ANTICANCER RESEARCH *24*: 1387-1392 (2004)

PHGPx Overexpression Induces an Increase in COX-2 Activity in Colon Carcinoma Cells

GUISLAINE BARRIÈRE, HÈLENE RABINOVITCH-CHABLE, JEANNE COOK-MOREAU, KARINE FAUCHER, MICHEL RIGAUD and FRANCK STURTZ

Laboratoire de Biochimie Médicale et de Génétique Moléculaire, Faculté de Médecine, 2 rue du Docteur Marcland, F-87025 Limoges Cedex, France

Abstract. *Background: Lipid peroxidation is a constant problem that eukaryotic cells have to face. Glutathione peroxidases (GPx) are among the most effective systems that protect cells from hydroperoxide toxicity. The objective of this study was to evaluate the relationship between GPx and cyclooxygenase 2 (COX-2), implicated in cancer pathogenesis, particularly in colon cancer cells. Materials and Methods: Phospholipid hydroperoxide glutathione peroxidase (PHGPx or GPx4), which metabolizes peroxidized phospholipids, was cloned in an expression plasmid, transfected in HT29 cl.19A colon carcinoma cells and the effects of PHGPx overexpression were measured on arachidonic acid metabolism by COX-2. Metabolites were studied by HPLC and EIA; COX-2 mRNA levels were analysed using semi-quantitative PCR. Results: Prostaglandins (PGE_2, $PGF_2\alpha$, 6 keto-$PGF_1\alpha$) and thromboxane (TXB_2) production were increased. COX-2 mRNA levels increased in PHGPx overexpressing cells. Conclusion: Surprisingly, our data suggest that PHGPx overexpression noticeably increases COX-2 metabolism.*

Hydrogen peroxide and several hydroperoxy derivatives of lipids are continuously being generated in aerobic cells by chemical or enzymatic reactions, called lipid peroxidation. Lipid peroxidation is a process involving the oxidative degradation of unsaturated lipids such as arachidonic acid (AA) and leads to the formation of many biologically active metabolites. Cyclooxygenases (COX) and lipoxygenases (LOX) are major enzymes involved in the formation of hydroperoxide metabolites and give rise to eicosanoids. These eicosanoids include prostaglandins, prostacyclins, thromboxane, hydroperoxyeicosatetraenoic acids, hydroeicosatetraenoic acids and leukotrienes (1, 2). Despite their biological activities, these peroxides must be degraded as they cause severe detrimental damage to membrane structure and functions.

Cytoprotection against lipid peroxides is accomplished by different means, especially by glutathione peroxidase and vitamin E. Glutathione peroxidases reduce cellular lipid hydroperoxides and play a major role in cell antioxidant systems. The most important types of selenium-dependent peroxidase are cellular glutathione peroxidase (cGPx also known as GPx1) and phospholipid hydroperoxide glutathione peroxidase (PHGPx also known as GPx4). PHGPx, first described in 1982 by Ursini *et al.* (3), is known to act on various kinds of lipid hydroperoxides and may have a role in determining the basal peroxide level. PHGPx has the ability to directly interact with peroxidized phospholipids even if the lipids are integrated in biomembranes. PHGPx also reduces ester lipid hydroperoxides such as hydroperoxy phospholipids (4) or hydroperoxy cholesterol esters (5). PHGPx activity has been detected in many rat tissues (6) as well as in human tumor cell lines (7) and normal human tissues (8).

These observations suggested a critical role for PHGPx in the antioxidant system, which is also considered to be involved in carcinogenic mechanisms. Oxygen radicals have been suggested as causative factors in carcinogenic processes and are implicated in degenerative diseases such as several types of human cancers and inflammatory diseases (9). In these diseases, COX enzymes are overexpressed, this being especially true for COX-2. COX have been shown to be a target of nonsteroidal anti-inflammatory drugs (NSAID). Various epidemiological studies have shown a correlation between NSAID use and a reduced risk of colon cancer (10, 11). NSAID reduced the size and the number of polyps in young patients with familial adenomatous polyposis (FAP) with regard to spontaneous polyp development (12). In human and rodent colorectal carcinomas, the expression of COX-2 is increased contributing to the amount of eicosanoids liberated. Logically, glutathione peroxidase reduces these hydroperoxides formed by AA metabolism. Morever, Battu *et al.* have shown that expression of COX-2 in HT29 cl.19A cells

Correspondence to: Prof. Franck Sturtz, Laboratoire de Biochimie Médicale et de Génétique Moléculaire, Faculté de Médecine, 2 rue du Docteur Marcland, F-87025 Limoges Cedex, France. Tel: +33 662 077 760, Fax: +33 555 056 402, e-mail: fsturz@club-internet.fr

Key Words: PHGPx, GPx4, cyclooxygenase, colon cancer cells.

