverse transcribed as described previously (21), and the entire coding region of sPLA₂ was amplified in three overlapping segments. The primers used for the amplification were as follows: segment 1 (200 bp): FP 5'-TGAGAGAGCCACCAAGGAGG-3', RP 5'-CTTCTCTGTCGCAACT-TGATC-3'; segment 2 (230 bp): FP 5'-GGTGAATTCACAGAATGATC-3', RP 5'-TTGCACAGGTGATTCTGCTC-3'; and segment 3 (180 bp): FP 5'-ACCTGTGCAAAACAGGACTC-3', RP 5'-AATTCAGCACTGGGTG-GAAG-3'. Each 20-µl PCR reaction consisted of 100 ng of each primer, 70 µM of each deoxynucleotide triphosphate, 100 ng of template DNA, 0.3 unit of Taq polymerase (Amplitaq Perkin-Elmer), and 0.2 µl of [α -³²P]dCTP (3000 Ci/mMol) in 1× PCR buffer [10 mM Tris (pH 8.3), 50 mM KCl, and 1 mM MgCl₂]. The amplification conditions involved an initial denaturation step at 94°C (4 min), followed by 30 cycles each consisting of 94°C for 1 min, 55–58°C for 1 min, and 72°C for 1 min. Samples were electrophoresed under nonreducing conditions on 6% polyacrylamide gels at 4°C with 10% glycerol for 2.5–3 h at 70 W. Gels were dried and autoradiographed for 6–24 h.

Immunoblot analysis of sPLA₂ protein expression was performed as follows. Normal and tumor tissue were homogenized in PBS containing a protease inhibitor cocktail (Boehringer Mannheim), and protein concentration for each of the samples was then determined using a Coomassie Protein Reagent Assay solution (Pierce). An equal volume of 2× Laemmli SDS sample buffer (22) without reducing agent was added to an aliquot of each sample, mixed, and heated at 95°C for 5 min. The samples (25 µg) were electrophoresed on 18% polyacrylamide SDS gels (Novex) and electrophoretically transferred to 0.05 µm nitrocellulose (23). The sPLA₂ antibody (kindly provided by Natalie Tremblay, Merck Frosst) used for detection of sPLA₂ protein is a monoclonal antibody that recognizes the unreduced form of the enzyme and was used at a dilution of 1:2000. The secondary antibody was a horseradish peroxidase-linked sheep anti-mouse IgG antibody (Amersham Life Sciences) used at a 1:4000 dilution. Immunodetection was performed using enhanced chemiluminescence (NEN Life Science).

RESULTS

Northern blotting and RT-PCR were used to investigate sPLA₂ mRNA expression and sequence in both normal mucosa and adenomatous polyp tissue from FAP patients. Based on the result of the frameshift mutation in the mouse sPLA₂ gene, which produces a very unstable and thus a virtually undetectable mRNA (10), it was thought that the level of sPLA₂ mRNA expression in the human samples could quickly indicate whether there was any mutation in the gene. Analysis of the sPLA₂ mRNA expression by Northern blot (Fig. 1) showed that sPLA₂ mRNA was highly expressed in all individuals, suggesting that it was unlikely that there was a mutation in the sPLA₂ gene. This was confirmed by sequencing and SSCP analysis of the RT-PCR product (see below) and is in agreement with a number of recent reports that did not find any mutation of the sPLA₂ gene in either sporadic (15) or FAP (16, 17) colorectal tumors. However, of interest was the increased level of expression of the sPLA₂ mRNA in the adenomatous polyp samples over the normal tissue. In 11 of 14 cases, there was a significant increase in sPLA₂ mRNA expression in the tumor sample.

