Institute for Pharmaceutical Technology[1], Johann Wolfgang Goethe-University, Frankfurt am Main; Institut für Lasertechnologien in der Medizin und Meßtechnik[2] an der Universität Ulm; Institute for Pharmaceutical Chemistry[3], Johann Wolfgang Goethe-University, Frankfurt am Main, Germany; Institute of Pharmaceutical Sciences[4], Karl-Franzens-University, Graz, Austria

Glutathione peroxidase isoenzymes in human tumor cell lines

T. Paukert[1], R. Sailer[2], W. S. L. Strauss[2], M. Schubert-Zsilavecz[3], A. Zimmer[4]

Received March 15, 2011, accepted April 15, 2011

Univ.-Prof. Dr. Andreas Zimmer, Institute of Pharmaceutical Sciences, Karl-Franzens-University, Humboldstr. 46, 8010 Graz, Austria
Andreas.Zimmer@uni-graz.at

Pharmazie 66: 894–898 (2011) *doi: 10.1691/ph.2011.1540*

A set of human tumor cell lines was characterized in terms of the GPx isoenzymes GPx1, -2, -3 and -4. Semiquantitative PCR was used to investigate the GPx mRNA transcripts and the GPx activity was determined photometrically. As a result of culturing under standard conditions, diverse distribution of GPx mRNA and basic GPx activity was found in the investigated cell lines. PCR results showed nearly ubiquitous existence of the isoenzymes GPx1 and GPx4. GPx2 mRNA transcript was only detected in the colonic cell line CaCo-2. After detection of the GPx3 mRNA transcripts in most of the tested cell lines, an ELISA was performed to investigate if the GPx3 protein is present as well. However, the GPx3 protein could not be detected. Glutathione peroxidases contain the amino acid selenocysteine in their active centre. Selenocysteine contains selenium instead of sulfur in cysteine. Therefore, the influence of selenium on GPx activity and GPx isoenzyme distribution was investigated. Cell culturing with additional selenium showed a clear elevation of GPx activity in Mono Mac 6 cells but no gain of mRNA transcripts or any change in the isoenzyme's distribution.

1. Introduction

Glutathione peroxidases (GPx) display an important selenoenzyme family which is involved in the detoxification of hydrogen peroxides and lipid - hydroperoxides (Brigelius Flohe 1999; Papp et al. 2007).
The GPx family comprises at least four distinct isoenzymes: GPx1 (cGPx), the classical or cellular enzyme is almost ubiquitous in mammalian cells. GPx2, the gastrointestinal GPx, is expressed almost exclusively tissue-specific in the gastrointestinal tract (Akasaka et al. 1990; Brigelius-Flohe et al. 2001; Chu et al. 1993) and possibly responsible for the detoxification of ingested lipid hydroperoxides (Chu et al. 1993; Esworthy et al. 1995; Wingler et al. 2000). GPx3, the extracellular plasma GPx (pGPx or eGPx), is present in extracellular fluids like plasma (Takahashi et al. 1987) and breast milk (Avissar et al. 1991). GPx3 is synthesized and secreted by kidney cells (Avissar et al. 1994; Maser et al. 1994; Yoshimura et al. 1991). GPx4, the phospholipid hydroperoxide glutathione peroxidase (PhGPx) is capable of metabolizing peroxidized phospholipids and cholesterol and appears to be ubiquitous (Gladyshev and Hatfield 1999; Himeno and Imura 2000; Mates and Sanchez-Jimenez 1999; Zhao 2001). There is also a membrane-associated form of GPx4 (Gladyshev and Hatfield 1999), which protects biomembranes from oxidative damage (Ursini et al. 1985; Zhao 2001). GPx1, GPx2 and GPx3 are tetramers; GPx4 is a monomer (Maiorino et al. 1991). In its active center, each monomer contains one molecule of the amino acid selenocysteine which is indispensable for catalytic properties (Brown and Arthur 2001; Flohe et al. 1973). Thus, depletion of selenium leads to a drastic impairment in enzyme activity (Chow and Tappel 1974) and cell culturing with additional selenium results in the rise of GPx activity (Wilke et al. 1992).
Since glutathione peroxidases play an important role in the metabolism of reactive oxygen species (ROS), we are planning to study the influence of GPx activity levels on the efficacy of photodynamic therapy (PDT). We intend to lower GPx activity levels by means of antisense oligonucleotides towards GPx to enhance the efficiency of PDT (Paukert et al., submitted). The antisense mechanism is a process in which an oligo nucleotide hybridizes specifically to a target RNA; thereby inhibiting the protein's translation (Aboul-Fadl 2005; Mizuno et al. 1984). Lowering of GPx activity through a decrease in GPx protein is expected to enhance PDT efficacy. In contrast, higher GPx activity is expected to increase cell viability after PDT-treatment due to a higher detoxification rate of ROS generated by PDT during light exposure. In order to find an adequate cell line for the PDT experiments, we investigated the GPx isoenzyme's distribution and GPx activity levels in different cell lines, preferentially human cancer cell lines.

