The 5-Lipoxygenase Promoter Is Regulated by DNA Methylation*

Received for publication, August 10, 2001, and in revised form, November 9, 2001 Published, JBC Papers in Press, November 12, 2001, DOI 10.1074/jbc.M107665200

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5-lipoxygenase (5-LO), the key enzyme in leukotriene biosynthesis, is expressed in a tissue- and cell differentiation-specific manner. The 5-LO core promoter required for basal promoter activity has a unique (G+C)-rich sequence that contains five tandem Sp1 consensus sequences. The mechanisms involved in the regulation of cell type-specific 5-LO expression are unknown. Here we show that 5-LO expression is regulated by DNA methylation. Treatment of the 5-LO-negative cell lines U937 and HL-60/TB with the demethylating agent 5-aza-2'-deoxycytidine (AdC) up-regulated expression of 5-LO primary transcripts and mature mRNA in a similar fashion, indicating that AdC stimulates 5-LO gene transcription. Analysis of the methylation status of the 5-LO promoter revealed that the core promoter region was methylated in U937 and HL-60/TB cells, whereas it was unmethylated in the 5-LO-positive parent HL-60 cell line. Reporter gene assays with 5-LO promoter constructs gave up to 68- and 655-fold repression of 5-LO promoter activity in HeLa and Mono Mac 6 cells by methylation. 1,25-dihydroxyvitamin D$_3$ and transforming growth factor-beta (TGFβ), potent inducers of the 5-LO pathway in myeloid cell lines, increased 5-LO RNA expression in HL-60/TB and U937 cells, but co-treatment with AdC was required to achieve 5-LO expression levels in HL-60/TB cell lines that were comparable with wild-type HL-60 cells. In reporter gene assays, 1,25-dihydroxyvitamin D$_3$ and TGFβ were unable to induce promoter activity when the 5-LO promoter constructs were methylated, which suggests that 5-LO promoter demethylation is a prerequisite for the high level induction of 5-LO gene expression by 1,25-dihydroxyvitamin D$_3$ and TGFβ and that the effects of both agents on 5-LO mRNA expression are not related to DNA methylation.

5-LO is expressed in a variety of immune competent cells including B-lymphocytes, granulocytes, monocytes, mast cells, and dendritic cells (3). Depending on the cell type, several cytokines have been shown to be inducers of the 5-LO pathway. In granulocytes 5-LO expression is stimulated by granulocyte-macrophage colony-stimulating factor (GM-CSF) (4), whereas interleukin-3 regulates the development of the 5-LO pathway in mouse mast cells (5). In the human myeloid leukemic cell lines HL-60 and Mono Mac 6, cell differentiation by 1,25-dihydroxyvitamin D$_3$ and transforming growth factor-beta (TGFβ) leads to a strong induction of the 5-lipoxygenase pathway (6, 7). In Mono Mac 6 cells, the induction of 5-LO protein expression and activity by TGFβ and 1,25(OH)$_2$D$_3$ was accompanied by a 64-fold up-regulation of mature 5-LO mRNA and an up to 5-fold increase in 5-LO primary transcripts (7, 8), whereas no significant induction of 5-LO transcription was found in nuclear run-off assays (9). The human 5-LO gene promoter was first characterized by Hoshiko et al. (10). Several features of the putative 5-LO promoter region (such as the lack of TATAA or CCAAT boxes and repeated (G+C)-rich elements) are characteristic for so-called housekeeping genes. Previous data suggest that the transcription factors Egr-1 and/or Sp1 are required for basal 5-LO transcription and that they functionally interact with the 5-LO promoter and activate it via repeated response elements located between positions −121 and −88 bp, relative to the translational start site (10, 11). Interestingly, naturally occurring mutations were found in the 5-LO promoter consisting of the deletion of one or two, or the addition of one Sp1 binding site (12). These mutations only slightly alter 5-LO promoter activity in reporter gene assays but have a significant impact on the response of asthma patients to 5-LO inhibitors (13).

As yet, no data are available on the mechanisms involved in the cell type-specific activation of the 5-LO promoter in response to cell differentiation signals and inflammatory stimuli. Expression of several genes with (G+C)-rich promoters has been shown to be regulated by DNA methylation (14). Whereas promoters of (G+C)-rich housekeeping genes are usually unmethylated, methylation of promoters of tissue-specific genes is usually linked with silencing of the respective genes.

