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Effect of Dietary Selenium and Cigarette Smoke on Pulmonary Cell Proliferation in Mice

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The objective of this study was to determine if dietary selenium could inhibit pulmonary cell proliferation in control and cigarette smoke-exposed female A/J mice. Selenium in the form of sodium selenite was supplemented to purified diets similar to the AIN-93M diet to yield 0.15, 0.5, or 2.0 mg selenium/kg diet. After 3 weeks, mice in each dietary group were divided into two subgroups; one used as control, whereas the other was exposed to cigarette smoke for five consecutive days. Mice from both groups were euthanized 3 days later. Mice were administered bromodeoxyuridine in the drinking water starting 5 days before the initiation of the smoke exposure and continuing until they were euthanized. After euthanasia, the left lung lobe was processed for histology and cell proliferation analysis. Cigarette smoke increased cell proliferation in the terminal bronchioles and large airways, but not in alveoli. High-selenium diets inhibited cell proliferation in the alveoli, terminal bronchioles and large airways areas in both control and smoke-exposed mice. Increasing the dietary selenium level led to increased selenium levels in the blood and lung, and increased glutathione peroxidase (GPx) activity in the lung. Cytochrome P-450 1A1 protein levels in the lung were increased by cigarette smoke but were not affected by dietary selenium. It is concluded that dietary selenium inhibits pulmonary cell proliferation in both control and cigarette smoke-exposed mice, indicating that selenium is inhibiting cell proliferation independently of smoke exposure, and that this inhibition may be related to selenium concentration and GPx activity in the lung.

Key Words: **selenium; cigarette; smoke; cell proliferation; lung.**

Lung cancer is the leading cause of death from cancer in the United States. The major risk factor for lung cancer development is cigarette smoking. Tobacco smoke is a complex chemical mixture which has been found to contain approximately 4800 different compounds (Hoffmann *et al*., 2001); approximately 100 of them are known carcinogens, co-carcinogens and/or mutagens. Polycyclic aromatic hydrocarbons (PAHs) and N-nitrosamines have received most attention in relation to tobacco carcinogenesis. Many other smoke constituents like free radicals, aromatic amines, catechols, aldehydes, and inorganics like nickel, chromium, and cadmium may be equally important, however, because some of these are present in smoke at substantially high levels. The presence of high levels of pro-oxidants like free radicals in smoke is well documented, and a puff of cigarette smoke is estimated to contain over 10^{15} free radicals, which are detectable in both mainstream and sidestream cigarette smoke (Pryor *et al*., 1983). Steady state reactions in smoke can prolong the half-lives of many radicals that continue to generate superoxide anions and other free radicals (Pryor, 1997), thus increasing the oxidative potential and possibly tumorigenic activity of cigarette smoke.

Although the tumorigenicity of cigarette smoke condensates on mouse skin was demonstrated long ago, it has been generally difficult to induce respiratory cancers in animals by inhalation exposure to cigarette smoke (Coggins, 1998, 2007). However, Witschi and coworkers (Witschi *et al*., 1997a, b, 2000, 2002) have conclusively demonstrated the carcinogenicity of inhaled cigarette smoke in the A/J strain of mice. The A/J mouse strain used in the smoking model is relatively more sensitive to carcinogens and has been extensively employed to examine the carcinogenicity of many structurally diverse chemicals and to develop chemopreventive interventions for lung tumorigenesis (Stoner, 1991; Stoner *et al*., 1993). Treatment of these mice with single carcinogens, some known to be present in tobacco smoke, for example, PAH and N-nitrosamines, has been found to significantly increase the number of lung tumors in this strain. These observations and others have led to the widely held belief that PAH metabolites like diolepoxides and tobacco specific N-nitrosamine metabolites like 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are the primary tobacco carcinogens (Hecht, 1999). However, studies of Witschi and coworkers (Witschi *et al*., 1997a, 1998, 2000) suggest that other smoke constituents may also be involved in tobacco carcinogenesis. Two observations in particular support this contention. First, chemopreventive agents which inhibited PAH- and NNK-induced lung tumors in this mouse model were found to be ineffective against

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smoke-induced lung tumors (Witschi *et al*., 1998, 2000). Second, exposure to gas phase of smoke alone, which is largely devoid of the particulate carcinogens, increased lung tumors in A/J mice to the same extent as whole smoke (Witschi *et al*., 1997a). More recent studies have implicated a role of other tobacco smoke constituents such as 1,3-butadiene in smoke-induced lung tumorigenesis (Witschi, 2005). Free radicals and other pro-oxidants of tobacco smoke, which induce oxidative stress, can also be expected to play a role in lung tumorigenesis. Overall, these studies demonstrate the utility of Witschi model for testing complex mixture like cigarette smoke and for examining the effects of putative chemopreventive agents.

