Glutathione Peroxidase 2 Inhibits Cyclooxygenase-2–Mediated Migration and Invasion of HT-29 Adenocarcinoma Cells but Supports Their Growth as Tumors in Nude Mice

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Abstract

The selenoprotein gastrointestinal glutathione peroxidase 2 (GPx2) is up-regulated in a variety of cancer cells with thus far unknown consequences. Therefore, two clones of a human colon cancer cell line (HT-29) in which GPx2 was stably knocked down by small interfering RNA (siRNA; siGPx2) were used to test whether cancer-relevant processes are affected by GPx2. The capacity to grow anchorage independently in soft agar was significantly reduced in siGPx2 cells when compared with controls (i.e., HT-29 cells stably transfected with a scramble siRNA). The weight of tumors derived from siGPx2 cells injected into nude mice was lower in 9 of 10 animals. In contrast, in a wound-healing assay, wound closure was around 50% in controls and 80% in siGPx2 cells, indicating an enhanced capacity of the knockdown cells to migrate. Similarly, invasion of siGPx2 cells in a Transwell assay was significantly increased. Migration and invasion of siGPx2 cells were inhibited by celecoxib, a cyclooxygenase-2 (COX-2)–specific inhibitor, but not by α-tocopherol. Selenium supplementation of cell culture medium did not influence the results obtained with siGPx2 cells, showing that none of the other selenoproteins could replace GPx2 regarding the described effects. The data show that GPx2 inhibits malignant characteristics of tumor cells, such as migration and invasion, obviously by countering COX-2 expression but is required for the growth of transformed intestinal cells and may, therefore, facilitate tumor cell growth. The data also shed new light on the use of selenium as a chemopreventive trace element: a beneficial effect may depend on the stage of tumor development.

Introduction

Selenium is one of the few micronutrients for which an anticarcinogenic effect has been proven in a large controlled clinical trial (1). This and other findings corroborate early epidemiologic studies that indicate an increased cancer incidence in selenium-deficient areas (2). Specifically, selenium ranks high in the list of most effective dietary agents to suppress colon cancer in rodents and humans (3). From supplementation studies, a “limited suggestive decreased risk” for lung and colorectal cancer and a “probable decreased risk” for prostate cancer have been deduced in the most recent report of the World Cancer Research Fund/American Institute for Cancer Research (4). In the same report, however, a “limited suggestive increased risk” for skin cancer has been declared. Thus, selenium might not be effective in the prevention of all types of cancers. Mechanisms underlying an anticarcinogenic function of selenium are not clear, although various links between selenium biochemistry and carcinogenesis have been discussed (2, 5, 6). Links point out to the particular activities of selenium compounds used and also to the relevance of the time point of intervention. Therefore, selenium supplements may be beneficial at some stages of cancer development and not in others.

In mammals, selenium exerts its physiologic functions as selenocysteine incorporated into the polypeptide chain of selenoproteins (7). From the about 30 selenoproteins present in humans (8), the enzymology of the glutathione peroxidase (GPx) family has been most extensively studied (9, 10). Although all mammalian GPxs reduce hydroperoxides by means of glutathione, their individual functions seem to be different. Whereas GPx1 most probably is simply an antioxidant device, GPx4 (phospholipid hydroperoxide GPx) is mandatory for male fertility (11) and indispensable for embryonic development (12). From GPx4-overexpressing L929 cells, smaller tumors were formed than from control cells when injected into nude mice (13). The gastrointestinal GPx2 is not uniformly expressed in the intestine but is highest in the crypt grounds and decreases gradually toward the luminal surface (14), indicating a role in proliferating cells. GPx2 expression is increased in human colorectal adenomas (14), carcinomas (15), and in Barrett’s esophageal mucosa (16). GPx2 expression, however, is not specific for the gastrointestinal tract. For example, its expression is also increased during the neoplastic transformation of squamous epithelial cells (17), in lung adenocarcinomas from smokers (18), and in rat breast cancer (19). In the context of tumorigenesis, a preventive role of GPx2 has been proposed because mice, in which both GPx1 and GPx2 had been knocked out, progressively developed ileocolitis and subsequently intestinal cancer, especially when not raised under specific pathogen-free conditions (20). The lack of GPx2 was more detrimental because one intact GPx2 allele was sufficient to prevent intestinal inflammation (21), thereby indicating that rather GPx2 acts as the anti-inflammatory and, thus, the anticarcinogenic enzyme. A protective role of GPx2 can also be inferred from the fact that its expression is regulated by Nrf2 (22), a transcription factor that...
induces enzymes that are generally believed to be cytoprotective and tumor preventive (23), and that GPx2 is able to counteract cyclooxygenase-2 (COX-2) expression and prostaglandin E2 (PGE2) production (24).