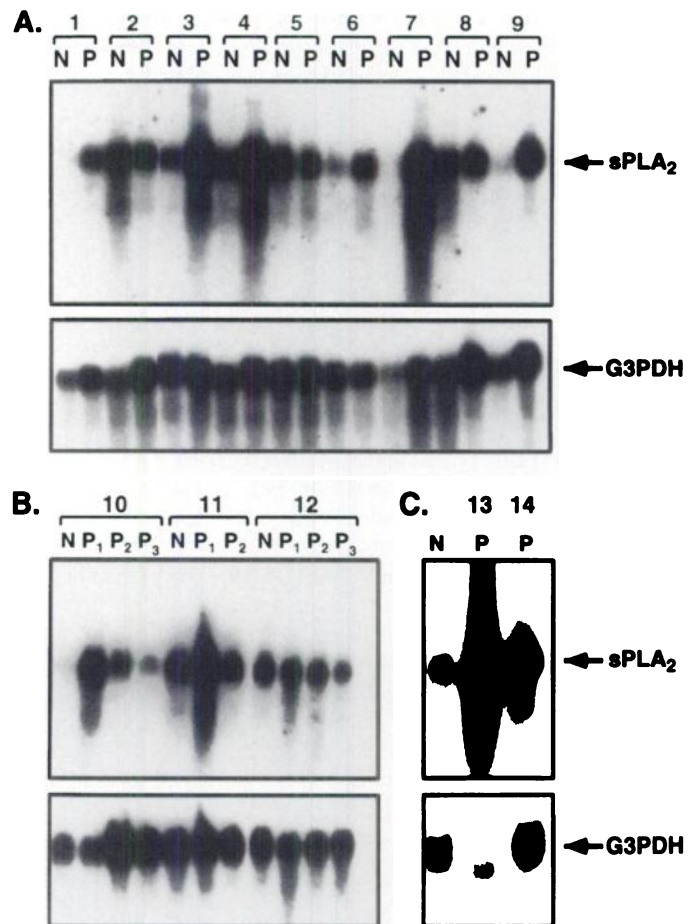


Fig. 1. Northern blot analysis of sPLA₂ mRNA expression in normal (N) mucosa and adenoma (P) from 14 FAP patients. A, sPLA₂ mRNA expression in matched normal and adenomatous polyp tissue from nine FAP patients. B, sPLA₂ mRNA expression in matched normal mucosa and multiple adenomas from three FAP individuals. C, sPLA₂ mRNA expression in nonmatched normal mucosa and adenomas from two different FAP patients. Total RNA (8 µg) was run on a 1% agarose/formaldehyde gel, transferred to nitrocellulose, hybridized first with a random-primed human sPLA₂ cDNA, stripped, and then rehybridized with a human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) probe.

In some samples (Fig. 1A, compare Lanes 5 and 6, 7 and 8, 11 and 12, 13 and 14; Fig. 1B, compare Lanes 1 and 2, 5 and 6; and Fig. 1C), there was a marked overexpression of sPLA₂ mRNA in the polyp. Multiple colorectal adenomatous polyps from three individual patients were also analyzed (Fig. 1B), and in two of the three patients, it was clearly evident that at least one of the polyps had a significant increase in sPLA₂ mRNA. However, integration of the blots by laser densitometry also showed that polyp sample P1 from patient 12 (Fig. 1B) had about a 3-fold increase in sPLA₂ mRNA over control. Recently, it has been shown that Cox-2 mRNA is also overexpressed in colorectal adenomas and adenocarcinomas (24). Because sPLA₂ has been implicated in inflammation and in some cases sPLA₂ and Cox-2 mRNA can be coordinately induced (25), the expression of Cox-2 mRNA in some of these samples was also examined. However, due to the low levels of Cox-2 mRNA, only very weak signals were obtained for Cox-2, even after 1 week of exposure. Nevertheless, an elevated level of Cox-2 expression was detectable in the polyp samples that expressed the highest level of sPLA₂ mRNA (data not shown). The low levels of Cox-2 mRNA in these samples were not surprising, because total RNA was used for this analysis, whereas the previous study used 5 µg of poly(A)⁺ and 1-week exposure to detect the Cox-2 mRNA (24). Although the polyp samples that express the highest

level of sPLA₂ mRNA also have detectable Cox-2 expression, there is a very large difference in the level of the sPLA₂ and Cox-2 mRNAs in these samples. The sPLA₂ hybridization was easily detectable after 2–3 h of exposure.

