Silibinin Down-regulates Expression of Secreted Phospholipase A_2 Enzymes in Cancer Cells

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Abstract. *Background: Silibinin, a naturally-occurring flavonoid produced by milk thistle, possesses antioxidant, anti-inflammatory and cancer-preventive activities. In the current study, we examined the effects of silibinin on the expression of secreted phospholipase A_2 ($sPLA_2$) enzymes, especially those of group IIA (hGIIA), which play a crucial role in inflammation and carcinogenesis. Materials and Methods: The effects of silibinin on $sPLA_2$ expressions in human HepG2 hepatoma and PC-3 prostate cancer cells were analyzed using quantitative reverse transcription-polymerase chain reaction and enzyme linked immunosorbent assay technique. Results: Silibinin inhibited the expression of hGIIA in unstimulated and cytokine-primed HepG2 and PC-3 cells. The mRNA levels of $sPLA_2$ of groups IB, III and V were also significantly decreased by silibinin. Analyses of transcription factor activation suggest that nuclear factor-κB, but not specificity protein 1 (SP1) is implicated in the silibinin-mediated down-regulation of hGIIA. Conclusion: Silibinin exhibits inhibitory effects on basal and cytokine-induced expression of $sPLA_2$s in cancer cells and therefore, may have the potential to protect against up-regulation of hGIIA and other $sPLA_2$ isoforms during inflammation and cancer.*

Secreted phospholipase A_2 ($sPLA_2$) plays crucial roles in a number of relevant physiological processes including defence mechanisms, production of bioactive lipids, and cell signaling (12). Among different isoforms, $sPLA_2$ of group IIA (hGIIA) is characterized as an acute-phase reactant contributing to the pathogenesis of diverse inflammatory diseases and cancer (2-4). Levels of this enzyme are strongly increased, not only in cancer-derived cell lines and cancer specimens, but also in serum and plasma samples from patients with cancer [for review see (4, 5)]. For this reason, serum levels of hGIIA are considered as prognostic marker in patients with different types of malignancies, such as prostate, breast, lung, bile duct, stomach, liver, oesophagus, colon, and pancreatic cancer (4, 6). Elevated serum levels of hGIIA in patients with inflammatory diseases and cancer are thought mainly to originate from liver cells (3, 7, 8). In comparison to group IIA, the pathophysiological aspects of other $sPLA_2$ isoforms, such as those of groups IB, III and V, are less known, but it was suggested that also they play a role in tumorigenesis and, therefore, represent important targets for anticancer therapy (4, 9).

Silibinin, a naturally-occurring flavonoid produced by milk thistle, possesses strong antioxidant, antiproliferative, anti-inflammatory, and anticancer activities (10-12). Silibinin inhibits multiple cancer cell signaling pathways related to proliferation, angiogenesis, epithelial-to-mesenchymal transition, invasion and metastasis (12). Suppression of pro-inflammatory signaling has been suggested as mechanisms explaining the silibinin-induced down-regulation of cytokines, inducible nitric oxide synthase and cyclooxygenase-2 expression during inflammation, oxidative stress and cancer (13-15). However, the effect of silibinin on the expression of hGIIA and other $sPLA_2$ enzymes remains unexplored.

In the present study, we describe for the first time, to our knowledge, the inhibitory effects of silibinin on the basal and cytokine-induced expression of hGIIA and other isoforms of $sPLA_2$ in HepG2 and PC-3 cancer cells.

Materials and Methods

Chemicals. Recombinant human interleukin-1β (IL1β), interleukin-6 (IL6), tumor necrosis factor-α (TNFα), and interferon-γ (IFNγ) were purchased from Roche Diagnostics Applied Science (Mannheim, Germany). Silibinin, mithramycin A, and caffeic acid phenethyl ester (CAPE) were obtained from Sigma-Aldrich (Deisenhofen, Germany).

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Cell culture and incubation. Human HepG2 hepatoma and PC-3 prostate cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA) and the German Collection of Microorganisms and Cell Cultures (Berlin, Germany), respectively. Cells were cultured in a standard cell culture medium RPMI-1640 supplemented with 10% fetal calf serum (FCS), as described previously (16, 17).

To analyze cell proliferation, HepG2 cells were plated in 96-well plates with a density of 5×10^3 cell/well. After attachment to the plate, cells were incubated in FCS-free RPMI-1640 medium containing different doses of silibinin for 48 h. Cell proliferation was determined by TACS™ XTT assay using the Cell Proliferation/Viability Assay kit (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany). The extinction of reduced XTT was measured at 490 nm.

