Recent studies suggest that secreted phospholipases A2 (sPLA2s) represent attractive potential tumour biomarkers and therapeutic targets for various cancers. As a first step to address this issue in human colorectal cancer, we examined the expression of the full set of sPLA2s in sporadic adenocarcinomas and normal matched mucosa from 21 patients by quantitative PCR and immunohistochemistry. In normal colon, PLA2G2A and PLA2G12A were expressed at high levels, PLA2G2D, PLA2G5, PLA2G10 and PLA2G12B at intermediate, and PLA2G1B, PLA2G2F and PLA2G3 at low levels. In adenocarcinomas from left and right colon, the expression of PLA2G3 was increased up to 40-fold, while that of PLA2G2D and PLA2G5 was decreased by up to 23- and 14-fold. The variations of expression for sPLA2-IID, sPLA2-III and sPLA2-V were confirmed at the protein level. The expression pattern of these sPLA2s appeared to be linked respectively to the overexpression of interleukin-8, defensin α6, survivin and matrilysin, and the down-regulation of SFRP-1 and RPLA-1, all these genes being associated to colon cancer. This original sPLA2 profile observed in adenocarcinomas highlights the potential role of certain sPLA2s in colon cancer and suggests that sPLA2-III might be a good candidate as a novel biomarker for both left and right colon cancers.

Keywords: colon cancer; secreted phospholipase A2; gene expression; qPCR; immunohistochemistry; biomarker
Secreted phospholipases A2 have been recently proposed as targets for anticancer drugs (Lay and Gill, 2003; Cummings, 2007), and there is increasing evidence for their involvement in various human cancers. Indeed, the expression levels of sPLA2-V and sPLA2-X are modified in human lung cancer (Masuda et al, 2005b), and those of sPLA2-V and sPLA2-XIIIA in human ovarian cancer (Gorozvet et al, 2006). In particular, the overexpression of human sPLA2-IIIA in gastric adenocarcinoma was proposed to be related to prolonged survival and less frequent metastasis (Leung et al, 2002; Aggarwal et al, 2006). By contrast, sPLA2-IIIA overexpression has been associated to oncogenic effects in prostate cancer (Sved et al, 2004) and is related to poor prognosis (Graff et al, 2001). The polymorphism of the sPLA2-IIIA gene, PLA2G2A, is also associated to some phenotypic features of patients with familial adenomatous polyposis (Yanaru-Fujisawa et al, 2007). More recently, human sPLA2-XIIIB was shown to be upregulated in 50% of patients with hepatitis C virus-associated hepatocellular carcinoma (Smith et al, 2003). Finally, a protective role for sPLA2-IIIA against ApoM-induced intestinal tumours has been established in mice (MacPhee et al, 1995; Cormier et al, 1997). However, data on sPLA2 and human cancers are still sparse, and often rely on only one or a few sPLA2s.

In this study, we have analysed the expression pattern of the full set of sPLA2s in tumour vs normal matched mucosa from patients with adenocarcinomas located in the left and right colon. Variation in the expression of a number of other genes associated with colon tumorigenesis and/or inflammation was also examined to establish possible gene coregulations. Our data provide the first comprehensive analysis of the expression pattern of all sPLA2s in normal human colon mucosa as well as adenocarcinomas. These data further support the fact that several sPLA2s may contribute to the initiation, progression or modulation of colon tumorigenesis, and may provide new potential tumour markers for this disease.

