Stimulatory Effect of Oral Administration of Green Tea or Caffeine on Ultraviolet Light-induced Increases in Epidermal Wild-Type p53, p21(WAF1/CIP1), and Apoptotic Sunburn Cells in SKH-1 Mice[1]

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ABSTRACT

Pretreatment of SKH-1 mice with p.o.-administered 0.6% green tea (6 mg of lyophilized tea solids/ml) or 0.044% caffeine (0.44 mg/ml; concentration present in 0.6% green tea) for 2 weeks enhanced UV-induced increases in the number of p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells in the epidermis. These effects of p.o.-administered green tea or caffeine on early adaptive responses to UV provide the first demonstration of *in vivo* up-regulation of a tumor suppressor gene by a chemopreventive agent. The stimulatory effect of green tea and caffeine on UV-induced increases in the number of p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells may play a role in the inhibitory effects of tea and caffeine on UV-induced carcinogenesis.

INTRODUCTION

Sunlight-induced nonmelanoma skin cancer is an important cancer in many temperate parts of the world (1–3). UVB light (280–320 nm) and to a much lesser extent UVA light (320–400 nm) are responsible for these cancers (4, 5), and molecular studies on mutations in human skin cancers have also implicated UV as the major causative agent (6–9).

The incidence of skin cancer is increasing, and additional increases are predicted because of an increase in recreational exposure to sunlight and also because of the depletion of the stratospheric ozone layer (7, 10). At the present time, skin cancer is the most common type of human cancer, and its appearance is often as frequent as all other cancers combined (11). Although most skin cancers seen by dermatologists are squamous cell carcinomas and basal cell carcinomas that are easily cured if detected early, many people suffer significant morbidity or die from these cancers as well as from the more dangerous sunlight-induced melanomas. The development of strategies to prevent UV-induced cancers would have a major impact in decreasing the total load of human cancer.

In 1991, Wang *et al.* (12) reported an inhibitory effect of a p.o.-administered green tea polyphenol fraction on UV-induced complete tumorigenesis in the skin of SKH-1 mice. Although the inhibitory effect of the green tea polyphenol fraction on UV-induced tumorigenesis was attributed to the polyphenols, the green tea polyphenol fraction used in this study also contained caffeine,[3] which could have contributed to the effect observed.[4] In additional studies, our laboratory reported inhibitory effects of p.o.-administered green tea, black tea, decaffeinated green tea, and decaffeinated black tea on UV-induced formation of papillomas, keratoacanthomas, and squamous cell carcinomas in mice initiated previously with 7,12-dimethylben-z(*a*)anthracene (13, 14). We observed that the decaffeinated teas were somewhat less effective than the regular teas (14). In a recent study, we evaluated the effects of p.o.-administered green tea, black tea, decaffeinated green tea, decaffeinated black tea, and caffeine on UV-induced complete carcinogenesis in the skin of SKH-1 mice. The results of this study indicated inhibitory effects of p.o.-administered green and black tea on UV-induced complete carcinogenesis, but the decaffeinated teas were either inactive (at moderate-dose levels) or they enhanced the tumorigenic effect of UV (at a high-dose level; Ref. 15). Oral administration of caffeine had a strong inhibitory effect on UV-induced complete carcinogenesis (15). In another study, SKH-1 mice were treated with UV for 23 weeks, and UV was discontinued to obtain tumor-free mice with a high risk of developing skin tumors. Subsequent treatment of these high-risk mice with green tea, black tea, or caffeine for several months inhibited carcinogenesis (16).