(colon adenocarcinoma cells) appeared to be at the same time inducible and constitutive (13). Chinery *et al.* suggested that coadministration of antioxidants and selective COX-2 inhibitors may be useful in the prevention and / or treatment of colorectal cancer (14).

The aim of the present work was to investigate whether PHGPx overexpression in HT29 cl.19A cells could modify AA metabolism and, more specifically, COX-2 which is implicated in colon cancer. Therefore, in this study we cloned the PHGPx gene in an expression plasmid and transfected it in HT29 cl.19A cells. After several days of selection, PHGPx-overexpressing clones were isolated and one clone was studied for its AA metabolism under basal conditions and after IL1β or TNFα stimulation. The results obtained were unexpected as they suggest a role for PHGPx as a regulator of COX activity.

Materials and Methods

Cell culture conditions. The HT29 cl.19A cell line is a human intestinal epithelial cell line derived from a human undifferentiated colon adenocarcinoma (15). Cells were grown at 37˚C in a humidified 6% CO_2, 94% atmosphere, in Dulbecco's Modified Eagle's Minimum Essential Medium supplemented with 10% FBS and 4 mM glutamine.

Cloning procedure. The PHGPx gene was inserted in an expression plasmid pcDNA3.1+ according to the method described by Dietmaier *et al.* (16). Human PHGPx cDNA was amplified by RT-PCR from MOLT4 cells. PHGPx cDNA coding the entire amino acid sequence was obtained with the following primers : 5'-ATCGCATTGGTCGGC TGGACG-3' and 5'-ATCGTATTCCCACAAGGTAGCCAG-3' specific for the published PHGPx sequence (17). The upper primer hybridizes to position -52 and the lower to position +785 with respect to the transcription initiation site. The length of the amplified region was 837 bp and included the selenocysteine insertion sequence (SECIS) in the 3'UTR. Cell clones which overexpressed PHGPx were isolated and cultured with 600 μg/ml G418. In order to study PHGPx activity and AA metabolism, the medium was supplemented for 10 days with 500 nM sodium selenite.

PHGPx activity. To measure PHGPx activity, the substrate L-α-phosphatidylcholine-β-linoleoyl-γ-palmitoyl was dissolved in 0.2 M borate buffer pH 9 and 10 mM deoxycholate. The reaction was started by adding 25 μg/ml of soybean lipoxygenase (type V) and incubated at 22˚C for 30 min. The formation of hydroperoxides was monitored spectrophotometrically at 234 nm and concentrations were calculated using a molar extinction coefficient of 25000 M^{-1} x cm^{-1}. PHGPx activity was measured by recording specific NADPH oxidation in the presence of GSH, glutathione reductase and phosphatidylcholine hydroperoxide (PCOOH) substrates at 340 nm and 37˚C (18). HT29 cl.19A cells (control and transfected) were washed twice with phosphate buffer saline and the resulting cell pellet was resuspended in lysis buffer (15 mM Tris-HCl pH 7.5, 2 mM $MgCl_2$) containing Complete™ protease cocktail (25/1 v/v) (Roche). Cells were lysed by freeze-thawing three times in a dry-ice ethanol bath and sonicated using a Scientific Vibra Cell 72434 (Bioblock, Illkirch, France). Sonicated samples were then ultracentrifuged at 100,000 x g for 1h. The supernatants were collected and total protein concentration was determined by the Lowry method (19). Briefly, the reaction mixture contained 50 mM potassium phosphate buffer, 0.5 mM EDTA pH 7, 2 mM GSH, 0.16 mM NADPH and 1U glutathione reductase. Protein (200 μg) from cells was added to 1 ml of reaction mixture and incubated for 5 min at 37˚C. The reaction was initiated by adding 20 μM PCOOH and monitored at 340 nm for 2 min with a thermostatic spectrophotometer (Safas, Monaco).