Therefore, it was of interest to study the role of DNA methylation in the regulation of 5-lipoxygenase expression. The human myeloid cell lines Mono Mac 6 and HL-60 show prominent 5-LO gene expression and 5-LO activity after differentiation by TGFβ and 1,25(OH)$_2$D$_3$ (6, 7), whereas U937 cells and the HL-60/TB cell line, a subline of the HL-60 cell line, show FLAP expression but lack expression of the 5-LO gene (15, 16).

Here we show that the suppression of 5-LO expression in U937 and HL-60/TB cells is a result of DNA methylation and that 5-LO promoter activity is regulated by methylation of CpG sites within the (G+C)-rich core promoter region.
MATERIALS AND METHODS

Reagents—Molecular biology reagents were from MBI Fermentas, Sigma, Life Technologies, Inc., Promega, or other sources indicated in the text. Insulin was a gift from Hoechst-Marion-Roussel (Frankfurt, Germany). Human TGFβ1 was purified from outdated platelets (according to Ref. 17). Nucleosipin extract columns for direct purification of pDNA were from Macherey-Nagel (Duren, Germany). HPLC solvents were purchased from Merck (Darmstadt, Germany).

Plasmid Constructs—Vectors pN10 and pN7 were obtained by insertion of 5-LO promoter fragments (~83 to ~128 (upstream of the 5-LO start codon) and ~1547 to ~128 into the promoterless luciferase reporter vector pGL3Basic (Promega), respectively. Plasmid constructs were analyzed by DNA sequencing. The pSG5VDR and pSG5RXR expression plasmids for the human vitamin D receptor (VDR) and the human retinoid X receptor α (RXRα) were obtained from Dr. Carsten Karbup (Köppen, Finland).

Cell Culture—HL-60 cells were obtained from ATCC; U937 and HL-60TB cells were from the Karolinska Institute (Stockholm) and grown at 37 °C in a humidified atmosphere with 6% CO₂ in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum (FCS), streptomycin (100 μg/ml), and penicillin (100 units/ml). For a cell culture of Mono Mac 6 cells, which were kindly provided by Dr. H. W. L. Zucker-Heitbrock (Munich), the growth medium was supplemented with 1× nonessential amino acids, sodium pyruvate (1 mM), oxalacetate (1 mM), and insulin (10 μg/ml) (18). HeLa cells were obtained from Dr. Muller (Pharmacological Institute, Frankfurt) and grown in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) FCS, 100 μg/ml streptomycin, and 100 units/ml penicillin.

DNA Demethylation by 5-Aza-deoxycytidine—U937 and HL-60TB cells (0.3 × 10⁶ cells/ml) were treated with 100 nm (U937 cells) or 30 nm AdC (HL-60TB cells) for 72 h. The medium containing AdC was replaced every 12 h as described (19). After 72 h, the cells were seeded at a density of 0.35 × 10⁶ cells/ml and grown without AdC for 4 days. AdC-treated cells are designated U937 AdC and HL-60TB AdC.

Cell Differentiation—Cells (0.3 × 10⁶ cells/ml) were differentiated by 1,25(OH)₂D₃ (0.5 nM) and TGFβ1 (5 ng/ml) (20). Mono Mac 6 cells, which were kindly provided by Dr. H. W. L. Zucker-Heitbrock (Munich), the growth medium was supplemented with 1× nonessential amino acids, sodium pyruvate (1 mM), oxalacetate (1 mM), and insulin (10 μg/ml) (18). HeLa cells were obtained from Dr. Muller (Pharmacological Institute, Frankfurt) and grown in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) FCS, 100 μg/ml streptomycin, and 100 units/ml penicillin.