The long-term effect of silibinin on cell growth was assessed using colony-forming activity test. For this purpose, HepG2 cells were plated in 6-well plates with a density of 500 cells/well. After attachment, cells were treated with different doses of silibinin and the cell medium was changed every three days. At the end of the eighth day, cells were washed with ice cold phosphate buffered saline, stained with crystal violet (CV) solution (0.5% CV in 30% ethanol and 3% formaldehyde) for 10 min, washed four times with water and finally dried. Colonies with more than 30 cells were counted under light microscopy.

By study of recombinant $sPLA_2$ enzymes IB, IIA, and V effects on cell proliferation, HepG2 cells were incubated for 48 h in 96 well plates containing 100 ng/ml $sPLA_2$ in FCS-free RPMI-1640 medium. Cell proliferation was determined by TACS™ XTT assay as described above. In case of bee venom $sPLA_2$-III, cell growth was determined by the CV staining. After washing and drying, cells were lysed with 1% solution of sodium dodecyl sulfate and the CV extinction was measured at 550 nm.

RNA extraction and quantitative real-time quantitative polymerase chain reaction analysis. RNA was isolated after lysis of cells in TRI Reagent (Sigma-Aldrich, Deisenhofen, Germany) according to the manufacturer's instructions. Isolated RNA was converted to cDNA using the GeneAmp RNA-PCR Kit (PerkinElmer LAS GmbH, Jügesheim, Germany). The reverse transcription reaction products were then amplified with polymerase chain reaction (PCR) for the identification of $sPLA_2$ isoforms and 40S ribosomal protein S14 (*RPS14*) as a reference gene. The applied primer pairs were for *RPS14* (143 bp): 5'-GGC AGA CCG AGA TGA ATC CTC A-3' (forward, f) and 5'-CAG GTC CAG GGG TCT TGG TCC-3' (reverse, r); for *PLA2G3* (250 bp): 5'-CTT CAG AGT CAG TGG GAG GC-3' (f)' and 5'-AGG GTG AGA TGT TCT GTG GG-3' (r); for *PLA2G5* (444 bp): 5'-AGG GAA GAA CGC CCT GAC AAA-3' (f) and 5'-CGT AGG TTT CTC TTG AGG CAG T-3' (r). For *PLA2G1B* and *PLA2G2A*, primers were used as described previously (18). Real-time PCR was performed on the Rotor-Gene Q in combination with QuantiTect SYBR Green RT-PCR kit (Qiagen, Hilden, Germany).

Immunoassays. Levels of released cellular hGIIA were determined using specific enzyme linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Cayman Chemicals, MI, USA). Levels of hGIIA were normalized to cell protein content, which was determined using bicinchoninic acid assay kit with bovine serum albumin as an internal standard (Sigma-Aldrich). For determination of transcription factor activation, HepG2 cells were cultured in 96-well microplates. Activation of nuclear factor-κB (NFκB) and specificity protein 1 (SP1) were quantified using cell-based ELISA technique; NF-κB p65 profiler kit (Active Motif, Carlsbad, CA, USA) and Phospho-SP1 kit (Immunoway Biotechnology Company, Newark, DE, USA), respectively.

Data analysis. For comparison of two groups, data were analyzed by two-tailed and unpaired Student's *t*-test. Multiple comparisons *versus* the control group were carried out using one-way analysis of variance, Holm-Sudak method. All statistical analyses were performed using the statistics module integrated in the SigmaPlot11 software (Systat Software GmbH, Erkrath, Germany). Differences were considered significant at *p*-values less than 0.05.

Results

In clonogenic assay, silibinin exerted a dose-dependent inhibitory effect on the growth of HepG2 cells (Figure 1A). Similar suppressive effect of silibinin on HepG2 cell proliferation was observed using the XTT assay, in which the exposure of HepG2 cells to silibinin led to a decreased cell growth with a half-maximal effect at the concentration of 40 μM silibinin (Figure 1B). In contrast to silibinin, human recombinant hGIIA, hGIB, hGV, and bee venom $sPLA_2$-III significantly increased HepG2 cell proliferation (Figure 1C). These data were reproduced using the clonogenic assay (data not shown), suggesting that silibinin and $sPLA_2$ enzymes exert opposing effects on the growth of HepG2 cells.

According to the results of real-time quantitative polymerase chain reaction (RT-qPCR), the highest mRNA levels of $sPLA_2$ isoforms were found in the case of hGIIA, whereas transcripts of hGIB, hGIII, and hGV were detected in lower amounts in HepG2 cells cultured in FCS-supplemented medium (Figure 2). Taking the level of hGIIA mRNA as 1.0, the corresponding values for hGIB, hGIII, and hGV were 0.145, 0.018, and 0.003, respectively. The constitutive expression of all $sPLA_2$ enzymes was significantly down-regulated by 100 μM silibinin (Figure 2).