However, the up-regulation of GPx2 by ΔNp63, a transcription factor necessary for cell proliferation, and the inhibition of oxidation-induced apoptosis in GPx2-overexpressing MCF7 cells (25), rather points out to a function of GPx in the normal self-renewal of intestinal epithelial cells, which might also facilitate tumor cell growth. Proliferation and differentiation of epithelial mucosa cells are regulated by the Wnt pathway (26), in which β-catenin is the main signal transducer. Disruption of β-catenin/T-cell factor-4 (TCF-4) activity in colorectal cancer cells led to a significant down-regulation of GPx2 mRNA (27), and indeed, the GPx2 promoter is activated by β-catenin/TCF (28).

Taken together, the role of GPx2 in the process of cancer development is far from being clear. Therefore, the potential dual role of GPx2 in carcinogenesis was studied in HT-29 clones, in which GPx2 was stably knocked down, thereby resulting in a high COX-2 expression (24). The approach revealed that GPx2 clearly inhibits cell migration and invasion evidently by counteracting COX-2 expression/activity but that it supports tumor cell growth. The findings strongly support the concept that manipulation of GPx2 might be either detrimental or beneficial depending on the stage of tumor development.

**Materials and Methods**

**Cell culture.** HT-29 cells (human colon adenocarcinoma cells; German Collection of Microorganisms and Cell Cultures) were grown in DMEM (high glucose) with 1% nonessential amino acids, 10% FCS (Sigma), 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies) at 37°C. Medium of cells stably transfected with GPx2 small interfering RNA (siRNA) or scramble siRNA contained 800 μg/ml geneticin (Calbiochem). Generation of siGPx2 clones and scramble controls is described by Banning and colleagues (24). To adjust the selenium status, thereafer, cells were treated for 24 h with celecoxib (10 μM/L) in serum-free medium. PGE2 released into the cell culture medium was measured with a PGE2 competitive enzyme immunoassay (Cayman Chemical Co.) with 50 μL undiluted sample according to the manufacturer’s protocol.

**Immunohistology.** Samples were prepared and stained as described (24, 29). Primary antibodies, rabbit anti-GPx2 (24) and rabbit anti-COX-2 antibodies (Cayman Chemical), were applied overnight at 4°C. A biotin-spacer–conjugated goat anti-rabbit IgG (Jackson) followed by a streptavidin–biotin–horseradish peroxidase complex (StreptABComplex/HRP Dako-Cytomation) and diaminobenzidine (DakoCytomation) were used for visualization.

**Cell growth in soft agar.** Cells (5 × 104 per well) were suspended in 2 ml of 24-h HT-29–conditioned medium ± selenium and 0.35% Bacto-agar (Becton Dickinson). The suspension was overlaid onto a 2 ml solidified bottom layer of conditioned medium containing 1% Bacto-agar in six-well plates. Plates were incubated at 37°C in 5% CO2. Once a week, 200 μL of the respective growth medium were added. After 2 wk, colony formation was visualized by staining with 0.01% crystal violet for 1 h. Plates were scanned with a GS-800 densitometer, and colonies were counted with the Quantity One software (Bio-Rad).

**In vivo tumor formation.** The tumorigenic potential of GPx2 knockdown and scramble control cells was determined in 10 male CD-1-nu/nu mice, aged 8 wk (Charles River). Cells (2 × 106/mL) were resuspended in growth medium without FCS and antibiotics. One million cells were injected s.c. into the hind legs of five different mice. In each case, a pair of a scramble and a siGPx2 clone was injected into the right and the left side, respectively. Animals were sacrificed 5 wk after injection. Tumors were dissected, weighed, divided, and frozen in liquid nitrogen or fixed in 4% buffered formaldehyde for further analysis. Animals were housed in standard barrier facilities according to the Federation of European Laboratory Animal Science Associations regulations. The experiment was approved by the governmental animal ethics committee (MLUV 32-44457+51).