2. Investigations and results

2.1. *Distribution of GPx isoenzymes under standard conditions*

Nine different human tumor cell lines were screened for the distribution of mRNA transcripts of the four GPx isoenzymes. Cells were cultured using standard culturing medium, harvested

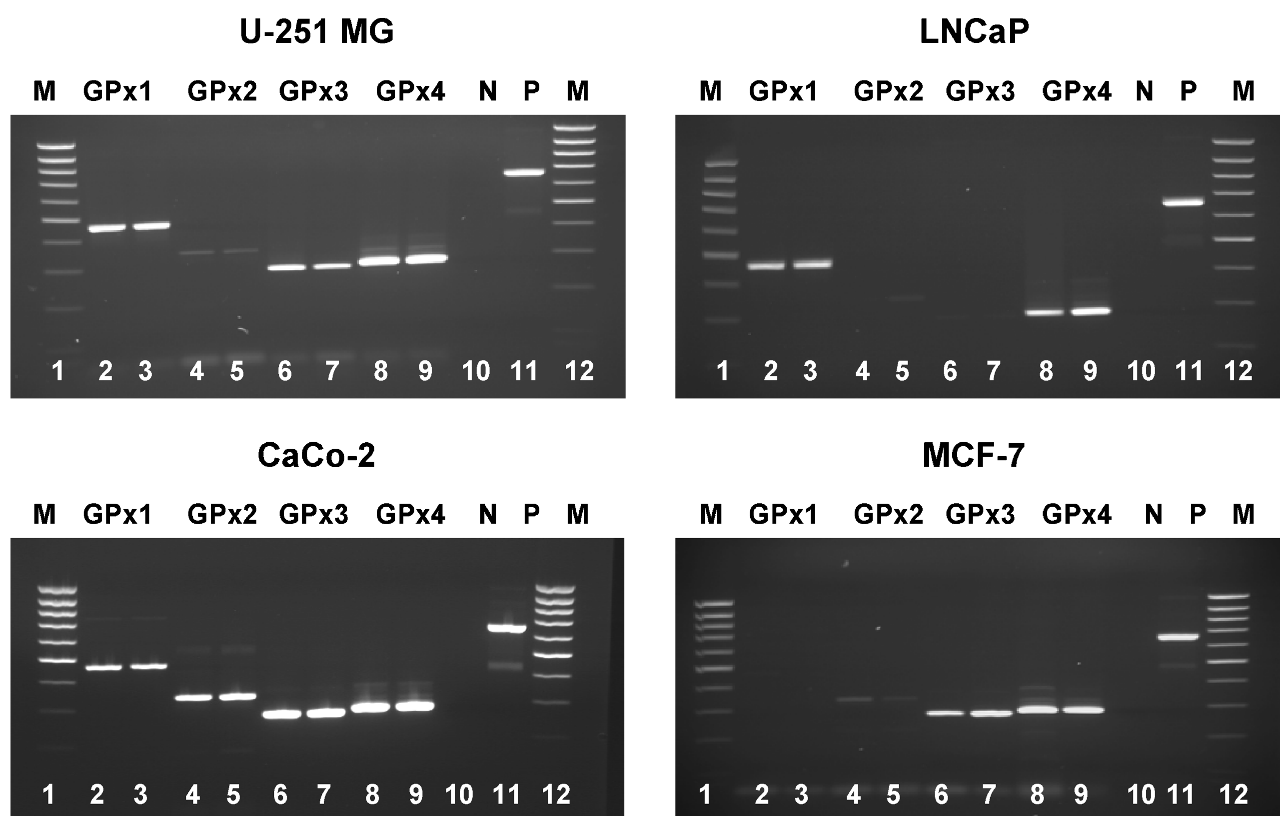

Fig. 1: GPx mRNA transcripts (standard culturing conditions) M: DNA ladder, N: negative control, P: positive control β-tin

and PCR was performed. The results show divers allocation and intensity for mRNA transcripts for each cell line (see Table 1 and Fig. 1). GPx1 mRNA transcript was detected in all tested cell lines except for MCF-7 and T47D. In our experiments GPx2 was found only in the cell line CaCo-2. Therefore, it is the only cell line comprising all four GPx isoenzymes investigated in this work. GPx3 mRNA transcripts are found in every cell type except LNCaP und BT-20. GPx4 mRNA exists in all tested cell lines.

2.2. *Estimation of GPx activity*

GPx activity was determined in the cell lines U-251-MG, MCF-7, Mono Mac 6 and CaCo-2. The impact of the selenium supplementation on Mono Mac 6 cells differs clearly from other cell lines tested. As shown in Fig. 2, the cell lines MCF-7 and CaCo-2 show no considerable gain in GPx-activity by selenium.

