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Selenoproteins Are Expressed in Fetal Human Osteoblast-like Cells

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Selenoproteins are involved in mechanisms of cell differentiation and defense. We investigated the expression of glutathione peroxidases, as well as other selenoproteins, in fetal human osteoblasts (hFOB-cells). Using 75-selenium metabolic labelling of viable hFOB-cells, we identified several selenoproteins in cell lysates of about 45-80 kDa and in the migration range of 14 kDa to 24 kDa. Cells expressed low mRNA levels of both cellular glutathione peroxidase and plasma glutathione peroxidase mRNA as analysed by Southern analysis of RT-PCR products. Basal cellular glutathione peroxidase enzyme activity in hFOB-cells (19.7 nmol NADPH oxidised per min and μg protein) was further increased 2.5-fold by the addition of 100 nM sodium selenite to the culture medium for 3 days. Furthermore, expression of selenoprotein P mRNA was demonstrated by RT-PCR. hFOB-cells did not show activities of the selenoproteins type I or type II 5′-deiodinase. In summary, we identified cellular glutathione peroxidase, plasma glutathione peroxidase and selenoprotein P among of a panel of several 75-selenium labelled proteins in human fetal osteoblasts. The expression of selenoproteins like glutathione peroxidases in hFOB-cells represents a new system of osteoblast antioxidative defense that may be relevant for the protection against hydrogen peroxide produced by osteoclasts during bone remodelling.

Key Words: **glutathione peroxidase; selenoproteins; osteoblasts; selenium; bone; selenoprotein P.**

Selenium is an essential trace element in higher vertebrates (1). Since its identification, the expression of several selenoproteins has been described in various tissues, especially endocrine and reproductive organs, gastrointestinal tract, muscle and brain (2, 3). In eukaryotic cells, incorporation of selenocysteine into proteins is encoded by the UGA-codon and is directed by a hairpin loop within the 3′-UTR of selenoprotein encoding mRNA's (4-7). In humans, severe selenium deficiency is associated with cardiac and muscle disease (Keshan disease) as well as osteoarthritis (Kashin-Beck disease) in some provinces of China and Central Africa (3, 8). Selenium deficiency and/or fulvic acid supplementation also altered the structural integrity of skeletal tissue in a mouse model (9). Severe selenium deficiency facilitates inflammatory reactions in Kashin-Beck disease, however, the involvement of bone cells such as osteoblasts to the progression of this disease is not clear.

The best characterised family of eukaryotic selenoproteins that contain Se as selenocysteine residues are gluthathione peroxidases (GPx) which are important for cellular antioxidative defense (11, 17). Four different isoforms have been described until now: cytosolic GPx (cGPx), plasma GPx (pGPx), gastrointestinal GPx (GI-GPx), and phospholipid hydroperoxide GPx (PHGPx) (10-12). GPx degrade hydrogen peroxide (cGPx, pGPx, GI-GPx) and lipid hydroperoxides (PHGPx) 13-16). Human cGPx is expressed in numerous tissues (11, 17, 18) whereas pGPx displays a more tissue specific expression pattern (19-22). Human GI-GPx is only expressed in the gastrointestinal tract and liver (16, 23). Human PHGPx expression was described in several tumor cell lines, endothelial cells and testis (24-26).

Another important family are the iodothyronine deiodinases which either activate (type I and II) or inactivate (type I and III) thyroid hormones (27). Recently thioredoxin reductase (TRR) was identified (28, 29), which is involved in the modulation of the redox balance of cells and protein folding (30, 31). Selenoprotein P, a plasma selenoprotein (32, 33), selenoprotein W (34), SelD2, (35), as well as other selenoproteins have not been characterised to date with respect to their function (2, 3).

Previously, we reported the expression and regulation of TRR by selenite and $1,25(OH)_2D_3$ in human osteoblasts (36). Here, we describe the expression of GPx enzyme activity and its regulation by the selenite-status in cultured human fetal osteoblast-like cells and osteosarcoma cell-lines. We also investigated the

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mRNA expression pattern of various other selenoproteins of unknown function. To our knowledge, this is the first report on the expression of GPx in human osteoblasts. Our data might indicate an important contribution of the selenite-dependent GPx-mediated antioxidative cellular defense vs. H_2O_2 and reactive oxygen intermediates (ROI) produced in the bone microenvironment under physiological conditions. Missing or decreased expression, therefore, could result in impaired osteoblast function and consecutive development of bone diseases such as osteoporosis.