**Tumor sample preparation and Western blotting.** For Western blotting, frozen tissues were extensively ground in liquid nitrogen. Tissue powder (20–30 mg) was suspended in 500 μL homogenization buffer...
[100 mmol/L Tris-HCl, 300 mmol/L KCl, 0.1% Triton X-100 (pH 7.6)] containing 4 μL of protease inhibitor cocktail (Calbiochem). Homogenization was achieved with a tissue lyser (Qiagen) for 2 min at 30 Hz. Cellular debris was removed by centrifugation at 15 min at 20,800 × g and 4°C. Protein content was assessed according to Bradford (30). Proteins (50 μg per lane) were loaded onto a 12.5% polyacrylamide gel, and the electrophoresis was run with 15 mA in 25 mmol/L Tris buffer containing 1% SDS. After gel electrophoresis, proteins were transferred to a nitrocellulose membrane. The membrane was blocked with 5% nonfat dry milk in TBS (50 mmol/L Tris, 150 mmol/L NaCl (pH 7.5), containing 0.1% Tween 20) for 1 h at room temperature. Primary antibodies were incubated with the membrane at room temperature. Thereafter, the membrane was washed three times with PBS. The signal was detected with the appropriate secondary antibodies conjugated to horseradish peroxidase. The signal was detected with a chemiluminescence detection system (ECL; Amersham). The density of the signal was quantified with a Synergy 2 Fluorescence Multiwell Plate Reader (Biotek Instruments) at excitation and emission wavelengths of 485 and 528 nm, respectively, and expressed as the mean ± SD. Significant differences were calculated by two-way ANOVA followed by Bonferroni's post test (GraphPad Prism version 5.0). A P value of <0.05 was regarded as statistically significant.

Results

**Gpx2 knockdown and control cells.** For all experiments, the recently created HT-29 cells stably knocked down for Gpx2 by siRNA ("siGpx2" clones) were used (24). Gpx2 was stably knocked down with two different siRNA constructs to exclude nonspecific effects. Two clones were selected for analysis: siGpx2-1b in which Gpx2 expression is almost zero and siGpx2-2b containing 10 ± 2.2% residual Gpx2 expression. HT-29 cells stably transfected with a plasmid producing a scramble siRNA without homology to any human sequence, 'scramble a' and 'scramble b', served as controls. In contrast to scramble cells, siGpx2 clones markedly overexpressed COX-2 (24).

**Inhibition of PGE2 production in Gpx2 knockdown cells by celecoxib.** Cell viability after 72-hour treatment of siGpx2-1b and scramble a with celecoxib remained unchanged up to a concentration of 30 μmol/L (Fig. 1A). At higher concentrations, a strong toxic effect was observed. Therefore, a concentration of 10 μmol/L was chosen for subsequent experiments. As shown in Fig. 1B, this concentration was sufficient to reduce PGE2 production in the siGpx2 clone by >90%. Even the tiny amount of PGE2 in scramble cells was reduced (Fig. 1B, inset). Neither the PGE2 production nor the celecoxib effect was influenced by selenium supplementation.

**Gpx2 supports growth of HT-29 cells in soft agar.** A characteristic feature of many cancer cells is their capability to grow anchorage independently, which is experimentally shown by their capacity to grow in soft agar. In both Gpx2 knockdown clones, siGpx2-1b and siGpx2-2b, the colony-forming capacity in

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**Figure 2.** Gpx2 facilitates colony formation in soft agar. A. colonies formed from controls (scramble a and scramble b) and from Gpx2 knockdown (siGpx2-1b and siGpx2-2b) clones grown in the presence of 50 nmol/L selenite for 2 wk were stained with 0.01% crystal violet for 1 h at 37°C and visualized with a GS-800 densitometer. Results obtained with cell lines grown without selenium supplementation were comparable. B. stained colonies were counted and recorded as the total number per well. *P < 0.05 versus scramble a; **P < 0.05 versus scramble a; ***P < 0.01 versus scramble a; ###P < 0.001 versus scramble b under the respective conditions (±Se). Columns, means (n = 2); bars, SD.
soft agar substantially decreased compared with the scramble controls (Fig. 2A). Quantification by counting colony numbers revealed the significance of the effect (Fig. 2B). Selenium supplementation did not influence colony formation in the siGPx2 clones, whereas scramble a seemed to grow less than scramble b in selenium deficiency.