To study the expression of the $sPLA_2$ enzymes under pro-inflammatory conditions, HepG2 cells were cultured in FCS-deprived medium containing a cytokine mixture (CM) of IFNγ, IL1β, TNFα, and IL6. The treatment of cells with the CM resulted in increased transcription of hGIIA and hGV, whereas levels of hGIB and hGIII decreased significantly or remained unchanged, respectively (Figure 3). The transcript levels of all studied $sPLA_2$ enzymes were strongly reduced by silibinin in a dose-dependent manner (Figure 3). The suppressive effect of silibinin on hGIIA expression was also demonstrated at the protein level (Figure 4A). In this experiment, the expression of hGIIA in HepG2 cells was up-regulated by treatment with 25 ng/ml IFNγ, which had a strong inducing effect on hGIIA expression in different cell lines (17). As shown in Figure 4A, silibinin effectively reduced the protein expression of hGIIA in HepG2 cells in a dose-dependent manner.

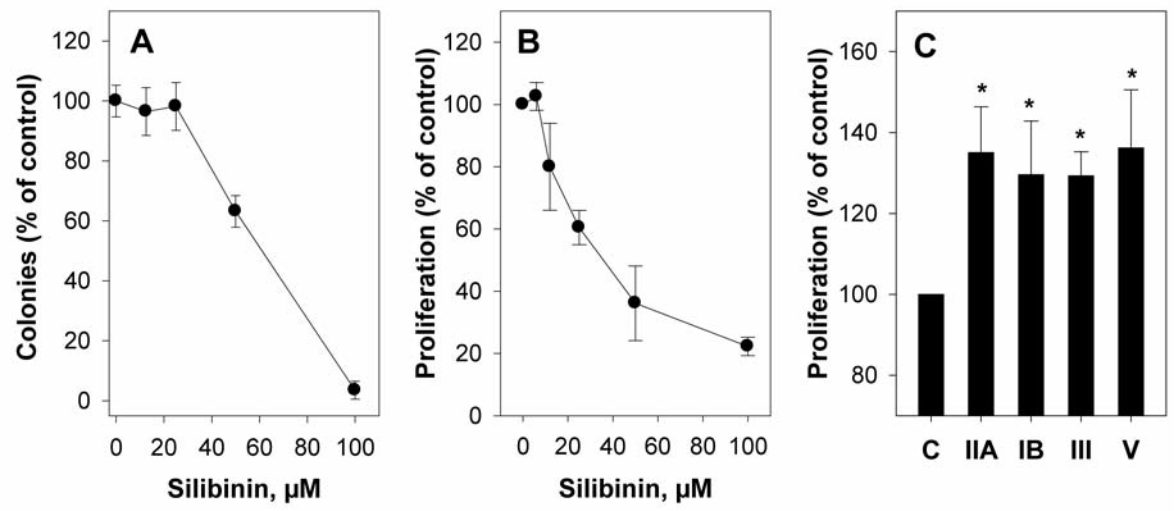

Figure 1. *Effects of silibinin and human recombinant phospholipase A_2 isoforms on the growth of HepG2 hepatoma cells. A: Colony-forming activity of HepG2 cells was measured as described in Materials and Methods section. The number of colonies in the untreated control was set to a value of 100%. The data shown are the means±SD of analyses in triplicate and are representative of two independent experiments. B: HepG2 cell proliferation was determined using the TACSTM XTT assay. The proliferation in the untreated control represents a value of 100%. The data shown are the means±SD of analysis in quadruplicate and are representative of two independent experiments. C: Human recombinant $sPLA_2$ enzymes of groups IB, IIA, and V and bee venom $sPLA_2$-III were added at final concentrations of 100 ng/ml for 48 h. Cell proliferation was assayed by TACSTM XTT assay (hGIIA, hGIB and hGV) or crystal violet staining ($sPLA_2$-III). Data shown are the means±SD of analysis in quadruplicate and are representative of three independent experiments. *Significantly different from the control at $p<0.05$.*

To study cell signaling pathways involved in these silibinin-mediated effects, HepG2 cells primed with IFNγ were treated with selective pharmacological inhibitors. Down-regulation of the IFNγ-induced hGIIA expression in HepG2 cells was observed with CAPE, a natural inhibitor of NF-κB activation, both at the protein (Figure 4A) and mRNA levels (data not shown). Treatment of HepG2 cells with mithramycin A, an inhibitor of SP1 binding to promoter, induced a down-regulation of hGIIA expression at mRNA (Figure 5A) and protein levels (data not shown)

The analysis of NF-κB p65 phosphorylation at Ser536 showed that treatment of HepG2 cells with 100 μM silibinin reduced NF-κB p65 phosphorylation after 30 min (not shown) and this suppressive effect of silibinin on NF-κB activation was maintained for up to 24 h (Figure 4B). A similar decrease of p65 phosphorylation was caused by 50 μM CAPE. In contrast to NF-κB p65 phosphorylation, levels of phosphorylated SP1 were similar in control and silibinin-treated HepG2 cells (Figure 5B). These results exclude SP1 as a possible mediator of silibinin-induced hGIIA down-regulation.