Dietary selenium (Se) has been found to inhibit the development of several types of cancer in epidemiological and experimental studies. The beneficial effect of Se in cancer chemoprevention has been recognized for nearly nine decades (Schrauzer, 2000). The Se supplementation trial by Clark *et al*. (1996), primarily designed to prevent skin cancer recurrence, demonstrated that treatment with Se decreased the risk of cancer of the prostate, lung, and colon and rectum. In addition, the concentration of Se in tobaccos from low lung cancer-incidence countries is significantly higher (0.49 ± 0.22 μg/g) than that in tobaccos from high lung cancer-incidence countries (0.16 ± 0.05 μg/g) (Bogden *et al*., 1981). However, the recently-published SELECT trial failed to detect inhibitory effects of Se on lung, colon, or prostate cancer (Lippman *et al*., 2009). Other clinical trials are currently ongoing (Facompre and El-Bayoumy, 2009). Although the Clark *et al*. study and similar clinical trials as well as epidemiologic studies and animal studies pointed to the potential use of Se for cancer prevention and therapy, the mechanisms by which Se could protect from cancer have not been well defined. Proposed mechanisms that may explain the anti-cancer effect of Se include antioxidant protection from glutathione peroxidases (GPx) and thioredoxin reductase (TrxR), cell proliferation inhibition, increased apoptosis, effects on the cell cycle, transcription factor activation, increased expression of the tumor suppressor gene p53, impaired glutathione (GSH) metabolism, and formation of Se metabolites that are anti-tumorigenic (Combs and Gray, 1998; Ganther, 1999; Ip, 1998). Selenium is an essential nutrient for cell growth, but in higher amounts can inhibit cell proliferation (Zeng and Combs, 2008).

In this study, we tested the hypothesis that dietary Se inhibits cell proliferation in the lungs of A/J mice exposed to cigarette smoke. Witschi *et al*. (1995, 1997b) previously observed that smoke exposure increased cell proliferation in the lungs of mice. Mice were fed diets containing the recommended level of Se as well as two higher levels to determine if supplemental Se would influence cell proliferation in the lung.

MATERIALS AND METHODS

Materials. The University of Kentucky reference research cigarettes 3R4F (Lexington, KY) were used. Materials for purified diets were obtained from Harlan Teklad (Madison, WI). Se was purchased from Sigma Chemical Company (St Louis, MO). The anti-CYP 1A1 antibody was purchased from XenoTech (Lenexa, KS) and the anti-rabbit horseradish peroxidase (HRP) antibodies were purchased from Sigma. All other chemicals, unless noted, were purchased from Sigma.

TABLE 1
Purified Diet Composition

Ingredient	% of diet
Torula yeast	30
Corn starch	36
Dextrose	19.95
Cellulose fiber	5
AIN-93M Mineral Mix	3.5
AIN-93 Vitamin Mix	1
Choline bitartrate	0.25
D,L-Methionine	0.3
Soybean oil	4
Total	100

Smoke exposure system. Inhalation exposures to smoke was carried out in a whole-body Hinners type stainless steel/glass chamber as described earlier (Gairola, 2006). Cigarette smoke was generated from 3R4F University of Kentucky research cigarettes. The concentration of smoke particulates in the exposure chamber atmosphere averaged 46 ± 3 mg TPM/m^3. The mice received smoke exposure for a total of 6 h each day for 5 days, Monday through Friday, and then were euthanized the next Monday morning after the weekend.

Experimental design. Female A/J mice, 7–8 weeks old, were purchased from the Jackson Laboratory (Bar Harbor, ME), and allowed to acclimate for one week before the beginning of the study. A total of 48 mice were split into six different groups containing eight mice per group and fed a purified diet similar to the AIN-93M diet formulation (Table 1), which contained Se at the concentration of 0.15 mg/kg (Reeves *et al*., 1993). Se (as sodium selenite) was added to the diet to obtain Se concentrations of 0.5 and 2.0 mg Se/kg diet, respectively (Table 1). Mice were fed the diets for 3 weeks before beginning smoke exposure, to allow them to adjust to the diets. At this time, one-half of the mice in each dietary group were exposed to cigarette smoke for 5 days (6 h/day). Mice were kept an additional 3 days (64 h) without being further exposed to smoke and then were euthanized, by anesthesia with isoflurane followed by exsanguination. Mice were administered bromodeoxyuridine (BrdU) in the drinking water (0.5 mg/ml) starting 5 days before the initiation of the smoke exposure and continuing until they were euthanized. The lungs were removed during autopsy with part of the lung frozen in liquid nitrogen and then stored at −80°C, whereas the remainder was fixed in buffered neutral formalin and then processed for histology. Blood was also collected during autopsy and serum was prepared and then stored at −80°C.