**GPx2 supports tumor growth from HT-29 cells in nude mice.**

The relevance of the surprising data from the soft agar experiments for tumor growth in vivo was scrutinized by monitoring tumor growth in nude mice. Pairs of scramble and siGPx2 cells were s.c. injected in the right and left hind leg of nude mice, respectively. Palpable tumors developed within 3 weeks; thereafter, animals were euthanized and tumors were analyzed (Fig. 3A and B). A reduction in tumor weight was clearly seen in four of five mice injected with the pair siGPx2-1b and scramble a, whereas in one mouse no tumor at all was detected with siGPx2-1b. The reduction in tumor weight in all five mice ranged from 28% to 100% and was statistically significant. In mice injected with the pair siGPx2-2b and scramble b, the weight of siGPx2-2b-derived tumors was lower than that of scramble b cell–derived tumors in four of five mice. Because in one animal the tumor derived from siGPx2-2b was larger than that from scramble b, the reduction in tumor weight did not reach statistical significance. Knockdown persistence up to the end of the experiment was verified by lysing tumor tissue and analyzing GPx2 expression in Western blots. In tumors derived from scramble a or b, GPx2 expression was high, whereas the analyzing GPx2 expression in Western blots. In tumors derived from control cells, COX-2 was distinctly lower than in scramble b cell–derived tumors in four of five mice. In contrast, selenium supplementation decreased migration in in vivo tumors derived from scramble a or b, GPx2 expression was high, whereas the expression was absent (clone 1b) or marginal (clone 2b) in tumors derived from scramble b cells (Fig. 3C). Maintenance of the enhanced COX-2 expression and the absence/reduction of GPx2 in siGPx2 cells were further verified by immunohistochemistry. In tumors derived from control cells, COX-2 was distinctly lower than in tumors derived from scramble b cells (Fig. 4). Thus, the knockdown of GPx2 associated with up-regulation of COX-2 persisted under conditions of in vivo tumor growth.

**GPx2 inhibits migration of HT-29 cells.**

The potential to migrate was tested in the commonly used in vitro wound-healing assay. Confluent monolayers of control and siGPx2 cells were disrupted (i.e., mechanically wounded by scraping them with a pipette tip). Wound closure was followed every 24 hours for up to 72 hours. Compared with scramble cells, which closed the wound by ~50% after 72 hours, wound closure was 80% in GPx2-deficient clones (Fig. 5A–C). Inhibition of cell proliferation by mitomycin C (10 μg/ml) did not influence migration of any clone during the observation period of 72 hours (data not shown), thus indicating that wound closure is a result of cell migration and not of cell proliferation. The role of the increased COX-2 expression in GPx2 knockdown cells was tested in clone siGPx2-1b versus scramble a. In the presence of celecoxib, motility of GPx2-deficient clone was significantly inhibited (Fig. 5D), whereas control cells were unaffected. This shows that GPx2 inhibits migration by keeping COX-2 expression and/or PGE2 production at a low level. To get an idea whether antioxidant functions of GPx2 play a role in the prevention of migration, the experiment was repeated in cells enriched with α-tocopherol, which, however, did not influence migration at all (Fig. 5E). Selenium supplementation did not affect the migration of siGPx2 clones, indicating that GPx2 could not be replaced by any other selenoprotein in inhibiting cell migration. In contrast, selenium supplementation decreased migration in controls cells, an effect that became significant after 48 hours (Fig. 5C–E).

**GPx2 inhibits invasion of HT-29 cells.**

Invasiveness was tested in the chemoinvasion assay in which cells cross a reconstituted basement membrane of Matrigel coated on top of Transwell filters. The ability of siGPx2 cells to invade the Matrigel layer, quantified by measuring the fluorescence being emitted by the cells, was facilitated invasion, the experiment was repeated with clone siGPx2-1b versus scramble a + celecoxib, and the invading cells were counted. Also with this readout, the number of invading cells was distinctly higher in GPx2-deficient cells than in control cells expressing GPx2 (Fig. 6B). Treatment with celecoxib rescued the low invasive potential in siGPx2 cells. In contrast, α-tocopherol had no effect at all (Fig. 6C).