HPLC analysis of arachidonic acid metabolites. After 10 days culture with 500 nM sodium selenite, cells were trypsinized, washed twice with 0.05 M sodium phosphate buffer (pH 8.2), resuspended in 500 μl of the same containing 2 mM $CaCl_2$ and 0.5 mM $MgCl_2$ and preincubated for 5 min at 37˚C. Calcium ionophore A23187 (0.5 μg) and exogenous [1-^{14}C] AA (1.5 μCi) were added in an ethanolic solution that never exceeded 0.4 % and did not affect AA metabolism. The same experiments were performed on cells preincubated for 30 min at 37˚C with COX inhibitors (10 μM indomethacin ; 278 μM aspirin; 1 μM or 100 μM meloxicam; 3.7, 15 or 30 μM resveratrol). After 15 min at 37˚C, the reaction was stopped by addition of 500 μl methanol containing 500 ng x ml^{-1} PGB_2 as internal standard. The extract was centrifuged for 5 min at 3000 g and the supernatant was acidified to pH 3-4 with phosphoric acid. The extracts were directly injected on a 5-μm Radial-Pack™ C_{18} cartridge (Waters-Millipore), protected by Waters C_{18}™Guard-Pak precolumn. COX metabolites were eluted at a flow rate of 2.0 ml x min^{-1}, using a tertiary methanol-acetonitrile-water gradient, as previously described (20). Exogenous [1-^{14}C] AA metabolism was analyzed by reverse-phase HPLC, which was carried out on a Merck system equipped with an L-6200A Intelligent pump and a 655A variable wavelength UV monitor. Radioactivity was monitored using a Packard FLO-ONE/Beta detector Series A-500, and the scintillation cocktail (ULTIMA-FLOTM AP, Packard) was pumped at a flow rate of 2 ml x min^{-1}.The identity of COX products (PGE_2, TXB_2, HHT) was confirmed by use of specific cyclooxygenase inhibitors and HPLC standards.

Quantification of arachidonic acid metabolites (EIA). Transfected HT29 cl.19A and control cells were cultured and the medium was centrifuged at 1000g for 5 min at 4˚C and stored at -80˚C until analysis. The amount of PGE_2, TXB_2, $PGF_2\alpha$ and 6 keto-$PGF_1\alpha$ released by a cell monolayer was measured by a commercially available EIA kit according to the manufacturer's instructions (Cayman) using serial dilutions of previously conditioned medium.

Semi-quantitative mRNA assay. The relative levels of PHGPx and COX-2 mRNA were determined using a semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). The expression of PHGPx and COX-2 was normalized to β-actin mRNA levels. The primers used for PHGPx and COX-2 were, respectively: sense 5'-TCGTGTGCATCGTCACCAAC-3', antisense 5'-CGTTCA CGCAG ATCTTGCTG-3', sense 5'-TTCTCCTGCCTACTGGAAGC-3', antisense 5'-TATCATCTAGTCCGGAGCGG-3'. The primers used for β-actin were: sense 5'-CTACAATGAGCTGCGTGTGG-3' ; antisense, 5'-AAGGAAGGCTGGAAGAGTGC-3'. For PCR, 1.5 μg cDNA (obtained after reverse transcriptase) was amplified in a reaction mixture containing 1xPCR buffer with 2mM $MgCl_2$, 20 mM dNTP, 20 pmole primers and 0.1 U Taq DNA polymerase (Qbiogene, Montreal, Canada). The PCR reaction was run on a Crocodile II thermal cycler (Appligene) for 22 cycles : 45 sec at 94˚C, 45 sec at 62˚C and 1 min at 72˚C. The lengths of the amplified regions for PHGPx,

