Inhibition of Activator Protein-1 by Sulforaphane Involves Interaction with Cysteine in the cFos DNA-Binding Domain: Implications for Chemoprevention of UVB-Induced Skin Cancer

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Abstract

Sulforaphane is an isothiocyanate derived from cruciferous vegetables that has been linked to decreased risk of certain cancers. Although the role of sulforaphane in the induction of the transcription factor Nr2 has been studied extensively, there is also evidence that inhibition of the transcription factor activator-protein-1 (AP-1) may contribute to the chemopreventive properties of this compound. In this study, we show for the first time that sulforaphane is effective at reducing the multiplicity and tumor burden of UVB-induced squamous cell carcinoma in a mouse model using cotreatment with the compound and the carcinogen. We also show that sulforaphane pretreatment is able to reduce the activity of AP-1 luciferase in the skin of transgenic mice after UVB. Chromatin immunoprecipitation analysis verified that a main constituent of the AP-1 dimer, cFos, is inhibited from binding to the AP-1 DNA binding site by sulforaphane. Electrophoretic mobility shift assay analysis of nuclear proteins also shows that sulforaphane and diamide, both known to react with cysteine amino acids, are effective at inhibiting AP-1 from binding to its response element. Using truncated recombinant cFos and cJun, we show that mutation of critical cysteines in the DNA-binding domain of these proteins (Cys$^{154}$ in cFos and Cys$^{272}$ in cJun) results in loss of sensitivity to both sulforaphane and diamide in electrophoretic mobility shift assay analysis. Together, these data indicate that inhibition of AP-1 activity may be an important molecular mechanism in chemoprevention of squamous cell carcinoma by sulforaphane.

Introduction

UV radiation is a known contributor to skin aging and carcinogenesis. Of the two types of UV light that penetrate our atmosphere, UVB (280-320 nm) and UVA (320-400 nm), UVB is much less abundant but exponentially more potent at inducing DNA damage and cell signaling events. The transcription factor activator protein-1 (AP-1) is known to be a key mediator of UV-induced nonmelanoma skin cancer, particularly squamous cell carcinoma. AP-1 is composed of homodimers of Jun family members (cJun, JunD, and JunB) or heterodimers of Jun and Fos family members (cFos, FosB, Fra1, and Fra2). In cultured keratinocytes, UVB treatment leads to dramatic up-regulation of cFos protein levels and an increase in cFos/JunD binding to the 12-O-tetradecanoylphorbol-13-acetate response element (TRE), a binding site in the promoters of AP-1 target genes (1). Binding of AP-1 to the TRE can be prompted by a variety of stimuli and its activation regulates responses such as proliferation, apoptosis, and differentiation. Inhibition of AP-1 activity through genetic or pharmacologic means has been shown to greatly reduce UVB-induced skin carcinogenesis in mice (2-4). AP-1 is therefore an intriguing target for chemoprevention of nonmelanoma skin cancer using topical agents.

Recent evidence indicates that UVB irradiation causes increased levels of reactive oxygen species in the cell, which contributes to damage and signaling events. These reactive oxygen species have been linked to UVB-induced AP-1 activation (5, 6). Abate and colleagues showed that DNA binding of the AP-1 dimer depends on a conserved lysine-cysteine-arginine motif present in the DNA-binding domain of each subunit of the transcription factor (7). The cysteine amino acid of this motif was shown to be the site of redox-mediated control of AP-1 TRE binding and therefore a major transcriptional control. Replacement of this cysteine with serine resulted in increased TRE binding and loss of sensitivity to thiol-reactive agents (7). Thus, the cysteine of the lysine-cysteine-arginine motif must be maintained in a reduced state in order for AP-1 to bind DNA. Because many stimulants such as UVB actually increase oxidant levels in the cell, the reduction of the AP-1 DNA-binding cysteine is maintained by the redox-sensitive Ref-1 protein (8).