DNA Determination—DNA was purified with NucleoTrap CR (Macherey-Nagel) according to the manufacturer’s protocol, and desulfonated by addition of 0.1 volumes of 3 N NaOH at 37 °C for 15 min. After 2 min on ice, 0.2 volumes of 3 M sodium acetate (pH 5.2) and 2.5 volumes of ethanol were added to precipitate the DNA. The precipitated DNA was washed once with 80% (v/v) ethanol, dried, and dissolved in 20–50 μl of water at 4 °C. The DNA solution was amplified by nested PCR with 35 cycles for each amplification using primers specific for the noncoding strand of the 5-LO gene with the sequences (R = reverse, F = forward primer): 5’-ATCCCTCCATAAATACCTCAGTCTC-3’ (R = 552/525); 5’-CTTCCCATAAAATCCTCAGTCTC-3’ (R = 549/525); 5’-TTTGAAGGGTTTGGTTAAGGTGG-3’ (F = 107/131); 5’-ATAATTGGTGTGTAGTTGAGTTGGG-3’ (F = 14/34). Annealing temperatures were 62 and 57 °C for the first and second amplification, respectively. The amplified fragments were directly sequenced (377 sequencer, PerkinElmer Applied Biosystems). Additional primers used only for sequencing were: 5’-TACCCCAATCCCCCTA-5’ (R = 306/290, sequences from 5’ → 3’); 5’-ACACACTAATAAATCAACCCACCC-5’ (R = 71/51, sequences from 3’ → 5’); 5’-TAGCTGGCCGCCG-ATT-5’ (F = 215/200, sequences from 5’ → 3’); 5’-TATGTTGTGGGGTTTGGG-5’ (F = 13/5, sequences from 5’ → 3’). The Big-Dye Terminator Cycle Sequencing Kit (PerkinElmer Applied Biosystems) has been used under the recommended conditions for RNA sequence determination.

In Vitro DNA Methylation—Plasmid DNA was incubated for 24 h at 37 °C with 100 ng/ml of Esen End BioStar DNA glycosylase (21) in 50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂, 1 mM dithiothreitol (pH 7.9) supplemented with 160 μM S-adenosylmethionine. For transfections, methylated pDNAs were purified by Nucleospin extract columns (Macherey-Nagel). Complete methylation at CG sites was confirmed by HpaII digestion of the plasmids.

Transfection of Mono Mac 6 Cells—Mono Mac 6 cells (0.3 × 10⁶ cells/ml) were grown for 48 h, centrifuged by centrifugation at 1000 g for 5 min at room temperature, and washed twice at room temperature with RPMI 1640 without FCS and L-glutamine. The cells were then resuspended at 46 × 10⁵ cells/ml in RPMI 1640 without supplements, and 0.3 ml of the cell suspension was placed into a 0.4-cm electroporation cuvette (Bio-Rad). 40 micrograms of supercoiled plasmid DNA in 30 μl of water were added to the cell suspension. After 5 min at room temperature, electroporation was performed at 975 μF and 200 V using a Bio-Rad GenePulser. The cuvettes were immediately iced on ice for 20 min. Then the cells were transferred into 10 ml of RPMI 1640 containing 10% FCS, insulin, glutamine, and nonessential amino acids. 1,25(OH)₂D₃ (50 nM) and TGFβ1 (1 ng/ml) were added immediately after the cell transfer as indicated. 6 h after transfection cells were harvested for luciferase assay.

Transfection of HeLa Cells—24 h prior to transfection, cells were plated into a 24-well tissue culture plate at a density of 6 × 10⁴ cells/well so that 60–80% of the cells were confluent at the time of transfection. Plasmid DNA (0.4 μg) was diluted into serum-free Dulbecco’s modified Eagle medium and precomplexed with 5 μl of PLUS reagent (Life Technologies, Inc.) by incubation at room temperature for 15 min. After complexation, plasmid DNA was mixed with 125 μl of 1.5% diluted Lipofectin reagent and incubated for 30 min at room temperature. The medium was then replaced by 200 μl of fresh serum-free medium, and the DNA-PLUS-Lipofectin reagent complexes were added to the cells and incubated for 5 h at 37 °C in 5% CO₂, 1 ml of medium containing 15% (v/v) FCS was added. 24 h after transfection, cells were washed once in phosphate-buffered saline (pH 7.4), and luciferase activity was determined as described below.

Luciferase Assays—After transfection of Mono Mac 6 (24 h) and HeLa (24 h), cells were washed once in phosphate-buffered saline containing 0.5 mM MgCl₂ and 0.5 mM CaCl₂ and lysed in 100 μl of lysis buffer (Luciferase Reporter Gene Assay constant light signal kit, Roche Molecular Biochemicals). Luciferase activity was determined by monitoring light emission with a Microlumat Plus LB980 EG&G Berthold luminescent. Light emission signal was integrated for 5 s. Transfection efficiency was monitored and normalized by cotransfection with 1 μg (MonoMa6) and 1 μg (HeLa) pCMVSEAP using the Phospha-Light™ kit (Tropix) to determine the secreted placental alkaline phosphatase (SEAP) activity. Expression vectors pSG5VDR and pSG5RXR were cotransfected in all reporter gene experiments. pCMVLuc was used as positive control.