Analysis of cell proliferation. After fixation and processing, 5-μm sections were prepared from paraffin blocks. The sections of paraffin-embedded tissue samples were deparaffinized, thoroughly washed in water, and then placed in 3% H_2O_2 in methanol for 10 min. The sections were then stained immunohistochemically by the avidin-biotin-peroxidase complex method using Vectastain ABC reagent (Vector Laboratories, Burlingame, CA) with monoclonal antibodies specific for BrdU (Bectin Dickinson Labware, Franklin Lakes, NJ), at 100 μl/slide using a 1:40 dilution of BrdU. The reaction product was then visualized using diaminobenzidine (Vector Laboratories Peroxidase Substrate Kit) and the slides were counterstained with hematoxylin. Having brown nuclei identified cells that had incorporated BrdU. Labeling indexes in lung epithelial cells were determined for three regions: the alveolar zone, the terminal bronchioles, and the intrapulmonary large airways. In the alveolar

TABLE 2
Body Weights

Selenium (mg/kg diet)	Treatment	Mice per group	Body weight (g) Beginning of exposure	Body weight (g) End of study
0.15	Control	8	18.85 ± 1.53[a,b]	19.29 ± 1.67
	Smoke	7	18.79 ± 1.01[a,b]	16.54 ± 1.48*
0.5	Control	8	19.54 ± 1.07[b]	20.00 ± 1.14
	Smoke	8	19.95 ± 1.03[b]	17.14 ± 1.04*
2.0	Control	8	18.90 ± 1.37[a]	19.50 ± 1.33
	Smoke	8	17.98 ± 1.14[a]	16.24 ± 0.82*
Results of two-way ANOVA (p values)				
Main effect for selenium			0.01	0.24
Main effect for smoking			0.59	< 0.001
Selenium × Smoking interaction			0.29	0.84

Note. Results are means ± SEM. Significant differences in smoke-exposed mice for the same selenium level are indicated with an asterisk (*); for differences due to dietary selenium, values with different superscript letters are significantly different ($p < 0.05$).

zone 1000 cells per slide were counted in random fields, and in the terminal bronchioles and large airways 500 cells per slide were counted. Terminal bronchioles were identified by their opening into the alveolar ducts and large airways by their diameter (0.5–1.5 mm).

Exposure markers. Microsomes were prepared from the frozen lung tissue by differential centrifugation (Fadhel *et al*., 2002), and were then used for determining CYP 1A1 protein levels by Western analysis (Subramaniam *et al*., 1999).

Determination of selenium status. GPx activity was measured in lung homogenates and serum using the method of Lawrence and Burk (1976), using hydrogen peroxide as the substrate, which is specific for the Se-dependent forms of GPx. The absorbance was measured at 340 nm in spectrophotometer. Immediately cumene hydroperoxide was added and the absorbance was measured again at 340 nm in spectrophotometer. Lung and serum Se was determined using the method of Spallholz *et al*. (1978). For the GPx and serum Se methods, tissues from every mouse in the group were used, with two tissues combined before analysis, for a total of four samples per group. For the lung Se analysis, four lungs per group were used with two lungs being combined before analysis, for a total of two samples per group.

Statistical analyses. All statistical analyses were conducted using SYSTAT V.8 (SPSS, Inc., Chicago, IL) software. Results were first analyzed by 2 × 3 ANOVA. Two-way ANOVA was used because the study was a two factorial study (Se level being one factor and smoke exposure being the other factor). Differences between means were determined using Bonferroni's *post hoc* test. The results are reported as means ± SEM. A probability level ≤ 0.05 was required for significance.

RESULTS

In this study, we examined if dietary Se could inhibit lung cell proliferation induced by cigarette smoke. Mice were initially fed diets varying in Se for 3 weeks. Mice fed the 0.5 mg/kg Se diet weighed significantly more than mice fed the 2.0 mg/kg diet at the end of this period (Table 2). One-half of the mice were then exposed to cigarette smoke for 5 days; mice were kept an additional 64 h without being further exposed to smoke and then were euthanized. All the mice survived cigarette smoke exposure except one (in the 0.15 mg/kg group). The control mice gained body weight, whereas the smoke-exposed mice lost body weight during the smoke exposure; mice exposed to cigarette smoke weighed less than control mice at the end of the study (Table 2). Final body weights were not affected by dietary Se.

