Functional and physical interaction between the selenium-binding protein 1 (SBP1) and the glutathione peroxidase 1 selenoprotein

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Selenium-binding protein (SBP) 1 is present in reduced levels in several cancer types as compared with normal tissues, and lower levels are associated with poor clinical prognosis. Another selenium-containing protein, glutathione peroxidase 1 (GPX1), has been associated with cancer risk and development. The interaction between these representatives of different classes of selenoproteins was investigated. Increasing SBP1 levels in either human colorectal or breast cancer cells by transfection of an expression construct resulted in the reduction of GPX1 enzyme activity. Increased expression of GPX1 in the same cell types resulted in the transcriptional and translational repression of SBP1, as evidenced by the reduction of SBP1 messenger RNA and protein and the inhibition of transcription measured using an SBP1 reporter construct. The opposing effects of SBP1 and GPX1 on each other were also observed when GPX1 was increased by supplementing the media of these tissue culture cells with selenium, and the effect of selenium on SBP1 was shown to be GPX1 dependent. Decreasing or increasing GPX1 levels in colon epithelial cells of mice fed a selenium-deficient, -adequate or -supplemented diet resulted in the opposing effect on SBP1 levels. These data are explained in part by the demonstration that SBP1 and GPX1 form a physical association, as determined by coimmunoprecipitation and fluorescence resonance energy transfer assay. The results presented establish an interaction between two distinct selenium-containing proteins that may enhance the understanding of the mechanisms by which selenium and selenoproteins affect carcinogenesis in humans.

Introduction

Mammalian selenium-containing proteins fall into three distinct categories (1,2). In one, selenium is erroneously substituted for sulfur in sulfur-containing amino acids due to the similarity in structure between these two elements. The second class consists of proteins in which selenium is a constituent of the amino acid selenocysteine, which is inserted cotranslationally into selenoproteins in response to a UGA codon in the corresponding messenger RNA (mRNA) (3). The third class is composed of selenium-binding proteins (SBPs), which bind selenium by a mechanism that has yet to be clarified. Given the wealth of data indicating that low non-toxic levels of selenium can prevent cancer in animal models, and human epidemiology indicating an inverse association between dietary selenium intake and the risk of several cancer types (4–10), there is considerable interest in investigating the role of selenium-containing proteins in cancer etiology, although the Selenium and Vitamin E Cancer Prevention Trial showed lack of efficacy of selenium (11) and other studies have indicated that higher selenium intake may be associated with increased risk of diabetes (11–13).

The human SBP gene (SBP1, SELENBP1 or hSP56) (14) is located on chromosome 1 at q21–22 and is the homologue of the mouse SP56 gene that was originally reported as a 56 kDa mouse protein that stably bound 75selenium (15,16). The human complementary DNA (cDNA) contains a 472 amino acid-encoding open reading frame (14) and protein resides both in the nucleus and in the cytoplasm (17). It is expressed in a variety of tissue types, including the heart, lung, kidney and tissues of the digestive tract. The function of SBP1 is unknown although it may be involved in intra-golgi transport (18). SBP1 was recently reported to interact with the von Hippel–Lindau protein (pVHL)-interacting debiquitinating enzyme 1 (VDU1) and might play a role in ubiquitination/debiquitination-mediated protein degradation pathways in a selenium-dependent manner (19). We and others have recently reported that the levels of SBP1 are significantly decreased in various epithelial cancers, including those of the prostate (20), stomach (21), ovary (22), lung (17) and colon (23,24). More recently, we found that SBP1 becomes silenced by methylation in colon cancers and can influence sensitivity to oxidative stress, cell migration and tumorigenesis (25). Decreased expression of SBP1 in lung and colorectal cancer is also associated with poor prognosis (17,23,24).