Table 1: Allocation of GPx mRNA transcripts determined by PCR in nine cell lines – no transcription + light, ++ medium, +++ strong transcription

	GPx1	GPx2	GPx3	GPx4
Mono Mac 6	+++	–	+	+++
U-251-MG	++	–	+	+++
MCF-7	–	–	+++	+++
CaCo-2	+	++	+++	+++
A431	++	–	+	+++
LNCaP	+++	–	–	+++
T47D	–	–	+++	+++
OV2774	+	–	+	++
BT-20	+	–	–	++

The basic GPx-activities of these two cell lines, as well as their activity after selenium supplementation, are too low to obtain reliable results (MCF-7: 12 and 32 mU/mg protein; CaCo-2: 39 and 97 mU/mg protein). U-251-MG cells show a 5-to 7-fold increase compared to the basic GPx-activity (50 mU/mg). Mono Mac 6 cells show the most obvious enhancement of GPx-activity. An about 10-fold increase compared to the basic GPx-activity (99 mU/mg to 996 mU/mg) can be measured in Mono Mac 6 cells after the addition of selenium.

2.3. *GPx3 protein*

In PCR experiments, GPx3 mRNA transcripts were found in every tested cell line except LNCaP und BT-20 (see above). Thus, an ELISA assay was performed to test the cell lines Mono Mac 6, U-251-MG, MCF-7 and CaCo-2 for the GPx3 protein. The testing of the cell lysates and the cell supernatants showed

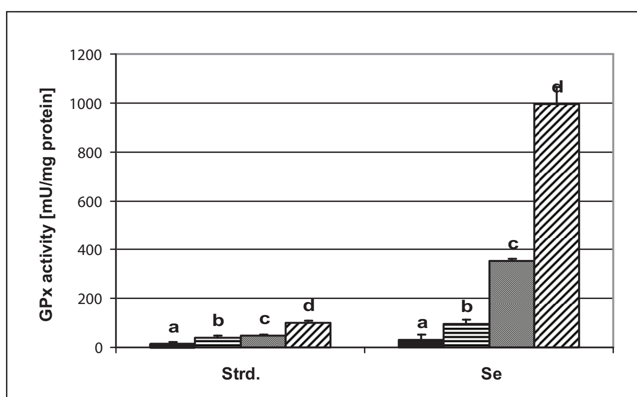

Fig. 2: GPx activity under standard (Strd.) culturing conditions and after supplementation with selenium dioxide (Se); a MCF-; b CaCo-2; c U-251-MG; d Mono Mac 6