**Figure 3.** GPx2 supports tumor cell growth in vivo. Cells (1 × 10⁶) were injected s.c. into the hind legs of nude mice. In all cases, pairs of scramble a and siGPx2-1b or scramble b and siGPx2-2b were injected: scrambles into the right and siGPx2 clones into the left hind legs. Five mice were used for each cell pair. Animals were sacrificed 3 wk after the injection of the cells. All tumors were dissected and weighed. A, representative photographs of a tumor from control and siGPx2 cells grown in the same mouse. B, histograms showing the average tumor weights ± SD of all five mice per group. Columns, mean (n = 5); bars, SD. C, stability of GPx2 knockdown in tumors was verified by Western blotting. Tumors were homogenized, and cell lysates were analyzed for GPx2 and β-actin. For further details, see Materials and Methods. *, P < 0.05 versus scramble a in a paired Student’s t test based on percentage of reduction in tumor weight.
compared with scramble sequence–transfected cells to rule out any
in GPx2 activity. The GPx2 knockdown cells were generated by
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In this study, a genetic approach was applied to ensure that
altered cellular behavior can selectively be attributed to variations
in GPx2 activity. The GPx2 knockdown cells were generated by
stable transfection with two different GPx2-specific sequences and
compared with scramble sequence–transfected cells to rule out any
artifactual disturbance of cell physiology. Scramble controls
behaved like wild-type cells in all in vitro experiments (data not
shown). The persistence of the induced genetic alteration was verified up to the end of the longest experiment (analysis of tumor
growth in nude mice). Furthermore, all cell culture experiments
were performed under selenium deprivation and supplementation
to detect a potential compensation of GPx2 deficiency by other
selenoproteins. The different siGPx2 clones yielded consistent
results that proved to be unresponsive to selenium supplementation. Because selenium-supplemented siGPx2 cells only differ from
controls by the absence of GPx2, the effects observed can only result
from the loss of GPx2. In contrast, selenium deficiency
influenced the outcome in control cells. Selenium deficiency
tended to decrease growth in soft agar, at least in one control
cloned (Fig. 2B), tended to increase invasion (Fig. 6A), and
significantly accelerated migration in the wound-healing assay
(Fig. 5B–E). The effects are small but are in line with a lower GPx2
expression in selenium deficiency. A decrease in total GPx activity
to which GPx2 contributed to 30% (24) was generally observed in
cells grown in unsupplemented medium where the only selenium
source is FCS. The FCS used in our studies usually contains 8 to
10 μg/L of selenium in an unknown form and does not supply cells
with an adequate amount of selenium (36). Under deficient
conditions, GPx1 protein always completely disappeared, whereas
a substantial residue of GPx2 remained and never reached the zero
status established in the siGPx2 clones (24). Because selenium
supplementation never influenced siGPx2 effects but restored GPx1
expression, GPx2 seems to play the dominant role also in the
system described here as observed in the GPx1/GPx2 double
knockout models.

Anticarcinogenic activities of GPx2. The common denomina-
tor of glutathione peroxidases is the inhibition of hydroperoxide-
dependent events. Oxidative stress is generally considered to
contribute to the initiation of carcinogenesis. Thus, an increase in
the expression of enzymes lowering the cellular oxidative state is
believed to be preventive. It is tempting to speculate that the
numerous reports on enhanced chemically induced carcinogenesis in selenium deficiency are due to low GPx1 activity because GPx1 is
one of the selenoproteins that most rapidly responds to selenium
depriavation (12). An analogous role of GPx2 in cancer initiation
cannot be deduced from the present investigation because the
cell lines studied here had already experienced malignant
transformation and an alternative antioxidant, α-tocopherol, did
not show any effect. It can, however, be inferred from the obvious
synergism of GPx1 and GPx2 in the gastrointestinal tract observed
in knockout studies. Whereas neither GPx1−/− nor GPx2−/− mice
displayed any obvious phenotype, the double knockout mice spontaneouly developed an acute inflammation in the colon and
distal ileum at the age of 27 to 70 days (37) and tumors at the age of
5 to 9 months (20). Notably, tumors were only observed in the
gastrointestinal tract where GPx2 represents the major cytosolic
GPx in the epithelial cells (38).