We are interested in characterizing the chemopreventive mechanisms of natural products that are potentially useful for preventing the development of squamous cell carcinoma. Sulforaphane is an isothiocyanate compound found in cruciferous vegetables, especially broccoli and broccoli sprouts. Sulforaphane or sulforaphane-containing extracts are effective at inhibiting lung adenocarcinomas (9), colon polyps (10), and skin cancer (11-13) in mouse models, but the molecular chemopreventive mechanism(s) employed by sulforaphane is poorly understood. One major mechanism through which sulforaphane mediates chemopreventive effects is through its ability to react with thiol groups such as those found on cysteine in target proteins. Interaction of sulforaphane with cysteines in the Keap1 protein is thought to be responsible for activation of the Nrf2 transcription factor and therefore up-regulation of antioxidant and detoxification genes in the cell (14, 15). Cells treated with sulforaphane also up-regulate rate-limiting enzymes in the biosynthesis of the thiol-based antioxidant glutathione and develop enhanced abilities to scavenge reactive oxygen species or react to xenobiotic stress (16, 17).

Recent work on the effects of sulforaphane on AP-1 activity suggests that UVB-stimulated AP-1 might be inhibited by...
sulforaphane due to changes in the redox potential of the cell (18). However, although sulforaphane significantly reduces AP-1 luciferase activity induced by UVB treatment, this occurs independently of glutathione levels in the cell (18). Therefore, we hypothesize that inhibition of AP-1 by sulforaphane is dependent on a chemical modification of the transcription factor by the compound and not simply due to a change in cellular redox status. Although sulforaphane is known to modify cysteines such as those known to be crucial for AP-1/DNA binding (7, 14), the exact mechanism of its inhibition of AP-1 remains uncertain.

**Materials and Methods**

**Reagents.** RS-sulforaphane [1-isothiocyanato-(4R,S)-(methylsulfinyl)butane] was purchased from LKT Laboratories and dialized in acetone or acetone from Sigma-Aldrich. Diamide (diacenedicarboxylic acid bis[N,N-dimethylamyl]) was purchased from Sigma.

**UVB skin carcinogenesis.** SKH-1 hairless female mice were purchased from Charles River Laboratories and housed in accordance with The University of Arizona Animal Care and Use Committee standards. Mice were split into four groups of 20 each: UVB alone, acetone + UVB, 1 µmol sulforaphane + UVB, and 2.5 µmol sulforaphane + UVB. Mice were exposed to UVB using six FS40T12 UVB lamps (National Biological Corporation) three times weekly for 25 weeks. Fluence was determined using a UVX radiometer (Ultraviolet Products). The UVB dose was initiated at 0.54 kJ/m² and increased 25% each week until the maximal dose of 1.65 kJ/m² was reached at week 5 and maintained for the remainder of the experiment. Mice were pretreated with drug or vehicle for 1 week before initiation of UVB exposure and then 1 h before each irradiation. Tumors were measured weekly, and the experiment was terminated at week 25. Tumor burden was calculated by multiplying diameter by height in millimeters (19). Average tumor burden was calculated by dividing the sum of individual tumor burdens each week by the number of mice in the treatment group.

**Mouse epidermal luciferase assay.** SKH-1 mice expressing TRE-driven luciferase (AP-1 luciferase mice; refs. 2, 20) were separated into two groups of 10 sex and age-matched mice and pretreated with 0.3 µmol sulforaphane/ear or vehicle. This dosage approximates the exposure of 1 µmol per back used in the above carcinogenesis experiment, given that the surface area of a mouse ear (front and back) is roughly one third the size of the mouse back treatment area. Both ears of each mouse were pretreated four times (Monday, Wednesday, Friday, and Monday) before an acute UVB treatment of 2.75 kJ/m² 48 h after (Wednesday). Mice were sacrificed 48 h post-UVB (Monday, Wednesday, Friday, and Monday) before an acute UVB treatment used in the above carcinogenesis experiment, given that the surface area of sequences and PCR conditions are available upon request.

**Cysteine to serine on tFos and cysteine 58 to serine on tJun.** Primer QuikChange site-directed mutagenesis kit was used to specifically mutate and cloning back into pET19b at the NdeI sites. A Stratagene Lambda phage containing protein were pooled, concentrated, evaluated using SDS-PAGE, under native conditions as described (QIAexpressionist handbook, June 2005-623, cFos and IgG: Santa Cruz Biotechnology, sc-53 and sc-2027, respectively) or had no more additions (No Antibody control) and was rotated at 4°C overnight. Next, 100 µL of fresh beads were added to each tube and rotated at 4°C for 2 h. Pelleted beads were transferred to Handee spin cup columns (Pierce Biotechnology) and washed as described in the kit. Chromatin was eluted from the beads by incubating two times with 250 µL elution buffer. Eluted chromatin was uncrosslinked, subjected to RNase A and proteinase K digestions, and purified using the Qiaquick PCR Purification kit. Cleaned immunoprecipitated DNA was eluted from the column using 50 µL nuclease-free H₂O.