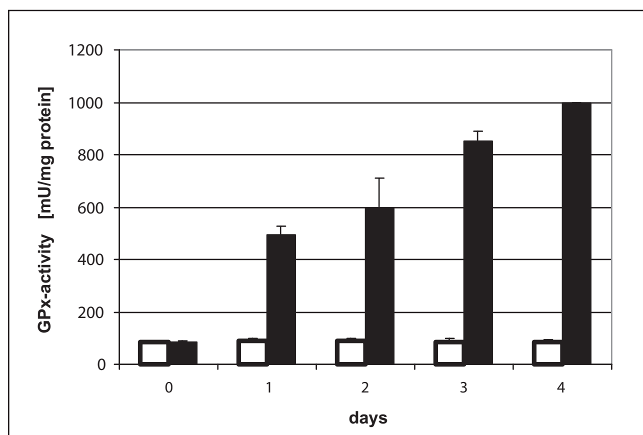

Fig. 3: Supplementation of Mono Mac 6 with selenium dioxide over 4 days.
■ standard culturing conditions; □ culturing with SeO_2

no GPx3 protein. Also, the culturing with additional selenium dioxide or hydrogen peroxide did not result in detectable GPx3 protein.

2.4. *Distribution of GPx isoenzymes after supplementation with selenium*

Mono Mac 6 cells show a considerable increase of GPx activity when cultured with additional selenium dioxide (see Fig. 2); therefore, it was further examined, whether the amount of the GPx mRNA transcripts changes in correspondence to the increased GPx activity. All samples were tested for GPx1, −2, −3, and −4 mRNA transcripts. The determination of GPx activity in the corresponding samples showed the same results as the previous experiments. Supplementation of selenium dioxide over a period of four days leads to a clear activation of GPx activity up to 996 mU/mg protein compared to standard culture conditions (87 mU/mg protein) (Fig. 3). Despite the fact that there is an enormous increase of GPx activity measurable in four days, there were no detectable changes in transcripts as GPx activity increased over the time elapsed (Fig. 4).

2.5. *Conclusion*

Semiquantitative PCR of nine cell lines showed varying distribution of GPx isoenzymes. Culturing with additional selenium did not result in detectable changes. After conducting a GPx enzyme activity assay only Mono Mac 6 cells showed a notable basic GPx activity which can be elevated considerably by culturing the cells with additional selenium. Therefore the Mono Mac 6 cell line will be the best cell model for the intended investigation of GPx activity's influence on PDT efficacy.

3. Discussion

First of all, we investigated the distribution of mRNA transcripts of the four GPx isoenzymes in nine different cell lines. As a result of culturing under standard conditions, diverse distribution was found (Fig. 2): According to published findings, GPx1 exists almost ubiquitous and is mainly found in the cytosol (Chambers et al. 1986; Flohe et al. 1973; Frampton et al. 1987; Mills 1957). We detected GPx1 in every cell line except MCF-7 and T47D (data not shown). GPx2, the gastrointestinal GPx, is solely transcribed in CaCo-2 cells, which is a colon adenocarcinoma cell line. These results align with publications that describe GPx2 as tissue-specific for the gastrointestinal tract (Akasaka et al. 1990; Brigelius-Flohe et al. 2001; Chu et al. 1993) for example in CaCo-2 cells (Avissar et al. 1994). In addition, GPx2 is found in human liver and large intestine as well (Chu et al. 1993). Like GPx1, GPx4 is expressed ubiquitously (Gladyshev and Hatfield 1999; Himeno and Imura 2000; Maiorino et al. 1991; Mates and Sanchez-Jimenez 1999; Roveri et al. 1994; Ursini et al. 1985). We were able to detect GPx4 transcript in each cell line. GPx3 is found predominantly in plasma and also in other extracellular fluids like milk (Avissar et al. 1991; Takahashi et al. 1987). It is synthesized mainly in the kidneys (Yoshimura et al. 1991) and secreted into the blood. We detected GPx3 mRNA in every cell line except LNCaP and BT-20. Because of these findings we performed an Enzyme-Linked ImmunoSorbent Assay (ELISA) to test if there is any GPx3 protein detectable in Mono Mac 6, CaCo-2, MCF-7 or U-251-MG. However, no protein was detectable in the cell lysate. Therefore, we tested the supernatant as well to examine if the GPx3 protein is secreted right after translation into extracellular surroundings, but no protein was provable. After supplementation with selenium dioxide to exclude a depletion of selenocysteine or the addition of H_2O_2 to create oxidative stress, no GPx3 protein was confirmable as well. These findings match published results that GPx3 mRNA is existent in most of the tested cell lines but the mature protein is primarily secreted by renal cell types (Bierl et al. 2004).