All GPxs investigated thus far are also able to inhibit the activity
of COXs by removal of hydroperoxides (12, 39). COXs require a
certain hydroperoxide tone for activity (40) and do no longer
produce PGE2 if the tone is reduced. In inflamed tissue, COX-2 is
up-regulated by PGE2 in a positive autocrine loop (13, 41). In
consequence, inhibition of COX-2 activity by GPxs will also lead
to decreased COX-2 expression. Such inhibition of COX-2 expres-
sion has been shown for GPx4 (13) and for GPx2 (24). Counter-
acting COX-2 activity and expression as well as PGE2 production
by GPx2 evidently is the reason for the distinctly enhanced migration

Discussion

The need to focus on individual selenoproteins. The ongoing
debate on the potential effect of the trace element selenium on
tumor prevention has to consider the function of individual
selenium compounds and selenoproteins. Particular selenium
compounds may interfere with the activity of selenoproteins, such as
thioredoxin reductases, or display other pharmacodynamic
properties of their own (2, 6, 32, 33). As source of selenoprotein
biosynthesis, their role is not easily predicted either because the
conditions of individual selenoproteins to tumorigenesis can
hardly be identical and, in fact, may be antagonistic. Among
functionally characterized selenoproteins, the thioredoxin reduc-
tases, because of their essential role in deoxynucleotide biosyn-
thesis and cell division, are being discussed as molecular target for
cancer therapy (32, 34). In contrast, GPxs are widely believed to
prevent carcinogenesis because of their ability to balance oxidative stress and associated inflammation, mutagenesis, and tumor
initiation (35). Despite their common enzymatic potential to
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Figure 4. Expression of GPx2 and COX-2 in a tumor from control and siGPx2
cells grown in the same mouse. Serial sections from the control (A–C) and
from the siGPx2 (D–F) tumor stained with H&E (A and D), GPx2 antibody
(B and E), or COX-2 antibody (C and F). Microphotographs are representative
for the staining at the periphery of the tumors.
(Fig. 5) and invasion (Fig. 6) observed with GPx2 knockdown cells. This interpretation is strongly supported by the observation that the control phenotype was restored in siGPx2 cells by celecoxib. Because overexpression of COX-2 as well as increased PGE2 production coincide with increased metastatic potential, GPx2 like other GPxs may be considered to be antimetastatic. In line with this conclusion, L929 cells overexpressing GPx4 developed less lung metastases in nude mice (13) and selenium supplementation reduced pulmonary metastases of melanoma cells in mice (42) as well as invasiveness of HT1080 cells (43).

An additional link between GPx-dependent hydroperoxide removal and invasiveness is the redox regulation of proteases required for matrix degradation. Metalloproteinases (MMP) are activated by hydroperoxides (44) while at the same time their physiologic inhibitors are inhibited (45). Selenite reduced the expression of MMP-2 and MMP-9 and of the urokinase-type plasminogen activator, all of which are enzymes required for matrix degradation (43). On the other hand, expression of the tissue inhibitor of metalloproteinase-1 was enhanced (43). Thus, the removal of hydroperoxides in the invasive phase is rather beneficial.

**Procarcinogenic activities of GPx2.** The ability of GPxs to remove H2O2 and other hydroperoxides, however, cannot generally be considered to be anticarcinogenic. A function that is shared by

