Biochemical and Biophysical Research Communications 445 (2014) 230–235

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Secreted phospholipases A_2 are differentially expressed and epigenetically silenced in human breast cancer cells

Vesna Brglez [a], Anja Pucer [a], Jože Pungerčar [a], Gérard Lambeau [b], Toni Petan [a,*]

[a] Department of Molecular and Biomedical Sciences, Jožef Stefan Institute, Ljubljana, Slovenia
[b] Institut de Pharmacologie Moléculaire et Cellulaire, CNRS et Université de Nice Sophia Antipolis, UMR 6097, Sophia Antipolis, Valbonne, France

ARTICLE INFO

Article history:
Received 27 January 2014
Available online 6 February 2014

Keywords:
DNA methylation
Epigenetics
Histone acetylation
Secreted phospholipase A_2
Lipid signalling
Breast cancer

ABSTRACT

Secreted phospholipases A_2 (sPLA$_2$s) have recently been associated with several cancers, but their role in breast cancer is unknown. Here we demonstrate that mRNA expression of group IIA, III and X sPLA$_2$s differs both *in vivo* in tumour biopsies and in breast cancer cells *in vitro*. Their expression is differentially regulated by DNA methylation and histone acetylation and, significantly, all three genes are silenced in aggressive triple negative cells due to both mechanisms. The transcription start site promoter region and the upstream CpG islands, exclusive to the group X sPLA$_2$ gene, have variable roles in the regulation of sPLA$_2$ expression. Our results suggest that the differential expression of hGIIA, hGIII and hGX sPLA$_2$s in breast cancer cells is a consequence of various degrees of epigenetic silencing due to DNA hypermethylation and histone deacetylation.

1. Introduction

Dysregulated expression of oncogenes and tumour suppressor genes in cancer is often the result of epigenetic modifications, particularly DNA methylation and histone modifications [1,2]. Aberrant DNA methylation of cytosines adjacent to guanines (CpG dinucleotides) is common in gene regulatory regions rich in CpG pairs, called CpG islands. Although altered DNA methylation in cancer has been mostly associated with hypermethylation of promoters, resulting in gene expression silencing, hypomethylation has also been observed [1,2]. Post-translational histone modifications, such as acetylation, constitute another set of epigenetic mechanisms that are a major contributor to tumourigenesis [1,2]. Histone acetylation/deacetylation cycles regulate the accessibility of DNA to transcription factors (TFs) and RNA polymerase II, thereby activating or repressing gene expression [1,2]. Importantly, epigenetic changes are reversible and thereby constitute a promising target for therapeutic intervention [1]. Inhibitors of DNA methylation, such as 5-aza-2′-deoxycytidine (decitabine, DAC), have a proven efficacy as anti-cancer drugs [1,2]. Several inhibitors of histone deacetylases (HDACi), including trichostatin A (TSA), induce differentiation and apoptosis in malignant cells [2]. Furthermore, DNA methylation is often associated with specific histone modifications that cooperatively modulate chromatin structure to alter gene expression and cell fate [1].

Secreted phospholipases A_2 (sPLA$_2$s)[1] hydrolyse the *sn*-2 ester bond of membrane glycerophospholipids to liberate free fatty acids and lysophospholipids [3]. They are secreted from various cells and act in an autocrine or paracrine manner on cell membranes and other extracellular phospholipids [3,4]. The eleven human sPLA$_2$ genes display different tissue expression patterns and are involved in lipid digestion, inflammatory and cardiovascular diseases, reproduction, host defence against infections and cancer [3,4]. Aberrant expression of human group IIA (hGIIA), III (hGIII) and X (hGX) sPLA$_2$s has been associated with the pathology of colorectal, breast, gastric, oesophageal, ovarian and prostate cancers, but the pro- or anti-tumourigenic role of a particular sPLA$_2$ seems to be dependent on cancer type [5]. Their role in tumourigenesis may be associated with, but is clearly not limited to, arachidonic acid release and stimulation of cyclooxygenase-2-dependent eicosanoid synthesis [5–7]. Although a link between sPLA$_2$s and breast cancer has not been established, increased levels of hGIIA sPLA$_2$ have been observed in

Abbreviations: DAC, 5-aza-2′-deoxycytidine; HDACi, histone deacetylase inhibitor; TF, transcription factor; TSA, trichostatin A; TSS, transcription start site.

* Corresponding author. Address: Department of Molecular and Biomedical Sciences, Jožef Stefan Institute, Jamova cesta 39, SI-1000 Ljubljana, Slovenia. Fax: +386 1 477 3594.
E-mail address: toni.petan@ijs.si (T. Petan).