Furthermore, GPx activity was determined. By using the substrate cumene hydroperoxide, the total GPx activity of all GPx isoenzymes was measured (Brigelius-Flohe et al. 2002). The basic activities on the basis of standard culturing conditions and after the supplementation with SeO_2 are given in Fig. 2. CaCo-2 and MCF-7 cells exhibited a low basic GPx activity and only a slight increase in GPx activity was found after SeO_2 supplementation. In the case of U-251-MG cells, GPx activity after SeO_2 supplementation was moderately enhanced as compared to its basic activity. The GPx activity of Mono Mac 6 cells was elevated about 10-times (Fig. 3) after addition of selenium dioxide to the culture medium. Thus, we primarily used Mono Mac 6 in our studies, owing to the very low levels of expression in CaCo-2, MCF-7 and U-251-MG cells.

Due to the drastic elevation of GPx activity in Mono Mac 6 cells, we decided to investigate if the elevation is accompanied by a change in mRNA level of the four GPx isoenzymes. Transcripts of cells grown with standard culture medium were compared to those cultured with supplemented selenium dioxide over four days. For all GPx isoenzymes, no detectable changes of the mRNA amounts in Mono Mac 6 cells were found (Fig. 4). Quite comparable results were obtained for U-251-MG (data not shown). Therefore, the increased GPx activity in Mono Mac 6 (and U-251-MG) cells mediated through selenium addition to the culture medium does not appear to be caused by mRNA upregulation. Thus, it is possibly due to a higher quantity of protein. A limitation of selenium implies a lack of selenocysteine, which leads to a translational stop and no active enzyme is produced (Berry et al. 1991, 2001; Birringer et al. 2002; Chambers et al. 1986).

The assay for the estimation of GPx activity is a useful tool to investigate changes in GPx activity. The culturing of Mono Mac 6 cells with SeO_2 elevates GPx activity considerably; thus, this cell line seems to be an excellent model system for the intended antisense experiments, which are supposed to result in a lowered GPx activity.

4. Experimental

4.1. *Cell culture*

Mono Mac 6 (acute monocytic leukemia, DSMZ: ACC 124), MCF-7 (mammary gland, ATCC: HTB-22), U-251-MG (glioma, ECACC: 09063001), CaCo-2 (colon, ATCC: HTB-37), A431 (vulva, ATCC: CRL-1555), LNCaP (prostate, DSMZ: ACC 256), BT-20 (mammary gland, ATCC: HTB-19),

Fig. 4: GPx mRNA transcripts in Mono Mac 6 cells after supplementation with SeO_2 over 4 days. M: DNA ladder, N: negative control, P: positive control CaCo-2 cells

OV2774 (ovary, D. G. Kieback, Maastricht, NL) and T47-D (mammary gland, ATCC: HTB-133) cells were maintained in standard culture medium (RPMI 1640 with 300 mg/l L-glutamine supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin sulfate, 250 ng/ml amphotericin B) at 37 °C in a humidified atmosphere (95% relative humidity) containing 5% CO_2. U-251-MG cells were supplemented additionally with 1x non-essential amino acids (NEAA). Mono Mac 6 cells were additionally supplemented with 1x NEAA, 1 mM oxal acetic acid, 10 μg/ml bovine insulin and 1 mM sodium pyruvate.