**Quantitative real-time PCR.** Chromatin immunoprecipitation products were tested for the presence of the TRE site found in the matrix metalloproteinase-1 promoter using primers designed to detect the specified region from Applied Biosystems. These custom probes were based on the promoter sequence first described by Angel and colleagues (25) and were confirmed against the human genome sequence. Chromatin immunoprecipitation DNA (4 µL) in triplicate was used for quantitative PCR using Applied Biosystems TaqMan Universal PCR Master Mix in an ABI Prism 7700 Sequence Detector (Applied Biosystems). Fold enrichment of the immunoprecipitated fragment was determined using the comparative Ct method using the following equation: 2^ΔΔCt (Input - IP) - Ct (Input). Fold enrichments for each experiment were normalized to the respective control sample. The glyceraldehyde-3-phosphate dehydrogenase promoter was also amplified from input samples and from the RNA polymerase II immunoprecipitation samples for each experiment using normal PCR primers and conditions supplied by the Upstate kit as a control (RNA polymerase data not shown).

**Electrophoretic mobility shift assay.** Two micrograms of recombinant heterodimers or 5 µg of nuclear proteins were subjected to electrophoretic mobility shift assay (EMSA) using established protocols (2, 18). Nuclear proteins were extracted from treated HaCaT human keratinocytes 12 h after a dose of 250 J/m² UVB. Nuclear or recombinant proteins were mixed with either sulforaphane, diamide, or vehicle in a final volume of 10 µL and incubated at 37°C for 1 h. Proteins were then mixed with 5× binding buffer [50 mmol/L HEPEs (pH 7.9), 250 mmol/L KCl, 65 mmol/L EDTA, 12.5 mmol/L DTT, 50% glycerol, 2.5% Triton X-100, 1 µg poly(deoxyinosinic-deoxyctydilic acid), and water to a final volume of 19 µL. This was incubated on ice for 20 min, at which time 1 µL 32P-labeled TRE probe (18) was added and the tubes were incubated at room temperature for 30 min. Products were loaded onto a 6% acrylamide, 0.25× TBE, and 2.4% glycerol nondenaturing gel. Finished gels were dried and exposed to film. All EMSAs displayed only one retention band in addition to the probe front.
Statistical analysis. Primary analyses compared average tumor burden (diameter × height in millimeters) and tumor count (multiplicity) at week 25 in the three treatment groups (acetone, 1 μmol sulforaphane, and 2.5 μmol sulforaphane). These cross-sectional analyses among the three treatment groups used the Kruskal-Wallis test. A nonparametric test for linear trend across treatment groups used Stata’s nptrend command. For these primary analyses, statistical significance was assessed at $P = 0.05$; 2×2 analyses used the Wilcoxon rank-sum test. These post hoc multiple comparisons used a Bonferroni corrected $P$ value of 0.025. For the luciferase assay, a two-tailed Student’s $t$ test was used to calculate significance between the fold induction of the acetone versus sulforaphane-treated mice. The same analysis was used for comparison of the normalized quantitative real-time PCR data. In both cases, significance was defined as $P < 0.05$.

Results

Sulforaphane inhibits UVB-induced skin carcinogenesis in SKH-1 mice. Treatment of mouse back skin with sulforaphane at either 1 or 2.5 μmol/mouse produced marked reduction in tumor multiplicity and tumor burden, although there were no differences noted in tumor type between the groups. An overall test for differences in tumor multiplicity among the four experimental groups at week 25 using the Kruskal-Wallis test was borderline significant. ($P = 0.06$). However, comparison of the acetone group to the 2.5 μmol sulforaphane group indicated a statistically significant difference at week 25 (58% fewer tumors with 2.5 μmol sulforaphane; $P = 0.03$). A nonparametric test for linear relationship between tumor count and increasing sulforaphane dose for weeks 15 to 25 was also statistically significant ($P = 0.007$; Fig. 1A). Tumor burden was not different among the experimental groups at week 25. However, there was a statistically significant trend toward lower tumor burden in the groups treated with sulforaphane ($P < 0.0001$; Fig. 1B). Thus, sulforaphane treatment is effective at inhibiting tumorigenesis in this model, especially when using the higher dose of sulforaphane.

