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Major differences among chemopreventive organo-selenocompounds in the sustained elevation of cytoprotective genes

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Abstract

Cytoprotective enzyme elevation through the Nrf2-keap1/ARE pathway has been promulgated for cancer prevention. This study compares the redox insult and sustained cytoprotective enzyme elevation by organo-selenocompounds and sulforaphane in lung cells. Sulforaphane, elicited a rise in reactive oxygen species (ROS) and drop in glutathione (GSH) at 2hrs, nuclear accumulation of Nrf2 at 4hrs, and a GSH rebound and elevation in NAD(P)H quinone oxidoreductase (NQO1), thioredoxin reductase (TR1), and glutamate-cysteine ligase (GCL) at 24 hrs. Selenocystine elicited a similar 24 hr response, despite lesser earlier time-point changes. 2-Cyclohexylselenazolidine-4-carboxylic acid effects were similar to selenocystine's but with a larger Nrf2 change and the largest 24hr-increase in GSH and GCL, TR1 and NQO1 of any compound investigated. Selenomethionine elicited a similar acute rise in ROS, but lesser depletion of GSH, no 4hr-increase in nuclear Nrf2, only minor 24hr-elevations in TR1 and NQO1 and a GCL elevation insufficient to elevate GSH.

Suggested Keywords

organoselenium; sulforaphane; cytoprotection; chemoprevention; Nrf2-Keap1

INTRODUCTION

Lung cancer is the leading cancer type for cancer deaths. An estimated 222,520 new cases of lung and bronchus cancer in the USA in 2010 make it the second most common cancer type (1). The link between cigarette smoke and lung cancer is well established. Cigarette smoke contains reactive oxygen and nitrogen species that can place a large oxidative burden on the lung. Oxidative stress contributes to the initiation and progression of lung carcinomas (2) and a chemoprevention strategy to combat oxidative insult is to increase the cytoprotective antioxidant defense system of the lung cells. Activation of the nuclear erythroid 2 p45-related factor 2 (Nrf2) pathway is one mechanism by which antioxidant capacity of a cell can be enhanced. Nrf2 is basic leucine zipper transcription factor that under basal conditions

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is tethered to the cytoplasm by its negative regulator Kelch-like ECH-associated protein 1 (Keap1), where it remains inactive and is targeted for proteasomal degradation by ubiquitination (3,4). Under conditions of oxidative or electrophilic stress, Nrf2 dissociates from Keap1 and can then translocate to the nucleus. Once in the nucleus, Nrf2 associates with small Maf proteins and binds to antioxidant response element (ARE) sequences located in many cytoprotective enzyme gene promoters to initiate transcription. Among the battery of cytoprotective enzymes recognized as being regulated by the Nrf2-Keap1/ARE pathway are the antioxidant enzymes NAD(P)H quinone oxidoreductase 1 (NQO1; (5)), thioredoxin reductase 1 (TR1; (6,7)), and glutathione peroxidase 2 (GPx2; (8)); the glutathione-S-transferase (GST) and UDP glucuronosyltransferase (UGT) detoxification enzymes (9-11); and the glutamate cysteine ligase (GCL_C) enzyme required for glutathione biosynthesis (12). Thus, Nrf2 activation serves as a means by which the inherent cellular antioxidant defense can be enhanced to attenuate oxidative stress, such as that implicated in lung cancer development.

Selenium, in various forms, has been investigated by many as an agent for cancer prevention. In previous work, we sought to identify novel organo-selenocompounds that exhibit efficacy in lung cancer chemoprevention. Selenocysteine demonstrates intriguing properties for chemoprevention, but can be chemically unstable. Selenazolidines, patterned after thiazolidine sulfur compounds (13), were designed to be selenocysteine delivery agents. This group of compounds comprises selenazolidine-4(R)-carboxylic acids with varying 2-substituents. Many of these compounds, including ChSCA, are thought to release selenocysteine through non-enzymatic spontaneous hydrolysis. Selenocystine (SECY), the selenocysteine dimer, and certain selenazolidines (selenocysteine prodrugs) have demonstrated efficacy in reducing the number of lung tumors formed in the 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) mouse model of lung tumorigenesis (14,15) while selenomethionine and Se-methyl-L-selenocysteine, the methylated cysteine congener of selenomethionine, have been found to be ineffective (15-17). These prior studies demonstrated that some selenium compounds can function as chemopreventive agents in the lung, but efficacy appeared very compound dependent. What determines selenocompound chemopreventive efficacy, and the mechanism responsible, has not been fully elucidated.