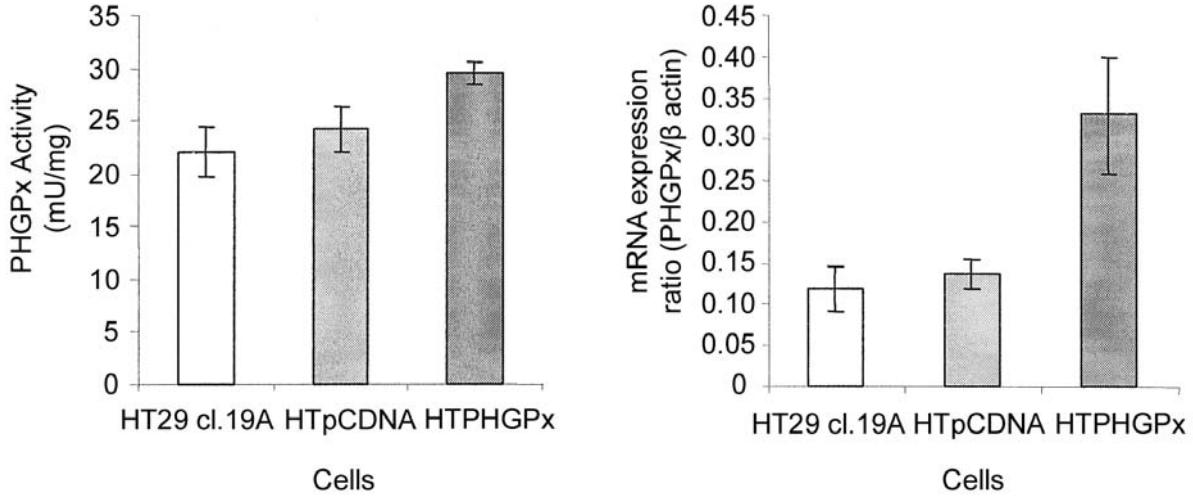

Figure 1. *PHGPx activity levels in transfected and wild-type HT29 cl.19A cells and level of PHGPX mRNA. PHGPx activity and level of PHGPX mRNA were determined on several HT29 cl.19A cell populations: the wild-type population, the population transfected with the empty expression plasmid and a clone overexpressing the PHGPx enzyme. One unit of activity of PHGPx is defined as the amount of enzyme catalysing the reduction of 1 μmole of hydroperoxide/min at 37˚C and pH 7.4. The mRNA levels of PHGPx were determined by reference to β-actin mRNA.*

COX-2 and β-actin were, respectively 224 pb, 194 pb and 528 pb. Amplification products were separated on 1.2% agarose gels and signal intensities were analyzed by densitometry using the Gel DOC 2000 Quantity One^R (BioRad) system.

Results

Effect of transfection on PHGPx activity. In the present study, HT29 cl.19A cells that overexpressed human PHGPx were established. We isolated G418-resistant stable transfectants after HT29 cl.19A had been transfected with the expression vector pcDNA3.1-PHGPx or pcDNA3.1+ (control vector). PHGPx recombinant cells were characterized by PCR using primers which hybridized to the vector on the 5'end and to the insert on the 3'end. Transfection was confirmed by overexpression of PHGPx mRNA in transfectants (data not shown). PHGPx is widely expressed in many cell types but PHGPx activity is much lower than other GPx activities. PHGPx activity was analysed after 10 days supplementation with 500 nM sodium selenite. HT29 cl.19A and control transfected cells showed similar activity. We isolated transfectant clones of HT29 cl.19A that overexpressed PHGPx and selected the clone with the highest expression of PHGPx. The expression of PHGPx in this clone was stable over time (data not shown), but limited to 33% (Figure 1) as compared to parent cells. The transfection efficiency was also verified by semiquantitative RT-PCR and the level of PHGPx mRNA in HTPHGPx cells was increased by 176% (Figure 1).

Arachidonic acid metabolism. When $4x10^7$ HT29 cl.19A cells expressing the PHGPx cDNA were incubated with exogenous [1-C^{14}] arachidonic acid, the metabolic profile shown in Figure 2 demonstrated a significant increase in cyclooxygenase activity when compared to control cells. These cells produced TXB_2 (peak 1), PGE_2 (peak 2), HHT (peak 3) and an unidentified compound (peak 4). This was confirmed by the use of standards and inhibitors of AA metabolism. In the presence of indomethacin, aspirin, meloxicam and resveratrol, we observed an inhibition of all four peaks mentioned in Table I, while the LOX pathway was not changed. Radioactive COX products were identified by extraction of eluted fractions and thin-layer chromatography with standards. The major changes in AA metabolism between control cells and positive PHGPx-expressing transfected clones were not the appearance of new peaks but only modification of the quantities of known products.

Metabolite quantification. Quantification of major cyclo-oxygenase metabolites was determined by EIA. This method evaluates the synthesis of PGE_2, TXB_2, $PGF_2\alpha$ and 6 keto-$PGF_1\alpha$ in the total absence of stimulation by ionophore A23187 and exogenous AA. The results, shown in Figure 3, display a large increase in metabolic rate. Concerning the four measured metabolites, PHGPx-overexpressing cells synthesized higher amounts of TXB_2, 6 keto-$PGF_1\alpha$, $PGF_2\alpha$ and PGE_2 which increased 1.7, 5, 5.5 and 4.17-fold, respectively. After 20-h treatment with IL1β and TNFα (10 ng/ml), AA metabolism was elevated in particular for the COX pathway (PGE_2, TXB_2, $PGF_2\alpha$, 6 keto-$PGF_1\alpha$). This difference was observed on HPLC profiles and measured by EIA in Figure 3.