4.2. *Semiquantitative PCR*

RNA isolation from cells was performed with RNeasy® MinEluteTM Cleanup Kit and QIAshredder® (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. RNA quantity was determined photometrically at 260 nm. The purity of RNA was checked with an agarose gel electrophoresis. For DNase digestion of the RNA, 5 μg RNA were used. Thereafter, cDNA was synthesized through reverse transcription with the Omniscript™ Reverse Transkriptase Kit (Qiagen GmbH). PCR was used for the determination of GPx mRNA levels. The method was performed according to standard protocols. We used the Taq PCR Master Mix Kit (Qiagen GmbH, Hilden, Germany), and peqGOLD 100 bp DNA ladder (PEQLAB GmbH, Erlangen, Germany) as DNA standard. Primers (see Table 1) were synthesized by Thermo Hybaid (Ulm, Germany). As a positive control, β-actin was employed. Equal amounts of samples were used.

4.3. *Detection of GPx3 protein*

An ELISA kit (BIOXYTECH® pl·GPx Enzyme Immunoassay™, Oxis-Research, Portland, USA) was used for the detection of the GPx3 protein in Mono Mac 6, U-251-MG, MCF-7 and CaCo-2 cells. Performance was according to the manufacturer's protocol. Human plasma was used as a positive control sample. U-251-MG, MCF-7 and CaCo-2 cells were detached with Accutase (PAA Laboratories, Cölbe, Germany). Cells were cultured in standard culture medium or additionally supplemented with SeO_2 (2.5 μM, Sigma) or H_2O_2 (50 nM, Sigma). Supernatants of the cell cultures and cell lysates (homogenized with ultrasound) were tested for GPx3 protein.

4.4. *Estimation of GPx activity*

To determine the activity of GPx, the decrease of NADPH was continuously measured photometrically (340 nm) at 37 °C for 30 min with a fluorescence plate reader (FluoStar Galaxy, BMG Labtechnologies Offenburg, Germany). This assay was first described by Paglia and Valentine (1967). The basic reactions of this assay (Wendel 1981) are given in Fig. 5. GSH (reduced glutathione) and NADPH were purchased from Serva Electro phoresis GmbH, Heidelberg, Germany); glutathione reductase from Sigma, Taufkirchen, Germany. Because the GPx activity is rate limiting, the decrease in absorbance of NADPH at 340 nm is directly proportional to the GPx concentration (Paglia and Valentine 1967; Wendel 1981). Published protocols (Günzler and Flohe 1985; Wendel 1981) were applied with a few adaptations. Standard reaction mixture contained 0.5 mM NADPH, 1 mM GSH, and 15 mU GSH-Red. in 0.25 M potassium phosphate buffer (pH 7.0) with 2.5 mM EDTA and 2.5 mM sodium azide. Total reaction mixture volume was 200 μl. The substrate was cumene hydroperoxide (12 μM, Sigma) and glutathione peroxidase from bovine erythrocytes (Sigma) was used as positive control. One unit GPx activity is defined as the conversion of 1 μmol NADPH to $NADP^+$ per minute at 25 °C and 1 mM GSH. Protein concentration of cells was determined with Bradford Reagent (Sigma). GPx activity is expressed in milli-units (mU) per mg total protein. Adherent cells were detached with EDTA 0.05%. All cells were washed with PBS 1x and homogenized with ultrasound (Sonopuls GM 70/MS 73, Bandelin electronic GmbH, Berlin, Germany). SeO_2 (2.5 μM, Sigma) was added to the culture

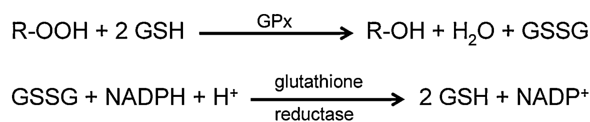

Fig. 5: GPx activity assay reactions.