[1] sPLA$_2$ enzymes are abbreviated with a lowercase letter indicating the species of origin (h, human) and with uppercase letters denoting the sPLA$_2$ group (GIIA, GIII and GX), encoded by genes *PLA2G2A*, *PLA2G3* and *PLA2G10*, respectively.

http://dx.doi.org/10.1016/j.bbrc.2014.01.182
0006-291X/

nipple aspirate fluids of breast cancer patients [8] and overexpression of the enzyme in breast tumours correlates with shorter disease-free and overall survival [9]. We have shown recently that hGX $sPLA_2$, but not the hGIIA enzyme, prevents starvation-induced cell death of invasive breast cancer cells through a novel mechanism involving alterations in fatty acid energy metabolism [10].

The aim of the present study was to determine the expression patterns of the full set of human $sPLA_2$s in tumour biopsies and in a panel of breast cancer cell lines, and to investigate the involvement of epigenetic mechanisms in the regulation of $sPLA_2$ expression. We have found that group IIA, III and X $sPLA_2$s are differentially expressed in breast cancer cells as a result of epigenetic silencing by DNA methylation and histone deacetylation. In highly tumourigenic triple negative cells both mechanisms act in concert to fully repress $sPLA_2$ expression. Our results suggest that different mechanisms of epigenetic regulation are responsible for hGIIA, hGIII and hGX gene expression silencing in various breast cancer cells.

2. Materials and methods

2.1. Materials

Cells (MDA-MB-231, T-47D, MCF7, SK-BR-3, MCF 10A) and culture media (RPMI-1640, MEM, McCoy's 5A) were obtained from ATCC (USA). Mammary epithelial cell growth medium (MEGM) was from Lonza (USA), and fetal bovine serum (FBS), Dulbecco's phosphate-buffered saline (DPBS), TrypLe Select and Opti-MEM from Life Technologies (USA). 5-aza-2′-deoxycytidine (DAC) and trichostatin A (TSA) were obtained from Sigma–Aldrich (USA). All other chemicals were of at least analytical grade and purchased from Sigma–Aldrich (USA) and Serva (Germany).

2.2. In silico analyses

The Oncomine platform (Compendia Bioscience, USA) was used for meta-analysis of DNA microarray datasets and visualisation of $sPLA_2$ gene expression in tumour biopsies [11]. The promoter region from 2000 bp upstream to 200 bp downstream of the putative transcription start site (TSS) of each $sPLA_2$ gene was analysed for the presence of CpGs and CpG islands using the MethPrimer software [12]. Putative TF binding sites in $sPLA_2$ promoter regions were identified using TFsearch (http://www.cbrc.jp/research/db/TFSEARCH.html) and TESS software (Transcription Element Search System; http://www.cbil.upenn.edu/cgi-bin/tess/tess).

2.3. Treatment of cells with DAC and TSA

Cell lines were cultured as described previously [10]. Cells were seeded in 6-well plates at a concentration of 1.5×10^5 cells/well and left to attach for 24 h. They were treated with either DAC (1 μM) for 72 h or TSA (200 nM) for 24 h. For combined treatment, cells were exposed to DAC (1 μM) for 72 h and to TSA (200 nM) for the last 24 h of the 72 h treatment. Due to the instability of DAC, media was replenished daily. Mock-treated cells were run in parallel with DPBS or dimethyl sulphoxide (DMSO) added as a control.

2.4. Real-time quantitative PCR (qPCR)

Total RNA extraction, first strand cDNA synthesis and qPCR were performed as described previously [10]. Primer sequences are listed in Table S1. For the basal expression experiment, C*t* values were rescaled to non-expressed genes, whose relative expression levels were assigned a value of 1, whereas for other experiments, they were rescaled to non-treated controls for each cell line.

2.5. Genomic DNA isolation and bisulphite modification

Genomic DNA (gDNA) was extracted from cells using the QIAamp DNA Mini Kit (Qiagen, Germany), and bisulphite modified using the EpiTect Bisulfite kit (Qiagen, Germany). PCR-amplified gDNA and gDNA, treated with the CpG methyltransferase M.SssI (New England Biolabs, USA), were used as fully unmethylated and fully methylated controls, respectively, and run in parallel.

2.6. Methylation-independent PCR (MIP) and sequencing

Bisulphite-modified gDNA was amplified by a touchdown standard or semi-nested PCR. Primer pairs are listed in Table S2 and the corresponding amplified regions shown in Fig. S1. Platinum *Taq* DNA polymerase High Fidelity (Life Technologies, USA) was used to amplify 20 ng bisulphite modified DNA or 1 μl of the first round PCR product for semi-nested reactions, with 250 nM of each set of primers and 5% DMSO. PCR products were gel-purified and sequenced using BigDye Version 3.1 chemistry (Eurofins MWG Operon, Germany). Fully methylated and non-methylated control samples were run in parallel. Methylation frequency at each CpG site was calculated as the ratio of C:T and G:A residues in the forward or reverse sequencing.

