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Sulforaphane, Erucin, and Iberin Up-Regulate Thioredoxin Reductase 1 Expression in Human MCF-7 Cells

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Isothiocyanates (ITCs) found in cruciferous vegetables are potentially important anticarcinogenic phytochemicals for many types of cancers including breast cancer. In this study, we have shown that three isothiocyanates, sulforaphane, erucin, and iberin, are potent inducers of thioredoxin reductase 1 (TrxR1) in human breast cancer MCF-7 cells. Sulforaphane, erucin, and iberin at 1 μM induce TrxR1 mRNA 2−3-fold within 8 h of treatment, and induce mRNA 5−7-fold with 12 μM ITC treatments. Selenium did not affect sulforaphane-induced TrxR1 mRNA levels, but significantly enhanced both TrxR1 protein expression (up to 9-fold in erucin treatment) and corresponding activities. These results suggest that dietary ITCs are important factors in the regulation of redox status through the induction of the selenoprotein thioredoxin reductase.

KEYWORDS: Isothiocyanates; sulforaphane; erucin; iberin; selenium; thioredoxin reductase

INTRODUCTION

A diet rich in cruciferous vegetables such as broccoli, Brussels sprouts, cabbage, and cauliflower correlates with a decreased risk of many common cancers including lung, colon, and breast (*1*−*3*). Crucifers contain a class of secondary metabolites termed glucosinolates, and the chemopreventative benefit of these vegetables is attributed to their relatively high glucosinolate contents (*4*). There are more than 100 glucosinolates and secondary metabolites present in cruciferous vegetables. Isothiocyanates (ITCs) are hydrolysis products derived from glucosinolates catalyzed by myrosinase (*5*, *6*). In broccoli, the primary glucosinolate is glucoraphnin [4-(methylsulfinyl)butyl glucosinolate], which is a precursor of sulforaphane (SFN) and SFN nitrile, the latter not being biologically active (*7*, *8*). SFN is a potent inducer of phase II detoxification enzymes such as quinone reductase (QR), UDP-glucoronosyl transferases (UGTs), and glutathione transferases (GSTs) (*9*−*11*). In addition to glucoraphanin, broccoli also contains two other glucosinolates, glucoerucin and glucoiberin, that give rise to erucin (ERN) and iberin (IBN), respectively (*12*, *13*). ERN was originally found in rocket (*Eruca sativa* Miller) (*14*). The chemical structures of SFN, ERN, and IBN are listed in **Figure 1**.

Glucosinolate contents vary from 20 mg/100 g in Pe-tsai Chinese cabbage to 389 mg/100 g in cress in the published food composition database (*15*). Therefore, glucosinolate intakes can be estimated on the basis of the consumption of cruciferous vegetables (*15*). For example, estimation for the consumption of total gluconsinolates in Germany was 36−46 (mg/person)/day (*16*). In a human intervention study, following a single dose of 200 μmol of isothiocyanates (largely SFN, with lesser amounts of ERN and IBN), the total plasma ITC levels reached 0.94−2.27 μM after 1 h of feeding (*13*).

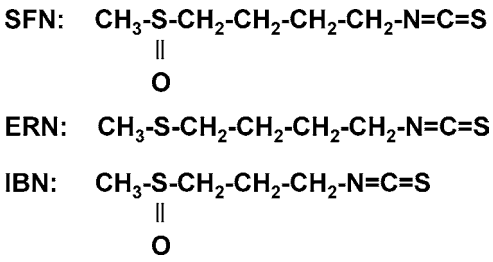

Figure 1. Chemical structures of SFN, ERN. and IBN.

Several mechanisms for the activities of ITCs in cancer chemoprevention have been proposed, including (i) inhibition of phase I carcinogen-activating enzymes (*17*, *18*), (ii) induction of phase II carcinogen detoxification enzymes (*8*, *19*), (iii) induction of G_2/M block and apoptosis (*20*−*22*), and (iv) reduction of DNA binding of NF-kB (*23*). SFN has also been reported to act as a breast cancer antiproliferative agent (*24*), disrupting microtubulin polymerization (*25*, *26*) and inhibiting histone deacetylase (*27*). We recently demonstrated that sulforaphane at low concentrations protects against heterocyclic

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amine (PhIP)−DNA adduct formation in human hepatocytes (*28*).