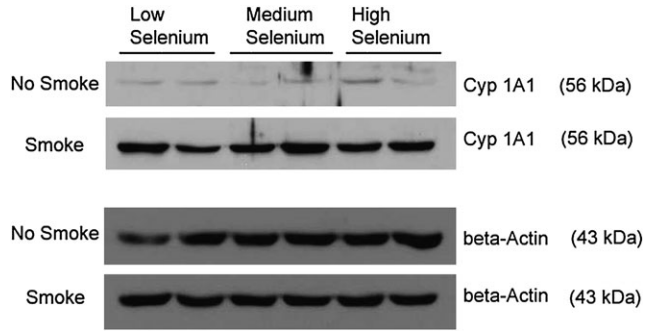

FIG. 1. Western blot of CYP 1A1 in the lung of mice exposed to smoke and fed different levels of selenium.

Lung CYP1A1 protein levels were quantified to verify smoke exposure. After 5 days exposure to cigarette smoke, the CYP1A1 protein levels of lung microsomes were higher than in the control mice in the three groups (Fig. 1). Dietary Se did not affect the CYP1A1 levels.

Se concentrations in the lung and serum as well as GPx activity in the lung were quantified to determine if tissue Se levels responded to dietary Se. The Se levels in lung and serum increased as the intake of dietary Se increased (Table 3). In the lung, each dietary group was significantly different from the other groups; in the serum the medium and high dose groups were significantly different from the low dose group but not from each other ($p < 0.05$). There were no significant differences in Se levels, however, between the control and smoke-treated mice in either the lung or serum. Se-dependent GPx activity in the lung of the medium dose group was higher than that for the low dose group, but there were no significant differences between the high dose group and either of the other two groups. Selenium-dependent GPx activity was significantly higher in mice exposed to cigarette smoke. Total GPx levels were not affected by either dietary Se or smoke exposure (Table 4).

Cell proliferation was measured in the alveolar zone, terminal bronchioles, and large airways after a 12-day exposure to BrdU in the drinking water. In mice exposed to cigarette smoke, cell proliferation was increased in the terminal bronchioles and large airways, but not in the alveolar zone. In all three zones, cell proliferation in the medium and high Se dose groups was significantly lower than the low dose group

TABLE 3
Selenium Concentrations in Lung and Serum

Selenium (mg/kg diet)	Treatment	Se concentration (ppm) Lung	Serum
0.15	Control	0.104 ± 0.008[a]	0.109 ± 0.026[a]
	Smoke	0.098 ± 0.011[a]	0.097 ± 0.023[a]
0.5	Control	0.117 ± 0.005[b]	0.119 ± 0.021[b]
	Smoke	0.134 ± 0.008[b]	0.148 ± 0.031[b]
2.0	Control	0.143 ± 0.021[c]	0.139 ± 0.036[b]
	Smoke	0.169 ± 0.013[c]	0.161 ± 0.034[b]
Results of two way ANOVA (p values)			
Main effect for Selenium		< 0.01	0.01
Main effect for Smoking		0.13	0.29
Selenium × Smoking interaction		0.22	0.35

Note. Results are means ± SEM. Significant differences in smoke-exposed mice for the same selenium level are indicated with an asterisk (*); for differences due to dietary selenium, values with different superscript letters are significantly different ($p < 0.05$).

but was not significantly different from each other, in both control and smoke-exposed mice ($p < 0.05$) (Table 5).

DISCUSSION

In this study, we tested the hypothesis that cell proliferation induced by cigarette smoking may be inhibited by dietary Se. We observed that increased dietary Se inhibited cell proliferation in the lung, and that these effects were consistent with higher levels of Se in the lung. Dietary Se appeared to inhibit cell proliferation independently of smoke exposure, because it significantly inhibited cell proliferation in both control and smoke-exposed mice, with no significant interactions in the ANOVA.

We observed that cigarette smoke induced increases in cell proliferation in the terminal bronchioles and large airways but had no effect in alveoli. The results in the large airways and alveoli are in agreement with the earlier study of Witschi *et al*. (1995), who used a similar protocol (6 h/day, 5 days/week exposure with euthanasia one wk after beginning exposure; 7-day Alzet pumps were used for BrdU administration). However, we observed a significant increase in the labeling index in the terminal bronchioles, whereas Witschi *et al*. did not. Witschi *et al*. did observe an 89% increase, but this was not listed as being statistically significant, and the P value obtained was not listed. However, Witschi *et al*. (1997b) observed increases in cell proliferation in large airways and terminal bronchioles with 1–6 weeks exposure and in alveoli with 1–2 weeks exposure, using the same protocol. Prolonged exposure to smoke in mice did not induce increases in cell proliferation in any of the cell types, however (Witschi *et al*., 1995, 1997b). In rats, after exposure to cigarette smoke for 6 h/day, 5 days/week, bronchiolar cell proliferation was increased after 5 days but not after 28 or 90 days of smoke exposure, using a 3-day Alzet pump; alveolar cell proliferation was not affected at any time (Ayres *et al*., 1995). March *et al*. (1999), however, observed that exposing rats to cigarette smoke for 6 h/day, 5 days/week for 2 weeks, with a 7-day BrdU exposure using Alzet pumps resulted in increased cell proliferation in the terminal bronchioles and axial airways. In another study using rats, Zhong *et al*. (2005) found that a 14-week exposure (6 h/day, 3 days/week) increased cell proliferation in the central and distal airways but not the parenchyma. In hamsters, exposure to cigarette smoke did not result in increased cell proliferation in lung epithelium (Takahashi *et al*., 1992; Witschi and Rajini, 1994). Therefore species differences clearly exist in the proliferative response to cigarette smoke exposure, and variation among studies also exists.