To show that the increased level of sPLA₂ mRNA expression also resulted in increased sPLA₂ enzyme levels, sPLA₂ protein in normal and tumor tissues was assayed by immunoblotting. The antibody used for the detection of sPLA₂ protein was a monoclonal antibody that recognizes the unreduced form of the enzyme. This results in the appearance of multiple sPLA₂ immunodetectable bands on SDS gels (Fig. 2) due to the different oxidized forms of the enzyme (Ref. 26; this *M_r* 14,000 group II sPLA₂ contains 14 cysteine residues). However, taking this into account, there was a detectable increase in the amount of immunodetectable sPLA₂ enzyme over normal tissue in 8 of 10 adenomas examined. Not all adenomas from the same patient had increased sPLA₂ protein levels, which is consistent with the result found for the mRNA expression (Fig. 1B). Therefore, the overexpressed sPLA₂ mRNA in colorectal adenomas is translated, resulting in increased levels of sPLA₂ enzyme.

The sequence of the sPLA₂ mRNA in colorectal polyps from the nine FAP patients analyzed in Fig. 1A was determined by RT-PCR (Fig. 3) and sequencing of the amplified product. SSCP analysis of an additional nine FAP duodenal adenomas (Fig. 4) was also performed, because it was hypothesized that these would be more analogous to the *Min* mouse small

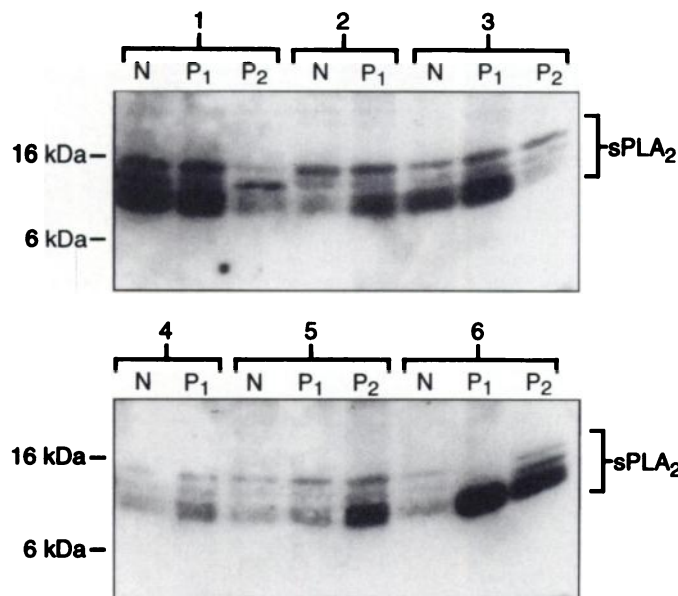


Fig. 2. Immunoblot analysis of sPLA₂ expression in normal (N) mucosa and adenoma (P) from six FAP patients. The multiple immunodetectable sPLA₂ bands are due to different oxidized forms of the enzyme (see "Results").

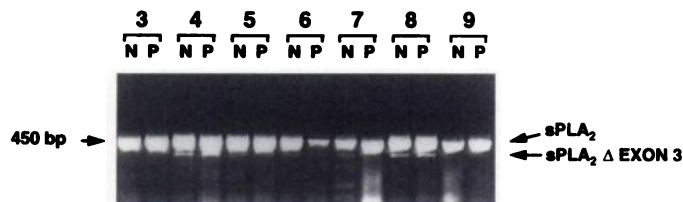


Fig. 3. RT-PCR amplification of sPLA₂ cDNA normal (N) mucosa and adenomatous polyp (P) samples. The numbering corresponds to patient number RNA samples in Fig. 1A. The RT-PCR reaction, carried out as described in "Materials and Methods," is not quantitative. The 450-bp sPLA₂ cDNA PCR product is easily amplified from all samples. The misspliced sPLA₂ mRNA with exon 3 deleted can be seen just below the sPLA₂ cDNA. The amplified products were excised from the gel and sequenced.