Sulforaphane inhibits UVB-induced AP-1 luciferase in vivo. Full-thickness skin punch biopsies from the ears of AP-1 luciferase

![Figure 1. Sulforaphane inhibits UVB-induced squamous cell carcinoma in mice. SKH-1 mice were pretreated with acetone, 1 μmol sulforaphane (SFN), 2.5 μmol sulforaphane, or nothing 1 h before exposure to UVB three times a week for 25 wk. Tumors were counted and measured weekly. The average tumors per mouse (multiplicity) in the 2.5 μmol group was significantly different than the acetone control at week 25 ($P = 0.03$), and trend analysis from weeks 15 to 25 showed strong significant inhibition of multiplicity with sulforaphane treatment ($A; P = 0.007$). Sulforaphane treatment also resulted in a significantly lower trend in the average tumor burden for this group ($B; P = 0.0001$).]
mice confirm significant activation of AP-1 luciferase by UVB ($P = 0.0002$; data not shown). However, the 24-fold luciferase induction by UVB was significantly reduced to 13-fold with sulforaphane pretreatment ($P = 0.01$; Fig. 2). Control punches taken 24 h before UVB exposure show luciferase expression levels in both vehicle and sulforaphane-treated ears to be very low overall (data not shown), in accordance with previous studies (21). Thus, sulforaphane is effective at reducing AP-1 activation after UVB in mouse skin. AP-1 luciferase mouse ears were pretreated with acetone or sulforaphane before acute treatment with UVB. Luciferase assays were done on pre-UV–treated and post-UV–treated ear punches, and fold activation by UVB was calculated for each mouse. *, $P < 0.01$, statistically significant difference between acetone- and sulforaphane-treated mice after UVB.

Sulforaphane inhibits nuclear binding of cFos to the TRE. Sulforaphane is known to influence many factors in the cell that could affect UVB-induced AP-1 luciferase activation. To confirm that the inhibition of AP-1 noted in our previous results was due to inhibition of AP-1 binding to DNA in the cell, we performed chromatin immunoprecipitation assays using keratinocytes in culture. Quantitative PCR to amplify the TRE in the collagenase-1 (matrix metalloproteinase-1) promoter, the same sequence as the promoter in the AP-1 luciferase construct, was done. Our data show ~2.5-fold activation of cFos DNA binding in cells treated with UVB ($P < 0.01$; Fig. 3A), in agreement with previous observations using other assays (1). In addition, our data clearly indicate that sulforaphane inhibits cFos from binding to the matrix metalloproteinase-1 TRE after UVB exposure. The level of cFos binding is significantly reduced by sulforaphane treatment when compared with the UVB-alone samples ($P < 0.01$). These results are supported by low levels of binding in all of the negative controls (no antibody or IgG controls), which are not affected by sulforaphane or UVB treatment. PCR of the glyceraldehyde-3-phosphate dehydrogenase promoter from each Input DNA sample confirms that the initial starting chromatin concentrations were equivalent (Fig. 3B).

Both sulforaphane and diamide, cysteine-oxidizing agents, effectively block binding of nuclear AP-1 to the TRE in vitro. Nuclear extracts from cells either mock-irradiated or exposed to 250 J/m$^2$ UVB were incubated with sulforaphane or diamide before the addition of TRE probe and EMSA analysis. The dose-dependent inhibition of AP-1 binding due to sulforaphane exposure in vitro (Fig. 4A) is typical of our previous results (18). At a dose of 1 mmol/L sulforaphane, the binding of nuclear AP-1 to the TRE was reduced to baseline levels. Diamide, another cysteine-oxidizing agent, was also very effective at inhibiting the binding of AP-1 under the same conditions (Fig. 4B).