The promising indications for the ability of certain but not all selenocysteine prodrugs, the selenazolidines, to diminish lung tumorigenicity in a mouse model prompted an investigation of the mechanism behind the most promising of them, ChSCA, and whether the efficacy was related to the degree to which the Nrf2-Keap1/ARE system was activated. To provide a context for the changes elicited by ChSCA, it was compared to selenocystine, the most common nutritional organo-selenium compound, selenomethionine (18-20) and the much studied redox modulator and known Nrf2-Keap1/ARE pathway agonist, sulforaphane (2,21).

MATERIALS AND METHODS

Materials

L-selenocystine and DMSO were purchased from Sigma–Aldrich (St. Louis, MO), L-selenomethionine was from Acros Organics (Morris Plains, NJ), R-sulforaphane [1-isothiocyanate-(4R)-(methylsulfinyl)butane] was from LKT Laboratories (St. Paul, MN), and ChSCA was synthesized as previously described (22). The BEAS-2B cell line, a model for a human non-malignant bronchial epithelial cell was a gift from Dr. Christopher Reilly (University of Utah). LHC-8 media, LHC basal media, First Strand cDNA synthesis kit with SuperScript III, 2,7-dichlorofluorescein diacetate (H₂DCFDA), propidium iodide (PI), NuPAGE Bis-Tris gels, Lipofectamine 2000, and Opti-MEM I reduced-serum media were from Invitrogen (Carlsbad, CA). SYBR Green I Master Mix and Complete protease inhibitor tablets were from Roche (Indianapolis, IN). Bovine serum albumin standard, Coomassie Plus Protein Reagent, SuperSignal chemiluminescence reagent, and tris(2-carboxyethyl)phosphine hydrochloride (TCEP HCl) were from Thermo Scientific (Rockford, IL). Primary monoclonal antibody against TR1 and primary polyclonal antibodies against Nrf2 (C-20), lamin B1 and GAPDH were from Santa Cruz Biotechnology (Santa Cruz, CA). NQO1 polyclonal primary antibody was purchased from Abcam (Cambridge, MA). Secondary horseradish peroxidase (HRP)-conjugated antibodies were from Santa Cruz Biotechnology or CalBioChem (San Diego, CA). GSH-Glo and CellTiter-Fluor kits were purchased from Promega (Woods Hollow, WI). Primers for QPCR and siRNA oligos were synthesized at the University of Utah DNA/Peptide synthesis core facility. All other reagents were purchased from established suppliers.

Cell Culture

BEAS-2B cells were cultured in LHC-8 media supplemented with 2.75 mM epinephrine and 0.33 nM retinoic acid. The normal human bronchial epithelial (NHBE) primary cell line with retinoic acid was purchased from Lonza and cultured in bronchial epithelial growth media (BEGM). Prior to seeding, BEAS-2B and NHBE cell culture surfaces were pretreated with a plate coat consisting of LHC basal media, 10 µg/ml bovine serum albumin, 10 µg/ml fibronectin, and 30 µg/ml collagen. NHBE cells at or below passage number 6 were utilized for experiments. For cell treatments, the highest non-cytotoxic concentrations of each compound (75% viability after 24 hrs (23-26)) was used and these were 100 µM for selenomethionine, 5 µM for selenocystine, 5 µM (NHBE) or 25 µM (BEAS-2B) for ChSCA, and 1 µM (NHBE), or 5 µM (BEAS-2B) for SF. DMSO served as vehicle for all treatments at a concentration 0.1%.