C 1 2 3 4 5 6 7 8 9

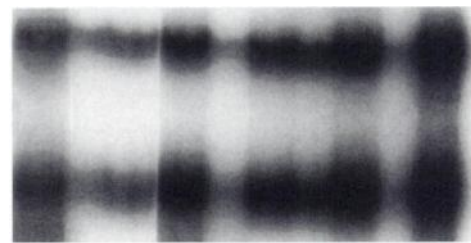


Fig. 4. SSCP analysis of segment 3 (180 bp) of sPLA₂ cDNA in duodenal adenomas from nine FAP patients (Lanes 1–9) and in one normal duodenal mucosa (C, control). There are no altered conformants.

intestinal adenomas. Essentially, no significant changes in the coding sequence of sPLA₂ mRNA were detected in either the colorectal or duodenal adenomas, which is in agreement with previous results (15–17). However, in a number of samples (both normal and adenomas), an additional faster-migrating PCR product was observed (Fig. 3). Sequencing of this product revealed that it coded for a misspliced sPLA₂ cDNA that was missing exon 3. The reason for this missplicing is not clear, because it is unlikely due to mutation of the gene. It is of interest to note that the frameshift mutation in exon 3 of the mouse sPLA₂ gene also generates a similar gene product (5, 10). Exon 3-deleted sPLA₂ cDNA in both human and mouse, if translated, terminates out of frame at the exact same position in exon 4 (10).

DISCUSSION

The results presented here show that sPLA₂ mRNA and protein can be overexpressed to very high levels in FAP adenomas. In the *Min* mouse model of adenomatous polyposis coli, sPLA₂ expression appears to confer resistance to the *Apc^{Min}* phenotype (5, 27), thus suggesting that the increased sPLA₂ gene expression in FAP may be a protective response to reduce polyp formation. Previous reports have shown that PLA₂ activity (11) and arachidonic acid levels (12) are increased in human colorectal cancer. It is also well-documented that sPLA₂ is involved in the inflammatory response and can provide arachidonic acid for eicosanoid production. Therefore, the increased PLA₂ activity and arachidonic acid levels in colorectal adenomas could be due to the overexpression of sPLA₂. Nonsteroidal anti-inflammatory drugs, which inhibit prostaglandin synthesis, have been reported to reduce polyp size and number (13, 14), as well as colon cancer cell growth (28). Furthermore, Cox-2 mRNA (24) and protein (29) are also induced in colorectal cancer and inhibition of Cox-2, either by gene knock-out or by a selective Cox-2 inhibitor, suppresses intestinal polyposis in *Apc^{Δ716}* mutant mice (14). Therefore, it is possible that this supposedly protective response of sPLA₂ overexpression in FAP could inadvertently be contributing substrate to the Cox-2 pathway for the increased production of prostaglandins. In this regard, the increased expression of sPLA₂ in adenomas is more likely contributing to tumor promotion. Overexpression of sPLA₂ has been observed in other cancers. For example, sPLA₂ overexpression in breast cancer has been correlated with increased malignant potential (30, 31). There is also increased sPLA₂ expression in gastric cancer (32), hepatocellular carcinomas (33), and in large bowel neoplasms of rats treated with carcinogen (34). Thus, the increased sPLA₂ expression in FAP could be contributing to tumor progression and not preventing polyp formation as appears to be the case in the *Min* mouse (5, 27). The absence of sPLA₂ gene mutations in sporadic and FAP colorectal neoplasms as observed by us and others (15–17) is also not consistent with sPLA₂ being a modifier

of *APC* mutations in humans. Therefore, it is somewhat difficult to reconcile the difference between the *Min* mouse model and FAP. There are a few possibilities that may account for this difference. Mouse physiology is different than that of humans; for example, *Apc* mutations in the mouse produce numerous intestinal adenomas, whereas in humans, polyps occur primarily in the colon and duodenum. The modifiers of *APC* mutations in humans are different than in the mouse, and the genetic backgrounds of humans are much more diverse than that of inbred mouse strains.