SFN up-regulates thioredoxin reductase 1 (TrxR1) expression in a dose-dependent manner in human cell cultures (*29*). TrxR1 is a multifunctional selenoenzyme with an FAD, a functional disulfide/dithiol, and a penultimate C-terminal selenocysteine residue (*30*, *31*). TrxR1 has broad substrate specificity, reducing many low molecular weight compounds such as hydrogen peroxide, lipid hydroperoxides, ascorbate, lipoic acid, and ubiquinone in addition to thioredoxin (*32*). There is growing evidence that redox regulation by the TrxR system plays a crucial role in the biological response against oxidative stress (*33*), and in cell growth promotion and apoptosis (*34*). In the study of SFN on apoptosis induction and microtubulin disruption, the concentration of SFN used was 15−30 μM or above. However, these levels are unlikely to be achieved in human plasma through a normal diet. In the present study, the effect of three structurally related dietary ITCs, sulforaphane, erucin, and iberin, on the induction of TrxR1 expression was examined using relatively low concentrations (1−12 μM) in MCF-7 adenocarcinoma cells from human breast.

MATERIALS AND METHODS

Cell Culture and Reagents. The MCF-7 human mammary epithelial cell line originally isolated from a malignant adenocarcinoma was purchased from ECACC. Sulforaphane, 1-isothiocyanato-4-(methylsulfinyl)butane, was purchased from ICN Biomedicals (U.K.). Iberin, 1-isothiocyanato-3-(methylsulfinyl)propane, and erucin, 1-isothiocyanato-4-(methylthio)butane, were from LKT Laboratories. TrxR (EC1.6.4.5) and Trx from *Escherichia coli* were purchased from Sigma (U.K.). Cells were cultured in DMEM supplemented with FBS (10%), penicillin (100 U/mL), and streptomycin (100 μg/mL) under 5% CO_2 in air at 37 °C. For the enzyme assays, cells were seeded in 10 cm dishes; for mRNA quantification, cells were cultured in six-well plates.

RNA Isolation. Total RNA was isolated from MCF-7 cells using the GenElute total mammalian RNA kit (Sigma, U.K.) according to the manufacturer's instructions. RNA concentration and purity were determined by absorbance measurement at 260 and 280 nm. After addition of RNase inhibitor (20 U/preparation) the total RNA was stored at −70 °C.

TrxR1 mRNA Quantification. TrxR1 mRNA was determined by real-time RT-PCR (TaqMan assay) using the ABI PRISM 7700 sequence detection system. Primers and the fluorogenic TaqMan probes were designed using Primer Express Software according to the human TrxR1 sequence. Primer/probe sets were homology-searched to ensure that they were specific for TrxR1 mRNA transcript using an NCBI BLAST search. The primer/probe set for TrxR1 (GenBank accession number AF208018) is (forward primer) 5′-CCACTGGTGAAAGACCACGTT-3′, (reverse primer) 5′-AGGAGAAAAGATCATCACTGCTGAT-3′, and (probe) 5′-CAGTATTCTTTGTCACCAGGGATGCCCA-3′.

The probes were labeled with a 5′ reporter dye, FAM (6-carboxyfluoroscein), and 3′ quencher dye, TAMRA (6-carboxytetramethylrhodamine). RT-PCR reactions were carried out in a 96-well plate in a total volume of 25 μL per well consisting of TaqMan one-step RT-PCR master mix reagents (Applied Biosystems), 100 nM probe, 200 nM forward and 300 nM reverse primers, and 10 ng of total RNA. A reverse transcription was performed for 30 min at 48 °C, then an AmpliTaq gold activation for 10 min at 95 °C, followed by 40 PCR cycles of denaturation at 95 °C for 15 s, and finally annealing/extension at 60 °C for 1 min. Reactions were carried out in triplicate. The data were analyzed by TaqMan software using a standard curve method as described in *User Bulletin No. 2* (ABI PRISM 7700 sequence detection system) to quantify the mRNA. The TaqMan threshold cycle number (C_t) was normalized into the fold of relative induction using the equation of ΔC_t method, fold of induction $= 2^{C_t(\text{control})-C_t(\text{treatment})}$. β-Actin mRNA was measured as an internal reference (*35*): forward primer, CCTGGCACC CAGCACAAT; reverse primer, GCCGATCCACACGGAGTACT; probe, ATCAAGATCATTGCTCCTCCTGAGCGC.