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**Figure 5.** GPx2 inhibits cell migration in a wound-healing assay. A, wounds were set into a confluent cell layer of controls (top) and GPx2 knockdown cells (bottom) grown ± selenium. Photographs at time 0 and after 72 h are shown. White bars, 500 μm. B, percentage of the wound opening left after 72 h. C, time-dependent closure of the wound. For clarity, only scramble a and siGPx2-1b grown ± selenium are shown. Identical results were obtained with clones scramble b and siGPx2-2b. D and E, percentage of the wound opening left in scramble a and siGPx2-1b cells after 72-h incubation in the presence of 10 μmol/L celecoxib or DMSO as vehicle (D), 50 μmol/L α-tocopherol (αToc), or ethanol (EtOH) as vehicle (E). *, P < 0.05; **, P < 0.01; ***, P < 0.001 versus scramble a and b ± selenium; +++, P < 0.001 versus DMSO control; †, P < 0.05 versus scramble a grown without selenium supplementation.
all GPxs and that is related to their peroxidase activity is their ability to prevent oxidant-induced apoptosis. Antiapoptotic activity has for long been established for GPx1 and GPx4 and was inferred from the GPx2 gradient in the gastrointestinal tract declining from the GPx2-rich proliferative crypt ground to the apoptotic tip of the villi/crypt (14). Further evidence is provided from the increased apoptosis in GPx1−/−/GPx2−/− mice in the crypt ground of the ileum (20) and has recently also been verified for GPx2 by overexpression in MCF7 cells (25). Therefore, GPxs may also counteract the immune surveillance by inhibiting apoptosis and thereby preventing the elimination of cancer cells. Accordingly, mice overexpressing GPx1 proved to be more susceptible to 7,12-dimethylbenz(a)anthracene–induced and 12-O-tetradecanoylphorbol-13-acetate–promoted skin cancer (46). However, the way GPx2 might interfere with these regulatory mechanisms remains to be elucidated.

Certainly, the decreased colony formation of GPx2 knockdown cells in soft agar (Fig. 2) as well as the lower weight of tumors derived from siGPx2 cells in nude mice (Fig. 3) contrasts the anticarcinogenic role of GPx2. The tumor-promoting potential of overexpressed COX-2 in siGPx2 clones is obviously not sufficient to completely compensate for the lack of GPx2 and its proliferative properties. Multiple hints for a role of GPx2 in proliferative processes already exist. (a) GPx2 is a target of ΔNp63 (25), a transcription factor preferentially expressed in undifferentiated, proliferating epithelial cells and suggested to be a useful marker for undifferentiated cells (25). (b) GPx2 is a target of β-catenin/TCF (28), which also activates genes required for proliferation (26). (c) GPx2 expression gradually decreases from the crypt ground where proliferation takes place toward the luminal surface where cells are eliminated by apoptosis (14). Therefore, the requirement of GPx2 for a balanced renewal and apoptotic disposal of the gastrointestinal epithelium is discussed as an essential physiologic function of this particular GPx. However, by counteracting apoptosis factors provided by the host, GPx2 may represent a survival factor for cancer cells.

At this stage, it must be questioned if the antiapoptotic activity that GPx2 shares with other GPxs fully explains its support of tumor growth. In contrast to GPx2, GPx4 proved to inhibit tumor growth in nude mice (see above). The peculiar behavior of GPx2 is, however, mimicked by another selenoprotein, thioredoxin reductase 1 (TrxR1). Like GPx2, TrxR1 is highly expressed in a variety of tumors (34), and a knockdown of TrxR1 resulted in a reduced tumor progression but also in reduced metastasis formation (47). On the other hand, the thioredoxin/TrxR system via peroxiredoxins (Prx) is linked to hydroperoxide metabolism (48) and has been implicated in the prevention of oxidative damage and cancer initiation (49). However, regarding peroxide removal, the TrxR/Trx/Ppx system can hardly compete with the glutathione-dependent system in terms of speed and capacity. More likely, specific redox regulations of cellular events have to be evoked to understand the roles of TrxR and GPx in carcinogenesis.

In conclusion, the present findings make clear how complex and even contrasting the functions of GPx2 and probably other selenoproteins may be, making an evaluation of their role in carcinogenesis difficult. Furthermore, the present findings are not unequivocally in line with an anticarcinogenic function of GPx2. A beneficial role of GPx2 in carcinogenesis may depend on the stage of the disease. In the cancer initiation phase, GPx2 may protect cells from oxidative damage and can further counteract COX-2 expression and the production of procarcinogenic PGE2. In cells having reached a transformed stage, GPx2 might still inhibit cell migration and invasion, both being first steps in metastasis. In the established tumor, however, GPx2 might adopt the role of a survival factor for cancer cells. It supports proliferation and may prevent elimination of cancer cells by inhibition of apoptosis. Interestingly, most experimental studies on the influence of selenium on carcinogenesis allow similar conclusions. Most consistently, an inhibitory effect of various selenium compounds on the initiation
References


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