The expression of sPLA₂ mRNA in the *Min* mouse and in FAP patients have in common the presence of a misspliced sPLA₂ mRNA that has exon 3 deleted. The C57BL/6 mouse strain in which the *Min* phenotype was established (6) carries a frameshift mutation in exon 3 of the sPLA₂ gene (5, 10). This mutation is thought to be responsible for the exon skipping, because similar exonic mutations in several other genes have been shown to result in aberrant mRNA splicing (10). The FAP patients' colorectal or duodenal adenomas are unlikely to have mutations in the sPLA₂ gene, because no mutations were observed in the sPLA₂ cDNA, and the mRNA is expressed at very high levels. Furthermore, the misspliced transcript is found in both normal and adenoma samples of FAP patients. In the mouse, it was only detected in the intestine mRNA of sPLA₂-deficient mouse strains and not in the wild-type sPLA₂ strains. Therefore, how and why this alternatively spliced sPLA₂ mRNA is produced in these samples is not known. It is unlikely to be a PCR artifact because exon 3 is specifically deleted. The fact that the mRNA is expressed at such high levels may attribute to missplicing. It is of interest to note that both the mouse and human exon 3-deleted sPLA₂ mRNA, if translated, terminate out of frame in exon 4 at the exact same position. The translated product, if produced, would contain 13 amino acids from the sPLA₂ signal peptide sequence and a new 22-amino acid sequence. Whether this product has some fortuitous function remains to be determined.

In conclusion, the results presented here are in agreement with previous results that showed the absence of sPLA₂ gene mutations in FAP adenomas. However, the high level of sPLA₂ mRNA expression in adenomas could account for the elevated levels of arachidonic acid in colorectal cancer, thus providing substrate for Cox-2 and leading to increased prostaglandin production and tumor promotion.