2.7. In vitro assay of $sPLA_2$ enzymatic activity using [^{3}H]-oleic acid-radiolabelled *Escherichia coli* membranes

$sPLA_2$ activity in cell supernatants was determined as described previously [10]. Samples (20 μl) were assayed at 37 °C for 24 h. Data were normalized to total protein content in cell lysates (660 nm Protein Assay, Thermo Scientific, USA).

2.8. Statistical analysis

Prism software (GraphPad Software, USA) was used for statistical analysis, using one-way ANOVA with Bonferroni adjustment for multiple comparisons. *p*-values below 0.05 were considered as statistically significant. All experiments were performed at least in duplicate.

3. Results

3.1. Several $sPLA_2$s are differentially expressed in human breast cancer and breast cancer cells

In order to determine whether $sPLA_2$ expression is altered in breast cancer, *in silico* analysis of microarray data from human primary tumours was performed using Oncomine [11]. We found that the expression of genes encoding hGIIA, hGIII and hGX $sPLA_2$s (*PLA2G2A*, *PLA2G3* and *PLA2G10*, respectively) in breast tumour biopsies differs from that in normal tissues (Table S3 and references therein). There were no significant differences in the expression of the other human $sPLA_2$ genes (data not shown). Oncomine data on *PLA2G2A* is conflicting with some studies pointing to up- and other to down-regulation (Table S3). *PLA2G3* and *PLA2G10* were, on the other hand, clearly up-regulated in breast cancer according to several studies (Table S3). However, the expression of *PLA2G10* was lower in triple negative samples relative to other tumours, and in basal relative to luminal tumours, both in biopsies and cultured cells (Table S3), suggesting that *PLA2G10* expression is dependent on the subtype of breast cancer [13].

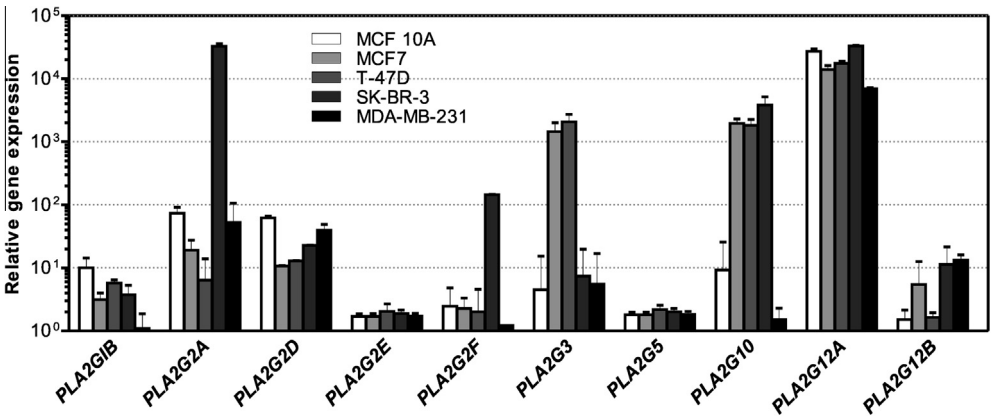

Fig. 1. The mRNA expression levels of sPLA$_2$s differ between mammary cell lines. Total RNA was isolated and the relative expression levels of the whole set of human sPLA$_2$s were determined by qPCR. Values on the graphs are means ± SD of three isolations.

We next chose five model cell lines, which differ in their phenotype and genotype and represent different types of breast cancer (Table S4), to study the expression and epigenetic regulation of sPLA$_2$s. Of the 10 human sPLA$_2$ genes analysed, *PLA2G2A, PLA2G3* and *PLA2G10* were differentially expressed in the cell lines, whereas the expression of other sPLA$_2$s was very low or did not differ between the cell lines (Fig. 1). The *PLA2G2A* gene was highly expressed only in the HER2-positive SK-BR-3 cells, while high *PLA2G3* mRNA levels were observed only in the luminal-like MCF7 and T-47D cells. The expression of *PLA2G10* mRNA was high in all luminal-like cells in comparison with that determined in the basal-like cell lines, which is in accordance with the *in vivo* and *in vitro* Oncomine data (Table S3). The expression of several genes functionally related to sPLA$_2$s also differed among cell lines (Fig. S2), but no correlation with the expression of sPLA$_2$s was found.

Thus, mRNA expression of hGIIA, hGIII and hGX sPLA$_2$s differs both *in vivo* in tumour biopsies and *in vitro* in cancer cell line models.

3.2. DNA methylation is involved in sPLA$_2$ gene silencing in breast cancer cells

To determine whether epigenetic mechanisms are involved in the regulation of sPLA$_2$ expression in breast cancer cells, we first assessed the importance of DNA methylation. Cell treatment with DAC, a potent inhibitor of DNA methyltransferases [1], led to an increase in hGIIA, hGIII and hGX sPLA$_2$ mRNA levels in several cell lines (Fig. 2), confirming the involvement of DNA methylation-driven epigenetic silencing and suggesting that the promoters of the three differentially expressed sPLA$_2$-encoding genes are hypermethylated in several breast cancer cell lines.