Isolation and characterization of recombinant truncated cFos and cJun. PCR products encoding the β-zip region of cFos and cJun were cloned into bacterial expression vectors encoding a NH$_2$-terminal His tag to create tFos and tJun. These truncated proteins contain only two cysteine residues each: one in the DNA-binding domain (Cys$^{154}$ for cFos and Cys$^{272}$ for cJun) and one proximal to the COOH terminus in the leucine zipper domain (Fig. 5A). Recombinant proteins were purified and visualized on a SDS-PAGE gel (Fig. 5B). After dimerization of tFos and tJun

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**Figure 2.** Inhibition of UVB-induced AP-1 luciferase by sulforaphane in mouse skin. AP-1 luciferase mouse ears were pretreated with acetone or sulforaphane before acute treatment with UVB. Luciferase assays were done on pre-UV–treated and post-UV–treated ear punches, and fold activation by UVB was calculated for each mouse. *, $P < 0.01$, statistically significant difference between acetone- and sulforaphane-treated mice after UVB.

**Figure 3.** Sulforaphane inhibits cFos binding to the TRE in the nucleus. HaCaT keratinocytes were pretreated and posttreated with acetone or 10 μM sulforaphane before exposure to UVB and harvest for chromatin immunoprecipitation assay. Samples were immunoprecipitated with an antibody against cFos, normal IgG, or no antibody. Real-time quantitative PCR was done using a probe specific for the TRE from the collagenase-1 promoter. Data are pooled from three independent experiments. *, $P < 0.001$, statistically significant difference between control and UVB alone–treated samples; **, $P = 0.0013$, UVB alone versus sulforaphane + UVB (A). Input samples were used for amplification of the glyceraldehyde-3-phosphate dehydrogenase promoter to show that each sample started with equivalent amounts of chromatin (B).
subunits to form tAP-1. EMSA analysis showed that these proteins bind tightly to the TRE. Preincubation of tAP-1 with cold wild-type TRE oligos reduced binding to 32P-labeled TRE in a dose-dependent manner, whereas cold mutant oligos did not (Fig. 6A). The tAP-1 dimer therefore specifically binds to the TRE. This binding was inhibited by sulforaphane pretreatment in a dose-dependent fashion, similar to that noted with nuclear extracts (Fig. 6B). Thus, the recombinant tAP-1 protein reacts to the TRE and to sulforaphane in a manner similar to that of its nuclear counterpart.

DNA-binding cysteine is required for sulforaphane- or diamide-induced inhibition in vitro. To test for direct interaction of sulforaphane with the DNA-binding cysteines in AP-1, we mutated the DNA-binding cysteines in tFos and tJun to serines, leaving the remaining cysteines near the COOH terminus intact. Both wild-type and mutant heterodimers were then treated with sulforaphane or diamide and analyzed for their ability to bind to the TRE via EMSA. As shown in Fig. 6C, mutation of the DNA-binding cysteine resulted in loss of sensitivity to treatment with either of these oxidizing agents. In fact, although binding to the TRE is completely blocked by pretreatment of the wild-type dimer with 7 mmol/L diamide, the mutant dimer is totally immune to this inhibition. The mutant form of tAP-1 is also completely resistant to inhibition by 1 mmol/L sulforaphane.

Discussion

To our knowledge, this is the first study to report an inhibitory effect of sulforaphane on UVB-induced skin carcinogenesis in mice exposed to both sulforaphane and UVB simultaneously. Other reports have described the effect of sulforaphane using chemically induced mouse skin carcinogenesis (12, 13) or a UVB model using a chemotherapeutic treatment protocol with broccoli extracts (UVB was stopped before the extract was applied; ref. 11). All of these studies described a protective effect of sulforaphane treatment. Therefore, the current data corroborate previous chemopreventive reports and do so using purified sulforaphane and a model of concurrent UVB/agent exposure, which may be more relevant to human outcomes. Sulforaphane does not absorb light in the UV spectrum or produce a sunscreen effect (26). This provides us with a positive framework for using sulforaphane as a topical chemopreventive agent in conjunction with studies to identify molecular mechanisms of sulforaphane chemopreventive effects in the skin.