Quantitative Real Time PCR

Total RNA was isolated from cells using the Qiagen RNeasy kit. First stand cDNA synthesis was performed using 1 µg total RNA and 150 ng/µL random primers with SuperScriptIII according to the manufacturer's protocol. QPCR was performed using a Roche LightCycler 480 for 40 cycles at 95°C for 10 s, 65°C for 5 s, and 72°C for 10 s. Each amplification reaction contained a 1:80 dilution of cDNA, 500 nM primer, and SYBR Green I Master Mix. Target gene mRNA expression was normalized to beta-2 microglobulin (B2M) mRNA expression. Melt curve analysis was performed after amplification to ensure

the generation of a single product. The following primer sets were utilized: TR1 forward: 5'-TTGGAATCCACCCTGTCTGT-3'; TR1 reverse: 5'-CATCCACACTGGCTTAAC-3'; NQO1 forward: 5'-ATGTATGACAAAGGACCCTTCC-3', NQO1 reverse: 5'-TCCCTTGCAGAGAGTACATGG-3'; glutamate cysteine ligase catalytic subunit (GCL_C) forward: 5'-ATGCCATGGGATTTGGAAT-3', GCL_C reverse: 5'-AGATATACTGCAGGCTTGAATG-3'; B2M forward: 5'-TTCTGGCCTGGAGGCTATC-3', B2M reverse: 5'-TCAGGAAATTTGACTTTCCATTC-3'.

Reactive Oxygen Species

Reactive oxygen species were assessed following 2 hr treatments by incubating cells with 20 μ M H₂DCFDA for 20 min at 37°C. 2',7'-dichlorofluorescein (DCF) and PI fluorescence concentrations were determined cytometrically as previously described, with a minimum of 10,000 events collected for each sample (24).

Intracellular Glutathione—Glutathione concentrations were determined after 2 hr treatments using the GSH-Glo kit as previously described (27). Reduced glutathione was directly measured. Total glutathione was measured by adding 1 mM TCEP. GSH concentrations were normalized to the live cell fluorescence values obtained using the CellTiter-Fluor reagent.

Immunoblot Analysis

Whole cell lysates were collected in passive lysis buffer as previously described (24). Nuclear lysates were obtained with the Active Motif Nuclear Extract Kit following the manufacturer's protocol. Protein concentrations were determined using the Bradford method. Equal amounts of protein were run out on 10% or 4-12% NuPAGE Bis-Tris gels and transferred to PVDF membranes. Membranes were blocked with 5% milk in T-BST and probed with the following primary antibody dilutions: 1:200 anti-Nrf2, 1:200 anti-lamin B1, 1:300 anti-TR1, 1:800 anti-NQO1, 1:500 anti-GAPDH. When probing for Nrf2, membranes were incubated with primary antibody overnight, fixed in 0.25% glutaraldehyde in cold PBS for 15 minutes at 4°C, and blocked again in 5% milk. Membranes were washed with T-BST, probed with HRP conjugated secondary antibodies, and washed again with T-BST. Protein was detected using chemiluminescence and blots were visualized on a Kodak ImageStation.

Nrf2 Knockdown by siRNA

BEAS-2B cells were seeded at 5.5×10^4 cells/well and allowed to recover overnight. Cells were transfected with 200 nM Nrf2 siRNA (28) or control siRNA that does not code for any known human gene (sense sequence: 5'-AGGCAAUCACGGUGUCCUtt-3') using Lipofectamine 2000 in OptiMEM serum free medium. After 24 hrs, the transfection mixture was removed and LHC-9 media containing the treatment compounds was added. Treatments were performed for 24 hrs, after which whole cell lysates for immunoblotting and total RNA for QPCR were collected as described above.

Statistical Analysis

Data are presented as mean $\pm$ standard deviation. Two-way ANOVA with Holm-Sidak post-hoc testing was used to analyze the mRNA expression data for BEAS-2B Nrf2 knockdown and control cells. All other data were evaluated using one-way or two-way ANOVA with Holm-Sidak post-hoc testing. $p < 0.05$ was considered significant. SigmaStat Version 3.5 was used for analysis.