Expression of COX-2 mRNA after PHGPx transfection. Semiquantitative RT-PCR analysis of COX-2 mRNA

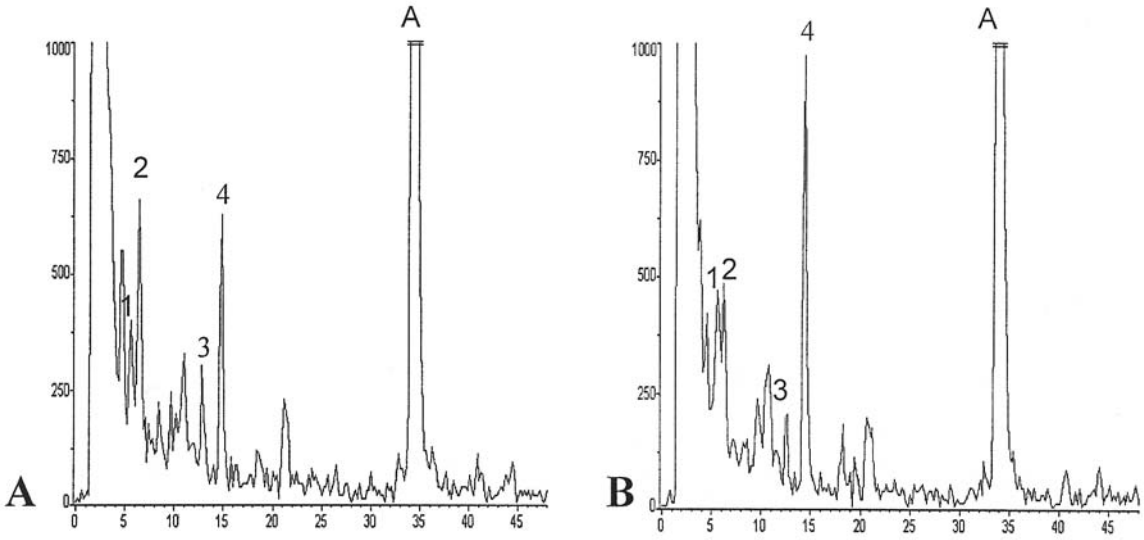

Figure 2. *Chromatograms of AA metabolism profiles. Panel A and B show, respectively, the AA metabolism of wild and PHGPx-overexpressing cells measured by HPLC (peak 1 TXB_2, peak 2 PGE_2, peak 3 HHT, peak 4 unidentified COX product). These data were representative of several independent experiments.*