TABLE 4
GPx Activities in the Lung

Dietary Selenium (mg/kg diet)	Treatment	n	Se-dependent GPx activity (nmol NADPH/min/mg protein)	Total GPx activity (nmol NADPH/min/mg protein)
0.15	Control	4	160.8 ± 51.8[a]	321 ± 62
	Smoke	4	227.8 ± 62.7*[a]	305 ± 51
0.5	Control	4	225.1 ± 26.3[b]	330 ± 37
	Smoke	4	262.6 ± 39.6*[b]	346 ± 19
2.0	Control	4	203.6 ± 31.6[ab]	299 ± 46
	Smoke	4	211.7 ± 49.0*[a,b]	302 ± 27
Results of two way ANOVA (p values)				
Main effect for selenium			0.10	0.24
Main effect for Smoking			0.05	0.96
Selenium × Smoking interaction			0.44	0.75

Note. Results are means ± SEM. Significant differences in smoke-exposed mice for the same selenium level are indicated with an asterisk (*); for differences due to dietary selenium, values with different superscript letters are significantly different ($p < 0.05$).

TABLE 5
Labeling Indices in Alveolar Zone and Airways

Selenium (mg/kg diet)	Treatment	*n*	Labeling Index (%)		
			Alveolar zone	Terminal bronchioles	Large airways
0.15	Control	8	8.9 ± 4.0[a]	9.8 ± 3.9[a]	7.7 ± 3.0[a]
	Smoke	7	7.7 ± 4.3[a]	11.3 ± 3.8*[a]	11.6 ± 3.8*[a]
0.5	Control	8	5.3 ± 1.8[b]	5.6 ± 2.1[b]	2.9 ± 1.5[b]
	Smoke	8	4.3 ± 2.3[b]	8.3 ± 2.7*[b]	10.3 ± 4.5*[b]
2.0	Control	8	3.4 ± 1.7[b]	5.6 ± 1.9[b]	2.4 ± 1.6[b]
	Smoke	8	2.5 ± 1.3[b]	7.4 ± 3.5*[b]	7.7 ± 2.7*[b]
Results of two-way ANOVA (*p* values)					
Main effect for selenium			< 0.001	< 0.01	< 0.001
Main effect for Smoking			0.22	0.04	< 0.001
Selenium × Smoking interaction			0.99	0.86	0.28

Note. Results are means ± SDs. Significant differences in smoke-exposed mice for the same selenium level are indicated with an asterisk (*); for differences due to dietary selenium, values with different superscript letters are significantly different ($p < 0.05$).

The role of cell proliferation in lung carcinogenesis remains unclear. The induction of lung tumors clearly involves increased cell proliferation and/or decreased apoptosis. The results of the present study as well as those of Witschi *et al*. (1995; 1997b) show that the rate of cell proliferation in terminal bronchioles and large airways correlates well with the induction of tumors by smoke exposure, although not as well as in alveoli, one of the sites of lung tumorigenesis. With other lung carcinogens, cell proliferation is often correlated with carcinogenicity, but not always (Witschi *et al*., 1987; Witschi, 1986). Several metabolic pathways that lead to enhanced cell proliferation have been found to be activated during lung carcinogenesis, including cyclooxygenase (COX)-2 and its downstream signal transduction pathways, the Wnt-signaling pathway, ras-mediated signaling, and the epidermal growth factor signaling pathway (Adjei, 2001; Capdevila *et al*., 2009; He *et al*., 2005; Huber and Stratakis, 2004; Lee *et al*., 2008).