METHODS

Cell culture. Fetal human immortalised osteoblasts (hFOB-cells) were kindly provided by Dr. Spelsberg, Mayo Clinic, Rochester, USA (37). Cells were cultured in Dulbecco's modified eagle medium nutrient mix F12 (DMEM/F12) containing antibiotics (penicillin, 10 000 U/l; streptomycin, 10 mg/l; amphotericin B, 25 μg/l; G418, 150 mg/l) and 10 % fetal calf serum (FCS; Gibco, Eggenstein, Germany) in a humidified atmosphere of 5 % CO_2 and 95 % air at 34°C. Medium change was done every two days. Human MG-63 and TE 85 osteosarcoma cells and human liver Hep G2 cells were obtained from the American Type Culture Collection (Rockville, MD, USA) and were cultured under similar conditions at 37°C without G418. Medium change was twice per week.

Selenium-75 metabolic labelling and separation of radiolabelled proteins. hFOB-cells were cultured under selenium deficient conditions (i.e. serum-free culture conditions for 3 days). 75-selenium (specific activity 1.9 Ci/μg; Research Reactor facility Univ. of Missouri, Columbia, USA) was added as a final concentration of 10 nM for 24 hours. Cells were briefly washed with phosphate buffered saline (PBS; sodium chloride 15 mM, potassium phosphate 100 mM, pH 7.0). Cells were harvested by incubation with versene (Gibco, Eggenstein) for 3 min and were lysed by three subsequent freeze/thaw cycles. Lysates were cleared by centrifugation (5 min, 10000 rpm) and cell lysates containing 20 000 cpm of incorporated 75-selenium were subjected to SDS/PAGE analysis on a 12 % separating gel. The dried gel was exposed to Kodak X-Omat XAR X-ray film for 24 h at −80°C. These cell lysates also contained equal total cellular protein as determined by the Bio-Rad protein assay (38).

GPx-activity assay. Cell homogenates were prepared as was described priviously (18). The supernatants (cytosols) were used for GPx activity measurements according to Beutler (39) with modifications (18, 40). 50 μg protein, as measured by the Bio-Rad protein assay (38) were added to the reaction mixture (final volume 1 ml, containing 0.1 M Tris, 0.5 mM EDTA pH = 8.0, 200 μM NADPH, 2 mM glutathione, 1 U/ml glutathione reductase; type IV from bakers's yeast, Sigma München, Germany). The reaction was started by the addition of the substrate tertiary butylhydroperoxide at 7 μM. Following an initial incubation of 1-1.5 min the reaction was monitored at 340 nm for 2-3 min at 25°C using a photometer (Uvicord III, Pharmacia, Freiburg, Germany). Unspecific NADPH oxidation was measured by completely inhibiting GPx activity with the addition of 100 mM mercaptosuccinate before the addition of the substrate. This inhibitor has negligible absorption at 340 nM and blocks completely and specifically cGPx and pGPx but not PHGPx activity (J. Köhrle, unpublished results). Unspecific oxidation values (5-25 % of total activity) were subtracted from the results obtained. The GPx activity was expressed in nanomoles NADPH oxidised per min and mg of total cellular protein.

Expression of 5′-deiodinases type I and II. Activities of 5′-deiodinases Type I and type II were quantitated from homogenates prepared as described above for the GPx-activity assay, using a highly sensitive enzyme activity assay (release of $^{125}I^-$ from 3,3′5′-[^{125}I]-triiodothyronine-reverse T3/rT3 in the presence of 10 nM nonradioactive rT3 and 20 mM DTT with and without 1 mM of propylthiouracil) as described by Leonard and Rosenberg (41) with modifications (42). The detection limit of this assay is 1 fmol $^{125}I^-$-released per min and per mg protein.

RNA isolation. RNA was isolated with a modified protocol of the guanidine thiocyanate/phenol extraction procedure described by Chomczynski and Sacchi (43). PolyA$^+$ RNA was purified from total RNA using the Pharmacia mRNA purification system (Pharmacia, Freiburg, Germany) according to the protocol provided by the manufacturer.