RESULTS

Induction of the 5-LO Pathway in HL-60TB and U937 Cells by 5-Aza-deoxycytidine—As shown previously, undifferentiated HL-60 and Mono Mac 6 cells do not exhibit significant 5-LO

Luciferase activity was determined by monitoring light emission with a Microlumat Plus LB980 EG&G Berthold luminescent. Light emission signal was integrated for 5 s. Transfection efficiency was monitored and normalized by cotransfection with 1 μg (MonoMa6) and 1 μg (HeLa) pCMVSEAP using the Phospha-Light™ kit (Tropix) to determine the secreted placental alkaline phosphatase (SEAP) activity. Expression vectors pSG5VDR and pSG5RXR were cotransfected in all reporter gene experiments. pCMVLuc was used as positive control.

RESULTS

Induction of the 5-LO Pathway in HL-60TB and U937 Cells by 5-Aza-deoxycytidine—As shown previously, undifferentiated HL-60 and Mono Mac 6 cells do not exhibit significant 5-LO-
activity. However, after differentiation with TGFβ and 1,25(OH)2D3 there is a strong up-regulation of cellular activity (Ref. 7 and Fig. 1). In contrast, HL-60TB and U937 cells have no significant 5-LO activity after differentiation with TGFβ and 1,25(OH)2D3. After 4 days of differentiation, 5-LO activity was determined. Values are given as mean ± S.E. of three independent experiments.

Similar effects were observed when 5-LO pre-mRNA and mature mRNA were analyzed by RT-PCR (Fig. 2). In HL-60 cells, TGFβ and 1,25(OH)2D3 up-regulated 5-LO pre-mRNA expression, but only modest levels of 5-LO pre-mRNA were observed in HL-60TB cells after differentiation with TGFβ and 1,25(OH)2D3. However, after pretreatment of HL-60TB cells with AdC, similar 5-LO pre-mRNA levels were found as in wild-type HL-60 cells. In U937 cells, AdC enhanced expression of 5-LO pre-mRNA and mature mRNA about 2- and 4-fold, respectively (Fig. 2). Stronger effects of AdC were observed in HL-60TB cells. AdC up-regulated 5-LO pre-mRNA and mature mRNA about 6.6- and 23-fold in undifferentiated cells and 4.8 and 8.5-fold in cells differentiated with 1,25(OH)2D3 and TGFβ, respectively. Thus, similar to Mono Mac 6 cells, the changes in 5-LO RNA levels were more pronounced when mature RNA was analyzed compared with pre-mRNA in HL-60 and U937 cells. Furthermore, the data indicate that all three cell lines respond to TGFβ and 1,25(OH)2D3 and show 5-LO RNA induction, although the achieved mRNA levels were lower in HL-60TB and U937 cells. Interestingly, AdC treatment also enhanced 5-LO RNA expression in undifferentiated cells, which suggests that its effects are at least in part independent of the cellular actions of TGFβ and 1,25(OH)2D3.

**Analysis of 5-LO Promoter Methylation Patterns**—Activation of gene expression by AdC is assumed to be caused by inhibition of DNA methylation, which subsequently leads to partial demethylation of CpG sites and up-regulation of the activity of methylation-sensitive gene promoters. Because basal 5-LO promoter activity is induced by multiple Sp1/Egr-1 binding sites located in a C+G-rich region with many putative CpG methylation sites, it was of interest to study the methylation pattern of the 5-LO core promoter in HL-60, HL-60TB, and U937 cells and to correlate it with the 5-LO expression data shown above.

Genomic DNA isolated from all three cell lines was digested with KpnI and subjected to DNA methylation analysis by genomic bisulfite sequencing as described under “Materials and Methods.” Fig. 3 shows the methylation pattern of the 5-LO core promoter region of the indicated cell lines. In HL-60 cells showing prominent 5-LO expression the 5-LO core promoter was completely unmethylated, whereas it was heavily methylated in HL-60TB and U937 cells that show only low 5-LO expression. In U937 cells all CpG sites of the core promoter required for basal activity were methylated, whereas CpG sites located more upstream were unmethylated. Thus, a good correlation exists between the methylation status of the 5-LO promoter and 5-LO gene expression in HL-60, HL-60TB, and U937 cells. In HL-60TB and U937 cells with AdC led to partial demethylation of the 5-LO promoter, although the demethylating effect of AdC was stronger in HL-60TB than in U937 cells (Fig. 3).