MATERIALS AND METHODS

Human tissues and RNA isolation

Fourteen colon adenocarcinoma specimens from the left colon (descending part of the colon) and seven colon adenocarcinoma specimens from the right colon (ascending part of the colon) were obtained from surgical resections according to the French and American institutional guidelines. No specimen was from rectum. The patients did not receive chemotherapy or radiotherapy prior to surgery. The specimens were from 42- to 85-year-old patients (median age: 71 years), 14 men and 7 women. Samples were well (3 out of 21), moderately (14 out of 21) or poorly (4 out of 21) differentiated sporadic adenocarcinomas, with pTNM classification (3 out of 21), moderately (14 out of 21) or poorly (4 out of 21) differentiated sporadic adenocarcinomas, with pTNM classification ranging pT2–4 N0–2 M0–1. Neither adenoma nor Duke’s D was examined in this study. All samples had an expression of hMLH1 and hMSH2 unchanged as evaluated by immunohistochemistry and quantitative PCR (qPCR) (data not shown). Tissues from the non-neoplastic part of the tumour and from distant normal mucosa were snap frozen in liquid nitrogen and stored at −80°C. Each tissue sample (20–100 mg) was mixed with 700 µl of ice-cold phenol–β-mercaptoethanol (nucleospin RNA II kit; Macherey-Nagel, Hoerdt, France) in a green-cap tube containing Lysing Matrix D (Q-BioGene, Illkirch, France), and tissue disruption was achieved with the fast prep instrument (FP220A; Q-BioGene). Total RNA was then isolated using the nucleospin RNA II kit, including DNase treatment. RNA concentration was determined by OD260 and RNA quality was evaluated by analysis on an Agilent Bioanalyzer (Agilent Technologies, Les Ulis, France).

Quantitative PCR

First-strand cDNA was synthesised from 5 µg of total RNA using 100 U of MMLV reverse transcriptase (#M170A; Promega, Charbonnières-les-bains, France) in a final volume of 50 µl with 500 ng of random primers (#C118A; Promega). Quantitative PCR was carried out in 96-well ABgene plates using the GENEAMP 5700 sequence Detection System apparatus (Applied Biosystems, Courtaboeuf, France) with the qPCR Master Mix Plus for SYBR® Green I (Eurogentec, Angers, France). All reactions were performed in a total volume of 16 µl and contained 50 ng of reverse transcribed RNA (based on the initial RNA concentration) and 250 nm of each primer set. The primer sets were designed using the Primer Express program from Applied Biosystems for the following human genes: PLA2G2A (NM_000300), sPLA2-IIIId gene (PLA2G2D) (NM_012400), sPLA2-IEE gene (PLA2G2E) (NM_014591), sPLA2-IIIf gene (PLA2G2F) (NM_022819), sPLA2-IIE gene (PLA2G1B) (NM_001071), sPLA2-IIF gene (NM_000929), sPLA2-X gene (PLA2G10) (NM_003561), sPLA2-XIIIA gene (PLA2G12A) (BC_017218), sPLA2-XIIIB gene (PLA2G12B) (NM_032562), iPLA2-VIB gene (PLA2G6) (NM_003560), pgis1 (BC_029840), COX-2 gene (pgs2) (NM_009635), pgs1 (NM_004878), pgs2 (NM_198797), IL-1x (NM_000575), IL-6 (NM_000600), IL-10 (NM_57627), PPARα (NM_138712), PPARδ (NM_006238), u-PA (NM_002658), u-PAR (NM_002659), IL-8 (NM_000584), EPR-1 (NM_002219), MMP-7 (NM_002423), SFRP-1 (NM_000312), PLA2R1 (NM_007366), TNF-α (NM_000594), MMP-9 (NM_004994), MSH2 (NM_00251), MLH1 (NM_000249). Most primer sets were designed to span an intron in order to avoid amplification from potential traces of genomic DNA in the total RNA preparations. Only the primer sets for MLH1, uPAR, EPR-1, MMP-7 and bcl-2 genes were not spanning an intron. For primer sets spanning an intron, we checked that no amplification signal was obtained using human genomic DNA as template in the qPCR (data not shown). The sequences of the designed primer sets are available on request.