Northern analysis. 15 μg total RNA or 5 μg polyA+ RNA were fractionated by denaturing formaldehyde gel electrophoresis and transferred to Hybond N nylon membranes (Amersham, Braunschweig, Germany) by capillary blotting. RNA from hFOB-cells was subsequently hybridised with cDNA probes for selenoprotein P, cGPx, pGPx and GAPDH labelled by random priming with [α^{32}P]dCTP and Klenow DNA Polymerase (Gibco, Eggenstein, Germany). Membranes were hybridised at 42°C in a solution containing 50 % formamide, 5× Denhardt's, 3× sodium chloride/sodium citrate (SSC), 0.1 % sodium dodecylsulfate (SDS) and 0.1 mg/ml calf thymus DNA. Filters were washed once in 3× SSC, 0.1 % SDS at room temperature for 20 min, followed by 0.5× SSC, 0.1 % SDS at 52°C for 30 min and a final wash with 0.5× SSC, 0.1 % SDS at 60-65°C for 30 min. Blots were exposed to Kodak X-Omat XAR X-ray films for 1-5 days at −80°C.

RT-PCR. Expression of human cGPx, pGPx and selenoprotein P was analysed by RT-PCR. 2 μg of total RNA was denatured (3 min, 94°C) and cDNA was synthesised using superscript reverse transcriptase (60 min, 42°C; Superscript, Gibco, Eggenstein, Germany). PCR was performed with a 10 μl aliquot (for selenoprotein P and cGPx analysis) or the total cDNA reaction (for pGPx analysis). cDNA was denatured (3 min, 94°C) and amplified as described below followed by a 5 min 72°C elongation step. Human pGPx forward primer was 5′-GGACAAGAGAAGTCGAAGATG-3′ (corresponding to position 70-90 of the pGPx cDNA, numbered according to (44)). The reverse primer was 5′-GATGTCGTGAACCTTCATGGGTTC-3′ according to position 537-514). PCR was done for 34 cycles (1.5 min, 94°C; 1.5 min, 62° C; 45 sec, 72°C). Human cGPx was amplified using the forward primer 5′-ATGTGTGCTGCTCGGCTAGC-3′ (corresponding to position 2558-2577 of the human cGPx genomic sequence as published by (45)) and the reverse primer 5′-GCCGGACGTACTTGAGGGAA-3′ according to position 3130-3111. This intron spanning primer combination results in a 279 bp product in RT-PCR analysis. PCR was performed for 34 cycles (1.5 min, 94°C; 1.5 min, 58°C; 45 sec, 72°C). Human selenoprotein P specific amplification was done with the forward primer 5′-GGCCCGTTGGAAGTGGTTGT-3′ and the reverse primer 5′-CCTAGGAGCCAACTCTGAAT-3′ (corresponding to position −33 to −14, with respect to the ATG-codon and position 894-875, respectively, of the cDNA sequence of Hill et al., (46)). Amplification was done for 34 cycles (1.5 min, 94°C; 1.5 min, 60°C; 45 sec, 72°C).

Specificity of PCR-amplicons was verified by Southern hybridisation. The cGPx-probe was an 850 bp probe obtained from rat liver cDNA (47) and the selenoprotein P probe was obtained by random priming from a cloned PCR-product (33). The pGPx probe was obtained by PCR, as described above, using human kidney RNA. The PCR product was TA-cloned according to the manufacturers instructions. To confirm the identity of the cloned probes, inserts were sequenced using the T7-sequencing system using M13 forward and reverse primers. For Southern hybridisation, aliquots of the PCR reactions were subjected to gel electrophoresis and processed for Southern hybridisation as described above for Northern hybridisation. Filters were washed once in 3× SSC, 0.1 % SDS at room temperature for 20 min, followed by 2 washes with 2× SSC, 0.1 % SDS at 60°C for 30 min. Blots were exposed to Kodak X-Omat XAR X-ray films at −80°C.