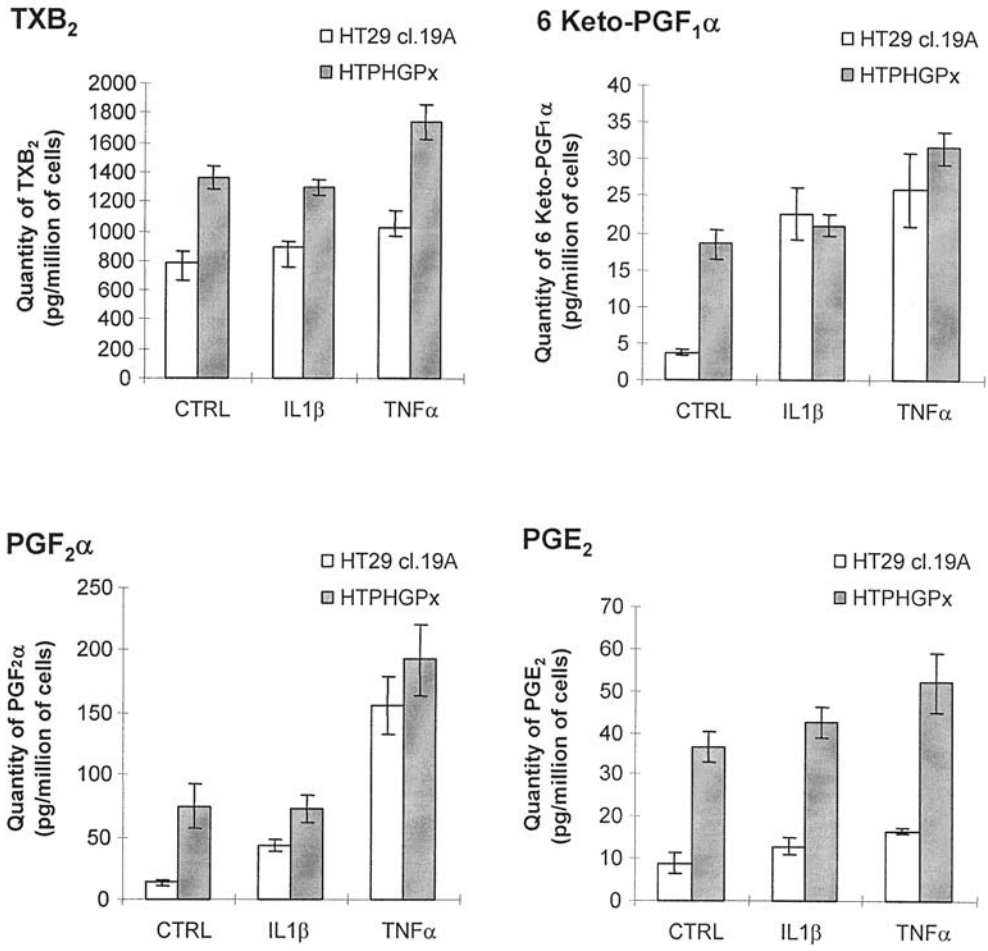

Figure 3. *Effects of PHGPx overexpression on cyclooxygenase metabolism. EIA measurements of TXB_2, 6 keto-$PGF_1\alpha$ $PGF_2\alpha$ and PGE_2 were performed in PHGPx-transfected and wild-type cells, grown under basal conditions or IL1β or TNFα stimulation (10 ng/ml during 20 hours). The results are expressed in picograms per million cells.*

Table I. *Percentage inhibition of several major peaks by different COX inhibitors. The results were obtained by HPLC after cells had been incubated for 30 min with different COX inhibitors. Similar results were obtained from several experiments.*

	TXB_2	PGE_2	HHT	Peak n°4
Inhibitors				
Indomethacin 10 μM	93.6	85.4	95	99
Aspirin 278 μM	90.1	78.1	86.5	91.3
Meloxicam 1 μM	38.8	0	0	62.2
Meloxicam 100 μM	11.7	0	0	53.6
Resveratrol 3.7 μM	56.1	16	26.2	76.3
Resveratrol 15 μM	98.5	33.4	67.4	84.1
Imidazol 5 mM	80.4	0	25.2	80

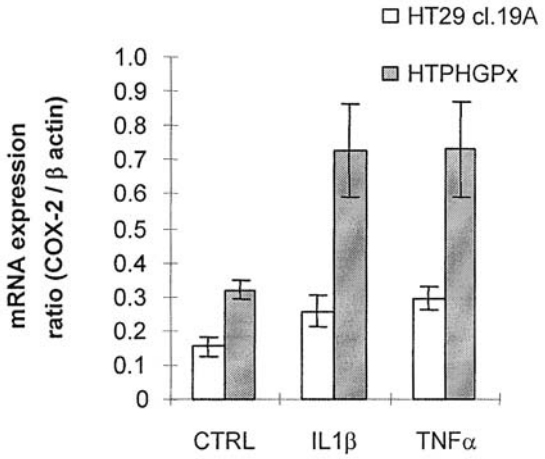

Figure 4. *Levels of COX-2 mRNA in PHGPx-transfected and wild-type cells. mRNA levels of COX-2 were determined by reference to β-actin mRNA in PHGPx-transfected and wild-type cells grown under different conditions. The effect of IL1β or TNFα stimulation (10 ng/ml) was measured after 20 hours on both cell types.*

expression in HT29 cl.19A transfected by PHGPx and control cells was studied using β-actin as an internal standard. As shown in Figure 4, significant increases in COX-2 mRNA levels were observed in transfected cells. As expected this phenomenon was amplified by IL1β or TNFα stimulation in similar proportions when compared to the enzymatic metabolism studied by HPLC and EIA quantification.