Se decreased cell proliferation in the alveolar zone, in the terminal bronchioles, and in the large airways, both in smoke-exposed and control mice. The medium dose level produced a significant decrease, with no further decrease in the high dose group. The concentration of Se in the lung was further increased in the high dose group, but this did not result in further reductions in cell proliferation. However, the highest activity of selenium-dependent GPx was observed in the medium dose group; the activity in the high dose group was not significantly different from that in the medium dose group. Therefore, the effects of Se on cell proliferation correlate better with selenium-dependent GPx activity than with Se concentrations. No previous studies have examined the effect of dietary Se on cell proliferation in the lung *in vivo*. Se has been found to inhibit the proliferation of lung cells *in vitro* (Ao *et al*., 1987; Chen *et al*., 2003; Swede *et al*., 2003).

These studies support a chemopreventive role for Se in lung carcinogenesis. 1,4-Phenylenebis(methylene)selenocyanate (p-XSC) but not sodium selenite or Se-enriched yeast was found to inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)–induced lung tumors in A/J mice (Das *et al*., 2003; el-Bayoumy *et al*., 1993). p-XSC but not selenomethionine was subsequently found to inhibit both the initiation and promotion phases of NNK-induced carcinogenesis in A/J mice (Prokopczyk *et al*., 1997). Li *et al*. (2005) found that 2-oxo-selenazolidine-4(*R*)-carboxylic acid (2-oxo-SCA) and selenocystine inhibited NNK-induced lung tumors in mice, whereas sodium selenite, L-selenomethionine, Se-methyl-L-selenocysteine, SCA, and 2-methyl-SCA did not. In addition, dietary Se as sodium selenite did not inhibit NNK-induced lung tumors in A/J mice (Castonguay *et al*., 1991). Therefore, some forms of dietary Se appear to be effective in inhibiting lung carcinogenesis in mice, whereas others do not.

In summary, cell proliferation in the lungs of A/J mice was inhibited by dietary Se. Se inhibited cell proliferation in both control and smoke-exposed mice, indicating that it acted independently of smoke exposure. The effect of dietary Se on the smoke-induced A/J mouse model of lung cancer (Witschi *et al*., 1997b) should be examined in future studies. In addition, further research needs to be carried out to determine the molecular mechanisms by which Se is inhibitory, and thus provide a mechanistic basis for possible dietary recommendations for the prevention of lung cancer.

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REFERENCES

Adjei, A. A. (2001). Blocking oncogenic Ras signaling for cancer therapy. *J. Natl. Cancer Inst.* **93,** 1062–1074.

Ao, P., Yu, S. Y., Zhao, M., and Sun, J. (1987). [Different effects of selenium on proliferation of human lung adenocarcinoma cells and human embryonic lung diploid cells in vitro]. *Zhonghua Zhong Liu Za Zhi* **9,** 408– 411, 22.

Ayres, P. H., McKarns, S. C., Coggins, C. R. E., Doolittle, D. J., Sagartz, J. E., Payne, V. M., and Mosberg, A. T. (1995). Replicative DNA synthesis in tissues of the rat exposed to aged and diluted sidestream smoke. *Inhal. Toxicol.* **7,** 1225–1246.

Bogden, J. D., Kemp, F. W., Buse, M., Thind, I. S., Louria, D. B., Forgacs, J., Llanos, G., and Moncoya Terrones, I. (1981). Composition of tobaccos from countries with high and low incidences of lung cancer. I. Selenium, polonium-210, Alternaria, tar, and nicotine. *J. Natl. Cancer Inst.* **66,** 27–31.

Capdevila, J., Elez, E., Macarulla, T., Ramos, F. J., Ruiz-Echarri, M., and Tabernero, J. (2009). Anti-epidermal growth factor receptor monoclonal antibodies in cancer treatment. *Cancer Treat. Rev.* **35,** 354–363.

Castonguay, A., Pepin, P., and Stoner, G. D. (1991). Lung tumorigenicity of NNK given orally to A/J mice: Its application to chemopreventive efficacy studies. *Exp. Lung Res.* **17,** 485–499.

Chen, W. X., Cao, X. Z., and Zhu, R. Z. (2003). [Effect of selenium dioxide on proliferation, apoptosis, and elomerase activity of human lung cancer cell line in vitro]. *Ai Zheng* **22,** 927–931.

Clark, L. C., Combs, G. F., Jr., Turnbull, B. W., Slate, E. H., Chalker, D. K., Chow, J., Davis, L. S., Glover, R. A., Graham, G. F., Gross, E. G., *et al.* (1996). Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA* **276,** 1957–1963.

Coggins, C. R. (1998). A review of chronic inhalation studies with mainstream cigarette smoke in rats and mice. *Toxicol. Pathol.* **26,** 307– 314; discussion 315

Coggins, C. R. (2007). An updated review of inhalation studies with cigarette smoke in laboratory animals. *Int. J. Toxicol.* **26,** 331–338.

Combs, G. F., and Gray, W. P. (1998). Chemopreventive agents: Selenium. *Pharmacol. Ther.* **79,** 179–192.

