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# Synergy between sulforaphane and selenium in protection against oxidative damage in colonic CCD841 cells

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ABSTRACT

Dietary isothiocyanates are potent inducers of the NF-E2–related factor 2 (Nrf2) pathway. Sulforaphane (SFN), a representative dietary isothiocyanate, has previously been shown to up-regulate antioxidant enzymes such as selenium (Se)–dependent thioredoxin reductase–1 (TrxR-1) in many tumor cell lines. In the present study, we hypothesized that a combination of SFN and Se would have a synergistic effect on the up-regulation of TrxR-1 and the protection against oxidative damage in the normal colonic cell line CCD841. Treatment of cells with SFN and Se significantly induced TrxR-1 expression. Pretreatment of cells with SFN protects against $H_2O_2$-induced cell death; this protection was enhanced by cotreatment with Se. The small interfering RNA knockdown of either TrxR-1 or Nrf2 reduced the protection afforded by SFN and Se cotreatment; TrxR-1 and Nrf2 knockdown reduced cell viabilities to 66.5% and 51.1%, respectively, down from 82.4% in transfection-negative controls. This suggests that both TrxR-1 and Nrf2 are important in SFN-mediated protection against free radical–induced cell death. Moreover, flow cytometric analysis showed that TrxR-1 and Nrf2 were involved in SFN-mediated protection against $H_2O_2$-induced apoptosis. In summary, SFN activates the Nrf2 signaling pathway and protects against $H_2O_2$-mediated oxidative damage in normal colonic cells. Combined SFN and Se treatment resulted in a synergistic up-regulation of TrxR-1 that in part contributed to the enhanced protection against free radical–mediated cell death provided by the cotreatment.



## 1. Introduction

Some early epidemiological studies suggest that intake of cruciferous vegetables is inversely correlated with the morbidity of various cancers including those of the lung, bladder, and colon [1,2]. However, the results of other epidemiological studies are inconsistent and inconclusive [3,4]. Since cruciferous vegetables are rich sources of glucosinolates, it is inevitable that their chemoprotective activity is attributed to the isothiocyanates (ITCs). ITCs, derived from the glucosinolates in cruciferous vegetables, have in themselves significant cancer chemopreventive potential [5]. Among all the ITCs, sulforaphane (SFN), which

*Abbreviations:* ITCs, isothiocyanates; Nrf2, nuclear factor-erythroid 2-related factor 2; Sam68, Src-associated in mitosis 68 kDa; Se, selenium; SFN, sulforaphane; TrxR-1, thioredoxin reductase.

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is derived from glucoraphanin—commonly found in broccoli and cauliflower—has been the most intensively studied ITC in relation to cancer prevention. Administration of crucifers or ITCs to experimental animals has been shown to inhibit the development of colonic aberrant crypt foci [6] and to reduce the incidence and multiplicity of chemical-induced tumors, including those of the bladder and colon [7,8]. Isothiocyanates are potent inducers of phase II enzymes, which are involved in detoxifying potential endogenous and exogenous carcinogens [9,10]. Importantly, ITCs have been shown to exert antioxidant effects via the regulation of NF-E2–related factor 2 (Nrf2) antioxidant responsive element pathways [11]. Nrf2 regulates a major cellular defense mechanism, and its activation is important in cancer prevention [12]. However, overexpression of Nrf2 in cancer cells protects them against the cytotoxic effects of anticancer therapies, thus promoting chemoresistance [13,14]. Thioredoxin reductase 1 (TrxR-1) is an Nrf2-driven antioxidant enzyme, and it has been shown to play a dual role in cancer [15]. We have previously shown that TrxR-1 plays an important role in the protection against free radical–mediated cell death in cultured normal and tumor cells [16,17]. Moreover, the induction of TrxR-1 and glutathione peroxidase 2 by SFN is synergistically enhanced by selenium (Se) cotreatment in colon cancer Caco-2 cells [18].