3.3. Proximal promoters of genes encoding hGIIA, hGIII and hGX sPLA$_2$s are differentially methylated in breast cancer cells

The increased expression of sPLA$_2$ genes on DAC treatment could be a consequence of DAC-induced re-expression of some other genes [1]. To confirm the direct involvement of DNA methylation in the regulation of sPLA$_2$ expression in breast cancer cells, we assessed the methylation level of sPLA$_2$ gene promoters. Potential methylation sites in the promoters of *PLA2G2A*, *PLA2G3* and *PLA2G10* were identified by an *in silico* analysis (Fig. S1). Those found in the vicinity of the putative TSS of each gene, as well as in the two CpG islands exclusive to *PLA2G10*, were further analysed by methylation-specific high resolution melt (Fig. S3) and bisulphite sequencing (Fig. 3). We found that the basal methylation level in the TSS region of the *PLA2G2A* gene, and its reduction upon DAC treatment, is highest in MCF 10A cells (Figs. 3A and S3A). A significant reduction in methylation frequency upon DAC treatment ($p < 0.05$) was observed at a CpG site located within the putative binding sites for several TFs (Fig. 3A). The average basal methylation level in the TSS region of the *PLA2G3* promoter was high and comparable in all cell lines (Figs. 3B and S3B). Three CpG sites in MCF 10A cells, overlapping with the putative binding sites for several TFs, displayed a reduction ($p < 0.05$) in methylation frequency upon DAC treatment. Interestingly, while the basal methylation

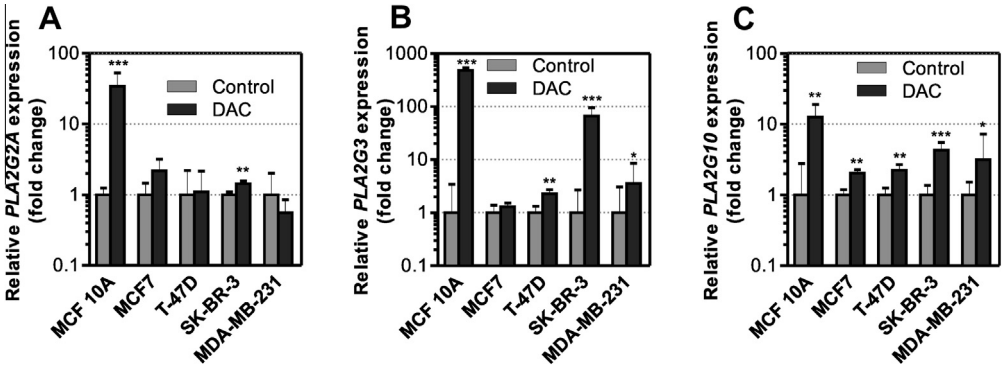

Fig. 2. Distinct patterns of DAC-induced stimulation of *PLA2G2A* (A), *PLA2G3* (B) and *PLA2G10* (C) mRNA expression. Cells were treated with 1 μM DAC for 72 h. Total RNA was isolated and the relative *PLA2G2A*, *PLA2G3* and *PLA2G10* mRNA expression levels were determined by qPCR. Values on the graphs are means ± SD of three experiments and statistically significant changes (relative to untreated controls) are indicated (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; one-way ANOVA with Bonferroni adjustment).