**5-LO Promoter Activity Is Methylation-sensitive**—To check whether 5-LO promoter activity is regulated by 1,25(OH)2D3 and/or DNA methylation, reporter gene assays were performed. The plasmids N10 and N7 and two deletion constructs (pN10ΔGC and pN7ΔGC) lacking the core region containing the five direct and two inverted GGGCGG repeats were used (Fig. 4). HeLa and Mono Mac 6 cells were transiently transfected with these plasmids, and promoter activities were determined as described under “Materials and Methods.”

Deletion of the region −258 to −96 containing the Sp1 and Egr-1 binding sites close to the transcription initiation site (plasmids N10ΔGC and N7ΔGC) significantly reduced 5-LO promoter activity in transfected HeLa cells (Fig. 5A) and al-
most abolished reporter gene expression in the 5-LO-positive monocytic cell line Mono Mac 6 (Fig. 5B). Surprisingly, 1,25(OH)_2D_3 and TGFβ did not increase 5-LO promoter activity in reporter gene assays in Mono Mac 6 cells (Fig. 5), although both agents strongly induced 5-LO gene expression in this cell line (7) and although at least one vitamin D response element was present in the 5-LO promoter (22). In control experiments, 1,25(OH)_2D_3 and TGFβ strongly activated (25-fold) a promoter construct containing the 4×-concatemerized DR3-type pig osteopontin vitamin D response element (core sequence AT-GGTCATAGGTTCA) in front of the thymidine kinase promoter. From these results it was concluded that there is no induction of 5-LO promoter activity by 1,25(OH)_2D_3 and TGFβ, at least under the experimental conditions of transient reporter gene assays.

To study the effect of DNA methylation on 5-LO promoter activity and to investigate possible relationships between DNA methylation and 1,25(OH)_2D_3/TGFβ signaling in the regulation of 5-LO promoter activity, the N10 and the N10ΔGC plasmids were methylated by SssI methylase in vitro. Subsequently, Mono Mac 6 and HeLa cells were transfected with the
methylated or unmethylated plasmids, and promoter activity was determined. Methylation completely suppressed 5-LO promoter activity in both cell lines, and transcriptional activities of the methylated N10 and N10ΔGC plasmids were comparable with the negative control (pGL3Basic promoterless plasmid) (Fig. 6). Interestingly, 1,25(OH)\\(_2\)D$_3$ and TGFβ could not activate transcription from the methylated constructs in HeLa and Mono Mac 6 cells, indicating that both hormones can not induce demethylation of the 5-LO promoter under these experimental conditions (Fig. 6). The relative changes in the 5-LO promoter activities by DNA methylation are summarized in Table I. Methylation of the 5-LO promoter plasmid pN10 led to a 68- and 655-fold repression of promoter activity in HeLa and Mono Mac 6 cells, respectively, whereas transcriptional repression by DNA methylation was much lower with the cytomegalovirus-driven pCMVluc vector (18- and 7-fold, respectively), which was used as positive control. Similar results were obtained when the 5-LO promoter insert of the pN7 plasmid was selectively methylated. Thus, the 5-LO promoter (−863 to −12) was excised from the pN7 plasmid, \textit{in vitro} methylated by SssI methylase, reinserted into the plasmid, and transfected into HeLa cells. Under these conditions, methylation led to a 38-fold repression of 5-LO promoter activity.

DISCUSSION

The 5-LO gene is expressed in a tissue- and cell differentiation-specific manner (3). In view of this, the features of the