Table 2: GPx primer sequences

Forward - Sense	Reverse - Antisense
GPx1 (amplicon 483 bp)	
5′-aaggtactacttatcgagaatgtg-3′	5′-gtcaggctcgatgtcaatggtctg-3′
GPx2 (amplicon 337 bp)	
5′-catcagcctggatggggagaa-3′	5′-gtaggggagcttgtccttcag-3′
GPx3 (amplicon 270 bp)	
5′-actacaggaagagcttgcacc-3′	5′-gaaccttcatgggttcccaga-3′
GPx4 (amplicon 287 bp)	
5′-tgtaaccagttcgggaagcag-3′	5′-tagaaatagtggggcaggtcc-3′

medium daily 24 h after cell adhesion for experiments with supplementary selenium. All cells were harvested 96 h later. Samples were prepared and total GPx-activity was determined as per description .

4.5. Effect of selenium dioxide on mRNA transcripts and GPx activity

Mono Mac 6 cells were cultured with standard culturing medium or with additional selenium. Cells were harvested daily for four days. Each sample was split into two aliquots. One sample was tested for the existence of GPx1, -2, -3, and -4 mRNA transcripts. The other sample was used for the determination of GPx activity. This procedure allows a direct correlation of GPx activity with mRNA transcription.

Acknowledgment: This work was supported by Deutsche Forschungsgemeinschaft (DFG) – Graduiertenkolleg 137/3 “Arzneimittel – Entwicklung und Analytik”. Also, we would like to thank Prof. Dr. Dieter Steinhilber, Frankfurt and Prof. Dr. Oliver Werz, Jena, for helpful discussions and support.