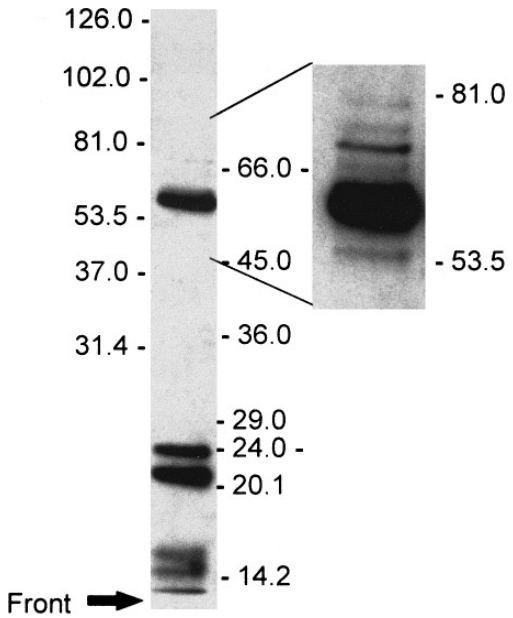

FIG. 1. SDS/PAGE analysis of 75-selenium labelled proteins. Labelled proteins from cellular extracts of hFOB-cells were separated as described in Materials and Methods. Positions of molecular weight markers are shown on the left and right, respectively. A prestained high molecular weight marker P 1677 (Sigma) and an unstained molecular weight marker SDS-7 (Sigma) were used. Following gel electrophoresis the dried gel was exposed to a Kodak X-Omat X-ray film for 24h.

Statistics. For statistical analysis of the results the Wilcoxon rank sum test was applied to GPx activity measurements.

RESULTS

Expression of Various Selenoproteins in hFOB-Cells Labelled with ^{75}Se

Using 75-Se metabolic labelling of hFOB-cells several bands corresponding to 75-Se-containing proteins were observed. As shown in Fig. 1 at least 4 bands in the migration range of 80-45 kDa and several bands in the range between 24 kDa and 14 kDa were visible on autoradiograms. No selenoprotein bands of the expected size of deiodinases (27-33 kDa) were observed. Also, no 5′-deiodinase type I or 5′-deiodinase type II enzyme activity was measurable from hFOB-cells, whereas human pituitary GX-cells analysed in parallel displayed significant quantities of $^{125}I^-$ release (48).

Expression of mRNAs for Selenoprotein P and GPx Isoforms

Selenoprotein P mRNA was not detectable in hFOB-cells by Northern hybridisation to 5 μg of poly A+ mRNA. RT-PCR/Southern blotting using SeP-specific primer oligonucleotides and labelled cDNA (See Materials and Methods) displayed a 928 bp specific labelled product as expected (Fig. 2c). An additional shorter product may have resulted from internal priming or represents a SeP isoform expressed in hFOB-cells (Fig. 2c).

Expression of GPx mRNAs initially was analysed by Northern hybridisation and no cGPx-specific or pGPx-specific hybridisation signals were observed. Using RT-PCR, products of the expected size were amplified from hFOB-cells and the respective positive controls (Fig. 2a and 2b). pGPx RT-PCR resulted in one product of 467 bp only (Fig. 2a). In contrast, cGPx RT-PCR resulted in a product of the expected size (279 bp) plus 2 additional products of 500/600 bp (Fig. 2b). These bands were negative in Southern hybridisation and thus represent unspecific amplification products. Specificity of the PCR products was further verified by Southern hybridisation using probes for either cGPx or pGPx. Therefore, both GPx-mRNA isoforms are expressed at low levels in hFOB-cells.