We used Qiagen commercial primer sets for cPLA2-IVA gene (PLA2G4A) (ref. QT000085813) and sPLA2-IB gene (PLA2G12B) (ref. QT0000637). We used published primer sets for RLP-1 and RLP-2 genes (Shida et al, 2004) and HD-6 gene (Andreu et al, 2005). The efficiency and specificity of each primer sets were validated using either serial dilutions of cloned human sPLA2 cDNAs or mixed human tissue cDNA for the other genes. Moreover, when enough total RNA was collected, negative controls without added reverse transcriptase were performed. Thermal cycling was performed for 40 cycles comprising each a denaturation step at 95°C for 15 s, and an annealing/extension step at 60°C for 1 min. Amplification of the appropriate product was verified by analysing the dissociation curve that was obtained after PCR with the following steps: 15 s at 95°C, 20 s at 60°C, and then a slow ramp of 20 min from 60 to 95°C. The abundance of the mRNA target was calculated relative to the expression of the reference gene TOP1 and is expressed as $2^{\Delta C_{t}}$, where $\Delta C_{t} = C_{t} (\text{gene of interest}) - C_{t} (\text{TOP1})$. The choice of TOP1 as a reference gene was determined using a Human GeNorm kit (PrimerDesign Ltd, Southampton, UK), allowing the determination of the best reference gene among 12 widely used reference genes. The data were also validated using GAPDH as a reference gene (data not shown). When the relative level of expression was plotted for normal mucosa (n) (Figure 1), we used the formula $\Delta C_{n} = C_{n}(\text{gene}) - C_{n}(\text{TOP1})$. The $C_{n}(\text{n})$ values for TOP1 were typically around 20. When the expression of each gene in normal mucosa was compared to the expression of each gene in normal mucosa, the decrease or increase factor $\phi$ in the tumour vs the normal mucosa (Figure 5A and B) was calculated with the formula $\phi = e^{\Delta C_{t}}$.
\[ \phi = 2^{\Delta C_t(n) - \Delta C_t(t) - 1} \, , \quad \text{with} \quad \Delta C_t(n) = C_t(n)_{\text{gene}} - C_t(n)_{\text{TOP1}}, \]
\[ \Delta C_t(t) = C_t(t)_{\text{gene}} - C_t(t)_{\text{TOP1}}, \quad \text{and} \quad I \quad \text{the absolute value of the term} \quad \Delta C_t(n) - \Delta C_t(t). \]

**Immunohistochemistry**

All experiments were performed using paraffin-embedded tissues from the same patients as those used for the qPCR experiments.

![Averaged relative expression level (arbitrary units)](image)

**Figure 1** Expression level of sPLA2 genes in human colon normal mucosa. The relative averaged expression level of all sPLA2 genes and the two reference genes used in this study (TOP1 and GAPDH) is shown for the 14 samples from left colon and the 7 samples from right colon. Arbitrary units are used (see Materials and Methods).

![Expression profile of sPLA2 genes in human adenocarcinomas and normal matched mucosa](image)

**Figure 2** Expression level of sPLA2 genes in human adenocarcinomas and normal matched mucosa. The expression level of sPLA2 genes and of the M-type sPLA2 receptor (PLA2R1) was measured in adenocarcinomas and normal matched mucosa for the 14 left-sided samples. The data were obtained after normalisation with TOP1 used as reference gene and using the formula \(-\Delta C_t = C_t_{\text{gene}} - C_t_{\text{TOP1}}\). N, normal tissues; T, tumour tissues.

The expression of sPLA2-IID, sPLA2-III, sPLA2-V and COX-2 proteins was analysed in tumours and normal matched tissues from three to four patients. Consecutive 4-μm tissue sections were depaaffinised in xylene and rehydrated in graded alcohol dilutions. Immunolabelling was performed using avidin–biotin–peroxidase technique (Vectastain ABC kit; Vector, Burlingame, CA, USA). Before immunostaining, endogenous peroxidase activity was inhibited with 0.1% hydrogen peroxide in methanol for 30 min. Colour development was achieved with 3-amino-9-ethylcarbazole, and sections were finally counterstained with haematoxylin. Specific rabbit polyclonal antibodies against recombinant human sPLA2-IID, sPLA2-III and sPLA2-V were produced as described (Haas et al., 2005), and used at working dilutions of 1/300, 1/250 and 1/100, respectively. All anti-sPLA2 antibodies were tested for specificity towards the various sPLA2s and were shown to be highly specific for each human sPLA2 (Haas et al., 2005).