Cell Extract Preparation. Adherent cells were washed twice with ice-cold PBS, and harvested by scraping in 0.1 M Tris−HCl, pH 7.4. Cell pellets were homogenized by sonication in 0.1 M Tris−HCl, pH 7.4, containing 0.1% digitonin, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride in an ice bath for 20 min and then centrifuged at 13000*g* for 10 min at 4 °C. The supernatant was collected, and the protein concentration was determined by the Brilliant Blue G dye-binding assay of Bradford (*36*) using bovine serum albumin as the standard.

TrxR1 Protein. The TrxR1 protein level was measured by an in-house RIA assay as described previously (*37*). Briefly, the tracer was ^{125}I-labeled human placental TrxR1, prepared using Bolton−Hunter reagent (Amersham International plc). Standards were prepared using purified placental human TrxR1 diluted in fetal bovine serum (Gibco, Paisley, Scotland). Standard or sample (100 μL) was added with 100 μL of [^{125}I]TrxR1 tracer (10000 dpm; 50 pg/tube) and primary antibody (100 μL). After an overnight incubation at 4 °C, preprecipitated second-antibody, donkey anti-rabbit reagent (100 μL) was added. After a further 1 h at room temperature with shaking, wash solution (0.05% Brij solution) was added to each tube, followed by centrifugation for 30 min at 1800*g* at 4 °C. The supernatant was decanted, and the precipitate was washed with a further 1.5 mL of wash solution. The ^{125}I radioactivity in the precipitate was counted in a multiwell γ-radiation counter, and the results were interpolated using the LKB 1224-RIACalc RIA evaluation program.

TrxR Activity. TrxR activity (including both TrxR1 and TrxR2) was measured spectrophotometrically using insulin as a substrate (*38*). A stock reaction mixture was made by mixing 200 μL of 1.0 M HEPES buffer (pH 7.6), 40 μL of NADPH (40 mg/mL), 40 μL of 0.2 M EDTA, and 500 μL of insulin (10 mg/mL). To each test tube were added 40 μL of the reaction mixture, 10 μL of 60 μM Trx, 20 μL sample, and water to a final volume of 120 μL. The samples were incubated at 37 °C for 20 min. The reaction was stopped by addition of 500 μL of 0.4 mg/mL DTNB/6 M guanidine hydrochloride in 0.2 M Tris−HCl (pH 8.0), and the absorbance at 412 nm was recorded. A nonenzyme reaction control was a sample of cell extract without addition of Trx. The absorbance of the control was subtracted from the absorbance of the sample. TrxR activity was calculated on the basis of the standard curve prepared using pure TrxR (88.1 U/mg of protein, Sigma).

Statistics. Data are presented as the mean ± SD. The differences between the groups were examined using the Student's *t* test. A *p* value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The effects of dose and treatment time of the three ITCs on TrxR1 expression were examined in MCF-7 cells. Induction of mRNA was observed at all doses and times tested including the lowest concentration of 1 μM. The inductions of TrxR1 mRNA by SFN, ERN, and IBN at 1 μM were 2.73-, 2.16-, and 2.68- fold, respectively (**Table 1**). There were dose-dependent inductions of TrxR1 mRNA at 8 h of treatment, and 12 μM SFN, ERN or IBN induced TrxR1 mRNA 5.36-, 7.26-, and 5.74-fold, respectively (**Table 1**). The dose−effect of the highest concentration (12 μM) at 4 and 24 h of treatment was not apparent probably due to the slight toxic effect (stress to the cells) and a slight inhibition of cell growth. The housekeeping gene β-actin showed no change under all three tested ITC (1−12 μM) treatments.

Selenium (Se) at 0.06, 0.2, 0.5, 1, and 2 μM had no effect on TrxR1 mRNA levels and no effect on the SFN-induced TrxR1 mRNA level. The results from 0.06 and 1 μM together with SFN (3 and 6 μM) cotreatment are shown in **Figure 2**. SFN at 3 and 6 μM induced TrxR1 mRNA 3.62- and 4.83-fold, respectively (**Figure 2**). Unlike in human heptoma HepG2 cells (*29*), Se had no effect on SFN-induced TrxR1 mRNA in MCF-7 cells. But, selenium either alone or together with ITC significantly induced/enhanced TrxR1 protein expression in MCF-7 cells (**Figure 3**). Since there was a lag phase in the