Among the 25 human selenocysteine-containing proteins, there is evidence that the cytosolic form of glutathione peroxidase 1 (GPX1) is associated with cancer risk. GPX1 is a ubiquitously expressed enzyme that detoxifies hydroperoxides using reducing equivalents from glutathione. Human studies have demonstrated that a functional GPX1 gene polymorphism at codon 198 resulting in either a proline (Pro) or a leucine (Leu) at that position is associated with increased risk for several types of cancer, including those of the head and neck, breast, colon, lung and prostate (26,27). In addition, allelic loss at the GPX1 locus is a common event in cancer development (28,29). This, as well as in vivo data indicating that reduced GPX1 expression can sensitize mammalian cells to DNA damage (30) and that overexpression can have the opposite effect (31), support a role for GPX1 levels in determining cancer risk. Given the cumulative data indicating possible roles of both SBP1 and GPX1 in cancer development and/or outcome, the interaction of these two selenium-associated proteins was investigated in several model systems in the present study.

Materials and methods

Cell lines and cell culture

The human colon cancer cell line HCT116 and breast cancer cell line MCF-7 were obtained from the American Type Culture Collection (Manassas, VA). Derivative MCF-7 cell lines obtained by transfection of a GPX1 expression construct (designed MCF-7-GPX1) and a control vector-only (pLNCX)-transfected cell line MCF-7 (designed MCF-7-vector) were previously generated (28). HCT116 cells were cultured in McCoy’s 5A medium with 10% fetal bovine serum and antibiotics (10 000 U/ml penicillin and 10 µg/ml streptomycin). The selenium concentration of the fetal bovine serum was determined to be 253 nM by the Analytical Services Laboratory at South Dakota State University. MCF-7 cells were cultured in modified Eagle’s medium supplemented with 10% fetal bovine serum and antibiotics (10 000 U/ml penicillin and 10 µg/ml streptomycin). Cells were cultured at 37°C in a humidified atmosphere containing 5% CO2. Sodium selenite (Sigma, St Louis, MO) was dissolved in phosphate-buffered saline and stored at −20°C until used.

Plasmids construction and establishment of SBP1 stable transfection cell line

A human SBP1 expression plasmid (pIRE2-SBP1) was generated using normal colon mucosa DNA as template for polymerase chain reaction (PCR) amplification and subcloning into the pIRE2 vector (Clontech Laboratory,}

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GPX1 and SBP1 interaction and regulation

SBP1 negatively regulates GPX1 activity

SBP1 and GPX1 represent distinct classes of proteins with the selenium moiety of GPX1 present as the amino acid selenocysteine. In order to determine whether the levels of SBP1 could influence protein GPX1 activity, we used a human colon cancer cell line (HCT116) that has undetectable levels of SBP1 but significant levels of GPX1 protein and enzyme activity (Figure 1). Increasing SBP1 levels in these cells by transfection of an expression construct resulted in an ~50% reduction in GPX1 activity as compared with cells transfected with parental pIRES2 vector alone (Figure 1A). The reduction in GPX1 activity observed in SBP1-transfected cells was considerably more than the amount seen when cells were transfected with the pRRES2 vector alone. Changes in GPX enzyme activity as a consequence of the transfection process of the magnitude shown have been observed previously (Yang, et al, unpublished results), indicating the necessity for this particular control. As seen in Figure 1, the observed reduction in GPX enzyme activity was posttranslational, not being associated with lowering either GPX1 protein (Figure 1B) or mRNA levels (Figure 1C).