Negative controls were performed by omission of the primary antibody. To further check for the specificity of labelling with sPLA2-IID-, sPLA2-III- and sPLA2-V-specific antibodies, competition experiments were performed in which the antibody was preincubated with the corresponding purified recombinant sPLA2, used for immunisation, prior to covering the tissue slides. The competition was performed by preincubating the relevant antibody solution with 200 nM of human recombinant sPLA2-III or 100 nM of human recombinant sPLA2-IID and sPLA2-V for 1 h at room temperature. Purified recombinant proteins were obtained as described (Rouault et al., 2007). Human COX-2 antibodies were from Cayman (Montigny-le Bretonneux, France) (ref. 160112, working dilution 1/500), human MLH1 antibodies were from BD Pharmingen (Le Pont-de-Claix, France) (clone G168-178, working dilution 1/100), and human MSH2 antibodies were from Calbiochem (Darmstadt, Germany) (clone FE-11, working dilution 1/125).

**Statistics**

We determined that the sample data from left and right colons followed a Gaussian distribution using the D’agostino–Pearson normality test. Normal mucosa and tumour paired data for each gene were analysed using the Bonferroni test, and statistical significances were represented by *\(P<0.05\), **\(P<0.01\) and **\(P<0.001\).
RESULTS

Expression levels of sPLA₂ genes in normal human colon mucosa

We first looked at the mRNA levels for the different sPLA₂ genes in normal mucosa and compared their relative expression levels. Our data show dramatic differences in the relative expression of sPLA₂. The lowest gene expression level was found for PLA2G3, and the highest gene expression level was found for PLA2G2A (Figure 1). The level of PLA2G2A expression was particularly high. It is above that of TOP1 and almost reaches that of GAPDH, which are both used as reference genes and are known to be highly expressed in many tissues, including colon. By contrast, the expression of PLA2G3 was generally very low, in fact below the limit of detection in many samples (Figure 1). The expression levels of PLA2G2F and PLA2G1B were also low, while that of other sPLA₂ genes were moderate. The expression of PLA2G2E was not detectable (data not shown). We also compared the expression levels in normal mucosa of left and right colon. A significant difference in gene expression levels was seen for PLA2G2F, PLA2G1B, PLA2G12B and PLA2G12A (respectively 30-, 13-, 24- and 40-fold lower expression in right-sided normal mucosa than in left-sided normal mucosa). No or minor differences were observed in gene expression for PLA2G2A, PLA2G2D, PLA2G5, PLA2G3 and PLA2G12A between right- and left-sided normal mucosa (Figure 1).

Expression profile of sPLA2s in human colon cancer

We next compared sPLA₂ gene expression in adenocarcinomas vs normal mucosa. The raw data for samples from the left colon are shown in Figure 2. The fold-increase or fold-decrease in the expression of each gene in adenocarcinomas vs normal mucosa is represented in Figure 3. Interestingly, we observed a 40-fold increase in PLA2G3 expression in the adenocarcinomas. In contrast, we found 23- and 14-fold decreases for PLA2G2D and PLA2G5, respectively. Similar data were obtained for samples from the right colon, with a 22-fold increased expression in tumour vs normal mucosa for PLA2G2A, respectively (Figure 4). No variation in expression between the tumours and normal mucosa was observed for PLA2G1B, PLA2G2A, PLA2G2F, PLA2G10, PLA2G12A and PLA2G12B and for the M-type sPLA₂ receptor (PLA2R1) (Figures 3 and 4). The expression of PLA2G2E

Figure 3  Expression level of sPLA₂, tumour-related and inflammation-related genes in human left colon adenocarcinomas. After normalisation with the TOP1 reference gene, the expression level for each gene in the tumour was compared to that in the normal matched mucosa. The zero value indicates no variation, a positive value indicates an increased expression level in tumour vs normal tissue and a negative value indicates a decreased expression level in tumour vs normal tissue (see Materials and Methods). The incidence of variation in the different samples is indicated.