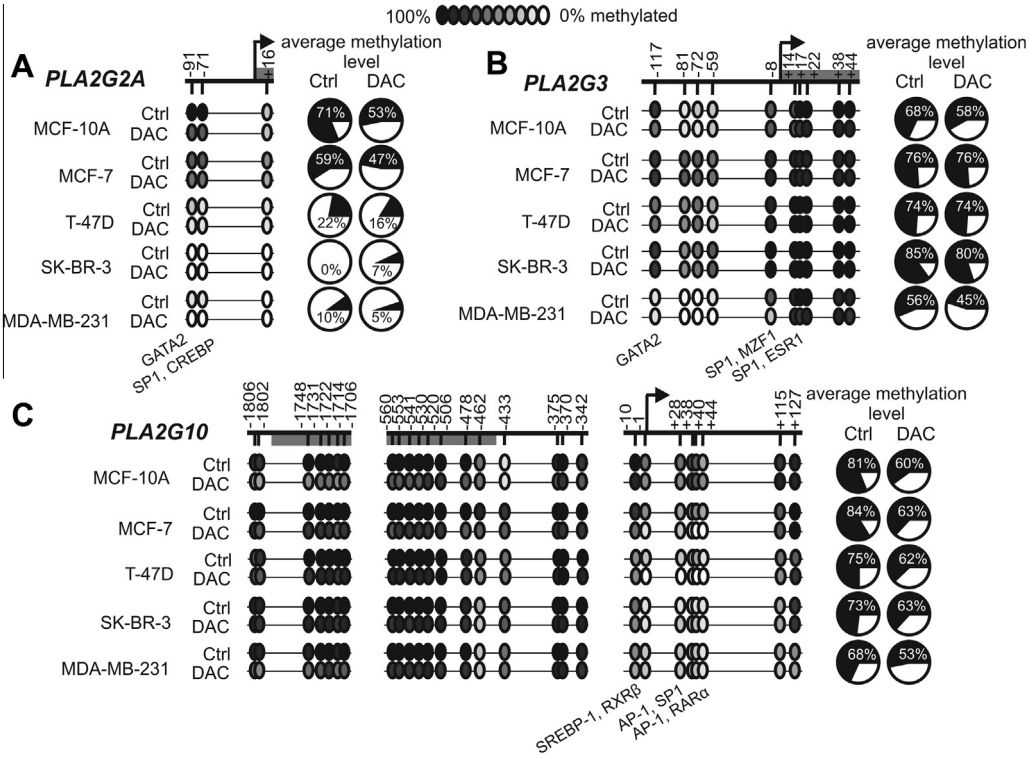

Fig. 3. Methylation frequencies in *PLA2G2A*, *PLA2G3* and *PLA2G10* promoter regions are altered upon DAC-treatment. Genomic DNA was bisulphite modified, amplified by MIP, agarose gel-purified and sequenced. Methylation frequency at each CpG site was calculated as the ratio of C:T and G:A residues in the forward or reverse sequencing. Arrows point to the putative transcription start sites. Grey areas represent CpG islands. Vertical bars and colour-coded circles indicate individual CpG sites. Black and white circles correspond to maximal and minimal frequency of methylation, respectively. Putative transcription factors binding sites overlapping the differentially methylated CpGs are shown. Values on the graphs are means of two experiments.

levels of the two CpG islands in the *PLA2G10* promoter (Fig. S1) were high in all breast cancer cells (Figs. 3C and S3D), the TSS region of the gene was highly heterogeneous in terms of methylation frequency (Figs. 3C and S3C). In the luminal-like cells, the majority of CpG sites displaying a significant DAC-induced reduction in methylation ($p < 0.05$) are in the vicinity of the TSS and correspond to putative binding sites for several lipid metabolism-associated TFs, including sterol regulatory element-binding protein 1 (SREBP-1), retinoic acid receptor alpha (RARα) and retinoid X receptor beta (RXRβ). In contrast to the luminal-like cells, a significant reduction in methylation frequency in the basal-like cells was observed within the CpG islands, but not in the TSS region of the *PLA2G10* promoter. In accordance, the −500 CpG island was not important for *PLA2G10* transcription in luminal-like SK-BR-3 cells (Fig. S4).

3.4. $sPLA_2$ genes are additionally epigenetically repressed by histone acetylation

Since restoring basal DNA methylation was not sufficient to increase mRNA expression of hGIIA, hGIII and hGX $sPLA_2$s, especially in MDA-MB-231 cells (Fig. 2), we next sought whether histone acetylation is responsible for their differential silencing in breast cancer cells. Interestingly, after treatment with the potent HDACi TSA, the most prominent increase in mRNA levels of all three $sPLA_2$s was observed in MDA-MB-231 cells (Fig. 4). The TSA treatment had no effect on $sPLA_2$ expression in the non-tumourigenic MCF 10A cells (Fig. 4), but it increased *PLA2G2A* and *PLA2G10* expression in all other cell lines.

Since both DNA methylation and histone deacetylation can act synergistically to repress gene expression in cancer [1], we next determined the levels of $sPLA_2$ mRNA after treatment with both DAC and TSA. Interestingly, the treatment did not affect the expression of any $sPLA_2$ in MCF 10A cells, but led to significant increases in the expression of hGIIA and hGX in the luminal-like cells and of all three $sPLA_2$s in MDA-MB-231 cells (Fig. 4). Thus, histone acetylation affects $sPLA_2$ expression primarily in tumorigenic breast cancer cells, but not in the non-tumorigenic MCF 10A cells.

Since $sPLA_2$s are produced in small quantities and are secreted from the cell, resulting in dilution of the enzyme, it is difficult to detect endogenously expressed $sPLA_2$ proteins in cell lines and tissues [3–5]. To confirm that the increase in $sPLA_2$ mRNA expression upon treatment with DAC and TSA corresponds to increased $sPLA_2$ protein levels, we used two sensitive and specific $sPLA_2$ assays, the time-resolved fluoroimmunoassay (TRFIA; Fig. S5) and the radioactive enzymatic assay (Figs. 4D and S6). We found that the treatment with DAC and TSA leads to increased secretion of active hGIIA protein from SK-BR-3 cells (Figs. 4D and S6). However, using both assays, we could not detect hGIIA, hGIII and hGX $sPLA_2$ proteins in the rest of the cell lines, suggesting that the released amounts of these $sPLA_2$s were below the limit of detection (data not shown). Nonetheless, the determination of hGIIA protein in SK-BR-3 cells provides a proof of principle that DAC- and TSA-induced $sPLA_2$ mRNA expression likely results in expression of the corresponding $sPLA_2$ protein in breast cancer cells.