RESULTS

Acute Effects of Organo-Selenocompounds on Human Lung Cell Redox Balance

Increased cellular oxidative tone is known to be one mechanism by which the Nrf2 enzyme induction pathway can be activated. The extent to which exposure to the various organo-selenocompounds elicit a change in oxidation that could lead to Nrf2 pathway activation was therefore determined. Utilizing BEAS-2B cells, all three organo-selenocompounds, and sulforaphane, produced a rapid (within 2 hrs) increase in reactive oxygen species (Figure 1A) and a decrease in intracellular reduced glutathione (GSH) content (Figure 1B). ChSCA and selenocystine elicited similar changes and these were greater than those of selenomethionine but less than those elicited by sulforaphane. For all compounds, changes in GSH were paralleled by changes in total glutathione (i.e., GSH plus GSSG), indicating that the compounds were depleting total glutathione and not simply oxidizing GSH to GSSG.

Effect of Organo-selenocompounds on Nrf2 Nuclear Localization

For Nrf2 to drive transcription of ARE genes, it must translocate from the cytosol to the nucleus. Western blotting of BEAS-2B cell nuclear lysates was utilized to determine if, after 4 hours, organo-selenocompounds had increased the amount of nuclear, and therefore active, Nrf2 protein. Nuclear Nrf2 was detectable in cells treated with DMSO, indicating that basal Nrf2 activity exists in BEAS-2B cells. ChSCA and selenocystine, but not selenomethionine, increased Nrf2 localization in the nucleus, ChSCA to a much greater extent than selenocystine (Figure 2). The increased nuclear localization by ChSCA was also greater than was seen with sulforaphane.

Organo-selenocompound Induction of Nrf2 Target Genes in Human Lung Cell Lines

All three organo-selenocompounds increased the mRNA expression of two antioxidant genes, TR1 and NQO1, at 24 hrs, with ChSCA increasing mRNA levels to the greatest extent (Figure 3A, 3B) and surpassing that elicited by sulforaphane. mRNA increases were accompanied by increases in TR1 and NQO1 protein expression for all compounds (Figure 3C).

The ability of the organo-selenocompounds to elevate glutathione biosynthesis at 24 hrs was also assessed. mRNA expression of GCLC, the catalytic subunit for the rate limiting step of glutathione synthesis (29) was significantly increased by selenocystine, ChSCA, selenomethionine, and the positive control, sulforaphane (Figure 4A). As with TR1 and NQO1 mRNA increases, the elevation with ChSCA was the highest. The intracellular glutathione content was also assessed as a more global indicator of the functional

consequences of GCL_C mRNA induction. Both total and reduced intracellular glutathione levels were determined to gain insight into the relative redox status of glutathione as a measure of overall oxidative tone at the 24 hr time point following its depression upon acute exposure (Figure 1B) to the compounds. Both selenocystine and ChSCA significantly increased glutathione levels, and to a greater extent than sulforaphane (Figure 4B). Similar increases were found whether determined as total or reduced glutathione; that is, most glutathione is present in the reduced state and the GSH/GSSG ratio was not markedly changed. Selenomethionine was unique in that the increased GCL_C mRNA expression (Figure 4A) did not result in increased glutathione content above baseline levels (Figure 4B). The responses of the antioxidant genes TR1 and NQO1 along with the changes in cellular GSH levels provide strong evidence that the organo-selenocompounds elicit an endogenous antioxidant response that could function in cancer prevention.