UV radiation exerts its carcinogenic and cytotoxic actions primarily through the direct formation of cyclobutane pyrimidine dimers (thymine dimers) and pyrimidine (6-4) pyrimidone photodimers (6-4 photoproducts) in DNA, but UV exposure also results in the formation of reactive oxygen species that damage DNA and non-DNA targets (17–22). To maintain genetic integrity after exposure to UV or other DNA-damaging agents, several responses are activated, including p53-dependent and -independent mechanisms for removal of DNA damage, cell cycle delay, and apoptosis. The *p53* tumor suppressor gene has an important role in protecting cells from DNA-damaging agents (23–29). DNA damage triggers a rapid increase in the level of cellular wild-type p53 protein, which enhances the synthesis of p21(WAF1/CIP1) and shuts off cell replication and DNA synthesis, thereby allowing more time for DNA repair and/or apoptosis. This blocking of the cell cycle by increased levels of wild-type p53 protein prevents the replication of damaged DNA templates. The increased level of p53 protein after DNA damage is also associated with enhanced programmed cell death (apoptosis), presumably in those cells that are too damaged for adequate DNA repair (30–34). Several studies have shown a transient stimulatory effect of UV light on the level of wild-type p53 in cultured cells and in mouse and human epidermis (35–40). An important function of wild-type p53 is to act as a transcription factor by binding to a p53-specific DNA consensus sequence in responsive genes (33, 41), which would be expected to increase the synthesis of p21(WAF1/CIP1) and Bax (42, 43). p21(WAF1/CIP1) is a potent inhibitor of cyclin-dependent kinase (42, 44), and the up-regulation of p21(WAF1/CIP1) should inhibit proliferation by blocking the G_1-to-S and the S-to-G_2 phases of the cell cycle (44). Increased levels of Bax should lead to increased apoptosis. In an earlier study, we found that administration of black tea to tumor-bearing mice inhibited proliferation and enhanced apoptosis in the tumors (45).

In the present article, we describe the effects of p.o.-administered green tea or caffeine on UV-induced early adaptive increases in

epidermal wild-type p53, p21(WAF1/CIP1), and apoptosis as well as effects on proliferation in SKH-1 mice. The results indicate that pretreatment of mice with p.o.-administered green tea or caffeine for 2 weeks enhances UV-induced increases in epidermal p53, p21(WAF1/CIP1), and apoptotic sunburn cells. A preliminary report of these studies appeared earlier (46).

MATERIALS AND METHODS

The methods used for the studies described in this article are similar to those described previously (40).

Exposure of Mice to UV and the Preparation of Serial Sections of Skin. Female SKH-1 hairless mice, 6–8 weeks of age, were fed a Purina Laboratory Chow 5001 diet and were irradiated with UV lamps that emit UVB (280–320 nm; 75–80% of total energy) and UVA (320–375 nm; 20–25% of total energy) as described in our earlier studies (13). The UV lamps used (FS72T12-UVB-HO; National Biological Corp., Twinsburg, OH) emitted little or no radiation that was <280 nm or >375 nm. The dose of UVB was quantified with a UVB Spectra 305 dosimeter (Daevlin Co., Bryan, OH) and then with a Model IL-1700 research radiometer/photometer (International Light, Inc., Newburyport, MA). Skin samples (20 mm long; 5 mm wide), which also contained associated dermis, were taken from the middle of the back and placed in 10% phosphate-buffered formalin at 4°C for 18–24 h. The skin samples were then dehydrated in ascending concentrations (80, 95, and 100%) of ethanol, cleared in xylene, and embedded in Paraplast (Oxford Labware, St. Louis, MO). Four-μm serial sections of skin containing epidermis and dermis were made, deparaffinized, rehydrated with water, and used for H&E staining or immunohistochemical staining. These sections were used for the measurement of morphologically distinct apoptotic sunburn cells and epidermal cells with p53, p21(WAF1/CIP1), and BrdUrd[4] incorporation into DNA.

All histological and immunohistochemical determinations were performed with 400-fold magnification and scored blind by two investigators (Y-P. L. and Y-R. L.), who evaluated coded samples randomly. Good agreement was obtained between the two investigators, and the mean value obtained from the examination of multiple fields by each investigator was determined for each mouse and used in the calculation of mean ± SE for the mice in each group. Each microscope field was approximately equivalent to a 0.5-mm length of epidermis.