Although colon-derived cells typically express both the GPX1 and the gastrointestinal-GPX, GPX2, HCT116 cells contain relatively little GPX2 (Yang, et al, unpublished results). To confirm that SBP1 overexpression could specifically inhibit GPX1 activity and extend these observations to a different cell type, SBP1 expression was introduced into the MCF-7 human breast carcinoma cell line by transfection of the SBP1 expression construct. MCF-7 cells do not express detectable levels of GPX1 mRNA and display low or undetectable total GPX1 enzyme activity (28). Using a previously generated derivative MCF-7 cell line engineered such that all of its detectable GPX activity is exclusively due to GPX1 (28) as the recipient of the SBP1 expression construct, it was shown that overexpression of SBP1 caused a dramatic reduction, ~90%, of GPX activity in these cells (Figure 1D).
the MCF-7-vector cells (Figure 2A). This reduction in SBP1 protein was associated with ~50% reduction in SBP1 mRNA levels as determined by Quantitative real-time PCR (Figure 2B). To determine whether the observed reduction in SBP1 RNA by GPX1 was due to either an effect on transcription or RNA stability, a reporter construct was generated using human SBP1 promoter sequences and luciferase as the reporter protein. As seen in Figure 2C, overexpression of GPX1 in MCF-7-GPX1 cells compared with the MCF-7-vector cells ($P < 0.01$) ($n = 3$). (C) GPX1 inhibited SBP1 promoter activity. MCF-7-vector and MCF-7-GPX1 cells were cotransfected with Renilla and the pGL4 control or pGL4-SBP1 promoter. Twenty-four hours after transfection, cells were harvested and luciferase activity determined ($P < 0.01$). (n = 3). Each bar represents the mean ± SD of samples obtained in triplicate.

Contrasting effects of selenium supplementation on SBP1 and GPX1

The data presented above indicated that increasing GPX1 by transfection resulted in reduction of SBP1 and increasing SBP1 by transfection resulted in the reduction of GPX1 activity. An alternative approach to enhance GPX1 is by the addition of sodium selenite. GPX1 activity in cell lines is generally inducible by low physiologically relevant doses of selenium (27). The media of HCT116-SBP1 and MCF-7-GPX1 cells were each supplemented with 25, 50, 100 or
250 nM of selenium in the form of sodium selenite for 48 h and levels of GPX1 and SBP1 were determined by western blotting (Figure 3A and B). As is typically observed, there was a dose–response with GPX1 enzyme activity increasing with selenium supplementation (data not shown). Consistent with what was observed in the transfection studies, there was an inverse association between the increase of GPX1 that occurred in a dose-dependent manner and the reduction of SBP1. Since MCF-7 is null for GPX1 (28), use of these cells allows one to determine whether the reduction in SBP1 that occurred with increasing selenium concentration was GPX1 dependent. MCF-7 cells transfected only with an empty vector were incubated with increasing selenium concentration under the same conditions described above for the GPX1 expressing lines and GPX1 and SBP1 were quantified. As expected, no GPX1 signal was detectable in the western blot (Figure 3C). In contrast to the results obtained with GPX1-expressing cells, the MCF-7 cells did not exhibit a selenium-dependent decline in SBP1, indicating that the selenium-mediated increment of GPX1 was responsible for the corresponding decline of SBP1.

The relationship between GPX1 and SBP1 was further examined by quantifying GPX1 enzyme activity in HCT116 transfected with either SBP1 vector or incubating the cells with increasing amounts of selenium (Figure 4A). It is apparent that baseline GPX1 activity was lower in the SBP1-expressing cells and the selenium-mediated increase in activity was attenuated at the tested doses. The obtained enzyme activity corresponded to the levels of protein obtained by western blotting (data not shown). One possible explanation for these results is that SBP1 and GPX1 are competing for the available cellular selenium pool. A selenium titration study was performed on HCT116-SBP1 cells using an expanded range of selenium supplementation, from 0 to 5 μM (Figure 4B). GPX1 levels increased and SBP1 declined with supplementation, reaching a maximum at 250 nM and then the trends reversed with increasing supplementation with GPX1 declining and SBP1 increasing at the higher selenium concentrations. Similar results were obtained with MCF-7-GPX1 transfectants (data not shown).