Das, A., Desai, D., Pittman, B., Amin, S., and El-Bayoumy, K. (2003). Comparison of the chemopreventive efficacies of 1,4-phenylenebis(methylene)selenocyanate and selenium-enriched yeast on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone induced lung tumorigenesis in A/J mouse. *Nutr. Cancer* **46,** 179–185.

el-Bayoumy, K., Upadhyaya, P., Desai, D. H., Amin, S., and Hecht, S. S. (1993). Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone tumorigenicity in mouse lung by the synthetic organoselenium compound, 1,4-phenylenebis(methylene)selenocyanate. *Carcinogenesis* **14,** 1111–1113.

Facompre, N., and El-Bayoumy, K. (2009). Potential stages for prostate cancer prevention with selenium: Implications for cancer survivors. *Cancer Res.* **69,** 2699–2703.

Fadhel, Z., Lu, Z., Robertson, L. W., and Glauert, H. P. (2002). Effect of 3,3′,4,4′-tetrachlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl on the induction of hepatic lipid peroxidation and cytochrome P-450 associated enzyme activities in rats. *Toxicology* **175,** 15–25.

Gairola, C. G. (2006). Animal models of 2nd hand smoking. In *Molecular Mechanisms for Tobacco-Induced Diseases* (X. L. Wang and D. Scott, Eds.), pp. 121–132. Nova Science Publishers, New York.

Ganther, H. E. (1999). Selenium metabolism, selenoproteins and mechanisms of cancer prevention: Complexities with thioredoxin reductase. *Carcinogenesis* **20,** 1657–1666.

He, B., Barg, R. N., You, L., Xu, Z., Reguart, N., Mikami, I., Batra, S., Rosell, R., and Jablons, D. M. (2005). Wnt signaling in stem cells and non-small-cell lung cancer. *Clin. Lung Cancer* **7,** 54–60.

Hecht, S. S. (1999). Tobacco smoke carcinogens and lung cancer. *J. Natl. Cancer Inst.* **91,** 1194–1210.

Hoffmann, D., Hoffmann, I., and El-Bayoumy, K. (2001). The less harmful cigarette: A controversial issue. A tribute to Ernst L. *Wynder. Chem. Res. Toxicol.* **14,** 767–790.

Huber, R. M., and Stratakis, D. F. (2004). Molecular oncology—Perspectives in lung cancer. *Lung Cancer* **45**(Suppl. 2), S209–S213.

Ip, C. (1998). Lessons from basic research in selenium and cancer prevention. *J. Nutr.* **128,** 1845–1854.

Lawrence, R. A., and Burk, R. F. (1976). Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem. Biophys. Res. Commun.* **71,** 952–958.

Lee, J. M., Yanagawa, J., Peebles, K. A., Sharma, S., Mao, J. T., and Dubinett, S. M. (2008). Inflammation in lung carcinogenesis: New targets for lung cancer chemoprevention and treatment. *Crit. Rev. Oncol. Hematol.* **66,** 208–217.

Li, L., Xie, Y., El-Sayed, W. M., Szakacs, J. G., Franklin, M. R., and Roberts, J. C. (2005). Chemopreventive activity of selenocysteine prodrugs against tobacco-derived nitrosamine (NNK) induced lung tumors in the A/J mouse. *J. Biochem. Mol. Toxicol.* **19,** 396–405.

Lippman, S. M., Klein, E. A., Goodman, P. J., Lucia, M. S., Thompson, I. M., Ford, L. G., Parnes, H. L., Minasian, L. M., Gaziano, J. M., Hartline, J. A., *et al.* (2009). Effect of selenium and vitamin E on risk of prostate cancer and other cancers: The selenium and vitamin E cancer prevention trial (SELECT). *JAMA* **301,** 39–51.

March, T. H., Kolar, L. M., Barr, E. B., Finch, G. L., Menache, M. G., and Nikula, K. J. (1999). Enhanced pulmonary epithelial replication and axial airway mucosubstance changes in F344 rats exposed short-term to mainstream cigarette smoke. *Toxicol. Appl. Pharmacol.* **161,** 171–179.

Prokopczyk, B., Amin, S., Desai, D. H., Kurtzke, C., Upadhyaya, P., and El-Bayoumy, K. (1997). Effects of 1,4-phenylenebis(methylene)selenocyanate and selenomethionine on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced tumorigenesis in A/J mouse lung. *Carcinogenesis* **18,** 1855–1857.

Pryor, W. A. (1997). Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. *Environ. Health Perspect.* **105**(Suppl. 4), 875–882.

Pryor, W. A., Prier, D. G., and Church, D. F. (1983). Electron-spin resonance study of mainstream and sidestream cigarette smoke: Nature of the free radicals in gas-phase smoke and in cigarette tar. *Environ. Health Perspect.* **47,** 345–355.