Measurement of Apoptotic Sunburn Cells. Identification of apoptotic sunburn cells was based morphologically on cell shrinkage and nuclear condensation attributable to fragmentation of the cells (6, 47). Earlier studies demonstrated that sunburn cells are indeed apoptotic cells (8). Apoptotic sunburn cells were identified in the epidermis by their intensely eosinophilic cytoplasm and small, dense nuclei, which were observed in H&E-stained histological sections of the skin using light microscopy. The percentage of apoptotic sunburn cells in the epidermis (basal plus suprabasal layers) was calculated from the number of these cells per 100 cells counted from the entire 20-mm length of epidermis for each skin section. Two separate nonadjacent skin sections from each mouse were analyzed, and an average value for the percentage of apoptotic cells was calculated.

p53 and p21(WAF1/CIP1) Immunostaining. Polyclonal rabbit NCL-p53 CM5p antibody purchased from Novocastra Laboratories Ltd. (Newcastle upon Tyne, United Kingdom) reacts with mouse wild-type or mutated p53 proteins (48, 49). Polyclonal rabbit anti-p21(WAF1/CIP1) antibody was purchased from Oncogene Research Products (Cambridge, MA). Skin sections were stained by the Biotin-Streptavidin Amplified System (alkaline phosphatase-conjugated streptavidin) using StrAviGen Super Sensitive Universal immunostaining kit purchased from Biogenex (San Ramon, CA), with some modifications. Paraffin sections were first treated with 0.01 M sodium citrate buffer (pH 6.0) in a microwave oven at high temperature for 10 min for p53 or p21 staining. The sections were then incubated with a protein block (normal goat serum) for 10 min at room temperature (this procedure was carried out for p21 staining but was omitted for p53). The sections were incubated with p53 antibody (1:500 dilution) or p21 antibody (1:100 dilution) for 1 h at room temperature. The samples were then incubated with a biotinylated antirabbit

secondary antibody for 5 min at 37°C, followed by incubation with conjugated streptavidin solution for 5 min at 37°C. Color development was achieved by incubation with New Fuchsin Substrate Pack (containing 0.6 mg/ml levamisole solution; Biogenex) for 20 min at room temperature. The slides were then counterstained with hematoxylin and dehydrated, and coverslips were added for permanent mounting.

A positive reaction was shown as a pink-to-red precipitate in the nuclei of the cells. The percentage of p53- or p21-positive cells in the epidermis (combined basal and suprabasal layers) was calculated from the number of stained p53- or p21-positive cells per 100 cells counted from the entire 20-mm length of epidermis for each skin section. The UV-induced transient increase in p53-positive cells was shown earlier to be caused by an increased level of wild-type p53 (39).

Western Blot Analysis. Dorsal skin samples were removed and immediately placed in a buffer solution containing 50 mM potassium phosphate (pH 7.4), 2 mM DTT, 0.1 mM EDTA, and a proteinase inhibitor cocktail (10 μg/ml aprotinin, 0.1 mM phenylmethylsulfonyl fluoride, and 2 μM pepstatin; Ref. 50) at 56°C–58°C for 20 s. The samples were then submerged immediately in an ice bath containing the same buffer, and the epidermis was scraped from the dermis and placed in 1 ml of the above buffer. The epidermis was homogenized with a Polytron homogenizer three times (each for 10 s at 4°C at 10-s intervals). Samples were centrifuged at 17,800 × g for 20 min at 4°C. Equal amounts of protein (40 μg) were separated by SDS-PAGE (4% stacking and 10–20% gradient) and electroblotted onto a poly(vinylidene difluoride) membrane. The blots were blocked in 5% nonfat milk in PBS-Tween 20 for 1 h and incubated with p53 primary antibody at 1:1000 dilution. Blots were washed in PBS-Tween 20 and then incubated with a 1:1500 dilution of peroxidase-conjugated secondary antibody (Amersham) in PBS-Tween 20 for 1 h at room temperature. Blots were again washed in PBS-Tween 20 and then developed by enhanced chemiluminescence (Amersham). α-Tubulin was used as an indicator for equality of lane loading. The levels of p53 protein were quantified using the Image-Pro PLUS imaging software (Media Cybernetics, Silver Spring, MD).