To examine whether the suppressive effect of silibinin on hGIIA expression is limited to hepatoma cells, the effect of silibinin was also analyzed in PC-3 prostate cancer cells. The basal expression of hGIIA was ~500-fold lower in PC-3 cells in comparison to HepG2 cells and the treatment of PC-3 cells with IFNγ resulted in a 30-fold increase of hGIIA protein levels (Figure 6). Silibinin dose-dependently reduced the basal and IFNγ induced hGIIA protein levels in PC-3 cells (Figure 6) similarly to HepG2 cells (Figure 2 and 4A).

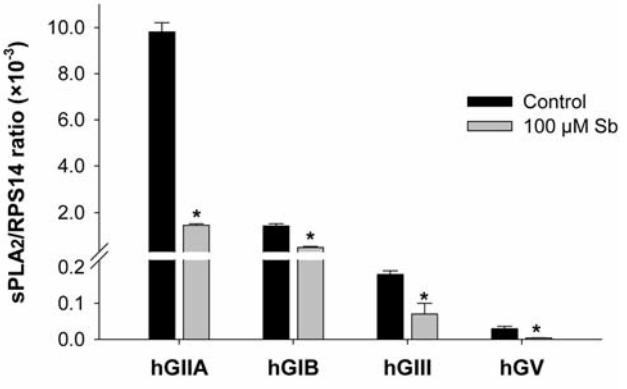

Figure 2. *Effects of silibinin on the basal expression of secreted phospholipase A_2 isoforms in HepG2 hepatoma cells. The levels of $sPLA_2$ hGIIA, hGIB, hGIII, and hGV transcripts were determined using RT-qPCR with 40S ribosomal protein S14 (RPS14) as reference gene. Cells were cultured in RPMI-1640 supplemented with 10% fetal calf serum in the absence (control) and presence of 100 μM silibinin (Sb) for 48 h. Results are shown as means±SD. Analyses were performed in duplicates and graphics are representative of three experiments with similar results. *Significantly different from the control at $p<0.05$.*

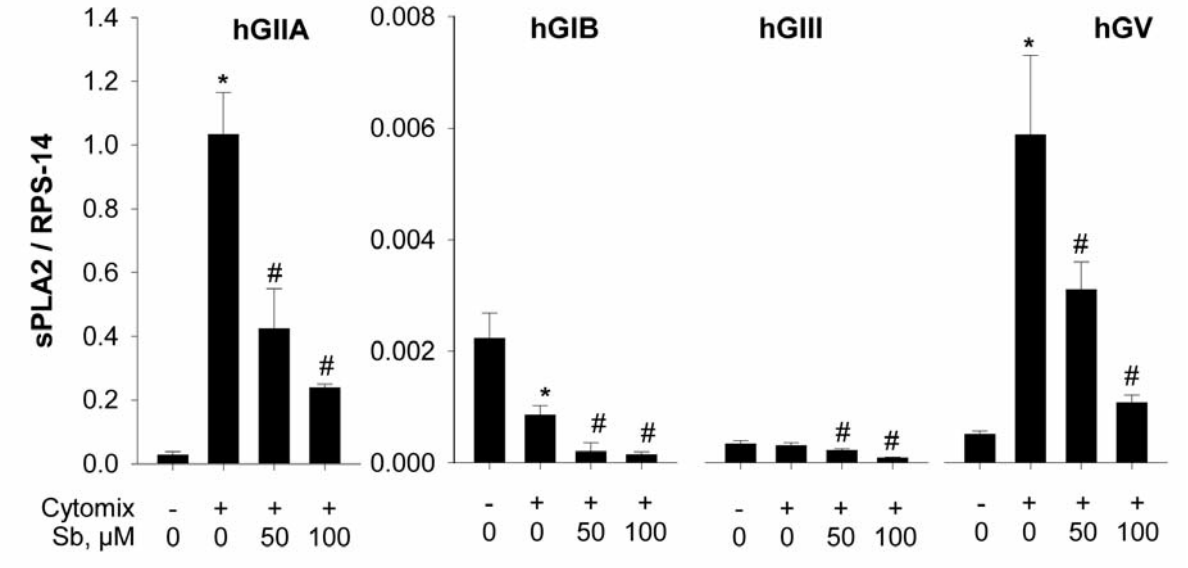

Figure 3. *Effects of silibinin on cytokine-induced expressions of secreted phospholipase A_2 isozymes in HepG2 cells. Cells cultured in serum-free RPMI-1640 medium were stimulated with a cytokine mixture (cytomix) consisting of interferon-γ, tumour necrosis factor-α, and interleukin-6 at a final concentration of each cytokine of 10 ng/ml for 24 h. The mRNA levels of $sPLA_2$ were determined using RT-qPCR with 40S ribosomal protein S14 (RPS14) as reference gene. Results are shown as means±SD. Analyses were performed in duplicates and are representative of three independent experiments. Significantly different at *p<0.05 versus control; #p<0.05 versus cytokine mix.*