***P < 0.001. We looked for correlation between all genes examined in this study by using the Mev3.1 software and hierarchical clustering to analyse different linkages to cluster genes and samples. Euclidean distance was used to calculate the distance between two genes.
Expression levels of genes involved in inflammation and tumorigenesis in left and right colon adenocarcinomas vs normal human colon mucosa

Since sPLA2s may exert a coordinate action with other genes in tumorigenesis and associated inflammation, we also examined the expression of a panel of genes known to be involved in inflammation and colorectal tumorigenesis (Figures 3 and 4). In left colon samples, we did not observe any differences in expression levels between adenocarcinomas and normal mucosa for most of the inflammation-related genes examined in this study, that is, ptgs2, ptges2, TNF-α, Il-1α, Il-6, Il-10, PPARγ, PPARδ, MMP-9 and PLA2G6. Only a slight decrease in the expression of ptgs1 and PLA2G4A was observed. With the exception of ptges2 and Il-1α, we found that the inflammation-related genes were already expressed at high levels in the normal mucosa.

We also looked at the expression of urokinase plasminogen activator (u-PA) and its receptor (u-PAR) that play a role in cell adhesion and cell migration, and are involved in late stages of tumour development, contributing to tumour cell invasion and metastatic spread (Terada et al, 2005). No changes were observed in u-PA and u-PAR gene expression levels.

RLPA-1 and RLPA-2 genes code for two distinct LPA receptors (Mills and Moolenaar, 2003). While the expression of RLPA-2 did not change, that of RLPA-1 was decreased by 20-fold in tumour vs normal matched mucosa.

IL-8, EPR-1, HD-6 and MMP-7 are target genes of the Wnt/β-catenin/tcf-4 pathway (Kolligs et al, 2002; Levy et al, 2002; Wheatley and McNeish, 2005). Interestingly, a marked increase in expression was found for IL-8, HD-6 and MMP-7 expression in the tumours (8-, 22- and 70-fold, respectively). The level of EPR-1 was not changed. SFRP-1 codes for an antagonist of the Wnt pathway, which binds to the frizzled receptor and blocks frizzled–Wnt interaction (Suzuki et al, 2004). Our results show a dramatic 130-fold decrease in the expression of SFRP-1 in the tumour.

Similar data were obtained for most genes in the right-sided samples (Figure 4). Indeed, no variation in expression was observed for most inflammatory-related genes, while a dramatic increase (280-fold) and a strong decrease (125-fold) were found for MMP-7 and SFRP-1, respectively. However, in contrast to left-sided samples, we observed no variation in HD-6 and IL-8 expression levels and a strong increase (45-fold) in EPR-1 expression.

Incidence and coregulation of studied genes

We first looked at the incidence of the above variations, that is, the number of samples showing an increase or decrease in the expression level of a particular gene within the 21 patients used in this study (Figures 3–5). The increase in PLA2G3 expression showed a high incidence (12 out of 14 for left-sided samples and 5 out of 7 for right-sided samples), which was similar to that of MMP-7 gene overexpression (10 out of 14 for left-sided samples and 6 out of 7 for right-sided samples). The incidence for the overexpression of HD-6 and IL-8 was lower, since these two genes were not significantly upregulated in right-sided samples. The decrease in PLA2G2D and PLA2G5 expression also showed a high incidence (12 out of 14 and 5 out of 7; and 11 out of 14 and 5 out of 7, respectively), which was similar to that of RLPA-1 and SFRP-1 gene expression (13 out of 14 and 6 out of 7; and 14 out of 14 and 7 out of 7, respectively). We did not observe any difference between...
female and male patients used in our study for the variations of expression of PLA2G3, PLA2G2D and PLA2G5 (data not shown).