Table 1. Effect of SFN, ERN, and IBN on TrxR1 mRNA Expression[a]

	4 h	8 h	24 h
control	1.0 ± 0.08	1.0 ± 0.07	1.0 ± 0.06
SFN			
1 μM	2.73 ± 0.21	3.41 ± 0.15	2.10 ± 0.18
3 μM	3.43 ± 0.33	4.11 ± 0.15	3.62 ± 0.55
6 μM	2.80 ± 0.10	4.76 ± 0.36	4.83 ± 0.43
12 μM	2.42 ± 0.40	5.36 ± 0.60	4.51 ± 0.39
ERN			
1 μM	2.16 ± 0.18	2.68 ± 0.21	1.47 ± 0.27
3 μM	2.73 ± 0.26	4.27 ± 0.24	2.92 ± 0.32
6 μM	3.13 ± 0.29	5.17 ± 0.67	5.62 ± 0.58
12 μM	2.32 ± 0.24	7.26 ± 0.25	6.22 ± 1.24
IBN			
1 μM	2.68 ± 0.21	3.66 ± 0.17	1.59 ± 0.12
3 μM	2.60 ± 0.21	4.35 ± 0.28	2.59 ± 0.38
6 μM	2.43 ± 0.24	4.95 ± 0.44	5.60 ± 1.0
12 μM	1.78 ± 0.02	5.74 ± 0.75	4.59 ± 0.12

[a] MCF-7 cells were cultured at approximately 60–70% confluence and then treated with ITCs. Total RNA was isolated using the GenElute total mammalian RNA kit. TrxR1 mRNA was quantified using TaqMan assay. Data are presented as the mean ± SD from triplicate treatments.

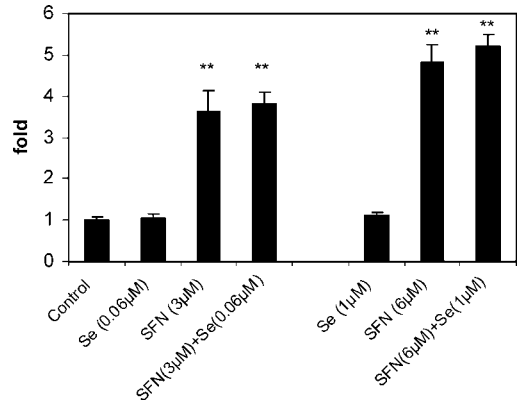

Figure 2. Effects of Se and SFN on TrxR1 mRNA expression. MCF-7 cells were treated with Se or SFN for 24 h. Control cells were treated with DMSO (0.1%). RNA was isolated as described in the Materials and Methods. Data are the mean ± SD of triplicate determinations. The double asterisks indicate $P < 0.01$ when compared with the control.

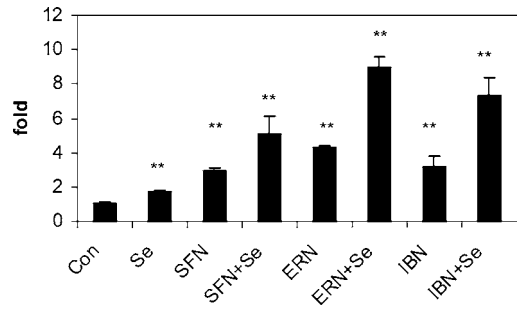

Figure 3. Effects of Se and ITCs on TrxR1 protein levels. MCF-7 cells were treated with ITCs (12 μM) either alone or with selenium (200 nM) for 48 h. Control cells were treated with DMSO (0.1%). Protein was isolated as described in the Materials and Methods. Data are the mean ± SD of triplicate determinations. Double asterisks indicate $P < 0.01$ when compared with the control.

increase of TrxR1 protein and activity relative to mRNA induction by SFN or Se (*29*), the cells in this study were treated for 48 h for the measurement of TrxR1 protein and activity. The induction of TrxR1 protein by Se (200 nM) was 1.7-fold, and the inductions by SFN, ERN, and IBN were 2.9-, 4.3-, and 3.2-fold, respectively; the cotreatment with both compounds increased the expression to 5.1-, 9.0-, and 6.3-fold, respectively.