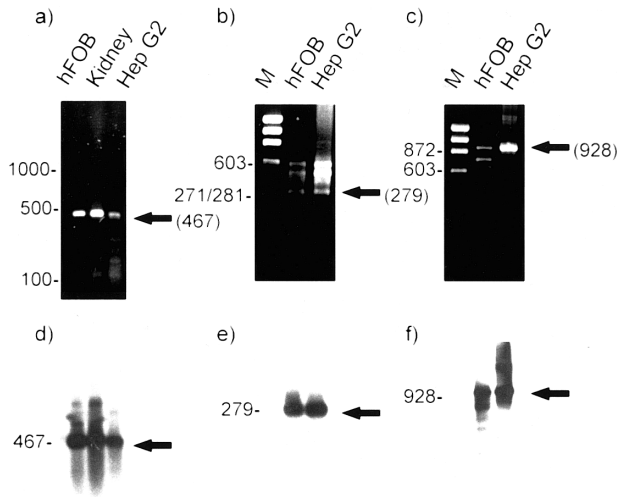

FIG. 2. RT-PCR for selenoprotein P, cGPx and pGPx. RT-PCR was performed using human gene-specific primers for pGPx (Fig. 2a), cGPx (Fig. 2b) or selenoprotein P (Fig. 2c). PCR products were separated on a 1 % agarose gel. A 100 bp ladder (Fig. 2a) or Φ174/Hae III (Fig. 2b and c) was used as a size marker. Specificity of the PCR-products was confirmed by Southern hybridisation with pGPx (Fig. 2d), cGPx (Fig. 2e) or selenoprotein P (Fig. 2f) cDNA probes. a) pGPx RT-PCR was performed using 2μg of total RNA from hFOB-cells, 2 μg of total RNA from human kidney, and 0.2 μg of polyA+ mRNA from HepG2-cells. Arrowhead indicates a 477 bp PCR product of the expected size. This product was in the position of a specific hybridisation signal obtained from Southern hybridisation with a pGPx probe (Fig. 2d). b) cGPx RT-PCR was done with 2μg of total RNA from hFOB-cells and 2 μg of total RNA from HepG2-cells. Arrowhead indicates a 279 bp PCR product of the expected size. This product migrated in the position of a specific hybridisation signal obtained from Southern hybridisation with a cGPx probe as shown in Fig. 2e. c) Selenoprotein P RT-PCR was performed using 2 μg of total RNA from hFOB-cells and 2 μg of total RNA from HepG2-cells. Arrowhead indicates a 928 bp PCR product of the expected size. This product was in the position of a specific hybridisation signal obtained from Southern hybridisation with a selenoprotein P probe as is shown in Fig. 2f.

Expression of GPx Activity and Regulation by Sodium Selenite

Total cellular GPx enzyme activity was determined form hFOB-cells using the NADPH-coupled photometric assay which is not able to discriminate between cGPx and pGPx. Activity levels were measured from cells grown in 10 % FCS or from selenium-deficient cells, i. e. cells grown serum-free for up to 3 days. Basal cellular GPx enzyme activity levels from cells grown with 10 % FCS were 19.7 ± 5.4 nmoles NADPH oxidised per min and mg protein (n = 7). After 3 days of supplementation of hFOB-cells with 100 nM sodium selenite to the culture medium, an increase of 1.43 ± 0.26-fold (n=4) was observed; as is shown in Fig. 3 ($p<0.025$).

Cells grown serum-free for 3 days expressed basal GPx activity levels of 14.7 ± 0.6 nmoles NADPH oxidised per min and mg protein, (n = 5) and responded to the treatment of 100 nM sodium selenite with a continuous rise in cellular GPx enzyme activity within 3 days as shown in Fig. 4. After 3 days the highest difference in GPx activity between selenite-depleted cells (i.e. serum-free grown cells) and selenite-repleted cells (cells supplemented with 100 nM sodium selenite) was observed as shown in Fig. 3 (2.5 ± 1.1 fold stimulation of GPx activity levels, n = 5 ($p<0.005$). A prolonged

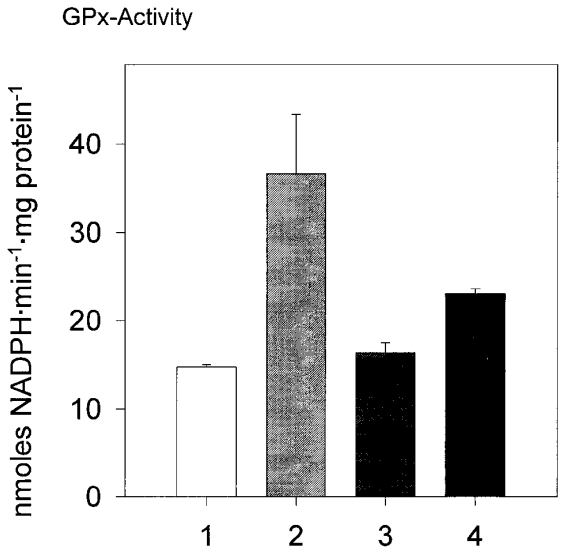

FIG. 3. GPx-activity in hFOB-cells treated with sodium selenite. GPx-activity was measured from cytosols prepared from hFOB-cells as described in Materials and Methods. Cells were grown and treated as follows: column 1, 10 % FCS, no selenite added; column 2, 10% FCS, 100 nM selenite added for 3 days; column 3, serum-free for 3 days, no selenite added; column 4, serum-free for 3 days, 100 nM selenite added during these 3 days. Results are expressed as nmoles NADPH oxidised per min and per mg protein ± SEM (n=5 for column 1 and 2, n=4 for columns 3 and 4). GPx-activity is increased by sodium selenite in serum-free medium ($p<0.005$) and using 10 % FCS ($p<0.025$).