Euclidean distance is commonly used to evaluate linkages in the expression of different genes. We used this method to point out linkages between the variations in expression level of the most relevant genes. In left-sided adenocarcinomas (Figure 5A), a first cluster (cluster I) of upregulated genes was observed containing PLA2G3 and several genes related to colon cancer such as HD-6, MMP-7 and IL-8. A second cluster (cluster II) of several downregulated genes, including SFRP-1, PLA2G2D, RLPA-1, PLA2G5, PLA2G4A and pigS1, was observed. These two clusters appeared most likely to discriminate adenocarcinomas from normal tissues. Remarkably, there are 3 sPLA2 genes out of 11 genes in these two clusters. The other genes did not show any linkage with colon tumorigenesis, since their expression levels were up- or downregulated or not changed among the different patients. When looking at right-sided samples (Figure 5B), similar patterns were observed even though the number of patients was lower.

Protein expression levels of sPLA2-IIID, sPLA2-III and sPLA2-V in colon adenocarcinomas and normal matched mucosa

To confirm that the variation of expression for PLA2G2D, PLA2G3 and PLA2G5 is also observed at the protein level, immunohistochemical analyses were performed on tissue sections from the same patients as those used for qPCR. For each sPLA2 protein analysed, tissue sections from three or four patients showing a significant increase or decrease of sPLA2 expression were selected and immunostained (Figure 6). The labelling for sPLA2-IIID and sPLA2-V was markedly decreased in tumour cells compared to the normal matched epithelium, in accordance with the qPCR data. By contrast, the labelling for sPLA2-IIID was absent or very low in the normal epithelium, with a significant increase in tumour tissue. The absence of labelling in competition experiments (data not shown) or when the primary antibody was omitted (Figure 6) demonstrated that the signals observed for the three sPLA2 proteins were specific. In addition, immunohistochemical analysis of COX-2 showed a more intense protein expression in tumours than in normal mucosa in a subset of patients (data not shown).

DISCUSSION

Several lines of evidence have been accumulated during the past decade to support the role of sPLA2s in cancer pathogenesis. Their role in human cancer, however, has not been clarified. As a first step towards addressing this issue, we have analysed the expression patterns of the full set of human sPLA2s in colorectal cancer tissue samples and normal matched mucosa. Our results indicate that (i) several sPLA2s, including sPLA2-IIIA, sPLA2-X and sPLA2-XIIA, are highly expressed in both normal and tumour colon tissues; (ii) the expression levels of sPLA2-III, sPLA2-IIID and sPLA2-V are dramatically altered in adenocarcinomas at both mRNA and protein levels; and (iii) their mRNA profiles are part of gene expression clusters with other genes associated with inflammation and cancer. Together, our data are further suggestive of a role of these sPLA2s in colorectal cancer and open the possibility that sPLA2s, in particular sPLA2-III, may provide novel cancer biomarkers.

sPLA2 gene expression levels in colon adenocarcinomas and normal human colon

Colon adenocarcinomas analysed in this study are likely cancers without high microsatellite instability. Indeed, we used immunohistochemical analysis as a reliable method for screening DNA mismatch repair defects (Lindor et al, 2002), and found that the expression of hMLH1 and hMSH2 was unchanged. Furthermore, we analysed the normal mucosa and the tumour tissues from the right and left colon separately because of their distinct gene expression patterns (Glebov et al, 2003; Birkenkamp-Demtroder et al, 2005). We observed similar levels of expression for most sPLA2s in left and right normal mucosa, with PLA2G2A having a high expression level, PLA2G3 being not detectable in most samples and the other sPLA2 genes showing intermediate levels of expression (Figure 1). Interestingly, we observed marked alterations in the expression levels of PLA2G2D, PLA2G5 and PLA2G3 in colon adenocarcinomas. In contrast, there was no difference in the expression levels of the other sPLA2 genes (Figures 2–4). The absence of tumour-related alterations in the gene expression of PLA2G2A and PLA2G10 in human sporadic colon cancer tissues is consistent with earlier studies (Dimberg et al, 1998; Osterstrom et al, 2002; Murakami et al, 2005). These data obtained in humans differ from those obtained in mice, which showed an increased expression of PLA2G2A and no variation in PLA2G5 expression in tumours from azoxymethane-treated mice (Islesy et al, 2003).
Expression level of genes involved in inflammation and tumorigenesis in colon adenocarcinomas and normal human colon