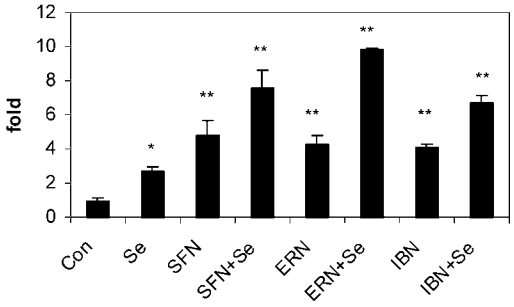

Figure 4. Effects of Se and ITCs on TrxR activity. MCF-7 cells were treated with ITCs (12 μM) either alone or with selenium (200 nM) for 48 h. Control cells were treated with DMSO (0.1%). Protein was isolated as described in the Materials and Methods. Data are the mean ± SD of triplicate determinations. A single asterisk indicates $P < 0.05$ and double asterisks indicate $P < 0.01$ when compared with the control.

The measured TrxR activities were consistent with the increase in TrxR1 protein expression in Se- and/or ITC-treated samples. The induction of TrxR activity by Se (200 nM) was 2.7-fold, and the inductions by SFN, ERN, and IBN were 4.7-, 4.3-, and 4.1-fold; the cotreatment with Se and ITC increased the expression to 7.6-, 9.8-, and 6.7-fold, respectively (**Figure 4**).

Although ERN was about one-third as active as SFN in quinone reductase induction in Hepa 1c1c7 cells (*9*) and iberin does not have significant effects on phase II enzyme induction in rats (*39*), we have demonstrated here that ERN and IBN possess activity similar (if not superior) to that of SFN in the induction of TrxR1 in MCF-7 cells. The different effects of ITCs may be dependent on the cell types used. We have also recently found that ERN is a more potent inducer than SFN in phase II enzyme induction and cell cycle arrest in Caco-2 cells and both ERN and IBN are more effective compounds than SFN in apoptosis induction in HL-60 and its multi-drug-resistant HL60/ADR or HL/60/VCR sublines (unpublished observation). There is also a report on the in vivo bioconversion of SFN to ERN, a reduced sulfur analogue, in rats (*40*). Epidemiological studies have suggested that both Se and cruciferous vegetables may play an important role in decreasing the risk of certain cancers including breast cancer (*3*, *41*−*42*). Isothiocyanates may be important dietary anticarcinogens on the basis of the evidence of their ability to inhibit chemically induced cancers in animal models and to strongly induce detoxification enzymes (*43*) and to regulate cell growth and apoptosis (*42*, *44*). Previously, we identified the potent activity of SFN in the induction of human TrxR1 in human hepatoma HepG2 cells, and this induction was found to be enhanced by Se (*29*). The interaction (synergistic, additive, or antagonistic) of dietary phytochemicals is an important area of research (*45*). A better understanding of the mechanisms of interactions may form a basis for sound dietary advice on healthy eating and prevention of chronic diseases using multiple dietary components.

There are reports that Se-enriched broccoli decreases intestinal tumorigenesis in multiple intestinal neoplasia mice (*46*), and enhances the binding of transcriptional factors p53, NF-kB, and AP-1 to their *cis*-acting elements (*47*). TrxR1 reduces oxidized thioredoxin to reduced thioredoxin, which protects cells from oxidative stress. Trx plays a pivotal role in cells not only as an antioxidant but also as a redox regulator (*48*). In this study, we have shown TrxR1 up-regulation by dietary ITCs at relatively low concentrations (1−12 μM). These concentrations could be

achieved in human plasma by consumption of ITC-rich vegetables (*15*) or SFN-enriched broccoli (*49*). There is also a direct induction of Trx mRNA (about 2-fold) by these three tested ITCs in human MCF-7 cells (data not shown). All three ITCs we tested contain moieties that bestow potent biological activity, unlike sulforaphane nitrile, which does not (*9*, *50*). These observations support the view that vegetables with enhanced levels of ITCs rather than of nitriles may be beneficial to human health. Accumulation of Se in broccoli enhances the anticarcinogenic potential of broccoli in the chemical-induced rat aberrant crypt model (*42*, *46*). ITCs from crucifers up-regulate selenoprotein TrxR1, and selenium is essential for the activity of TrxR1.

In summary, we have compared three dietary ITCs in the induction of TrxR1 in MCF-7 cells. SFN, ERN, and IBN are all potent inducers of TrxR1 mRNA, and selenium can enhance the TrxR1 protein expression and activity levels. This study indicates that an ITC-rich diet may play a role in the modulation of antioxidant enzyme TrxR1 and a role in maintaining redox in cell homeostasis.