Discussion

Because hydroperoxide metabolism seems to be of crucial importance in colon cancer, we wanted to explore the effects of PHGPx overexpression in colon cancer cells. After transfection of an expression plasmid, we isolated clones of HT29 cl.19A cells that overexpressed PHGPx to study the effects of PHGPx on AA metabolism. COX metabolites, quantified by EIA, were the principal components observed on HPLC profiles from control and PHGPx-transfected cells. The metabolic specificities of HT29 cl.19A cells have been previously described by Battu *et al.* (21). In agreement with these previous results, we noted an unidentified peak (n°4) on the HPLC profile. Interestingly, this unidentified peak (n°4) was noticeably increased in our study following PHGPx transfection. In addition, peak 4 was decreased from 53.6% to 99% in the presence of COX inhibitors, but not by LOX inhibitors and only slightly by cytochrome inhibitors (20%) (data not shown), indicating that this product is most probably derived from COX metabolism or derived from the action of COX on a hydroperoxide. Attempts to identify peak n°4 by isolating this molecule were not successful. In PHGPx-overexpressing clones, an increase in COX pathway products was observed and verified by EIA quantification of PGE_2, TXB_2, $PGF_2\alpha$ and 6 keto-$PGF_1\alpha$. Thus PHGPx seems to be involved in COX regulation and seems to increase the amount of COX products.

We also examined COX-2 mRNA expression because COX-2 is known to be inducible while COX-1 is constitutive. In our study, COX-2 mRNA levels correlated well with the increase in AA metabolites and cells that overexpressed PHGPx had increased quantities of COX-2 mRNA. Surprisingly, COX-2 mRNA increased 176% when the increase in enzyme activity was only 33%. This may be due to a lack of selenocysteine needed for protein synthesis or to a lack of post-translational modifying enzymes. This may also be due to the enzymatic method which did not include a full purification of PHGPx but rather a crude membrane preparation. Nevertheless, PHGPx seems to have an impact on COX-2 metabolism and could be a key regulatory element of COX-2 metabolism. In contrast, Huang *et al.* observed that partially purified PHGPx directly reduced lipid hydroperoxides and then down-regulated the activity of LOX and COX, but these studies were performed in acellular models using purified enzymes (PHGPx, LOX, COX) (22). To our knowledge our data are the first regarding the influence of PHGPx on COX metabolism in a whole cell system. Imai *et al.* showed, in a whole cell system, that LOX pathway products such as 5-hydroxyeicosatetraenoic acid, leukotrienes C4 and D4 were strongly decreased in PHGPx-overexpressing rat basophilic leukemia RBL-2H3 cells (23), but in HT29 cl.19A cells there was no difference in LOX metabolism. In this study, the authors did not measure the COX metabolism.

IL1β and, even more so, TNFα are known to induce COX-2 metabolism (24). Therefore we stimulated our PHGPx-overexpressing cells by IL1β or TNFα. This resulted in an even greater increase in COX metabolism in the PHGPx cells compared to normal cells. In fact, in all but one sample of stimulated PHGPx cells, COX metabolites (TXB_2, 6 keto-$PGF_1\alpha$, $PGF_2\alpha$, PGE_2) were present in higher quantities when compared to stimulated

wild-type HT29 cl.19A cells. The level of increase was of variable intensity. For instance, TNFα stimulated $PGF_2\alpha$ production quite strongly while PGE_2 was more moderately increased. This could indicate that TNFα has a different stimulatory action on the secondary metabolism of endoperoxides. Nevertheless, IL1β or TNFα stimulation did not alter the action of PHGPx on COX metabolite production but rather amplified it.

The results presented here were obtained on a colon cancer cell line (HT29 cl.19A) that is sensitive to Celecoxib, a selective COX-2 inhibitor. This inhibitor strongly suppresses the proliferation of HT29 cells (25).

Numerous studies have shown that COX-2 is a target for colon cancer prevention and treatment. In this study, a link between PHGPx expression and COX-2 metabolism in a colon carcinoma cell line was demonstrated. This unexpected finding needs to be confirmed in several colon cancer cell lines and, if possible, on primary colon cancer cells, as it might be useful in understanding the mechanisms of the oncogenic transformation of colon cells and the potential involvement of PHGPx in the regulation of COX-2.

References

1 Smith WL: The eicosanoids and their biochemical mechanisms of action. Biochem J *259*: 315-324, 1989.

2 Funk CD: Molecular biology in the eicosanoid field. Prog Nucleic Acid Res Mol Biol *45*: 67-98, 1993.

3 Ursini F, Maiorino M, Valente M, Ferri L and Gregolin C: Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. Biochim Biophys Acta *710*: 197-211, 1982.

4 Ursini F, Maiorino M and Gregolin C: The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. Biochim Biophys Acta *839*: 62-70, 1985.

5 Thomas JP, Maiorino M, Ursini F and Girotti AW: Protective action of phospholipid hydroperoxide glutathione peroxidase against membrane-damaging lipid peroxidation. *In situ* reduction of phospholipid and cholesterol hydroperoxides. J Biol Chem *265*: 454-461, 1990.