4. Discussion

The mechanisms underlying aberrant $sPLA_2$ expression in various cancers [5] are poorly understood, constituting a major obstacle in the understanding of their role in cancer. Apart from a few studies [8,9], the expression of $sPLA_2$s in normal breast tissue and cancer cells has not been investigated. We demonstrate here that the mRNA expression of $sPLA_2$s, namely the group IIA, III

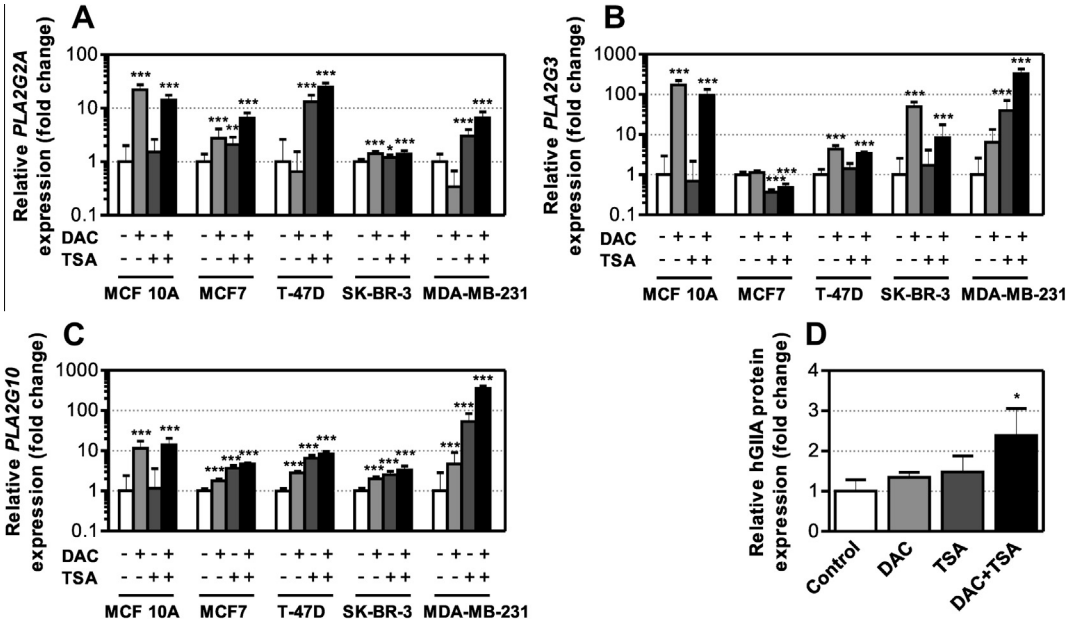

Fig. 4. Histone acetylation and DNA methylation regulate $sPLA_2$ expression in breast cancer cells. Cells were treated with 1 μM DAC for 72 h and/or 200 nM TSA for 24 h. Total RNA was isolated and the relative expression levels of *PLA2G2A* (A), *PLA2G3* (B) and *PLA2G10* (C) were determined by qPCR. For protein quantification (D), $sPLA_2$ enzymatic activity in the culture medium of SK-BR-3 cells was determined with *in vitro* enzymatic assay using [^{3}H]-oleic acid-radiolabelled *E. coli* membranes. Values on the graphs are means ± SD of three experiments and statistically significant changes (relative to untreated controls) are indicated (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; one-way ANOVA with Bonferroni adjustment).

and X $sPLA_2$s, differs *in vivo* in tumour biopsies and *in vitro* in breast cancer cell lines, and that both DNA methylation and histone acetylation are involved in the regulation of their gene expression in the latter.

Abnormal patterns of DNA methylation have been well documented for a number of genes in different types of cancer [1]. Although data on epigenetic regulation of $sPLA_2$s in cancer are limited, it has been shown that hGIIA is silenced by DNA methylation in several gastric cancer cell lines [14], Jurkat and U937 leukaemia [15] and DU-145 prostate cancer cells [16]. We show here that hGIIA, hGIII and hGX $sPLA_2$s are differentially expressed in breast cancer cells *in vivo* and *in vitro*, and are silenced by DNA hypermethylation in breast cancer cell lines (Table S1 and Figs. 1 and 2). Interestingly, the average level of *PLA2G2A* promoter methylation (Figs. 3A and S3A) was inversely proportional to tumourigenicity of the cell lines (Table S4). This is in accordance with recent findings showing that *PLA2G2A* is hypomethylated in invasive relative to non-invasive breast cancer cells [17], in lung tumours relative to normal lung [18] and in mouse intestinal adenoma [19]. On the other hand, the methylation levels of the *PLA2G3* and *PLA2G10* promoter were comparable in all cell lines (Figs. 3 and S3) and did not correlate with basal mRNA expression (Fig. 1) or response to DAC (Fig. 2B). Interestingly, the DAC-induced reduction of methylation in the CpG islands and the putative TSS region of *PLA2G10* were different in basal- and luminal-like cells (Figs. 3C, S3C and S3D). Along with the observed subtype specific expression of *PLA2G10* mRNA (Fig. 1 and Table S4), this suggests a differential regulation of *PLA2G10* expression in luminal- and basal-like breast cancer cells.