ABBREVIATIONS USED

TrxR, thioredoxin reductase; ITCs, isothiocyanates; SFN, sulforaphane; ERN, erucin; IBN, iberin; Se, selenium.

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LITERATURE CITED

(1) Heber, D.; Bowerman, S. Applying science to changing dietary patterns. *J. Nutr.* **2001**, *131* (Suppl.), 3078S−3081S.

(2) Verhoeven, D. T.; Goldbohm, R. A.; van Poppel, G.; Verhagen, H.; van den Brandt, P. A. Epidemiological studies on brassica vegetables and cancer risk. *Cancer Epidemiol., Biomarkers Prev.* **1996**, *5*, 733−748.

(3) Ambrosone, C. B.; McCann, S. E.; Freudenheim, J. L.; Marshall, J. R.; Zhang, Y.; Shields, P. G. Breast cancer risk in premenopausal women is inversely associated with consumption of broccoli, a source of isothiocyanates, but is not modified by GST genotype. *J. Nutr.* **2004**, *134*, 1134−1138.

(4) Fenwick, G. R.; Heaney, R. K.; Mullin, W. J. Glucosinolates and their breakdown products in food and food plants. *CRC Crit. Rev. Food Sci. Nutr.* **1983**, *18*, 123−201.

(5) Rask, L.; Andreasson, E.; Ekbom, B.; Eriksson, S.; Pontoppidan, B.; Meijer, J. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. *Plant Mol. Biol.* **2000**, *42*, 93−113.

(6) Mithen, R. Glucosinolates-biochemistry, genetics and biological activity. *Plant Growth Regulat.* **2001**, *34*, 91−103.

(7) Kushad, M. M.; Brown, A. F.; Kurilich, A. C.; Juvik, J. A.; Klein, B. P.; Wallig, M. A.; Jeffery, E. H. Variation of glucosinolates in vegetable crops of Brassica oleracea. *J. Agric. Food Chem.* **1999**, *47*, 1541−1548.

(8) Basten, G. P.; Bao, Y. P.; Williamson, G. Sulforaphane and its glutathione conjugate but not sulforaphane nitrile induce UDP-glucuronosyl transferase (UGT1A1) and glutathione transferase (GSTA1) in cultured cells. *Carcinogenesis* **2002**, *23*, 1399−1404.

(9) Zhang, Y.; Talalay, P.; Cho, C. G.; Posner, G. H. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399−2403.

(10) McMahon, M.; Itoh, K.; Yamamoto, M.; Chanas, S. A.; Henderson, C. J.; McLellan, L. I.; Wolf, C. R.; Cavin, C.; Hayes, J. D. The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. *Cancer Res.* **2001**, *61*, 3299−3307.

(11) Thimmulappa, R. K.; Mai, K. H.; Srisuma, S.; Kensler, T. W.; Yamamoto, M.; Biswal, S. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res.* **2002**, *62*, 5196−5203.

(12) Zhang, Y. S. Cancer chemoprevention with sulforaphane, a dietary isothiocyanates. In *Phytochemicals in Health and Disease*; Bao, Y. P., Fenwick, R., Eds.; Marcel Dekker: New York, 2004; pp 121−141.

(13) Ye, L.; Dinkova-Kostova, A. T.; Wade, K. L.; Zhang, Y.; Shapiro, T. A.; Talalay, P. Quantitative determination of dithiocarbamates in human plasma, serum, erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans. *Clin. Chim. Acta* **2002**, *316*, 43−53.

(14) Fimognari, C.; Nusse, M.; Berti, F.; Iori, R.; Cantelli-Forti, G.; Hrelia, P. Isothiocyanates as novel cytotoxic and cytostatic agents: molecular pathway on human transformed and non-transformed cells. *Biochem. Pharmacol.* **2004**, *68*, 1133−1138.

(15) McNaughton, S. A.; Marks, G. C. Development of a food composition database for the estimation of dietary intakes of glucosinolates, the biologically active constituents of cruciferous vegetables. *Br. J. Nutr.* **2003**, *90*, 687−697.

(16) Holst, B.; Williamson, G. A critical review of the bioavailability of glucosinolates and related compounds. *Nat. Prod. Rep.* **2004**, *21*, 425−447.