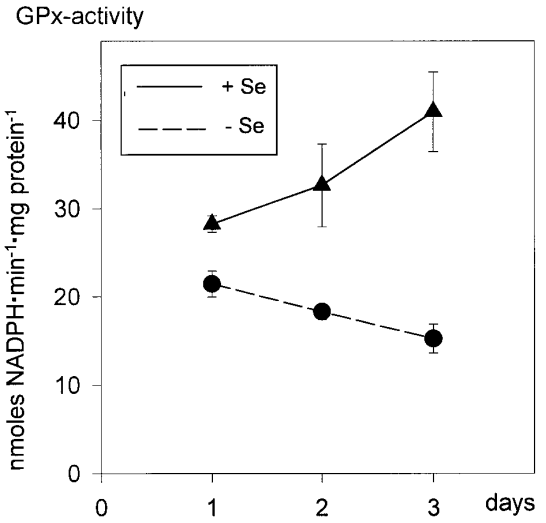

FIG. 4. Time course of stimulation of GPx-activity by selenite in hFOB-cells. GPx activity was measured from hFOB-cells as described in Materials and Methods. Cells were initially grown with 10 % FCS. Then, cells received serum-free medium for up to 3 days and were treated or not with 100 nM sodium selenite. GPx enzyme activity levels were measured from cytosolic extracts prepared after 24 hours, 48 hours and 72 hours of treatment. Data represent the mean of two replicate measurements. Results are representative for two independent experiments.

100 nM selenite treatment of 6 days using serum-free culture conditions did not further increase GPx activity (data not shown).

Expression of GPx Activity in Osteosarcoma Cells

Similar to hFOB-cells, osteosarcoma cell lines MG-63 and TE 85 were analysed for GPx expression using the photometric assay. MG-63 cells responded to a 100 nM sodium selenite supplementation (3 days) even when 10 % FCS containing cell culture medium was used: Basal GPx activity levels of 20.2 ± 1.0 nmoles NADPH oxidised per min and mg protein (n = 2) were stimulated 2.0-fold by the additional presence of 100 nM sodium selenite to 39.7 ± 6.1 nmoles NADPH oxidised per min and mg protein (n = 2).

TE-85 cells expressed 2-fold lower basal GPx activity levels compared to MG-63 cells and responded 2.5-fold to 100 nM sodium selenite (treatment of 3 days).

DISCUSSION

Expression of selenoproteins has been described in various tissues, especially endocrine and reproductive organs, gastrointestinal tract, muscle and brain (2, 3). In order to study the osteoblast-specific pattern of selenoproteins, we used 75-Se metabolic labelling. One band of about 58 kDa was in the migration range of selenoprotein P and TRR. We assume that this signal most likely corresponds to TRR, since Northern hy-

bridisation to poly A+ mRNA did not result in a specific hybridisation signal for selenoprotein P mRNA. Only using RT-PCR, a specific selenoprotein P amplification product was obtained, indicating low levels of expression. A specific selenoprotein P amplification product of slightly smaller size was also obtained from hFOB cells. This indicates the possible existence of a selenoprotein P isoform or a related protein in these cells, which has been reported for bovine cells (49). In contrast to the low selenoprotein P RNA levels, TRR was detectable by Northern hybridisation to total RNA and TRR enzyme activity was measurable in hFOB cells, as reported previously (36). From the 75-Se metabolic labelling several additional bands were visible. At present, we do not have indications on the nature of these putative selenoproteins. Since 75-Se metabolic labelling might also identify selenium-methionine containing proteins, potentially, individual bands could correspond to proteins not containing selenium-cysteine, but among many coomassie-stained proteins only a very limited number is visible by autoradiography.