So far, only one study has investigated the involvement of histone acetylation in $sPLA_2$ gene expression [20] and $sPLA_2$s have been found incidentally as hits in microarray analyses assessing gene expression changes in HDACi-treated cancer cells [21,22]. We show here that the HDACi TSA induces the expression of hGIIA, hGIII and hGX $sPLA_2$s in breast cancer cells (Figs. 4, S5 and S6) and that histone deacetylation is important for $sPLA_2$ gene expression silencing in tumourigenic cells, particularly in the most aggressive MDA-MB-231 cells. A combined DAC and TSA treatment resulted in a synergistic increase in the expression of all three $sPLA_2$s in these cells (Fig. 4), confirming that both histone deacetylation and DNA methylation are responsible for the low $sPLA_2$ expression. Our results suggest that the differential expression of $sPLA_2$s in breast cancer cells can be attributed, at least in part, to varying degrees of epigenetic regulation: in non-tumourigenic basal-like cells, DNA methylation appears to play a dominant role for all $sPLA_2$s, in the weakly and moderately tumourigenic luminal-like cell lines the effects of both mechanisms on the expression of a particular $sPLA_2$ are heterogenous, whereas in the highly tumourigenic basal-like cells histone deacetylation dominates over DNA methylation.

We have shown recently that exogenously added hGX $sPLA_2$, but not the hGIIA enzyme, induces lipid droplet formation and prevents starvation-induced apoptosis in MDA-MB-231, but not in MCF 10A cells, suggesting that the pro-tumourigenic effect of the enzyme is typical for invasive breast cancer cells [10]. In accordance, hGX $sPLA_2$ had a weak negative effect on the viability of non- and weakly tumourigenic cells, but increased the viability of highly tumourigenic cells (Fig. S7). The differential epigenetic regulation of $sPLA_2$ expression in basal- and luminal-like mammary cells reported here is in line with a differential, cell type-dependent role for $sPLA_2$s in cancer. This may explain the contradictory reports and difficulty in assigning a clear role for a particular $sPLA_2$ in various cancers. For example, the role of GIIA $sPLA_2$ in colon cancer is still controversial, and several groups have found opposing effects on tumour progression, suggesting that GIIA may suppress carcinogenesis at early stages of tumour development, while promoting it at later stages [23,24]. Given that the biological roles of $sPLA_2$s are diverse and depend on the enzyme studied, its expression level and the tissue involved, the differential epigenetic regulation, shown here for breast cancer cells, adds another layer of complexity to their role in cancer and human (patho)physiology in general.

In summary, we show in this study that hGIIA, hGIII and hGX $sPLA_2$s are differentially expressed in breast tumours and cell lines, with high expression in luminal-like cells and very low in basal-like cells. The differential expression of $sPLA_2$s in breast cancer

cells is associated with a differential role of DNA methylation and histone deacetylation, the former being the major cause of transcriptional repression in non-tumourigenic cells and the latter in highly tumourigenic cells, while both mechanisms affect $sPLA_2$ expression to varying degrees in weakly and moderately tumourigenic cell lines. The different DNA methylation patterns and the varying importance of DNA methylation and histone deacetylation mechanisms suggest a cell type-dependent epigenetic regulation of $sPLA_2$ expression in normal and neoplastic mammary cells.

Acknowledgments

We are grateful to Dr. Roger H. Pain for critical reading of the manuscript, Dr. Borut Jerman for preparation of recombinant hGX, Petra Kaferle for assistance with qPCR data analysis and statistics, and Christine Payré for preparation of radiolabelled *E. coli* membranes and technical help with *E. coli* assay and TRFIA. This work was supported by grant P1-0207 from the Slovenian Research Agency, by the French-Slovene partnership project BI-FR/12-13-PROTEUS-006, and by CNRS, the Association for International Cancer Research and the Fondation ARC pour la recherche sur le cancer to G.L.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.182.