(17) Langouet, S.; Furge, L. L.; Kerriguy, N.; Nakamura, K.; Guillouzo, A.; Guengerich, F. P. Inhibition of human cytochrome P450 enzymes by 1,2-dithiole-3-thione, oltipraz and its derivatives, and sulforaphane. *Chem. Res. Toxicol.* **2000**, *13*, 245−252.

(18) Barcelo, S.; Gardiner, J.; Gescher, A.; Chipman, J. K. CYP2E1-mediated mechanism of anti-genotoxicity of the broccoli constituent sulforaphane. *Carcinogenesis* **1996**, *17*, 277−282.

(19) Nestle, M. Broccoli sprouts as inducers of carcinogen-detoxifying enzyme systems: clinical, dietary, and policy implications. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11149−11151.

(20) Gamet-Payrastre, L.; Li, P.; Lumeau, S.; Cassar, G.; Dupont, M. A.; Chevolleau, S.; Gasc, N.; Tulliez, J.; Terce, F. Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. *Cancer Res.* **2000**, *60*, 1426−1433.

(21) Parnaud, G.; Li, P.; Cassar, G.; Rouimi, P.; Tulliez, J.; Combaret, L.; Gamet-Payrastre, L. Mechanism of sulforaphane-induced cell cycle arrest and apoptosis in human colon cancer cells. *Nutr. Cancer* **2004**, *48*, 198−206.

(22) Singh, S. V.; Herman-Antosiewicz, A.; Singh, A. V.; Lew, K. L.; Srivastava, S. K.; Kamath, R.; Brown, K. D.; Zhang, L.; Baskaran, R. Sulforaphane-induced G2/M phase cell cycle arrest involves checkpoint kinase 2-mediated phosphorylation of cell division cycle 25C. *J. Biol. Chem.* **2004**, *279*, 25813−25822.

(23) Heiss, E.; Herhaus, C.; Klimo, K.; Bartsch, H.; Gerhauser, C. Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. *J. Biol. Chem.* **2001**, *276*, 32008−32015.

(24) Jackson, S. J.; Singletary, K. W. Sulforaphane: a naturally occurring mammary carcinoma mitotic inhibitor, which disrupts tubulin polymerization. *Carcinogenesis* **2004**, *25*, 219−227.

(25) Smith, T. K.; Lund, E. K.; Parker, M. L.; Clarke, R. G.; Johnson, I. T. Allyl-isothiocyanate causes mitotic block, loss of cell adhesion and disrupted cytoskeletal structure in HT29 cells. *Carcinogenesis* **2004**, *25*, 1409−1415.

(26) Jackson, S. J.; Singletary, K. W. Sulforaphane inhibits human MCF-7 mammary cancer cell mitotic progression and tubulin polymerization. *J. Nutr.* **2004**, *134*, 2229−2236.

(27) Myzak, M. C.; Karplus, P. A.; Chung, F. L.; Dashwood, R. H. A novel mechanism of chemoprotection by sulforaphane: inhibition of histone deacetylase. *Cancer Res.* **2004**, *64*, 5767−5774.

(28) Bacon, J. R.; Williamson, G.; Garner, R. C.; Lappin, G.; Langouet, S.; Bao, Y. P. Sulforaphane and quercetin modulate PhIP-DNA adduct formation in human HepG2 cells and hepatocytes. *Carcinogenesis* **2003**, *24*, 1903−1911.

(29) Zhang, J.; Svehlikova, V.; Bao, Y. P.; Howie, A. F.; Beckett, G. J.; Williamson, G. Synergy between sulforaphane and selenium in the induction of thioredoxin reductase 1 requires both transcriptional and translational modulation. *Carcinogenesis* **2003**, *24*, 497−503.

(30) Zhong, L.; Holmgren, A. Mammalian thioredoxin reductases as hydroperoxide reductases. *Methods Enzymol*. **2002**, *347*, 236−243.

(31) Sandalova, T.; Zhong, L.; Lindqvist, Y.; Holmgren, A.; Schneider, G. Three-dimensional structure of a mammalian thioredoxin reductase: implications for mechanism and evolution of a selenocysteine-dependent enzyme. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9533−9538.

(32) Xia, L.; Nordman, T.; Olsson, M.; Damdimopoulos, A.; Bjorkhem-Bergman, L.; Nalvarte, I.; Eriksson, L. C.; Arner, E. S.; Spyrou, G.; Bjornstedt, M. The mammalian cytosolic selenoenzyme thioredoxin reductase reduces ubiquinone. A novel mechanism for defense against oxidative stress. *J. Biol. Chem.* **2003**, *278*, 2141−2146.