\begin{table}[h]
\centering
\caption{Effects of DNA methylation by SssI methylase on luciferase reporter gene activity}
\begin{tabular}{|c|c|c|}
\hline
Cells & Plasmid & Effects of DNA methylation (fold repression \pm S.E., n = 3) \\
\hline
Mono Mac 6 cells & pN10 & 655 \pm 120 \\
& pCMVluc & 7 \pm 1 \\
HeLa cells & pN10 & 68 \pm 8 \\
& pCMVluc & 18 \pm 4 \\
\hline
\end{tabular}
\end{table}
Much more knowledge has been gained about the connection between the methylation status of gene promoters and transcriptional activity of the respective genes. Methylation of CpG sites are recognized by a variety of methyl-CpG-binding proteins (e.g. MBDs1–3, MeCP2), which are associated either directly or indirectly with histone deacetylases (27). Thus, chromatin condensation mediated by recruitment of histone deacetylases seems to be one mechanism of gene silencing by CpG methylation. Concerning 5-LO, inhibition of histone deacetylases by trichostatin A (at 330 nm) did not lead to activation of 5-LO gene expression in U937 and HL-60TB cells, although general cellular histone acetylation by trichostatin A was detectable by AUT-gel electrophoresis (data not shown), suggesting that repression of 5-LO gene expression by DNA methylation does not depend primarily on recruitment of trichostatin A-sensitive histone deacetylases but seems to be mediated by other mechanisms. For 15-lipoxygenase, it has been shown with reporter gene assays that in contrast to DNA methylation, inhibition of histone deacetylases by trichostatin A (at 330 nm) did not affect 5-LO promoter activity under these conditions (8, 9), whereas no significant effects of both agents were found on 5-LO promoter activity in Mono Mac 6 cells with reporter gene assays (this study) and nuclear run-on assays (9). Here we have shown with reporter gene assays that in contrast to 1,25(OH)2D3 and TGFβ, DNA methylation regulates 5-LO promoter activity to about 15% of the control (29).

Recently, we have shown that up-regulation of 5-LO mRNA expression in Mono Mac 6 cells by 1,25(OH)2D3 and TGFβ is in part due to the induction of transcript elongation and mRNA maturation (8, 9), whereas no significant effects of both agents were found on 5-LO promoter activity in Mono Mac 6 cells with reporter gene assays (this study) and nuclear run-on assays (9). Here we have shown with reporter gene assays that in contrast to 1,25(OH)2D3 and TGFβ, DNA methylation regulates 5-LO promoter activity. First, there was a strong inhibition of 5-LO promoter activity by DNA methylation. Second, AdC treatment of U937 and HL-60TB cells induced 5-LO expression and led to 5-LO promoter demethylation. AdC-induced 5-LO promoter demethylation was much stronger in HL-60TB cells than in U937 cells, which correlated with a stronger induction of 5-LO pre-mRNA (1.8-versus 6.6-fold) and mature mRNA (3.9-versus 23-fold), respectively. It has to be considered that modest changes in the methylation status of CpG-sites (e.g. from 90 to 70%) that could already affect 5-LO promoter activity are hardly detectable by the methylation-specific DNA sequencing method.

Taken together, our experiments suggest that demethylation of the 5-LO promoter is required for high 5-LO gene transcription. Both AdC treatment and 1,25(OH)2D3/TGFβ stimulated 5-LO mRNA expression and the combination of both treatments had additive effects. In contrast to DNA methylation, 1,25(OH)2D3/TGFβ did not affect 5-LO promoter activity under our experimental conditions but rather seemed to be related to post-transcriptional effects such as transcript elongation and maturation. Furthermore, in reporter gene assays we found no evidence that 1,25(OH)2D3 and TGFβ can induce promoter activity when 5-LO promoter constructs are methylated, suggesting that both effects are independent from each other. Our data obtained with HL-60TB and U937 cells demonstrate that 5-LO promoter demethylation is required for the high level induction of 5-LO gene expression by inducers such as 1,25(OH)2D3 and TGFβ.

Acknowledgments—We thank Dagmar Szellas and Astrid Brüggerhoff for expert technical assistance.