6 Roveri A, Maiorino M and Ursini F: Enzymatic and immunological measurements of soluble and membrane-bound phospholipid-hydroperoxide glutathione peroxidase. Methods Enzymol *233*: 202-212, 1994.

7 Maiorino M, Chu FF, Ursini F, Davies KJ, Doroshow JH and Esworthy RS: Phospholipid hydroperoxide glutathione peroxidase is the 18-kDa selenoprotein expressed in human tumor cell lines. J Biol Chem *266*: 7728-7732, 1991.

8 Esworthy RS, Baker MA and Chu FF: Expression of selenium-dependent glutathione peroxidase in human breast tumor cell lines. Cancer Res *55*: 957-962, 1995.

9 Halliwell B and Gutteridge JMC: Free Radicals in Biology and Medicine. 2nd Ed. London: Oxford University Press 1989.

10 Thun MJ, Namboodiri MM and Heath CWJr: Aspirin use and reduced risk of fatal colon cancer. N Engl J Med *325*: 1593-1596, 1991.

11 Giovannucci E, Egan KM, Hunter DJ, Stampfer MJ, Colditz GA, Willett WC and Speizer FE: Aspirin and the risk of colorectal cancer in women. N Engl J Med *333*: 609-614, 1995.

12 Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF and Taketo MM: Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell *87*: 803-809, 1996.

13 Battu S, Chable-Rabinovitch H, Rigaud M and Beneytout JL: Cyclooxygenase-2 expression in human adenocarcinoma cell line HT29 cl.19A. Anticancer Res *18*: 2397-2403, 1998.

14 Chinery R, Beauchamp RD, Shyr Y, Kirkland SC, Coffey RJ and Morrow JD: Antioxidants reduce cyclooxygenase-2 expression, prostaglandin production, and proliferation in colorectal cancer cells. Cancer Res *58*: 2323-2327, 1998.

15 Augeron C and Laboisse CL: Emergence of permanently differentiated cell clones in a human colonic cancer cell line in culture after treatment with sodium butyrate. Cancer Res *44*: 3961-3969, 1984.

16 Dietmaier W, Fabry S and Schmitt R: DISEC-TRISEC: di- and trinucleotide-sticky-end cloning of PCR-amplified DNA. Nucleic Acids Res *21*: 3603-3604, 1993.

17 Esworthy RS, Doan K, Doroshow JH and Chu FF: Cloning and sequencing of the cDNA encoding a human testis phospholipid hydroperoxide glutathione peroxidase. Gene *144*: 317-318, 1994.

18 Paglia DE and Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med *70*: 158-169, 1967.

19 Lowry OH, Rosebrough NJ, Farr AL and Randall R: Protein measurements with the folin phenol reagent. J Biol Chem *193*: 265-275, 1951.

20 Borgeat P, Fruteau de Laclos B, Rabinovitch H, Picard S, Braquet P, Hebert J and Laviolette M: Eosinophil-rich human polymorphonuclear leukocyte preparations characteristically release leukotriene C4 on ionophore A23187 challenge. J Allergy Clin Immuno *l74*: 310-315, 1984.

21 Battu S, Clement G, Heyman M, Wal JM, Cook-Moreau J, Desjeux JF and Beneytout JL: Production of arachidonic acid metabolites by the colon adenocarcinoma cell line HT29 cl.19A and their effect on chloride secretion. Cancer Lett *116*: 213-223, 1997.

22 Huang HS, Chen CJ, Suzuki H, Yamamoto S and Chang WC: Inhibitory effect of phospholipid hydroperoxide glutathione peroxidase on the activity of lipoxygenases and cyclooxygenases. Prostaglandins Other Lipid Mediat *58*: 65-75, 1999.

23 Imai H, Narashima K, Arai M, Sakamoto H, Chiba N and Nakagawa Y: Suppression of leukotriene formation in RBL-2H3 cells that overexpressed phospholipid hydroperoxide glutathione peroxidase. J Biol Chem *273*: 1990-1997, 1998.

24 Jones DA, Carlton DP, McIntyre TM, Zimmerman GA and Prescott SM: Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines. J Biol Chem *268*: 9049-9054, 1993.

25 Yamazaki R, Kusunoki N, Matsuzaki T, Hashimoto S and Kawai S: Selective cyclooxygenase-2 inhibitors show a differential ability to inhibit proliferation and induce apoptosis of colon adenocarcinoma cells. FEBS Lett *531*: 278-284, 2002.

Received December 9, 2003
Revised January 19, 2004
Accepted February 24, 2004