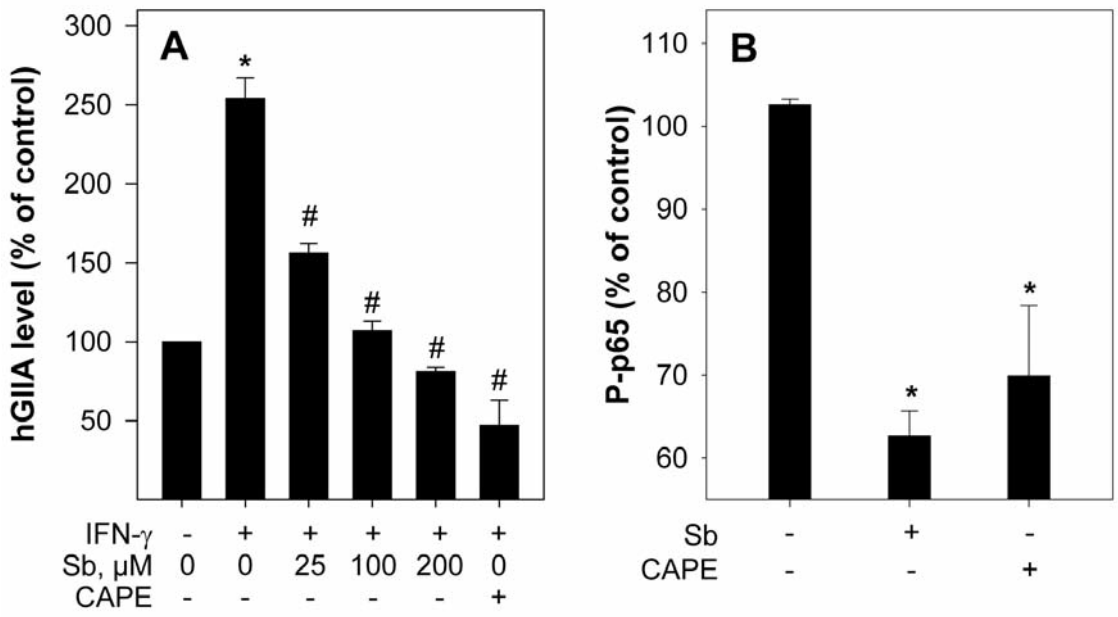

Figure 4. *Transcription factor nuclear factor-κB (NFκB) as a possible target mediating the inhibitory effect of silibinin on human group IIA phospholipase A_2 (hGIIA) expression. A: The hGIIA protein amounts released by HepG2 cells into the medium were measured after 48 h of incubation in RPMI-1640 supplemented with 10% fetal calf serum. Caffeic acid phenethyl ester (CAPE, 25 μM) and silibinin (Sb) were added simultaneously with interferon-γ (IFNγ, 25 ng/ml) as indicated. Results are expressed as changes relative to the control, which was assigned a value of 100%. The data shown are means±SD of analyses in triplicate and are representative of three independent experiments. B: Cells were incubated in the absence (control) or presence of 100 μM silibinin (Sb). Amounts of total and phosphorylated forms of NFκB p65 (Ser536) were determined using a cell-based ELISA. Data are expressed as changes relative to the control, which was assigned a value of 100% at the beginning of cellular silibinin treatment. Results are the means±SDs of analyses in triplicate and are representative of three independent experiments. Significantly different at *p<0.05 versus control; #p<0.05 versus IFNγ.*