No significant changes between adenocarcinomas and normal colon were observed for most inflammation-related genes. It should be noted that many of these genes were expressed at high levels within the normal mucosa, in particular, MMP-9, PLA2G6 and ptgs2. A recent study by Chen et al (2004) has also shown high mRNA expression levels of several inflammation-related genes in normal-appearing colon mucosa of patients with colon cancer. Therefore, our data would suggest a possible pre-existing inflammatory condition within the normal mucosa distant from the tumours, and likely explain the absence of increased expression in adenocarcinomas vs normal mucosa for several inflammation-related genes. The slight decrease in ptgs1 (the COX-1 gene) appeared in agreement with that observed in colon adenocarcinomas of stage III (Duke’s C) patients (Church et al, 2004). The COX-1 protein is considered to exert dual opposing effects in cancer, acting as either a tumour suppressor or a tumour initiator (Chulada et al, 2000). The slight decrease in PLA2G4A expression that we observed may be related to the dual opposing effects of sPLA2-JVA in cancer, that is, proliferative effects via metabolism of AA into eicosanoids, such as PGE2, and anti-proliferative effects via AA-dependent ceramide production leading to apoptosis (Ilsley et al, 2005). The absence of increase in ptgs2 (the COX-2 gene) levels in our set of patients is in accordance with two recent qPCR studies also performed on patients with colon cancer (Church et al, 2004; Gustafsson et al, 2007), and may be related to the inflammatory status of the normal mucosa discussed above. The fact that we did not observe an increase of COX-2 at the mRNA level, while we detected an overexpression at the protein level by immunohistochemistry as previously reported (Wendum et al, 2007), is in line with the post-transcriptional regulation of COX-2 expression (Dixon et al, 2003).

Therefore, mRNA and protein levels of COX-2 may not be closely linked, as recently proposed in colon cancer tissues (Gustafsson et al, 2007) and colon cancer cells (Dixon et al, 2003).

Downregulation of sPLA2-IID and sPLA2-V expression in human colon adenocarcinomas

A marked decrease in the expression level of PLA2G2D and PLA2G5 was observed in both left- and right-sided adenocarcinomas (Figures 2–4). These variations were also observed at the protein level by immunohistochemical analyses. Indeed, sPLA2-IID and sPLA2-V were found to be present in epithelial cells of normal mucosa, and their expression was markedly reduced in tumours (Figure 6). The decreased expression of sPLA2-IID and sPLA2-V in tumours may suggest a protective role of these sPLA2s, as it has been proposed for sPLA2-IIA in mice (MacPhee et al, 1995; Cormier et al, 1997). A similar decrease in expression of sPLA2-IID and sPLA2-V was also described in gastric tissues with signet-ring cell carcinoma (Masuda et al, 2005a). Interestingly, the PLA2G2A, PLA2G2C, PLA2G2D, PLA2G2E, PLA2G2F and PLA2G5 genes reside within the same region of human chromosome 1 at p35–36.1 (Valentin et al, 2000), a region frequently altered in colorectal cancer (Spirio et al, 1996). The decreased expression for PLA2G2D and PLA2G5 appears to be linked (cluster II, Figure 5) to that observed for PLA2G4A, ptgs1, SFRP-1 and RLPA-1, which have been proposed as tumour suppressor genes in colon cancer (Chulada et al, 2000; Shida et al, 2004; Suzuki et al, 2004; Dong et al, 2005).