References

[1] M.A. Dawson, T. Kouzarides, Cancer epigenetics: from mechanism to therapy, Cell 150 (2012) 12–27.
[2] R. Connolly, V. Stearns, Epigenetics as a therapeutic target in breast cancer, J. Mammary Gland Biol. Neoplasia 17 (2012) 191–204.
[3] G. Lambeau, M.H. Gelb, Biochemistry and physiology of mammalian secreted phospholipases A2, Annu. Rev. Biochem. 77 (2008) 495–520.
[4] M. Murakami, G. Lambeau, Emerging roles of secreted phospholipase A2 enzymes: an update, Biochimie 95 (2013) 43–50.
[5] K.F. Scott, M. Sajinovic, J. Hein, et al., Emerging roles for phospholipase A2 enzymes in cancer, Biochimie 92 (2010) 601–610.
[6] Y. Morioka, M. Ikeda, A. Saiga, et al., Potential role of group X secretory phospholipase A2 in cyclooxygenase-2-dependent PGE2 formation during colon tumorigenesis, FEBS Lett. 487 (2000) 262–266.
[7] F. Surrel, I. Jemel, E. Boilard, et al., Group X phospholipase A2 stimulates the proliferation of colon cancer cells by producing various lipid mediators, Mol. Pharmacol. 76 (2009) 778–790.
[8] F. Mannello, W. Qin, W. Zhu, et al., Nipple aspirate fluids from women with breast cancer contain increased levels of group IIa secretory phospholipase A2, Breast Cancer Res. Treat. 111 (2008) 209–218.
[9] S. Yamashita, J. Yamashita, M. Ogawa, Overexpression of group II phospholipase A2 in human breast cancer tissues is closely associated with their malignant potency, Br. J. Cancer 69 (1994) 1166–1170.
[10] A. Pucer, V. Brglez, C. Payré, et al., Group X secreted phospholipase A2 induces lipid droplet formation and prolongs breast cancer cell survival, Mol. Cancer 12 (2013) e111.
[11] D.R. Rhodes, S. Kalyana-Sundaram, V. Mahavisno, et al., Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles, Neoplasia 9 (2007) 166–180.
[12] L.C. Li, R. Dahiya, MethPrimer: designing primers for methylation PCRs, Bioinformatics 18 (2002) 1427–1431.
[13] R.M. Neve, K. Chin, J. Fridlyand, et al., A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes, Cancer Cell 10 (2006) 515–527.
[14] K. Ganesan, T. Ivanova, Y. Wu, et al., Inhibition of gastric cancer invasion and metastasis by PLA2G2A, a novel beta-catenin/TCF target gene, Cancer Res. 68 (2008) 4277–4286.
[15] M. Menschikowski, A. Hagelgans, H. Kostka, et al., Involvement of epigenetic mechanisms in the regulation of secreted phospholipase A2 expressions in Jurkat leukemia cells, Neoplasia 10 (2008) 1195–1203.
[16] M. Menschikowski, A. Hagelgans, E. Gussakovsky, et al., Differential expression of secretory phospholipases A2 in normal and malignant prostate cell lines: regulation by cytokines, cell signaling pathways, and epigenetic mechanisms, Neoplasia 10 (2008) 279–286.
[17] D.I. Rodenhiser, J. Andrews, W. Kennette, et al., Epigenetic mapping and functional analysis in a breast cancer metastasis model using whole-genome promoter tiling microarrays, Breast Cancer Res. 10 (2008) R62.
[18] H.H. Nelson, C.J. Marsit, B.C. Christensen, et al., Key epigenetic changes associated with lung cancer development: results from dense methylation array profiling, Epigenetics 7 (2012) 559–566.
[19] C. Grimm, L. Chavez, M. Vilardell, et al., DNA-methylome analysis of mouse intestinal adenoma identifies a tumour-specific signature that is partly conserved in human colon cancer, PLoS Genet. 9 (2013) e1003250.
[20] Y. Miki, S. Mukae, M. Murakami, et al., Butyrate inhibits oral cancer cell proliferation and regulates expression of secretory phospholipase A2-X and COX-2, Anticancer Res. 27 (2007) 1493–1502.
[21] S. Claerhout, J.Y. Lim, W. Choi, et al., Gene expression signature analysis identifies vorinostat as a candidate therapy for gastric cancer, PLoS One 6 (2011) e24662.
[22] C.S. Ward, P. Eriksson, J.L. Izquierdo-Garcia, et al., HDAC inhibition induces increased choline uptake and elevated phosphocholine levels in MCF7 breast cancer cells, PLoS One 8 (2013) e62610.
[23] R.J. Fijneman, J.R. Peham, M.A. van de Wiel, et al., Expression of Pla2g2a prevents carcinogenesis in Muc2-deficient mice, Cancer Sci. 99 (2008) 2113–2119.
[24] G.S. Belinsky, T.V. Rajan, E.A. Saria, et al., Expression of secretory phospholipase A2 in colon tumor cells potentiates tumor growth, Mol. Carcinog. 46 (2007) 106–116.