The mechanisms by which ITCs act in cancer prevention may involve multiple targeted effects, including the induction of phase II antioxidant enzymes, cell cycle arrest, and apoptosis [19,20]. Other potential targets include kinases, transcriptional factors, transporters, and receptors [21–24]. Since both Nrf2 and TrxR-1 can play dual roles in cancer [25–28], the benefits or risks of Nrf2 activation or TrxR-1 induction may depend upon the nature of the cells (normal vs tumor). Therefore, it is important to investigate the effects of ITCs on normal cells. We hypothesized that a combination of SFN and Se would have a synergistic effect on the up-regulation of TrxR-1 and on the protection against oxidative damage in the normal colonic cell line CCD841. Recently, we demonstrated that SFN promoted cancer cell proliferation, migration, and angiogenesis at low concentrations (<2.5 μmol/L), whilst demonstrating opposite effects at high concentrations (>10 μmol/L) [29]. Activation of Nrf2 signalling and TrxR-1 in normal cells may be beneficial, and this effect is associated with the chemoprotective activity of SFN. In the present study, we have demonstrated that the pretreatment of cells with SFN and Se protects against free radical–mediated cell death in normal colon epithelial CCD841 cells.

## 2. Methods and materials

### 2.1. Materials

Sulforaphane (1-isothiocyanato-4-[methylsulfinyl]-butane, purity 98%) was purchased from Alexis Biochemicals (Exeter, UK). Sodium selenite (purity 98%), dimethyl sulfoxide (DMSO), TrxR, hydrogen peroxide, and Bradford reagent were all purchased from Sigma-Aldrich (Dorset, UK). Complete protease inhibitors were obtained from Roche Applied Science (West Sussex, UK). Rabbit polyclonal primary antibodies to Nrf2 and TrxR-1, goat polyclonal primary antibody to β-actin, rabbit polyclonal primary antibody to the RNA-binding protein, Sam68 (Src-associated in mitosis 68 kDa), and HRP-conjugated goat anti-rabbit and rabbit anti-goat IgG were all purchased from Santa Cruz Biotechnology (Santa Cruz, Germany). The small interfering RNAs (siRNAs) for Nrf2 (Cat No. SI03246950; target sequence, 5′-CCCATTGATGTTTCTGATCTA-3′), TrxR-1 (Cat No. SI00050876; target sequence, 5′-CTGCAAGACTCTCGAAATTAT-3′), and AllStars Negative Control siRNA (AS) were all purchased from Qiagen (West Sussex, UK). The Annexin V-FITC apoptosis detection kit was purchased from eBioscience (Hatfield, UK). Electrophoresis and Western blotting supplies were obtained from Bio-Rad (Hertfordshire, UK), and the chemiluminescence kit was from GE Healthcare (Little Chalfont, UK).

### 2.2. Cell culture

CCD841 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10%), 2 mmol/L glutamine, penicillin (100 U/mL), and streptomycin (100 μg/mL) under 5% $CO_2$ in air at 37°C. When the cells achieved 70% to 80% confluence, they were exposed to various concentrations of SFN and/or Se for different times with DMSO (0.1%) as control.

### 2.3. Cell viability and apoptosis assays

The cell proliferation 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay was employed to determine the toxicity of SFN (1-160 μmol/L) and $H_2O_2$ (100-1600 μmol/L) towards cultured CCD841 cells. Cells were seeded on 96-well plates at a density of $1.0 \times 10^4$ per well in DMEM with 10% fetal calf serum (FCS). When cells were at approximately 70% to 80% confluence, they were exposed to SFN at various concentrations for different times, using DMSO (0.1%) only as control. After all treatments, the medium was removed and then MTT (5 mg/mL) was added and incubated with the cells at 37°C for 1 hour to allow the MTT to be metabolized. Then supernatant was removed and the produced formazan crystals were dissolved in DMSO (100 μL per well). The final absorbance in the wells was recorded using a microplate reader (BMG Labtech Ltd, Aylesbury, Buckinghamshire, UK), at a wavelength of 550 to 570 nm and a reference wavelength of 650 to 670 nm.

For apoptosis analysis, CCD841 cells were seeded on 12-well plates at a density of $5.0 \times 10^4$ cells per well and incubated at 37°C for 48 hours. After treatment with 2.5 μmol/L SFN and/or 0.1 μmol/L Se for 24 hours, cells were exposed to 100 to 150 μmol/L $H_2O_2$ for 24 hours. Cells were then trypsinized and collected by centrifugation at 180×*g* for 5 minutes at room temperature. The pellet was washed with cold phosphate-buffered saline (PBS) before being resuspended in binding buffer at a cell density adjusted to 2.0 to $5.0 \times 10^5$/mL according to the instructions from the Annexin V-FITC apoptosis detection kit (eBioscience). The Annexin V-FITC (fluorescein isothiocyanate) was used to stain for the apoptotic cells and propidium iodide (PI) used to stain the necrotic cells. For each sample, 10000 events were collected and the data were analyzed using the FlowJo software (Tree Star Inc, Ashland, OR, USA).