Reeves, P. G., Nielsen, F. H., and Fahey, G. C. (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A diet. *J. Nutr.* **123,** 1939–1951.

Schrauzer, G. N. (2000). Anticarcinogenic effects of selenium. *Cell. Mol. Life Sci.* **57,** 1864–1873.

Spallholz, J. E., Collins, G. F., and Schwarz, K. (1978). A single test tube method for the fluorometric microdetermination of selenium. *Bioinorg. Chem.* **9,** 453–459.

Stoner, G. D. (1991). Lung tumors in strain A mice as a bioassay for carcinogenicity of environmental chemicals. *Exp. Lung Res.* **17,** 405–423.

Stoner, G. D., Adam-Rodwell, G., and Morse, M. A. (1993). Lung tumors in strain A mice: Application for studies in cancer chemoprevention. *J. Cell. Biochem. Suppl.* **17F,** 95–103.

Subramaniam, S., Srinivasan, S., Bummer, P. M., and Gairola, C. G. (1999). Perinatal sidestream cigarette smoke exposure and the developing pulmonary surfactant system in rats. *Hum. Exp. Toxicol.* **18,** 206–211.

Swede, H., Dong, Y., Reid, M., Marshall, J., and Ip, C. (2003). Cell cycle arrest biomarkers in human lung cancer cells after treatment with selenium in culture. *Cancer Epidemiol. Biomarkers Prev.* **12,** 1248–1252.

Takahashi, M., Imaida, K., Mitsumori, K., Okamiya, H., Shinoda, K., Yoshimura, H., Furukawa, F., and Hayashi, Y. (1992). Promoting effects of cigarette smoke on the respiratory tract carcinogenesis of Syrian golden hamsters treated with diethylnitrosamine. *Carcinogenesis* **13,** 569–572.

Witschi, H. P. (1986). Separation of early diffuse alveolar cell proliferation from enhanced tumor development in mouse lung. *Cancer Res.* **46,** 2675–2679.

Witschi, H. (2005). Carcinogenic activity of cigarette smoke gas phase and its modulation by beta-carotene and N-acetylcysteine. *Toxicol. Sci.* **84,** 81–87.

Witschi, H., Espiritu, I., Dance, S. T., and Miller, M. S. (2002). A mouse lung tumor model of tobacco smoke carcinogenesis. *Toxicol. Sci.* **68,** 322–330.

Witschi, H., Espiritu, I., Maronpot, R. R., Pinkerton, K. E., and Jones, A. D. (1997a). The carcinogenic potential of the gas phase of environmental tobacco smoke. *Carcinogenesis* **18,** 2035–2042.

Witschi, H., Espiritu, I., Peake, J. L., Wu, K., Maronpot, R. R., and Pinkerton, K. E. (1997b). The carcinogenicity of environmental tobacco smoke. *Carcinogenesis* **18,** 575–586.

Witschi, H., Espiritu, I., Yu, M., and Willits, N. H. (1998). The effects of phenethyl isothiocyanate, N-acetylcysteine and green tea on tobacco smoke-induced lung tumors in strain A/J mice. *Carcinogenesis* **19,** 1789–1794.

Witschi, H., Godfrey, G., Frome, E., and Lindenschmidt, R. C. (1987). Pulmonary toxicity of cytostatic drugs: Cell kinetics. *Fundam. Appl. Toxicol.* **8,** 253–262.

Witschi, H., Oreffo, V. I., and Pinkerton, K. E. (1995). Six-month exposure of strain A/J mice to cigarette sidestream smoke: Cell kinetics and lung tumor data. *Fundam. Appl. Toxicol.* **26,** 32–40.

Witschi, H. P., and Rajini, P. (1994). Cell kinetics in the respiratory tract of hamsters exposed to cigarette sidestream smoke. *Inhal. Toxicol.* **6,** 321–333.

Witschi, H., Uyeminami, D., Moran, D., and Espiritu, I. (2000). Chemoprevention of tobacco-smoke lung carcinogenesis in mice after cessation of smoke exposure. *Carcinogenesis* **21,** 977–982.

Zeng, H., and Combs, G. F., Jr. (2008). Selenium as an anticancer nutrient: Roles in cell proliferation and tumor cell invasion. *J. Nutr. Biochem.* **19,** 1–7.

Zhong, C. Y., Zhou, Y. M., Douglas, G. C., Witschi, H., and Pinkerton, K. E. (2005). MAPK/AP-1 signal pathway in tobacco smoke-induced cell proliferation and squamous metaplasia in the lungs of rats. *Carcinogenesis* **26,** 2187–2195.