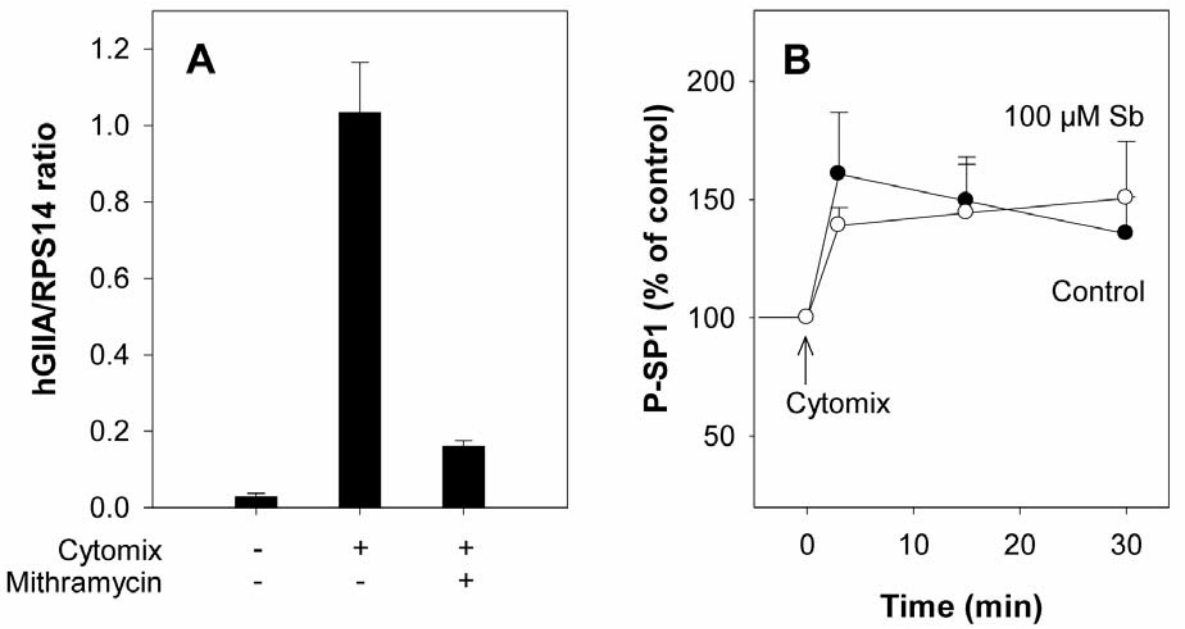

Figure 5. *Transcription factor specificity protein 1 (SP1) implicated in the regulation of human group IIA phospholipase A_2 (hGIIA) expression is not affected by silibinin. A: Effect of inhibitor of the SP1 binding, mithramycin A on the level of hGIIA mRNA in cytokine-primed HepG2 cells. Cells cultured in serum-free RPMI-1640 medium were stimulated with a cytokine mixture (cytomix) consisting of interferon-γ, tumour necrosis factor-α, and interleukin-6 at a final concentration of each cytokine of 10 ng/ml for 24 h. When indicated, 1 µM mithramycin A was added. B: Cells were incubated in serum-free medium in the absence (control) or presence of 100 µM silibinin (Sb). Amounts of the phosphorylated form of SP1 (T453) were determined using a cell-based ELISA Phospho-SP1 kit. Data are expressed as changes relative to the control, which was assigned a value of 100% at 0 min. Results are the means±SDs of analyses in triplicate and are representative of two independent experiments.*

Discussion

The present study shows that silibinin effectively down-regulates the basal and cytokine-induced expression of a number of different $sPLA_2$ isoforms such as hGIIA, IB, III, and V in HepG2 hepatoma and PC-3 prostate cancer cells. In addition to the cytosolic PLA2, pro-oncogenic effects are suggested for hGIIA and V, which seem to be mediated by arachidonic acid (AA) release and eicosanoid production through mechanisms involving cytosolic phospholipase A2, cyclooxygenase and lipoxygenase (1, 4, 9). The effects of $sPLA_2$s and AA-derived metabolites are mediated through corresponding receptors and subsequent activation of extracellular signal-regulated kinases 1 and 2, phosphoinositid-3 kinase, protein kinase C, and NF-κB pathways (20-22). For this reason, PLA2 enzymes, especially hGIIA and cytosolic PLA2, are considered novel targets for anticancer drug development (5, 9).

The expression of hGIIA itself is regulated by NF-κB, Janus kinase/signal transducer and activator of transcription-1, SP1, and CAAT-enhancer-binding protein-β (C/EBP-β) (2, 23). Among these transcription factors and related signaling pathways, NF-κB is considered the most important molecular