**Effects of selenium status on SBP1 and GPX1 in mouse intestinal epithelial cells**

The inverse association between SBP1 and GPX1 observed in tissue culture cells was examined in intestinal epithelial cells obtained from C57Bl/6 mice fed a selenium-deficient (0 p.p.m.), adequate (0.1 p.p.m.) or -enriched (0.4 p.p.m.) diet. The levels of selenium in the diet were selected as the lowest will result in the a dramatic reduction in GPX1 in most tissues, the mid range corresponds to the amount of selenium recommended for human intake and the highest dose is one used for chemoprevention studies and would correspond to the level used in human chemoprevention trials (35). Animals were fed the respective diets for 10 weeks and colon and duodenal epithelial cells were isolated for western blotting analysis using anti-SBP1 and anti-GPX1 antibodies. As shown in Figure 5, there were very low levels of GPX1 in cells derived from the animals maintained on the selenium-deficient diet, much higher levels in those fed the ‘adequate’ diet and GPX1 protein was the highest in cells obtained from mice on selenium-supplemented diet. Consistent with the cell culture studies, there was an inverse association between GPX1 levels and SBP1 levels in intestinal epithelial cells derived from both colon and duodenum.

**Direct interaction between SBP1 and GPX1**

The ability of SBP1 and GPX1 to influence each others abundance and/or activity raised the possibility that there were direct interactions between these two proteins. To investigate this possibility, a plasmid that expressed an HA-tagged SBP1 (pcDNA3-HA-SBP) was cotransfected with an expression construct containing a GFP-GPX1 fusion protein expression construct (pECFP-GPX1), mutant GPX1 plasmid (pECFP-GPX1-CYS) or the empty vector (pECFP) into HCT116 cells. Extracts from transfectants were analyzed by western blotting (Figure 6A). Using antibodies directed against GFP, it is apparent that the vector-only transfected expressed GFP, whereas the cells expressing the GFP-GPX1 fusion protein revealed a larger protein whose molecular weight was consistent with that expected from the fusion (49 KD). As seen with the earlier studies, cells expressing the GFP-GPX1 fusion also expressed less SBP1 as indicated by the reduced signal seen with anti-HA antibodies. In order to determine whether SBP1 and GPX1 physically bound each other, extracts from the three transfectants were immunoprecipitated using anti-GFP antibodies and probed with anti-HA to probe for the SBP1-HA-tagged protein in the precipitates. As seen in Figure 6B, HA-tagged SBP1 was only present in immunoprecipitates from cells that were also expressing GFP–GPX1 fusion proteins, including a derivative GPX1 in which the selenocysteine coding UGA triplet was changed to the codon of cysteine (GPX1-Cys), indicating the binding of SBP1 to GPX1. This also excluded the possibility that the interaction occurs by the binding of SBP1 to the selenium moiety of GPX1. The interaction between SBP1 and GPX1 was further supported by fluorescence resonance energy transfer assay (supplementary Figure S1 is available at Careinogenesis Online).

**Discussion**

The goal of the work presented in this manuscript was to investigate whether two proteins representing different classes of selenium-containing molecules functionally interacted. The first of these, SBP1, binds selenium and low levels of SBP1 are associated with
cancer and poor clinical outcome (17,23,24). The second, GPX1, contains selenium as the amino acid selenocysteine (2), and increasing or decreasing GPX1 levels has an inverse effect of susceptibility of cells to DNA damage (31,36–39). Human data have indicated that functional polymorphisms in GPX1 are associated with increased cancer risk and allelic loss of GPX1 frequently occurs during cancer development (26,28,29). SBP1 and/or GPX1 are therefore potential mediators of the chemopreventive effects of selenium identified by human epidemiology and animal supplementation studies.

Increasing the levels of SBP1 in either colon-derived HCT116 cells or MCF-7 breast carcinoma cells resulted in a consequential decline in GPX1 enzyme activity. How SBP1 affected GPX1 activity remains to be determined, but neither GPX1 RNA nor protein levels were changed (Figure 1). One possible mechanism to explain these results would involve a direct physical interaction between the two proteins resulting in reduced GPX activity. Support for this notion can be seen in the coprecipitation data presented in Figure 6 showing that SBP1 and GPX1 do form a complex. How these proteins interact remains to be determined, but it is unlikely that this interaction occurs by the binding of SBP1 to the selenium atom at the GPX1 active site as the selenium is probably to be inaccessible to a molecule the size of SBP1 and SBP1 is able to bind mutant GPX1 (Figure 6B).