References

Aboul-Fadl T (2005) Antisense oligonucleotides: the state of the art. Curr Med Chem 12: 2193–2214.
Akasaka M, Mizoguchi J, Takahashi K (1990) A human cDNA sequence of a novel glutathione peroxidase-related protein. Nucleic Acids Res 18: 4619.
Avissar N, Kerl EA, Baker SS, Cohen HJ (1994) Extracellular glutathione peroxidase mRNA and protein in human cell lines. Arch Biochem Biophys 309: 239–246.
Avissar N, Slemmon JR, Palmer IS, Cohen HJ (1991) Partial sequence of human plasma glutathione peroxidase and immunologic identification of milk glutathione peroxidase as the plasma enzyme. J Nutr 121: 1243–1249.
Berry MJ, Banu L, Larsen PR (1991) Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. Nature 349: 438–440.
Berry MJ, Tujebajeva RM, Copeland PR, Xu XM, Carlson BA, Martin GW, 3rd, Low SC, Mansell JB, Grundner-Culemann E, Harney JW, Driscoll DM, Hatfield DL (2001) Selenocysteine incorporation directed from the 3’UTR: characterization of eukaryotic EFsec and mechanistic implications. Biofactors 14: 17–24.
Bierl C, Voetsch B, Jin RC, Handy DE, Loscalzo J (2004) Determinants of human plasma glutathione peroxidase (GPx-3) expression. J Biol Chem 279: 26839–26845.
Birringer M, Pilawa S, Flohe L (2002) Trends in selenium biochemistry. Nat Prod Rep 19: 693–718.
Brigelius-Flohe R (1999) Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med 27: 951–965.
Brigelius-Flohe R, Muller C, Menard J, Florian S, Schmehl K, Wingler K (2001) Functions of GI-GPx: lessons from selenium-dependent expression and intracellular localization. Biofactors 14: 101–106.
Brigelius-Flohe R, Wingler K, Muller C (2002) Estimation of individual types of glutathione peroxidases. Methods Enzymol 347: 101–112.
Brown KM, Arthur JR (2001) Selenium, selenoproteins and human health: a review. Public Health Nutr 4: 593–599.
Chambers I, Frampton J, Goldfarb P, Affara N, McBain W, Harrison PR (1986) The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the ’termination’ codon, TGA. Embo J 5: 1221–1227.
Chow CK, Tappel AL (1974) Response of glutathione peroxidase to dietary selenium in rats. J Nutr 104: 444–451.
Chu FF, Doroshow JH, Esworthy RS (1993) Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. J Biol Chem 268: 2571–2576.
Esworthy RS, Baker MA, Chu FF (1995) Expression of selenium-dependent glutathione peroxidase in human breast tumor cell lines. Cancer Res 55: 957–962.
Flohe L, Gunzler WA, Schock HH (1973) Glutathione peroxidase: a selenoenzyme. FEBS Lett 32: 132–134.
Frampton J, Conkie D, Chambers I, McBain W, Dexter M, Harrison P (1987) Changes in minor transcripts from the alpha 1 and beta maj globin and glutathione peroxidase genes during erythropoiesis. Nucleic Acids Res 15: 3671–3688.
Gladyshev VN, Hatfield DL (1999) Selenocysteine-containing proteins in mammals. J Biomed Sci 6: 151–160.
Günzler W, Flohe L (1985) Glutathione Peroxidase. In: Greenwald (ed) Handbook of Methods for Oxygen Radical Research, vol. CRC Press, Boca Raton, pp 185–290.
Himeno S, Imura N (2000) New aspects of physiological and pharmacological roles of selenium. J Health Sci 46:393–398.
Maiorino M, Chu FF, Ursini F, Davies KJ, Doroshow JH, Esworthy RS (1991) Phospholipid hydroperoxide glutathione peroxidase is the 18-kDa selenoprotein expressed in human tumor cell lines. J Biol Chem 266: 7728–7732.
Maser RL, Magenheimer BS, Calvet JP (1994) Mouse plasma glutathione peroxidase. cDNA sequence analysis and renal proximal tubular expression and secretion. J Biol Chem 269: 27066–27073.
Mates JM, Sanchez-Jimenez F (1999) Antioxidant enzymes and their implications in pathophysiologic processes. Front Biosci 4: D339–345.
Mills GC (1957) Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. J Biol Chem 229: 189–197.
Mizuno T, Chou MY, Inouye M (1984) A unique mechanism regulating gene expression: translational inhibition by a complementary RNA transcript (micRNA). Proc Natl Acad Sci USA 81: 1966–1970.
Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70: 158–169.
Papp LV, Lu J, Holmgren A, Khanna KK (2007) From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxid Redox Signal 9: 775–806.
Roveri A, Maiorino M, Ursini F (1994) Enzymatic and immunological measurements of soluble and membrane-bound phospholipid-hydroperoxide glutathione peroxidase. Methods Enzymol 233: 202–212.
Takahashi K, Avissar N, Whitin J, Cohen H (1987) Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. Arch Biochem Biophys 256: 677–686.
Ursini F, Maiorino M, Gregolin C (1985) The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. Biochim Biophys Acta 839: 62–70.
Wendel A (1981) Glutathione peroxidase. Methods Enzymol 77: 325–333.
Wilke BC, Vidailhet M, Favier A, Guillemin C, Ducros V, Arnaud J, Richard MJ (1992) Selenium, glutathione peroxidase (GSH-Px) and lipid peroxidation products before and after selenium supplementation. Clin Chim Acta 207: 137–142.
Wingler K, Muller C, Schmehl K, Florian S, Brigelius-Flohe R (2000) Gastrointestinal glutathione peroxidase prevents transport of lipid hydroperoxides in CaCo-2 cells. Gastroenterology 119: 420–430.
Yoshimura S, Watanabe K, Suemizu H, Onozawa T, Mizoguchi J, Tsuda K, Hatta H, Moriuchi T (1991) Tissue specific expression of the plasma glutathione peroxidase gene in rat kidney. J Biochem (Tokyo) 109: 918–923.
Zhao L (2001) Phospholipid Hydroperoxide Glutathione Peroxidase (PhGPx) - An often overlooked antioxidant enzyme. In: Free Radical and Radiation Biology Graduate Program, University of Iowa, Iowa City, p 10.