Many of the molecular studies of sulforaphane have focused on its effects on the Nrf2 transcription factor pathway (12, 27, 28). Nrf2...
and its effector proteins help to protect cells from oxidative insults. Although Nrf2 is implicated in protecting the skin against carcinogenic chemicals, its role in UV-induced carcinogenesis is unclear. In cell culture, different doses or wavelengths of UV can induce or reduce Nrf2 levels depending on the cell type (29–31). Some have suggested that transient Nrf2 activation in the skin by electrophilic compounds (such as sulforaphane) may be protective against tumorigenesis, but constitutive activation of Nrf2 may lead to malignant conversion (32). Protection from UV-induced carcinogenesis by sulforaphane might also involve modulation of the inflammatory response by Nrf2 (33–37). However, a recent report discovered that although Nrf2 knockout mice had increased oxidative DNA damage, inflammation, and sunburn cell formation compared to wild-type mice after acute UVB exposure, chronic UVB treatment revealed no difference in the incidence rate or mean number of tumors between wild-type and Nrf2 knockout mice (38). Therefore, the ability of sulforaphane to inhibit UVB-induced nonmelanoma skin cancer may be due to factors other than Nrf2 stimulation.

The experiments reported here show for the first time the ability of sulforaphane to inhibit AP-1 activity in vivo. Earlier luciferase assays and EMSAs indicated that sulforaphane could regulate the DNA binding of AP-1 in vitro (18). In our transgenic mouse model, we have successfully shown that sulforaphane inhibits UVB-induced AP-1 luciferase activity in the skin. We have also confirmed through chromatin immunoprecipitation analysis that UVB causes increased binding of cFos to the TRE and that this binding is inhibited by sulforaphane. Although others have reported that sulforaphane slightly increases AP-1 luciferase activity at low doses, our results do not indicate increased basal AP-1-luciferase activity with sulforaphane pretreatment (data not shown; ref. 39). These differences may be due to cell type–specific reactions. The data in Figs. 2 and 3 support our hypothesis that inhibition of AP-1 may be a contributing factor to the ability of topical sulforaphane to block UVB-induced squamous cell carcinoma in mice.

Sulforaphane is known to bind to reactive thiol groups, especially cysteines, and may affect protein function through this mechanism (14). Diamide, another thiol oxidative agent, has been used previously to inhibit recombinant AP-1 binding in vitro (7). Treatment of nuclear extracts with either diamide or sulforaphane showed dose-dependent inhibition of AP-1 DNA binding. Because both compounds interact with cysteines, the inhibition of TRE binding is likely a result of cysteine oxidation of the AP-1 transcription factor. We have noted this reaction to sulforaphane previously at the same dose levels (18), which are likely to be physiologically relevant (40). Although the doses needed for inhibition of AP-1 binding in vitro are higher than those used in luciferase assays, sulforaphane is known to accumulate in the cell when added to culture medium. Treatment of mouse hepatoma cells with micromolar concentrations of sulforaphane yielded millimolar concentrations in cellular lysates (40). The data in Fig. 4 suggest a cysteine-specific chemical reaction, because nuclear extracts were exposed to both of these oxidants in a test tube where transcriptional or translational input is minimal.

We next addressed the importance of the specific DNA-binding cysteines of AP-1 (Cys154 in Fos and Cys272 in Jun) in the reaction with sulforaphane and diamide. Mutations of the DNA-binding cysteine in AP-1 results in a loss of activity of both sulforaphane and diamide on the TRE binding response of these proteins (C).

Figure 6. Truncated recombinant AP-1 binds specifically to the TRE and is inhibited by sulforaphane in a manner dependent on the presence of the DNA-binding cysteine. Recombinant Fos and Jun were heterodimerized to form functional IAP-1. The binding specificity of IAP-1 was confirmed through EMSA analysis in which wild-type cold competitor oligos successfully inhibited IAP-1 from binding to the labeled TRE in a dose-dependent fashion, but mutant (mut.) competitors did not (A). IAP-1 was also incubated with sulforaphane for 1 h at 37°C before being mixed with buffer and labeled probe. The ability of sulforaphane (SFN) to inhibit IAP-1 TRE binding in a dose-dependent fashion was therefore confirmed (B). Wild-type IAP-1 was then compared with mutant IAP-1 using EMSAs in which both dimers were pretreated with 1 mmol/L sulforaphane or 7 mmol/L diame as above. Mutation of the DNA-binding cysteine in IAP-1 results in a loss of activity of both sulforaphane and diame on the TRE binding response of these proteins (C).
after those used by Abate and colleagues (7). The truncated AP-1 proteins were able to dimerize and bind specifically to the TRE in a manner consistent with that observed using nuclear extracts. The fact that sulforaphane could dose-dependently inhibit truncated AP-1 from binding to the TRE suggests functional similarity between the recombinant form and its endogenous counterpart. However, the complete lack of response to cysteine oxidation by either sulforaphane or diamide when the DNA-binding cysteines are mutated supports our hypothesis: sulforaphane chemically oxidizes Cys134 in Fos and Cys272 in Jun to inhibit binding of AP-1 to the TRE. The cysteine-to-serine mutation creates a “permanently reduced” DNA-binding domain, which is unaffected by sulforaphane and leads to enhanced TRE binding (Fig. 6C).