As seen in Figure 2, overexpression of GPX1 resulted in the reduction of SBP1 in the same cell lines used to investigate the effects of SBP1 overexpression. This effect is due, at least in part, to the inhibition of SBP1 transcription, as determined by the GPX1-mediated reduction in both SBP1 mRNA (Figure 2B) and expression from an SBP1 promoter-driven reporter gene (Figure 2C). Little is known about the regulation of SBP1 transcription, but it is possible that SBP1 expression is dependent on reactive oxygen-responsive transcriptional elements, and the reduction in peroxides that is expected to be achieved with increased GPX1 activity results in the attenuation of transcription. A search for an antioxidant response element (40) in the DNA region upstream of the SBP1 transcription start site revealed two sequences (GTGACCCTGGC and TGACACCAGC) that are very similar to the consensus antioxidant response element recognition motif (40), located just 3' of the transcription start site, although the functionality of this sequence as an antioxidant response element remains to be established.

Also consistent with the studies using ectopic expression of either SBP1 or GPX1, selenium titration experiments indicated an inverse association in the expression of these proteins. GPX1 protein levels rose with selenium supplementation of HCT116- and MCF-7-derived cell lines with a corresponding decline in SBP1. This effect was dependent on the presence of GPX1 and not some other consequence or function of selenium. This was established by showing the selenium-associated decline in SBP1 did not occur in GPX1 null MCF-7 cells (Figure 3C). The relationship between SBP1 and GPX1 was also apparent in mice fed a selenium-deficient, -adequate or -supplemented diet. Selenium deficiency resulted in a dramatic reduction of GPX1 in colonic and duodenal epithelial cells, whereas selenium supplementation resulted in elevated GPX1 as compared with those animals fed a selenium-adequate diet. For each level of dietary intake, there was a corresponding decline in SBP1 levels.

![Fig. 4.](https://academic.oup.com/carcin/article-abstract/31/8/1360/2477298)
repeated at least three times with representative blots shown. Equal amount of protein were added to the gel and all experiments were performed with cell lysate from cells cotransfected with HA-SBP1 and pECFP-GPX1-Cys. Lane 2, immunoprecipitation of pECFP, pECFP-GPX1 and pECFP-GPX1-Cys. Lane 3, immunoprecipitation of pECFP, pECFP-GPX1-Cys and then probed with anti-HA antibody to detect the HA-tagged SBP1. Non-specific peptide (NSP) in the same blot showed the equal loading efficiency and downregulation of SBP1 by GPX1. (Fig. 5).

Collectively, the work presented herein establishes an interaction among selenium availability, the amount of a protein that binds selenium—SBP1 and another protein in which selenium is present as the amino acid selenocysteine. These data raise questions as to whether higher levels of GPX1 in tumors promote carcinogenesis by suppressing SBP1 and whether SBP1 has biological functions beyond its interaction with GPX1. Furthermore, there may be biological consequences of the regulatory interaction between GPX1 and SBP1 that reflect on the potential health consequences of selenium dietary intake. An inverse association between dietary selenium intake and cancer risk has been reported for several organ systems (42).

Encouraged by the results that were initially presented for the Nutritional Prevention of Cancer selenium supplementation trial (43,44), the large cancer prevention study selenium and vitamin E cancer prevention trial that investigated the efficacy of selenium, both in concert with vitamin E and alone, in the prevention of prostate cancer was initiated (45). This study was terminated early, in part due to the lack of efficacy of selenium (11), although the analysis of the cohort following stratification by selenium status and genetic polymorphisms has not yet been reported. Data from selenium and vitamin E cancer prevention trial, as well as an additional recent studies, have indicated that higher selenium intake may also be associated with increased risk of diabetes (11–13). Whether any of the possible benefits or risks of selenium intake are due to consequential effects on GPX1 and SBP1 remains to be determined.

Supplementary material

Supplementary FigureS1 can be found at http://carcin.oxfordjournals.org/

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