### 2.4. Knockdown of Nrf2 and TrxR-1 by siRNA

CCD841 cells were seeded on 12-well plates in DMEM with 10% FCS. After 24 hours, the cells were transfected with siRNA targeting Nrf2 or TrxR-1. Briefly, the cell medium was replaced

with 1000 μL 12% FCS medium, then 20 nM siRNA and 6 μL HiPerFect transfection reagent were combined in 100 μL medium (without serum and antibiotics) and incubated at room temperature for 10 minutes, and then gently added dropwise to the cells; AS was used as a negative control (this siRNA has no homology to any known mammalian gene). After 24 hours incubation with siRNA, SFN, and Se were added in fresh medium for a further 24 hours, then the effects of $H_2O_2$ (150 μmol/L, 24 hours) on cell viability and apoptosis were measured using flow cytometric analysis.

### 2.5. *Protein extraction and immunoblotting of Nrf2 and TrxR-1*

To extract total protein, CCD841 cells were washed twice with ice-cold PBS, then harvested by scraping in 20 mmol/L Tris-HCl (pH 8), 150 mmol/L NaCl, 2 mmol/L EDTA, 10% glycerol, 1% Nonidet P-40 containing mini-complete proteinase inhibitor, and 1 mmol/L phenylmethanesulfonyl fluoride, in an ice bath for 20 minutes to lyse cells. The lysate was then centrifuged at 12000×*g* for 15 minutes at 4°C and the protein-containing supernatant was collected. To extract nuclear protein, the Nuclear Extract Kit (Active Motif, Rixensart, Belgium) was used, following the manufacturer's instructions. Protein concentrations were determined by the Brilliant Blue G dye-binding assay of Bradford, using bovine serum albumin as a standard.

Protein extracts were heated at 95°C for 5 minutes in loading buffer and then loaded onto 10% sodium dodecyl sulfate polyacrylamide gels together with a molecular weight marker. After routine electrophoresis and transfer to polyvinylidene fluoride membranes, membranes were blocked with 5% fat free milk in Phosphate Buffered Saline Tween-20 (PBST) (0.05% Tween 20) for 1 hour at room temperature, and then with specific primary antibodies against Nrf2 or TrxR-1 (diluted in 5% milk in PBST) overnight at 4°C. Membranes were then washed 4 times for 40 minutes with PBST, then incubated with secondary antibodies (diluted in 5% milk in PBST) for 1 hour at room temperature. After 4 further washes for 40 minutes with PBST, antibody binding was detected using an ECL kit (GE Healthcare), and the density of each band was measured with the FluorChem Imager (Alpha Innotech, San Leandro, CA, USA), or the Li-Cor Odyssey Imager (Li-Cor Biotechnology UK Ltd., Cambridge, UK).

### 2.6. *Statistical analyses*

Data are represented as means ± SD. The differences between the groups were examined using the 1-way analysis of variance/least significant difference test, or the Student *t* test. $P < .05$ was considered statistically significant. The $IC_{50}$ values of SFN and $H_2O_2$ were determined using the CalcuSyn Software (Biosoft, Cambridge, UK).

## 3. Results

### 3.1. *Effect of sulforaphane on cell growth*

Sulforaphane has been shown to promote the growth of some tumor cell lines at low concentrations but to be toxic to the same cells at higher concentrations through the induction of stress-related cell cycle arrest and apoptosis [29–31]. CCD841 cells were cultured in 96-well plates (seeding $5.0 \times 10^3$ cells per well) and treated with SFN for 24 or 48 hours once they reached 70% to 80% confluence. In this study, 2.5 μmol/L and 5 μmol/L SFN moderately stimulated the growth of CCD841 cells. Treatment with 2.5 μmol/L and 5 μmol/L SFN for 24 hours increased cell viability by 13% and 15%, respectively, vs control, while treatment for 48 hours with the same concentrations did so by 25% and 11%, respectively (Fig. 1). Treatment with higher concentrations of SFN (20-160 μmol/L) significantly reduced cell viability. Sulforaphane had $IC_{50}$ values of 30.0 μmol/L (24 hours) and 40.4 μmol/L (48 hours) for CCD841 cells. In contrast, the $IC_{50}$ values of SFN for Caco-2 were 47.1 μmol/L (24 hours) and 50.6 μmol/L (48 hours) as reported previously [18], suggesting that normal colonic cells are more susceptible to SFN-induced cell death.