Antioxidant gene mRNA expression was also assessed in NHBE cells, another model of the non-malignant lung, to determine broad applicability of organo-selenocompound effects (Table 1). The changes in gene expression occurred earlier in the NHBE cells for all selenocompounds compared to the BEAS-2B cells and were monitored after 12 hrs. While selenocystine did not change mRNA expression of TR1, NQO1, and GCL_C in this cell line, the selenocysteine pro-drug, ChSCA produced a robust response in all three genes (Table 1). Selenomethionine also did not induce any of these genes in NHBE cells. With the positive control, sulforaphane, the only mRNA increase that was statistically significant was the increase in TR1. The greater induction of cytoprotective enzymes by ChSCA in NHBE cells, suggests that its cytoprotective and chemopreventive properties are present in primary cells. The greater degree of difference between ChSCA and other compounds in NHBE cells, may indicate that the experiments in BEAS-2B cells, although clearly differentiating ChSCA, may have considerably underestimated its likely superior cytoprotective properties.

Organo-selenocompound ARE Gene Induction in Cells Low in Nrf2

To determine if Nrf2 plays a role in the mechanism by which the increased mRNA expression of ARE-responsive genes by selenocompounds occurs, we used small interfering RNA (siRNA) to transiently depress Nrf2 expression. Low basal protein expression of Nrf2 in BEAS-2B cells necessitated stimulation with sulforaphane to observe protein attenuation with the use of siRNA. In cells transfected with control siRNA, a small amount of Nrf2 protein was present in DMSO vehicle treated cells. Following sulforaphane treatment, Nrf2 protein expression was increased ~15 fold (Figure 5A). In the cells transfected with Nrf2-specific siRNA, the induction of Nrf2 protein by sulforaphane was attenuated by greater than 95%. Confirmation that a functional knockdown had been achieved came with the observations of TR1 and NQO1 protein expression levels. Both proteins show increased expression with sulforaphane in cells transfected with control siRNA, but the induction of TR1 and NQO1 was not observed in the cells transfected with Nrf2-specific siRNA. Levels of GAPDH, the protein employed as a loading control, did not change with sulforaphane stimulation.

The mRNA levels of TR1, NQO1 and GCL_C were assessed following treatment with the siRNAs as well as the organo-selenocompounds or sulforaphane. Nrf2 knockdown

decreased basal TR1, NQO1, and GCL_C mRNA expression (Figure 5 B, C, and D) to 0.35, 0.20, and 0.45 that of “control” siRNA cells, respectively. The increases in mRNA expression of TR1, NQO1, and GCL_C in cells transfected with control siRNA and treated with the various compounds (Figure 5B, C, and D) followed the same pattern as in non-transfected BEAS-2B cells (Figures 3A, 3B, and 4A) with ChSCA eliciting the greatest response. The compounds did not increase the expression of TR1, NQO1, and GCL_C significantly in cells transfected with Nrf2-specific siRNA, but markedly attenuated the expression from that seen in control siRNA-treated cells, most especially that elicited by ChSCA.

The diminished response to ChSCA in cells where the abundance of Nrf2 has been severely compromised by Nrf2-specific siRNA and the parallelism to sulforaphane is further evidence in support of ChSCA acting through the Nrf2-Keap1/ARE pathway in which mechanism an initial redox insult (Figure 1) acts on Keap1 to release Nrf2, allowing it to translocate to the nucleus (Figure 2) rather than be channeled to ubiquitination and degradation. In the nucleus, it interacts with the response element of a battery of cytoprotective genes to promote their transcription (Figures 3 & 4).