Protein bands in the molecular mass range of 5′-deiodinases were not observed. We measured 5′-deiodinase type I and type II activity and obtained no indication for the presence of these enzymes in hFOB-cells. Since the detection limit of our assay is 1 fmole of deiodinase enzyme activity per mg protein, we conclude that 5′-deiodinase type I and type II are not expressed in hFOB-cells. This lack of expression of enzymes needed for the activation (type I and II) or inactivation (type I) of thyroid hormones does not rule out direct effects of thyroid hormones on osteoblasts, since clinical evidence suggests contributions of thyroid hormones on bone metabolism (50, 51) and thyroid hormone receptors are present in bone (52).

No GPx activity assays available to date are able to discriminate between the relative contribution of cGPx and pGPx. We assume that both isoforms contributed to H_2O_2 degrading activity measurements. This assumption is supported by RT-PCR results, since low levels of both pGPx and cGPx mRNA were expressed in hFOB-cells. We cannot rule out the expression of PHGPx in our cells. PHGPx activity, however, did not interfere with our results, since we used mercaptosuccinate as an inhibitor of GPx activity. Reported levels of PHGPx specific enzyme activity are about 100-fold lower compared to GPx enzyme activity measurements. In contrast to cGPx, pGPx is a secreted form of GPx. The presence of pGPx enzyme activity in conditioned cell culture medium indicates a contribution of extracellular antioxidative pGPx activity to hFOB defense systems (our unpublished results).

The treatment of hFOB-cells and osteosarcoma cells with sodium selenite resulted in a 2-4 fold stimulation of GPx activity. So far no reports on Se-dependent GPx expression in human osteoblast-like cells are available. However, several studies on selenite-dependent increase of GPx enzyme activity were reported for human cells such as ECV 304 umbilical cord vein endothelial cells, HepG2 hepatocellular carcinoma, THP-1 (53) Hep3B and HepG2 (54) and for HL-60 leukemia myeloid cells (55).

hFOB basal GPx activity levels correspond to a Se deficient state, since the 10 % FCS containing cell culture medium has a low concentration of 7.5 nM Se, as kindly measured by Gawlik and Behne (HMI, Berlin). This almost corresponds to the value of 5.5 nM Se which has been termed "Se deficient medium" by Brigelius-Flohé et al. (56). Se content of the constituents of serum-free medium are negligible as analysed by neutron activation analysis.

Diseases associated with Se deficiency such as Kashin-Beck disease display signs of osteoarthritis (8). Interestingly, a mouse model was established which upon selenite deficiency or fulvic acid supplementation (main causes of Kashin-Beck disease) developed an impaired structural integrity of the skeletal tissue due to impaired bone formation i.e. irregular mineralisation (9). Thus, expression of GPx in osteoblasts, may be relevant for normal bone remodelling. In addition, osteoclasts produce ROI at the interface between the bone surface and the osteoclast membrane (57-60). Superoxide anion or ROI resulting from superoxide production stimulate bone resorption (61). NADPH-oxidase expressed on the osteoclast membrane is involved in this superoxide production (62). Most investigators favor the quantitative importance of the superoxide anions or NO during bone remodeling (60, 62, 63). Research of the past years has indicated that radical production at the osteoclast bone interface is an integral part of normal bone degradation (60, 64). Human osteoclast-like cells express a 150 kDa superoxide dismutase-related glycoprotein (65), which uses superoxide anion and releases H_2O_2. Fraser et al. (66) reported that H_2O_2 and not superoxide is the stimulator of bone resorption in the mouse. In a human cell model this superoxide dismutase related glycoprotein is regulated by phorbol ester, $1,25(OH)_2$-D_3 and osteoblast-derived soluble factors (65), indicating a possible osteoblast-dependent control of superoxide dismutase related protein expression and in turn H_2O_2 production by the osteoclast. Since osteoblasts reside in the direct vicinity of osteoclasts these reports indicate the need for a H_2O_2 scavenging enzyme system in human osteoblasts, which might be represented by both intracellular and extracellular GPx. The sensitivity of osteoblasts to exposure of radicals is also shown by the cytotoxic effect of extensive release of radicals during polymerisation of cements used for orthopaedic implants (67).

To our knowledge, expression of GPx in osteoblasts has not been described. Our data demonstrate that osteoblasts express an antioxidative enzyme systems to protect themselves from H_2O_2 following osteoclast-mediated bone resorption. GPx-expression, therefore, rep-

resents an osteoprotective system. Lack of expression of GPx could be related to impaired osteoblast function and/or bone remodelling and could be involved in the pathogenesis of metabolic bone diseases such as osteoporosis.

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