Upregulation of PLA2G3 and Wnt target genes in human colon adenocarcinomas

A marked increase in sPLA2-III expression level was observed in both left- and right-sided adenocarcinomas, a finding that was confirmed by immunohistochemical analysis. No or very weak sPLA2-III expression was observed in normal epithelial cells, but a robust expression was observed in tumours (Figure 6). Our findings are consistent with the recent observation that sPLA2-III can trigger the proliferation of human colon cancer cells in vitro (Murakami et al, 2005). Parallel to the increase in PLA2G3 gene expression level, we observed an increase in the expression level of four target genes of the Wntβ-catenin/Tcf-4 pathway: II-8 (Levy et al, 2002), HD-6 (also called defensin z6) (Kolligs et al, 2002), MMP-7 (also called matrilysin) (Nelson et al, 2000) and EPR-1 (also called survivin) (Kolligs et al, 2002), as well as a concomitant dramatic decrease in SFRP-1 gene expression level. Therefore, our data are consistent with the activation of the Wntβ-catenin/Tcf-4 pathway in the development of colon adenocarcinomas. It will be of interest to determine whether the expression of PLA2G3 is related to the activation of the Wntβ-catenin/Tcf-4 pathway, as already suggested for PLA2G2A in gastric cancer (Aggarwal et al, 2006).

Our data further support the role of LPA in the pathogenesis of colon cancer. LPA exerts its effects through at least three different receptors: RLPA-1, RLPA-2 and RLPA-3 (Mills and Moolenaar, 2003). It has been demonstrated that the Wntβ-catenin/Tcf-4 pathway is involved in the proliferative effects of LPA through binding to RLPA-2 (Yang et al, 2005). We observed a decreased expression of RLPA-1 and a sustained expression of RLPA-2, which is in agreement with the predominant expression of RLPA-2 in adenocarcinomas (Shida et al, 2004). Whether sPLA2-III plays a role in the production of LPA, which in turn activates RLPA-2, remains to be established.

The sPLA2-III as a novel potential biomarker of human colon cancer

Because of their upregulation during colorectal carcinogenesis, Wnt target genes and their associated products have been examined for their potential use as biomarkers. The level of II-8 protein was found to be increased in colorectal cancer patients (Terada et al, 2005). A significant increase in the level of defensin z6 has also been found in patients with colon cancer (Nam et al, 2005). Although II-8 and defensin z6 levels have been recently proposed as markers of human colorectal cancer (Nam et al, 2005; Terada et al, 2005), our data show that they would detect only left-sided adenocarcinomas (Figures 3–5). In contrast, the increased expression of sPLA2-III was observed in both left and right colon adenocarcinomas, indicating that the analysis of sPLA2-III levels would detect cancers located in both left and right colon. MMP-7 is a matrix metalloprotease that has been associated with tumour invasion and metastasis (Nelson et al, 2000). We found that the expression pattern of PLA2G3 was more similar to that of MMP-7, which was also increased in both left and right colon adenocarcinomas (Figures 3–5).

In conclusion, this work is the first comprehensive analysis of the expression pattern of the full set of sPLA2s in human colon cancer. The distinct expression pattern observed for sPLA2 genes suggests that mRNA profiling of the full set of human sPLA2s may be useful to detect colon tumours either by analysing their expression pattern in tumours (Barrier et al, 2005), in circulating blood cells (Burczynski et al, 2005) or directly in serum on circulating mRNA, as recently proposed for other genes (Li et al, 2006). Moreover, we have observed a dramatic increase in sPLA2-III expression in both left- and right-sided adenocarcinomas, suggesting that sPLA2-III may represent a novel broad molecular biomarker of colon cancer. It will be of interest to determine whether the expression level of sPLA2-III also increases at earlier stages of tumorigenesis, including adenomas. It will also be useful to detect the sPLA2-III protein in human colon biopsies, stools or serum using the recently time-resolved fluoroimmunoassays developed for the different human sPLA2s (Nevalainen et al, 2003).
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