### 3.2. *Effect of sulforaphane on nuclear accumulation of Nrf2*

Untreated CCD841 cells exhibited very low Nrf2 levels in both the cytoplasm and the nucleus, consistent with the degradation of Nrf2 by proteasomes in a Keap1-dependent manner under homeostasis [32]. However, upon SFN treatment (1.25-40 μmol/L for 4 hours), a significant increase of Nrf2 in the nucleus was observed, suggesting the rapid liberation of Nrf2 from Keap1-coupled suppression and its subsequent nuclear translocation (Fig. 2A) [33]. However, SFN at 40 μmol/L showed less effect in this regard than at lower doses (2.5-20 μmol/L), indicating a toxic effect at high concentrations. Sulforaphane at 1.25 to 20 μmol/L induced a significant and dose-dependent translocation of Nrf2 into the nucleus, resulting in nuclear levels 5.3- to 8.4-fold in magnitude vs controls. In the time course experiment, the level of Nrf2 in the nucleus peaked at 1 hour following SFN (10 μmol/L) treatment, at which point it was 7.0-fold of the control level. The level of Nrf2 in the nucleus started to decrease after 12 hours. However, at 24 hours the Nrf2 level was still 3.7-fold that of the control (Fig. 2B).

### 3.3. *Effect of SFN and/or Se on TrxR-1 expression*

Sulforaphane induced TrxR-1 expression in a dose-dependent manner in CCD841 cells. These data are consistent with

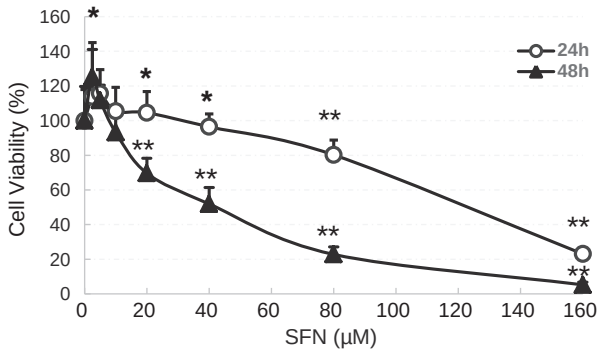


**Fig. 1 – Effect of SFN on CCD841 cell growth. Cells at 70% to 80% confluence were treated with SFN (0-160 μmol/L) in cell culture medium for 24 to 48 hours. Control cells were treated with DMSO (0.1%) only. Cell viability was determined by the MTT cell proliferation assay. Each data point represents the means ± SD (n = 5). *P < .05; **P < .01.**

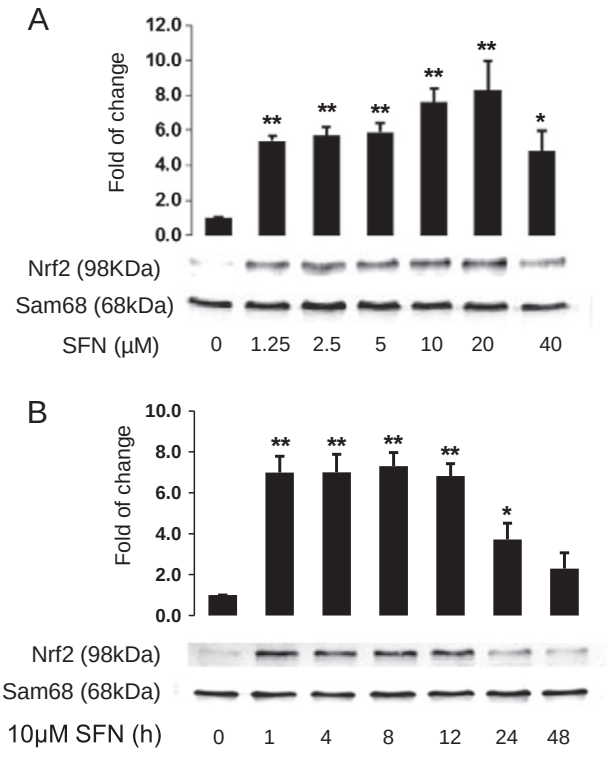


**Fig. 2 – Effect of SFN on the translocation of Nrf2 into the nucleus. CCD841 cells were exposed to SFN (with DMSO [0.1%] only as control). Nuclear protein fractions were isolated as described in Methods and materials. Nrf2 was detected and quantified by Western blot analysis. Nrf2 band densities were normalized against Sam68 (68 kDa), and results were expressed as fold induction relative to controls. Data are expressed as means ± SD (n = 3). A, Dose-response, SFN (0-40 μmol/L) for 4 hour. B, Time course, SFN (10 μmol/L) for 0 to 48 hours. *P < .05; **P < .01.**