DISCUSSION

The present study focused on organo-selenocompounds and their ability to elicit endogenous antioxidant responses via the Nrf2 protective pathway. Several studies have demonstrated the importance of Nrf2 in protecting the lung from oxidative damage. Nrf2 knockout mice are more susceptible to developing airway diseases such as asthma (30), emphysema (31-33), and pulmonary fibrosis (34). Lungs of Nrf2 knockout mice were more sensitive to cigarette smoke (33), butylated hydroxytoluene (35), diesel exhaust (36), and hypoxia (37), and developed more spontaneous and benzo(a)pyrene-induced genomic DNA mutations (38). Lung epithelial cells isolated from Nrf2 knockout mice had increased basal ROS and decreased basal GSH levels, and demonstrate enhanced sensitivity to oxidants in comparison to cells from wild type mice (39). Strategies that genetically enhance Nrf2 activity have further indicated a protective role of Nrf2-controlled events in the airway. Lung cells with decreased expression of the negative regulator Keap1 exhibit greater antioxidant capacity as demonstrated by increased expression of cytoprotective antioxidant and phase II genes and decreased ROS generated from H₂O₂ treatment (40). Pharmacological treatments to increase Nrf2-controlled activities in the lung have also demonstrated benefits (41,42). Studies in both mice and humans have shown Nrf2 activation and the downstream phase II gene induction occur in the airway in response to cigarette smoke (33,43,44), presumably to negate its oxidative effects. A protective role for Nrf2 in the susceptibility of several cancers has been shown (reviewed in (45)), but has yet to be explicitly tested in a lung cancer model. Still, cytoprotective and phase II enzyme induction are recognized mechanisms of lung cancer prevention (2,45).

Clinical selenium supplementation trials have demonstrated variable benefits for lung cancer. The NPC trial, utilizing selenized yeast, found a decrease in lung cancer incidence and mortality (46) especially among participants with low plasma selenium levels (47). The Selenium and Vitamin E in Cancer Prevention Trial (SELECT) (48) found no benefit of

selenomethionine, the most prevalent selenocompound found in the selenized yeast, with regard to lung cancer incidence (or all cancer mortality).

In the present study *in vitro* experiments with non-malignant lung cell lines, cells that approximate the pre-initiation step of carcinogenesis where chemopreventive agents can be beneficial, were used to investigate the likely mechanism of action and relative efficacy of organo-selenocompounds, especially compared to selenomethionine, in increasing cytoprotection. Of the three selenocompounds investigated, ChSCA invariably generated the most robust response in the direction of greatest cytoprotection. Selenomethionine invariably produced the weakest response and would likely result in the least cytoprotection. The beneficial effects seen in lung cells upon ChSCA exposure were also generally of greater magnitude than those seen with the chemoprotective organosulfur compound derived from cruciferous vegetables, sulforaphane. While not producing the greatest depletion of GSH and elevated presence of reactive oxygen species upon acute exposure and thereby creating a vulnerable environment for cell damage (sulforaphane was the most prominent in this regard) (Figure 1), ChSCA was able to produce the highest migration of Nrf2 to the nucleus (Figure 2), and downstream, elicit the greatest induction of cytoprotective enzymes (Figures 3 & 4) and the highest elevation of the important cellular nucleophile, glutathione (Figure 4).

The present studies indicate that ChSCA, one of a chemical series of selenocysteine prodrugs, has attributes that could make it a compound potentially worth pursuing as a chemopreventive/cytoprotective agent.

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Fig. 1. Effects of organo-selenocompounds on ROS levels and intracellular glutathione in BEAS-2B cells at 2 hrs

(A) DCF fluorescence concentration. Data are presented as mean % DMSO control and are for the PI negative population. Organo-selenocompound treatments include selenomethionine (SEM), selenocystine (SECY), 2-cyclohexylselenazolidine-4-carboxylic acid (ChSCA) and the positive control, sulforaphane (SF). Significant differences are denoted by: '*' indicates $p < 0.05$ and '***' indicates $p < 0.001$ versus DMSO control, $n=3$ for all treatments. (B) Reduced and total (GSH plus GSSG) intracellular glutathione content. Data are normalized to cell viability fluorescence measurements and are presented as mean % DMSO total glutathione. $n=4$ for all treatments. '***' indicates $p < 0.001$ versus the DMSO control.

Fig. 2. Effect of organo-selenocompounds on Nrf2 nuclear localization

Immunoblotting of Nrf2 in BEAS-2B nuclear lysate fractions after 4 hrs of treatment. 20 μ g nuclear lysate was loaded per sample. Selenocompound treatments are as denoted in Figure 1. Lamin B1 served as nuclear protein loading control. Nrf2/lamin B1 band density ratios, normalized to 1 for DMSO were 1.1, 2.6, 4.4, and 3.3 for SEM, SECY, ChSCA and SF, respectively. The immunoblot shown is representative of two independent experiments.