(33) Tanaka, T.; Nakamura, H.; Nishiyama, A.; Hosoi, F.; Masutani, H.; Wada, H.; Yodoi, J. Redox regulation by thioredoxin superfamily; protection against oxidative stress and aging. *Free Radical Res.* **2001**, *33*, 851−855.

(34) Soderberg, A.; Sahaf, B.; Rosen A. Thioredoxin reductase, a redox-active selenoprotein, is secreted by normal and neoplastic cells: presence in human plasma. *Cancer Res.* **2000**, *60*, 2281−2289.

(35) Svehlikova, V.; Wang, S.; Jakubikova, J.; Williamson, G.; Mithen, R.; Bao, Y. P. Interactions between sulforaphane and apigenin in the induction of UGT1A1 and GSTA1 in CaCo-2 cells. *Carcinogenesis* **2004**, *25*, 1629−1637.

(36) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein, utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(37) Miller, S.; Walker, S. W.; Arthur, J. R.; Nicol, F.; Pickard, K.; Lewin, M. H.; Howie, A. F.; Beckett, G. J. Selenite protects human endothelial cells from oxidative damage and induces thioredoxin reductase. *Clin. Sci.* **2001**, *100*, 543−550.

(38) Holmgren, A.; Bjornstedt, M. Thioredoxin and thioredoxin reductase. *Methods Enzymol.* **1995**, *252*, 199−208.

(39) Kore, A. M.; Jeffery, E. H.; Wallig, M. A. Effects of 1-isothiocyanato-3-(methylsulfinyl)-propane on xenobiotic metabolizing enzymes in rats. *Food Chem. Toxicol.* **1993**, *31*, 723−729.

(40) Kassahun, K.; Davis, M.; Hu, P.; Martin, B.; Baillie, T. Biotransformation of the naturally occurring isothiocyanate sulforaphane in the rat: identification of phase I metabolites and glutathione conjugates. *Chem. Res. Toxicol.* **1997**, *10*, 1228−1233.

(41) Whanger, P. D. Selenium and its relationship to cancer: an update dagger. *Br. J. Nutr.* **2004**, *91*, 11−28.

(42) Keck, A. S.; Finley, J. W. Cruciferous vegetables: cancer protective mechanisms of glucosinolate hydrolysis products and selenium. *Integr. Cancer Ther.* **2004**, *3*, 5−12.

(43) Talalay, P.; Fahey, J. W. Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *J. Nutr.* **2001**, *131* (Suppl.), 3027S−3033S.

(44) Conaway, C. C.; Yang, Y. M.; Chung, F. L. Isothiocyanates as cancer chemopreventive agents: their biological activities and metabolism in rodents and humans. *Curr. Drug Metab.* **2002**, *3*, 233−255.

(45) Bao, Y. P.; Fenwick, R. Future perspectives in phytochemicals and health research. In *Phytochemcials in Health and Disease*; Bao, Y. P., Fenwick, R., Eds.; Marcel Dekker Inc.: New York, 2004; pp 331−342.

(46) Davis, C. D.; Zeng, H.; Finley, J. W. Selenium-enriched broccoli decreases intestinal tumorigenesis in multiple intestinal neoplasia mice. *J. Nutr.* **2002**, *132*, 307−309.

(47) Zeng, H.; Davis, C. D.; Finley, J. W. Effect of selenium-enriched broccoli diet on differential gene expression in min mouse liver. *J. Nutr. Biochem.* **2003**, *14*, 227−231.

(48) Nakamura, H.; Kondo, N.; Hirota, K.; Masutani, H.; Yodio, J. J. Thios and thioredoxin in cellular redox control. In *Rodox-Genome Interactions in Health and Disease*; Fuchs, J., Podda, M., Packer, L., Eds.; Macel Dekker Inc.: New York, 2004; pp 61−77.

(49) Faulkner, K.; Mithen, R.; Williamson, G. Selective increase of the potential anticarcinogen 4-methylsulphinylbutyl glucosinolate in broccoli. *Carcinogenesis* **1998**, *19*, 605−609.

(50) Matusheski, N. V.; Jeffery, E. H. Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. *J. Agric. Food Chem.* **2001**, *49*, 5743−5749.

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