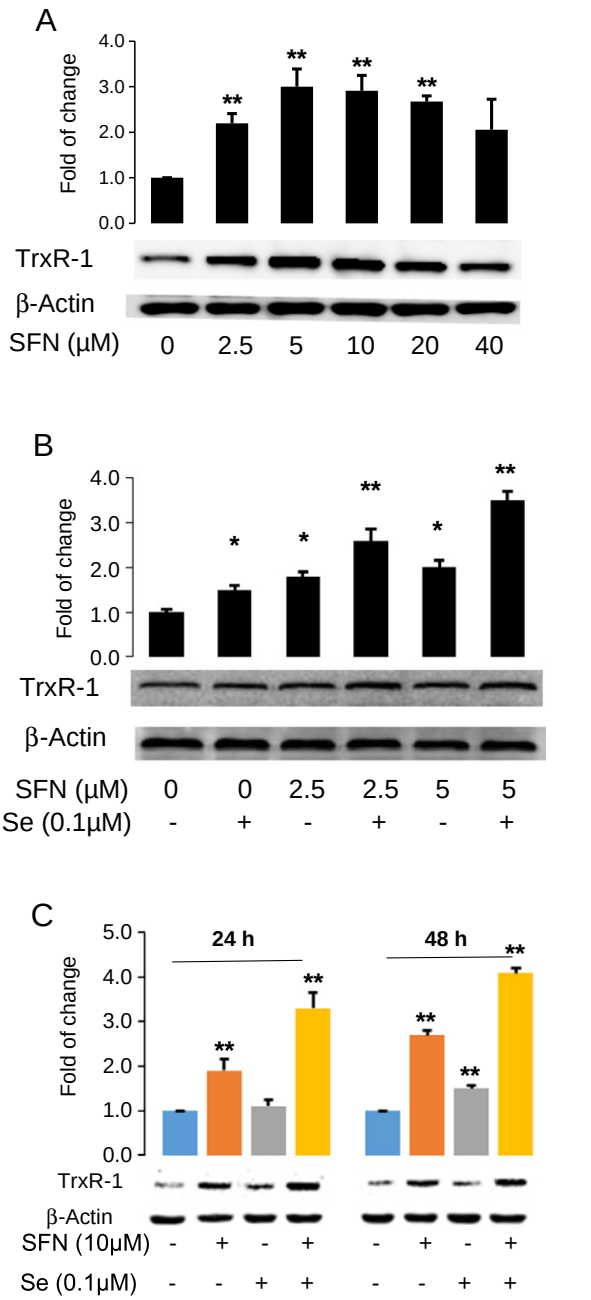


**Fig. 3 – Effect of SFN on TrxR-1 protein expression. A, CCD841 cells were exposed to SFN (2.5-40 μmol/L) for 24 hour (DMSO [0.1%] only was used as a control). Synergistic effect of SFN with Se: dose response (B), and time response (C). Folds of change were determined by Western blot analysis, from the average TrxR-1 band densities (normalized to those of ß-actin). Data are expressed as means ± SD (n = 3). *, P < .05; **P < .01.**

previous publications on tumor cell lines such as colon cancer Caco-2 and breast cancer MCF-7 cells [34,35]. The effect of SFN and/or Se on TrxR-1 protein expression in CCD841 was determined using Western blot analysis. A dose-dependent response was observed in cells exposed to 2.5 to 20 μmol/L SFN (with 0.1% DMSO only as control) (Fig. 3A). Co-treatment with SFN (2.5 μmol/L) and Se (0.1 μmol/L) produced a synergistic effect especially after 24 and 48 hours. Sulforaphane (2.5 μmol/L) alone induced TrxR-1 1.8-fold, and Se (0.1 μmol/L) alone induced it 1.4-fold, whereas the combination of 2.5 μmol/L SFN and 0.1 μmol/L Se induced it 2.6-fold (Fig. 3B). Moreover, cotreatment with 10 μmol/L SFN and 0.1 μmol/L Se also produced a synergistic effect, especially after 24 and 48 hours. SFN alone (10 μmol/L) induced TrxR-1, 3.7- and 2.6-fold at 24 and 48 hours, respectively; 0.1 μmol/L of Se alone induced it 1.9- and 1.5-fold at 24 and 48 hours respectively, whereas the combination of 10 μmol/L SFN and 0.1 μmol/L Se induced it 4.3- and 4.0-fold at 24 and 48 hours, respectively (Fig. 3C).