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REFERENCES

- Vadas, P., Browning, J., Edelson, J., and Pruzanski, W. Extracellular phospholipase A₂ expression and inflammation: the relationship with associated disease states. *J. Lipid Mediators*, 8: 1-30, 1993.
- Kudo, I., Murakami, M., Hara, S., and Inoue, K. Mammalian non-pancreatic phospholipases A₂. *Biochem. Biophys. Acta*, 1170: 217-213, 1993.
- Glaser, K. B., and Vadas, P. Phospholipase A₂ in Clinical Inflammation: Molecular Approaches to Pathophysiology. Boca Raton, FL: CRC Press, 1995.
- Hara, S., Kudo, I., and Inoue, K. Augmentation of prostaglandin E₂ production by human mammalian phospholipase A₂ added exogenously. *J. Biochem.*, 110: 163-165, 1991.
- MacPhee, M., Chepenik, K. P., Liddell, R. A., Nelson, K. K., Stracusa, L. D., and Bachberg, A. M. The secretory phospholipase A₂ gene is a candidate for the *Mom1* locus, a major modifier of the *APC*^{Min}-induced intestinal neoplasia. *Cell*, 81: 957-966, 1995.
- Moser, A. R., Pitot, H. C., and Dove, W. F. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science* (Washington DC), 247: 322-324, 1990.
- Su, L. K., Kinzler, K. W., Vogelstein, B., Preisinger, A. C., Moser, A. R., Luongo, C., Gould, K. A., and Dove, W. F. A germline mutation of the murine homolog of the *APC* gene causes multiple intestinal neoplasia. *Science* (Washington DC), 256: 668-670, 1992.
- Nishisho, I., Nakamura, Y., Miyoshi, Y., Miki, Y., Ando, H., Horii, A., Koyama, K., Utsunomiya, J., Baba, S., Hedge, P., Markham, A., Krush, A. H., Petersen, G., Hamilton, S. R., Nilbert, M. C., Levy, D. B., Bryan, T. M., Preisinger, A. C., Smith, K. J., Su, L.-K., Kinzler, K. W., and Vogelstein, B. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* (Washington DC), 253: 665-669, 1991.
- Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., Albertsen, H., Joslyn, G., Stevens, J., Spiro, L., Robertson, M., Sargeant, L., Krapcho, K., Wolf, E., Randall, B., Hughes, J. P., Warrington, J., McPherson, J., Wasmuth, J., LePaslier, D., Abderemhamn, H., Cohen, D., Leppert, M., and White, R. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell*, 66: 589-600, 1991.
- Kennedy, B. P., Payette, P., Mudgett, J., Vadas, P., Pruzanski, W., Kwan, M., Tang, C., Rancourt, D. E., and Cromlish, W. A. A natural disruption of the secretory group II phospholipase A₂ gene in inbred mouse strains. *J. Biol. Chem.*, 270: 22378-22385, 1995.
- Neoptolemos, J. P., Husband, D., Imray, C., Rowley, S., and Lawson, N. Arachidonic acid and docosahexaenoic acid are increased in human colorectal cancer. *Gut*, 32: 278-281, 1991.
- Hendrickse, C. W., Radley, S., Donovan, I. A., Keighley, M. R. B., and Neoptolemos, J. P. Activities of phospholipase A₂ and diacylglycerol lipase are increased in human colorectal cancer. *Br. J. Surg.*, 82: 475-478, 1995.
- Rao, C., Rivenson, A., Simi, B., Zang, E., Kelloff, G., Steele, V., and Reddy, B. Chemoprevention of colon carcinogenesis by sulindac, a nonsteroidal anti-inflammatory agent. *Cancer Res.*, 55: 1464-1472, 1995.
- Oshima, M., Dinchuk, J. E., Kargman, S. L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J. M., Evans, J. F., and Taketo, M. M. Suppression of intestinal polyposis in *Apc*^{Δ716} knockout mice by inhibition of cyclooxygenase 2 (Cox-2). *Cell*, 87: 803-809, 1996.
- Riggins, G. J., Markowitz, S., Wilson, J. K., Vogelstein, B., and Kinzler, K. W. Absence of secretory phospholipase A₂ gene alterations in human colorectal cancer. *Cancer Res.*, 55: 5184-5186, 1995.
- Spirio, L. N., Kutcher, W., Winstead, M. V., Pearson, B., Kaplan, C., Robertson, M., Lawrence, E., Burt, R. W., Tischfield, J. A., Leppert, M. F., Prescott, S. M., and White, R. Three secretory phospholipase A₂ genes that map to human chromosome 1p35-36 are not mutated in individuals with attenuated adenomatous polyposis coli. *Cancer Res.*, 56: 955-958, 1996.