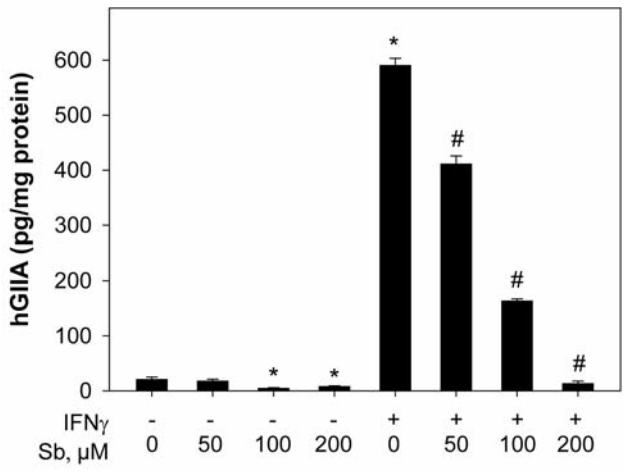

Figure 6. *Effects of silibinin on basal and interferon-γ (IFNγ)-induced human group IIA phospholipase A_2 (hGIIA) expression in PC-3 prostate cancer cells. Cells were cultured in RPMI-1640 supplemented with 10% fetal calf serum. Amounts of hGIIA protein released into the medium were measured after 48 h incubation using a hGIIA-specific ELISA. IFNγ was added at a final concentration of 25 ng/ml. The data shown are the mean±SD of analysis in triplicate and are representative of three independent experiments. Significantly different at *$p<0.05$ versus control (no addition); #$p<0.05$ versus IFNγ.*

target of silibinin action (13, 14, 24-26). Consistent with this, the silibinin-mediated inhibition of the NF-κB pathway in HepG2 cells was shown in our study. In contrast, silibinin did not affect the cytokine-induced activation of SP1 in these cells. A similar insensitivity of SP1 to silibinin was described in LNCaP prostate cancer cells (27). In addition, the possible role of C/EBP-β in silibinin-mediated down-regulation of hGIIA seems to be unlikely because of the earlier described activating effect of silibinin on this transcription factor (28). All data above suggest that the inhibition of hGIIA expression by silibinin is mediated predominantly through the modulation of NF-κB activation. NF-κB is also suggested to be a crucial factor in the control of hGV expression (29).

In conclusion, this study established that the expression of secreted PLA2 IB, -IIA,-III, and -V enzymes in HepG2 cells is effectively inhibited by silibinin. Given the proven pathophysiological relevance of these enzymes in cancer and inflammation, the demonstrated effect of silibinin on their expression can be used to develop novel therapeutic strategies for these diseases.

Conflicts of Interest

There were no conflicts of interest.

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References

1 Lambeau G and Gelb MH: Biochemistry and physiology of mammalian secreted phospholipases A2. Ann Rev Biochem *77*: 495-520, 2008.

2 Menschikowski M, Hagelgans A and Siegert G: Secretory phospholipase A_2 of group IIA: Is it an offensive or a defensive player during atherosclerosis and other inflammatory diseases? Prostaglandins Other Lipid Mediat *79*: 1-33, 2006.

3 Crowl RM, Stoller TJ, Conroy RR and Stoner CR: Induction of phospholipase A_2 gene expression in human hepatoma cells by mediators of the acute-phase response. J Biol Chem *266*: 2647-2651, 1991.

4 Scott KF, Sajinovic M, Hein J, Nixdorf S, Galettis P, Liauw W, de Souza P, Dong Q, Graham GG and Russell PJ: Emerging roles for phospholipase A_2 enzymes in cancer. Biochimie *92*: 601-610, 2010.

5 Laye JP and Gill JH: Phospholipase A_2 expression in tumours: A target for therapeutic intervention? Drug Discovery Today *8*: 710-716, 2003.

6 Menschikowski M, Hagelgans A, Schuler U, Froeschke S, Rosner A and Siegert G: Plasma levels of phospholipase A2-IIA in patients with different types of malignancies: prognosis and association with inflammatory and coagulation biomarkers. Pathol Oncol Res *19*: 839-846, 2013.

7 Bode JG, Albrecht U, Häussinger D, Heinrich PC and Schaper F: Hepatic acute phase proteins–regulation by IL6-and IL1-type cytokines involving STAT3 and its crosstalk with NFκB-dependent signaling. Eur J Cell Biol *91*: 496-505, 2012.

8 Nevalainen TJ, Kallajoki M, Pesonen E, Andersson S, Kärkkäinen P and Höckerstedt K: Origin of circulating group II phospholipase A_2 in hepatocytes in a patient with epitheloid hemangio-endothelioma of the liver. Lab Invest *74*: 585-591, 1996.

9 Cummings BS: Phospholipase A_2 as targets for anticancer drugs. Biochem Pharmacol *74*: 949-959, 2007.

10 Hackett ES, Twedt DC and Gustafson DL: Milk thistle and its derivative compounds: A review of opportunities for treatment of liver disease. J Vet Intern Med *27*: 10-16, 2013.

11 Singh RP and Agarwal R: Mechanisms and preclinical efficacy of silibinin in preventing skin cancer. Eur J Cancer *41*: 1969-1979, 2005.

12 Deep G and Agarwal R: Targeting tumor micro environment with silibinin: Promise and potential for a translational cancer chemopreventive strategy. Curr Cancer Drug Targets *13*: 486-499, 2013.