REFERENCES
1. Samuelsson, B., Dahlén, S.-E., Lindgren, J.-Å., Rouzer, C. A., and Serhan, C. N. (1987) Science 237, 1171–1176
2. Ford-Hutchinson, A. W., Gresser, M., and Young, R. N. (1994) Annu. Rev. Biochem. 63, 385–417
3. Steinhilber, D. (1999) Curr. Med. Chem. 6, 69–83
4. Stankova, J., Roš-Pleszczynski, M., and Dubois, C. M. (1995) Blood 85, 3719–3726
5. Murakami, M., Kuntz, K. F., Bingham III, C. O., Friend, D. S., Penrose, J. F., and Andonian, A. P. (1995) J. Biol. Chem. 270, 22853–22856
6. Brungs, M., Rådmark, O., Samuelsson, B., and Steinhilber, D. (1994) Biochem. Biophys. Res. Commun. 205, 1572–1580
7. Brungs, M., Rådmark, O., Samuelsson, B., and Steinhilber, D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 107–111
8. Harlé, D., Rådmark, O., Samuelsson, B., and Steinhilber, D. (1998) Eur. J. Biochem. 254, 275–281
9. Harlé, D., Rådmark, O., Samuelsson, B., and Steinhilber, D. (1999) Adv. Exp. Med. Biol. 469, 105–111
10. Hoshiko, S., Rådmark, O., and Samuelsson, B. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9073–9077
11. Silverman, E. S., Du, J., De Sanctis, G. T., Rådmark, O., Samuelsson, B., Drazen, J. M., and Collins, T. (1998) Am. J. Respir. Cell Mol. Biol. 19, 316–323
12. In, K. H., Asano, K., Beier, D., Grobholz, J., Finn, P. W., Silverman, E. K., Silverman, E. S., Collins, T., Fischer, A. R., Keith, T. P., Serino, K., Kim, S. W., De Sanchis, G. T., Yandava, C., Pillari, A., Rabin, P., Hemp, J., Israel, E., Busse, W., Ledford, D., Murray, Jr., J. S., Segal, A., Tinklenberg, D., and Drazen, J. M. (1995) J. Clin. Invest. 95, 1130–1137
13. Drazen, J. M., Yandava, C. N., Dubé, L., Szczeklik, A., Hippsteiner, S., Pillari, A., Israel, E., Schork, N., Silverman, E. S., Katz, D. A., and Dräges, J. (1999) Nat. Genet. 22, 168–170
14. Singal, R., and Ginder, G. D. (1999) Blood 93, 4059–4070
15. Karmang, S., Rousseau, P., Reid, G. K., Rouzer, C. A., Mancini, J. A., Rands, E., Dixon, R., Diehl, R. E., Leveillee, C., Nathanial, D., Vickers, P. J., and Evans, J. F. (1998) J. Lipid Mediators 7, 31–45
16. Claesson, H. E., Jakobsson, P.-J., Steinhilber, D., Odlander, B., and Samuelsson, B. (1993) J. Lipid Mediators 6, 15–22
17. Werz, O., Brungs, M., and Steinhilber, D. (1996) Pharmazie 51, 893–896
18. Ziegler-Heitbrock, H. W. L., Thiel, E., Futterer, A., Herzog, V., Wirtz, A., and Richtmüller, G. (1988) Int. J. Cancer 41, 456–461
19. Attadia, V. (1995) Leukemia 9 Suppl 1, 9–16
20. Chomozynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
21. McDonald, L. E., and Kay, G. F. (1997) BioTechniques 22, 272–274
22. Carlberg, C. (1993) Eur. J. Biochem. 215, 517–527
23. Funk, C. D., Hoshiko, S., Matsumoto, T., Rådmark, O., and Samuelsson, B. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2587–2591
24. Silverman, E. S., and Drazen, J. M. (2000) Am. J. Respir. Crit. Care Med. 161, S77–S80
25. Antequera, F., Bayes, J., and Bird, A. (1990) Cell 62, 503–514
26. Bird, A. P., and Wolfe, A. P. (1999) Cell 99, 451–454
27. Ballestas, E., and Wolfe, A. P. (2001) Eur. J. Biochem. 268, 1–6
28. Kamitsuru, H., Tanoue, S., Ikawa, H., Watanabe, T., Kelavkar, U. P., and Eling, T. E. (2001) Carcinogenesis 22, 187–191
29. Kato, K., Yokomizo, T., Izumi, T., and Shimizu, T. (2000) J. Exp. Med. 192, 413–420

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J. Biol. Chem. 2002, 277:4374-4379.
doi: 10.1074/jbc.M107665200 originally published online November 12, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M107665200

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Vol. 277 (2002) 4374–4379

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Page 4375: In line 9 of the last paragraph under “Materials and Methods,” “1 /H9262 g (HeLa) pCMVSEAP” should be changed to “0.01 /H9262 g (HeLa) pCMVSEAP.”

Vol. 275 (2000) 5416–5424

NAD(P)H:quinone oxidoreductase (NQO1) activity is the principal determinant of β-lapachone cytotoxicity.

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Page 5420, Fig. 6A: The legend should be changed to show that the open circles denote cells treated with β-lapachone alone and the closed circles denote cells treated with β-lapachone with 50 μM dicoumarol. Therefore, the sentence beginning on line 6 of the legend should read: “Cells were exposed to a 4-h pulse of a range of β-lap doses either alone (○) or with 50 μM dicoumarol (●) . . . .”

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.