### 3.4. *Protective effect of SFN and/or Se against $H_2O_2$-induced cell death*

Hydrogen peroxide is known to activate the mitochondrial apoptotic pathway, decrease Nrf2 expression, and increase reactive oxygen species levels, leading to cell death [36]. CCD841 cells were cultured in 96-well plates (seeding $5.0 \times 10^3$ cells per well), and when they reached 70% to 80% confluence, they were treated with a concentration series (0-1600 μmol/L) of $H_2O_2$ for 24 hours. The $IC_{50}$ value of $H_2O_2$ for CCD841 cells was 64.1 μmol/L. $H_2O_2$ treatment (100 μmol/L) decreased cell viability to 11.4% of the control (Fig. 4). Pretreatment with SFN at 2.5 or 5 μmol/L for 24 hours significantly protected against

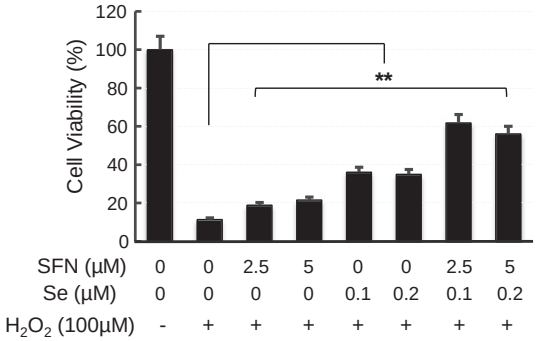


**Fig. 4 – Effect of cotreatment with SFN and Se on $H_2O_2$-induced cell death. CCD841 cells were cultured in 96-well plates (seeding 7.0 × $10^3$ cells per well) and when they reached 70% to 80% confluence, were pre-treated with SFN (2.5 or 5 μmol/L) (or DMSO [0.1%] only as control) and/or Se (0.1 or 0.2 μmol/L) for 24 hours, and were then exposed to $H_2O_2$ (100 μmol/L) in serum-free medium for a further 24 hours. Cell viability was measured by the MTT assay. *P < .05; **P < .01.**

the reduction in cell viability induced by 100 μmol/L $H_2O_2$. After 2.5 and 5 μmol/L SFN pretreatment, the 100 μmol/L $H_2O_2$ treatment only reduced cell viabilities to 18.9% and 21.6%, respectively. When the cells were pretreated with 0.1 and 0.2 μmol/L Se for 24 hours, the 100 μmol/L $H_2O_2$ treatment only reduced cell viabilities to 36% and 35%, respectively. For cells that were co-treated with 2.5 μmol/L SFN and 0.1 μmol/L Se, or with 5 μmol/L SFN and 0.2 μmol/L Se, subsequent 100 μmol/L $H_20_2$ treatment only reduced cell viabilities to 61.8 or 56.1%, respectively. Moreover, in a separate experiment using siRNA to knock down TrxR-1 or Nrf2, the protection afforded by pretreatment with SFN (2.5 μmol/L) and Se (0.1 μmol/L) against the induction of apoptosis by 150 μmol/L $H_2O_2$ treatment was reduced such that the proportion of viable cells, as indicated by Annexin V/PI staining, was reduced from 82.4% in transfection negative controls to 66% or 51% in TrxR-1 or Nrf2 knockdowns, respectively (Fig. 5A). This suggests that Nrf2 and TrxR-1 play important roles in SFN-mediated protection against $H_2O_2$-induced cell death in normal colonic cells. $H_2O_2$ caused a concomitant rise in early- (single positive) and late-stage (double positive) apoptotic cells, as indicated by Annexin V/PI staining. $H_2O_2$ induced a 59.9% proportion of