13 Giorgi VS, Peracoli MT, Peracoli JC, Witkin SS and Bannwart-Castro CF: Silibinin modulates the NFκB pathway and pro-inflammatory cytokine production by mononuclear cells from pre-eclamptic women. J Reprod Immunol *95*: 67-72, 2012.

14 Kim BR, Seo HS, Ku JM, Kim GJ, Jeon CY, Park JH, Jang BH, Park SJ, Shin YC and Ko SG: Silibinin inhibits the production of pro-inflammatory cytokines through inhibition of NFκB signaling pathway in HMC-1 human mast cells. Inflamm Res *62*: 941-950, 2013.

15 Tyagi A, Agarwal C, Dwyer-Nield LD, Singh RP, Malkinson AM and Agarwal R: Silibinin modulates TNFα and IFNγ mediated signaling to regulate COX2 and iNOS expression in tumorigenic mouse lung epithelial LM2 cells. Mol Carcinog *51*: 832-842, 2012.

16 Menschikowski M, Hagelgans A, Heyne B, Hempel U, Neumeister V, Goez P, Jaross W and Siegert G: Statins potentiate the IFN-gamma-induced up-regulation of group IIA phospholipase A_2 in human aortic smooth muscle cells and HepG2 hepatoma cells. Biochim Biophys Acta *1733*: 157-171, 2005.

17 Menschikowski M, Hagelgans A, Gussakovsky E, Kostka H, Paley EL and Siegert G: Differential expression of secretory phospholipases A_2 in normal and malignant prostate cell lines: Regulation by cytokines, cell signaling pathways, and epigenetic mechanisms. Neoplasia *10*: 279-286, 2008.

18 Menschikowski M, Hagelgans A, Kostka H, Eisenhofer G and Siegert G: Involvement of epigenetic mechanisms in the regulation of secreted phospholipase A_2 expressions in Jurkat leukemia cells. Neoplasia *10*: 1195-1203, 2008.

19 Menschikowski M, Hagelgans A, Hempel U and Siegert G: Glycogen synthase kinase-3β negatively regulates group IIA phospholipase A_2 expression in human aortic smooth muscle and HepG2 hepatoma cells. FEBS Lett *577*: 81-86, 2004.

20 Kandouz M, Nie D, Pidgeon GP, Krishnamoorthy S, Maddipati KR and Honn KV: Platelet-type 12-lipoxygenase activates NFκB in prostate cancer cells. Prostaglandins Other Lipid Mediat *71*: 189-204, 2003.

21 Hernández M, Martín R, García-Cubillas MD, Maeso-Hernández P and Nieto ML: Secreted PLA2 induces proliferation in astrocytoma through the EGF receptor: another inflammation-cancer link. Neuro Oncol *12*: 1014-1023, 2010.

22 Yu JA, Kalatardi S, Dohse J, Sadaria MR, Meng X, Fullerton DA and Weyant MJ: Group IIa $sPLA_2$ inhibition attenuates NFκB activity and promotes apoptosis of lung cancer cells. Anticancer Res *32*: 3601-3607, 2012.
23 Andreani M, Olivier JL, Berenbaum F, Raymondjean M and Béréziat G: Transcriptional regulation of inflammatory secreted phospholipases A2. Biochim Biophys Acta *1488*: 149-158, 2000.
24 Singh RP and Agarwal R: Prostate cancer chemoprevention by silibinin: Bench to bedside. Mol Carcinog *45*: 436-442, 2006.
25 Raina K, Agarwal C and Agarwal R: Effect of silibinin in human colorectal cancer cells: targeting the activation of NFκB signaling. Mol Carcinog *52*: 195-206, 2013.
26 Polyak SJ, Morishima C, Lohmann V, Pal S, Lee DY, Liu Y, Graf TN and Oberlies NH: Identification of hepatoprotective flavonolignans from silymarin. Proc Natl Acad Sci USA *107*: 5995-5999, 2010.
27 Zhu W, Zhang JS and Young CY: Silymarin inhibits function of the androgen receptor by reducing nuclear localization of the receptor in the human prostate cancer cell line LNCaP. Carcinogenesis *22*: 1399-1403, 2001.
28 Zhang J, Harrison JS, Uskokovic M, Danilenko M and Studzinski GP: Silibinin can induce differentiation as well as enhance vitamin D_3-induced differentiation of human AML cells *ex vivo* and regulates the levels of differentiation-related transcription factors. Hematol Oncol *28*: 124-132, 2010.
29 Ao C, Huo Y, Qi L, Xiong Z, Xue L and Qi Y: Pioglitazone suppresses the lipopolysaccharide-induced production of inflammatory factors in mouse macrophages by inactivating NFκB. Cell Biol Int *34*: 723-730, 2010.

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