- Dobbie, Z., Muller, H., and Scott, R. J. Secretory phospholipase A₂ does not appear to be associated with phenotypic variation in familial adenomatous polyposis. *Hum. Genet.*, 98: 386-390, 1996.
- Maniatis, T., Sambrook, J., and Fritsch, E. F. *Molecular Cloning: A Laboratory Manual*, Ed. 2, pp. 7.43-7.52. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.
- Tremblay, N. M., Kennedy, B. P., Street, I. P., Kaupp, W. J., Laliberte, F., and Weech, P. K. Human group II phospholipase A₂ expressed in *Trichoplusia ni* larvae: isolation and kinetic properties of the enzyme. *Protein Expression Purif.*, 4: 490-498, 1993.
- Cromlish, W. A., Payette, P., Culp, S. A., Ouellet, M., Percival, M. P., and Kennedy, B. P. High-level expression of active human cyclooxygenase-2 in insect cells. *Arch. Biochem. Biophys.*, 314: 193-199, 1994.
- Xia, L., St. Denis, K. A., and Bapat, B. Evidence for a novel exon in the coding region of the adenomatous polyposis coli (*APC*) gene. *Genomics*, 28: 589-591, 1995.
- Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (Lond.), 227: 680-685, 1970.
- Towbin, H., Staehelin, T., and Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA*, 75: 4350-4354, 1979.
- Eberhart, C. E., Coffey, R. J., Radhika, A., Giardiello, F. M., Ferrenbach, S., and Dubois, R. N. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology*, 107: 1183-1188, 1994.
- Vadas, P., Stefanski, E., Wloch, M., Groulx, B., Van den Bosch, H., and Kennedy, B. P. Secretory non-pancreatic phospholipase A₂ and cyclooxygenase-2 expression by tracheobronchial smooth muscle cells. *Eur. J. Biochem.*, 235: 557-563, 1996.
- Shalkwijk, C. G., Marki, F., Wiesenberger, I., and van den Bosch, H. The detection of multimeric forms of phospholipase A₂ upon sodium dodecyl sulfate-polyacrylamide electrophoresis. *J. Lipid Mediat.*, 4: 83-96, 1991.
- Gould, K. A., Luongo, C., Moser, A. R., McNeely, M. K., Borenstein, N., Shedlovsky, A., Dove, W. F., Hong, K., Dietrich, W. F., and Landers, E. S. Genetic evaluation of candidate genes for the *Mom1* modifier of intestinal neoplasia in mice. *Genetics*, 144: 1777-1785, 1996.
- Sheng, H., Shao, J., Kirkland, S. C., Isakson, P., Coffey, R. J., Morrow, J., Beauchamp, R. D., and Dubois, R. N. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J. Clin. Invest.*, 99: 2254-2259, 1997.
- Kargman, S. L., O'Neill, G. P., Vickers, P. J., Evans, J. F., Mancini, J. A., and Johty, S. Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res.*, 55: 2556-2559, 1995.
- Yamashita, S., Yamashita, J., Sakamoto, K., Inada, K., Nakashima, Y., Murata, K., Saishoji, T., Nomura, K., and Ogawa, M. Increased expression of membrane-associated phospholipase A₂ shows malignant potential of human breast cancer cells. *Cancer* (Phila.), 71: 3058-3064, 1993.
- Yamashita, S., Yamashita, J., and Ogawa, M. Overexpression of group II phospholipase A₂ in human breast cancer tissues is closely associated with their malignant potency. *Br. J. Cancer*, 69: 1166-1170, 1994.
- Murata, K., Egami, H., Kiyohara, H., Oshima, S., Kurizaki, T., and Ogawa, M. Expression of group II phospholipase A₂ in malignant and non-malignant human gastric mucosa. *Br. J. Cancer*, 68: 103-111, 1993.
- Ying, Z., Tojo, H., Komatsubara, T., Nakagawa, M., Inada, M., Kawata, S., Matsuzawa, Y., and Okamoto, M. Enhanced expression of group II phospholipase A₂ in human hepatocellular carcinoma. *Biochem. Biophys. Acta*, 1226: 201-205, 1993.
- Yoshimi, N., Ino, N., Suzui, M., Tanaka, T., Nakashima, S., Nakamura, M., Nozawa, Y., and Mori, H. The mRNA overexpression of inflammatory enzymes, phospholipase A₂ and cyclooxygenase, in the large bowel mucosa and neoplasms in F344 rats treated with naturally occurring carcinogen, 1-hydroxyanthraquinone. *Cancer Lett.*, 97: 75-82, 1995.

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