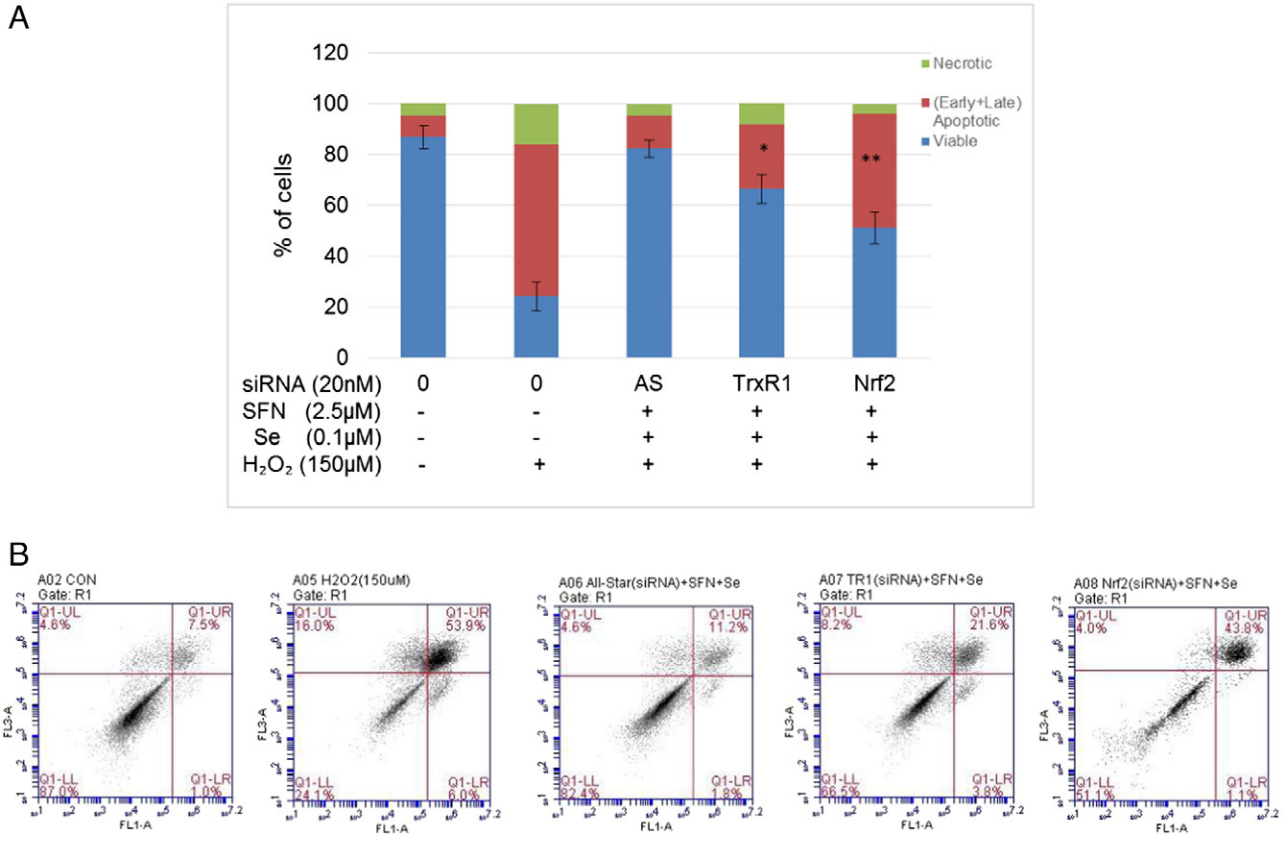


**Fig. 5 – Effect of small interfering RNA (siRNA) knockdown of TrxR-1 and Nrf2 on the protection against $H_2O_2$-induced cell death mediated by SFN and Se cotreatment. CCD841 cells were pretreated with SFN (2.5 μmol/L) and Se (0.1 μmol/L) for 24 hours, then siRNA (20 nM) knockdown of TrxR-1 or Nrf2 (with AS as negative control) was performed. Then the cells were exposed to $H_2O_2$ (100μ M) for 24 hours. The cells were then stained with annexin V and PI, and flow cytometric analysis was carried out. The $H_2O_2$-treated cells have a higher percentage of apoptotic cells (annexin V positive), as indicated by the percentage of gated cells (B). SFN and Se pre-treatment afforded significant protection against $H_2O_2$; siRNA against TrxR-1 or Nrf2 abrogated this protection. Early and late apoptotic data (red bars) are expressed as means ± SD (n = 3). *P < .05; **P < .01 in comparison to the AS control.**

apoptotic cells; cotreatment with SFN and Se reduced the proportion of apoptotic cells to 13.0% (Fig. 5A). The siRNA knockdown of either TrxR-1 or Nrf2 abrogated the protection afforded by SFN and Se cotreatment, and increased the apoptotic cell population to 25.4% or 44.9%, respectively, suggesting that Nrf2 signaling is important in the protection against free radical–mediated apoptosis in normal colonic cells.