Sulforaphane and other isothiocyanates are gaining credibility as potential “natural” chemopreventive agents. These natural agents are present in our diet and are easily tolerated by our metabolism. Orally administered broccoli sprout extracts containing sulforaphane have been safely tolerated by volunteers (44). Other groups are testing the efficacy of sulforaphane or related compounds for possible use in humans to prevent hepatocarcinoma, breast cancer, and nonmelanoma skin cancer (26–28, 45), although to date there are no published studies regarding oral administration of sulforaphane and the prevention of skin carcinomas. Due to the current focus on sulforaphane-induced Nrf2 activation in chemoprevention, many of these studies justifiably turn to markers of Nrf2 activity to measure the potential efficacy of this compound. Many other molecular targets of sulforaphane have been identified in the cell, including transcription factors such as nuclear factor-κB, which may be subject to a similar form of thiol-mediated redox regulation as AP-1 (46). The results described here suggest that inhibition of AP-1 is also important to consider when studying the properties of sulforaphane in human skin cancer chemoprevention trials.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References

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33. Mochizuki M, Ishii Y, Itoh K, et al. Role of 15-deoxy-
Δ(12,14) prostaglandin J₂ and Nrf2 pathways in
protection against acute lung injury. Am J Respir Crit
34. Braun S, Hanselmann C, Gassmann MG, et al. Nrf2
transcription factor, a novel target of keratinocyte
growth factor action which regulates gene expression
and inflammation in the healing skin wound. Mol Cell
35. Woo KJ, Kwon TK. Sulforaphane suppresses lipo-
saccharide-induced cyclooxygenase-2 (COX-2) ex-
pression through the modulation of multiple targets in
COX-2 gene promoter. Int Immunopharmacol 2007;7:
1776–83.
Sulforaphane suppressed LPS-induced inflammation in
mouse peritoneal macrophages through Nrf2 dependent
37. Cheung KL, Khor TO, Kong AN. Synergistic
effect of combination of phenethyl isothiocyanate
and sulforaphane or curcumin and sulforaphane in
the inhibition of inflammation. Pharm Res 2009;26:
UVB-induced sunburn reaction and oxidative DNA
damage with no alterations in UVB-induced skin
carcinogenesis in Nrf2 gene-deficient mice. J Invest
AP-1 by natural chemopreventive compounds in
human colon HT-29 cancer cell line. Pharm Res
40. Zhang Y. Role of glutathione in the accumulation of
anticarcinogenic isothiocyanates and their glutathione
conjugates by marine hepatoma cells. Carcinogenesis
2002;23:1175–82.
41. Okuno H, Akahori A, Sato H, Xanthoudakis S,
Curran T, Iba H. Escape from redox regulation
enhances the transforming activity of Fox. Oncogene
1993;8:695–701.
42. Chida K, Vogt PK. Nuclear translocation of viral Jun
but not of cellular Jun is cell cycle dependent. Proc Natl
43. Maki Y, Bos TJ, Davis C, Starbuck M, Vogt PK. Avian
sarcoma virus 17 carries the jun oncogene. Proc Natl
Safety, tolerance, and metabolism of broccoli sprout
glucosinolates and isothiocyanates: a clinical phase I
45. Kenden TW, Chen JG, Egner PA, et al. Effects of
glucosinolate-rich broccoli sprouts on urinary levels of
aflatoxin-DNA adducts and phenanthrene tetraols in a
randomized clinical trial in He Zuo township, Qidong,
People's Republic of China. Cancer Epidemiol Bio-
46. Heiss E, Herhaus C, Klimo K, Bartsch H, Gerhauser C.
Nuclear factor κB is a molecular target for sulforaphane-
mediated anti-inflammatory mechanisms. J Biol Chem
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