Fig. 3. Organo-selenocompound induction of Nrf2 regulated antioxidant genes in BEAS-2B cells following 24 hrs of treatment

(A) TR1 and (B) NQO1 mRNA expression. Data are presented as mean fold change relative to DMSO control. $n=3$ for all treatments. Organo-selenocompound treatments are as denoted in Figure 1. Significant differences are denoted by: ‘*’, ‘**’, and ‘***’ indicate $p<0.05$, $p<0.01$, and $p<0.001$ versus DMSO control, respectively. (C) TR1 and NQO1 protein expression as determined by Western blotting. GAPDH was probed as protein loading control. TR1/GAPDH band density ratios, normalized to 1 for DMSO, were 2.75, 2.4, 5.4, and 2.8 for SEM, SECY, ChSCA and SF, respectively. NQO1/GAPDH band density ratios, normalized to 1 for DMSO, were 1.2, 1.6, 1.4, and 2 for SEM, SECY, ChSCA and SF, respectively. Immunoblots shown are representative of at least two experimental replicates.

Fig. 4. Glutathione concentrations in BEAS-2B cells after 24 hrs of organo-selenocompound treatment

(A) GCL_C mRNA expression. Data are presented as mean fold change relative to DMSO control. n=3 for all treatments. Selenocompound treatments are as denoted in Figure 1. Significant differences are denoted by: ‘*’, ‘**’, and ‘***’ indicate p<0.05, p<0.01, and p<0.001 versus DMSO control, respectively. (B) Reduced and total (GSH plus GSSG) intracellular glutathione levels. Data are normalized to cell viability fluorescence measurements and are presented as mean % total glutathione DMSO control. n=4 for all treatments. ‘**’ indicates p<0.01 and ‘***’ indicates p<0.001 versus respective DMSO control.

Fig. 5. Attenuation of organo-selenocompound-induced antioxidant gene expression with Nrf2 knockdown

BEAS-2B cells were transfected with Nrf2 or control siRNA for 24 hrs and then treated with compounds for 24 hrs. (A) Knockdown of Nrf2 and Nrf2 target genes as assessed by Western blot. 25 μ g whole cell lysate proteins were loaded per sample. GAPDH was probed as loading control. The blot shown is representative of two independent experiments. (B) TR1, NQO1, and GCLC mRNA expression in control and Nrf2 siRNA cells. Selenocompound treatments are as denoted in Figure 1. Data are presented as fold change relative to DMSO control siRNA. $n=3$ for all treatments and the data was analyzed by two-way ANOVA. Selenocompounds significantly increased the gene expression in the control siRNA treated cells but not in the si-Nrf2 treated cells. ‘***’ indicates $p<0.01$ and ‘***’ indicates $p<0.001$ versus DMSO control siRNA. ‘#’ indicates $p<0.05$, ‘##’ indicates $p<0.01$ and ‘###’ indicates $p<0.001$ versus matched control siRNA treatment.

Table 1

Induction of Nrf2 target genes by selenocompounds in normal human bronchiolar epithelial (NHBE) cells.

Treatment	TR1	NQO1	GCL _C
Vehicle (DMSO)	1 ± 0.23	1 ± 0.07	1 ± 0.17
Selenomethionine	1.28 ± 0.21	1.05 ± 0.14	1.14 ± 0.12
Selenocystine	1.45 ± 0.20	0.87 ± 0.05	0.94 ± 0.13
ChSCA	4.18 ± 0.13 [*]	9 ± 2.5 [*]	11 ± 2.9 [*]
sulforaphane	3.94 ± 0.16 [*]	1.99 ± 0.05	2.62 ± 0.37

Abbreviation: 2-cyclohexylselenazolidine-4-carboxylic acid (ChSCA).

Data are presented as mean fold change relative to DMSO control in NHBE cells following a 12 hr treatment.

^{*} Significant differences are denoted by: ‘*’ indicating p<0.001 versus DMSO control.