## 4. Discussion

Oxidative stress is one of the most critical factors implicated in many gastrointestinal diseases, including inflammatory bowel disease and colon cancer [37]. Many selenoproteins including TrxR-1 are involved in cellular homeostasis and are protecting normal and tumor cells against oxidative stress [38]. Fruits and vegetables are rich in various antioxidants. Increasing the consumption of fruits and vegetables may inhibit certain cancers [39]. Although the results from many epidemiological studies are inconsistent and inconclusive, one exception is the Netherlands Cohort Study on Diet and Cancer, in which women (but not men) who had a high intake of cruciferous vegetables were shown to have a reduced risk of colon cancer [3]. Cruciferous vegetables are rich sources of glucosinolates, which can be broken down to ITCs under the action of myrosinases when the plant tissue is damaged or cooked. Several studies have demonstrated that dietary ITCs possess significant cancer chemopreventive potential [24]. However, ITCs have been shown to exert both chemopreventive and oncogenic activities. Overexpression of Nrf2 and/or TrxR-1 in cancer cells might be undesirable; high constitutive levels of Nrf2 occur in many tumors and can promote chemoresistance [13]. On the other hand, the induction of Nrf2 and TrxR-1 by ITCs in normal cells could be beneficial in cancer prevention [29]. There are over 1000 genes driven by Nrf2, many of which possess antioxidant or chemopreventive potential [40,41]. Apart from TrxR-1, other enzymes such as glutathione transferases (GSTs), quinone reductase (QR), and heme oxygenase (HO-1) might also be involved in chemoprevention [42,43]. Glutathione transferases are key enzymes in the metabolism of ITCs in cells. A recent comprehensive meta-analysis demonstrated an increased cancer risk in white populations conferred by GSTM1 and GSTT1 null genotypes [44]. Conversely, results from another study reveal statistically significant protective effects of crucifer consumption against colorectal neoplasms that are stronger among individuals with a single null GSTT1 genotype [45]. To better understand the mechanisms behind the role of Nrf2 in the chemoprevention of colorectal cancer, more studies, especially into the genetic aspects of responses to ITCs, are required.

The up-regulation of antioxidant enzymes by ITCs is one of the most important factors in chemoprevention. TrxR-1 is an important Se-dependent enzyme involved in the regulation of cell redox [46]. Similarly to Nrf2 activation, TrxR-1 induction may protect against carcinogenesis in normal cells, but TrxR-1 overexpression has been reported in a large number of human tumors [15]. A very recent study suggested that both TrxR-1 and 15 kDa selenoprotein (Sep15) participate in interfering regulatory pathways in colon cancer cells [38]. The relationship between Se and cancer is complex; an optimal intake may promote health [47]. In general, individuals who have low serum Se levels may benefit from Se supplementation, but those with high serum Se levels are at increased risk for other diseases [48]. The cancer-preventive properties of Se in colon cancer are believed to be mediated by both selenoproteins and low molecular weight selenocompounds [49]. Although TrxR-1 has been suggested as a novel target for cancer therapy [50], the function of TrxR-1 in tumor cell growth, migration, and invasion warrants further in vitro and in vivo studies.

In the present study, we have demonstrated that SFN can activate the Nrf2 signaling pathway and interact with Se in the up-regulation of TrxR-1 in normal colonic cells. Cotreatment of colonic cells with SFN and Se resulted in a synergistic induction of TrxR-1 expression and provided a greater protective effect against hydrogen peroxide–induced cell death than treatments with either component individually. An optimal combination of Se and SFN may be able to achieve the same level of gene expression using relative less concentration of each compound than when they are used alone. A limitation of this study is that the synergy was identified in in vitro cell cultures. Further in vivo studies could consider positive interactions between bioactives and nutrients to test if they result in greater protection against oxidative stress and stronger chemopreventive activities. It would be interesting to identify more synergistic or antagonistic interactions between food components and whole foods, to help inform healthy dietary recommendations. An optimal combination of different bioactive phytochemicals, vitamins, and minerals may be able to up-regulate chemoprotective enzymes, reduce oxidative stress, and improve gut health. In conclusion, combined SFN and Se treatment synergistically upregulated TrxR-1, which plays a significant role in maintaining intracellular redox homeostasis and contributed to the SFN-induced protection against free radical-mediated oxidative damage in normal colonic cells.

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There are no conflicts of interest for any of the authors.

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