BrdUrd Incorporation into DNA and Mitotic Index. BrdUrd, a thymidine analogue that is incorporated into proliferating cells during the S-phase, is detected by a biotinylated monoclonal anti-BrdUrd antibody and visualized using streptavidin-peroxidase and 3,3′-diaminobenzidine, which stains BrdUrd-containing nuclei a dark brown (staining kit from Oncogene Research Products; Refs. 45, 48, and 51). Briefly, all animals received injections of BrdUrd (50 mg/kg) i.p. and were killed 1 h later. Endogenous peroxidase was blocked by incubating the tissue sections in 3% hydrogen peroxide in methanol for 10 min at room temperature. The tissue sections were then incubated in a moist chamber with 0.125% trypsin for 10 min at 37°C, rinsed in distilled water, and incubated at room temperature for 30 min with denaturing solution (Oncogene Research Products). The sections were incubated with blocking solution for 10 min at room temperature and covered with biotinylated mouse monoclonal anti-BrdUrd antibody (Oncogene Research Products) at room temperature for 90 min. Sections were rinsed with PBS and incubated with streptavidin-peroxidase for 10 min. Color development was achieved by incubation for 5 min at room temperature with a substrate solution containing 0.02% 3,3′-diaminobenzidine tetrahydrochloride and 0.02% hydrogen peroxide. The slides were weakly counterstained in Mayer's hematoxylin (Sigma Chemical Co.) for 2 min, cleared with xylene, mounted with a coverslip, and scored under a light microscope. The percentage of BrdUrd-labeled cells in the basal layer of the epidermis was calculated from the number of stained BrdUrd-positive cells per 100 basal cells counted from the entire 20-mm length of epidermis for each section (40, 48). The mitotic index (cells with mitotic figures per 100 basal cells) was determined as described earlier (45).

RESULTS

Stimulatory Effect of Oral Administration of Green Tea on UV-induced Increases in Epidermal p53, p21(WAF1/CIP1), and Apoptotic Sunburn Cells in SKH-1 Mice. Oral administration of 0.6% green tea as the sole source of drinking fluid for 2 weeks before a single application of 30 mJ/cm^2 of UV enhanced the UV-induced increase in the number of epidermal p53-positive cells by 115% (above that which occurred with UV alone), enhanced the UV-

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[4] The abbreviations used are: BrdUrd, bromodeoxyuridine; EGCG, (−)-epigallocatechin gallate; AP-1, activator protein-1; DTT, dithiothreitol.
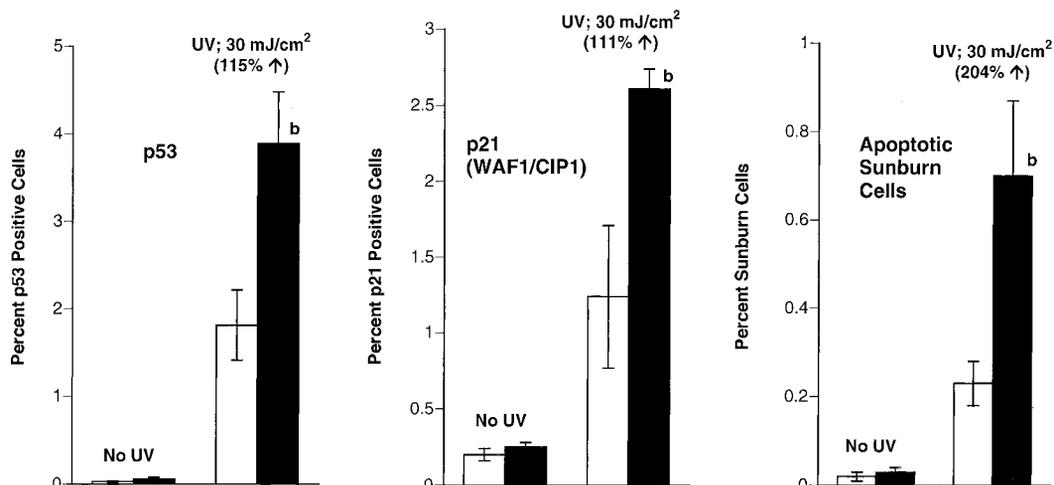
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Fig. 1. Effect of oral administration of green tea on UV-induced increases in p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells. Female SKH-1 mice were treated with water ($\square$) or green tea (6 mg of solids/ml; $\blacksquare$) as their sole source of drinking fluid for 2 weeks. The mice were then treated with UV (30 mJ/cm^2) and were killed 10 h later. Each value represents the mean from five mice; *bars,* SE. *b*, $P < 0.05$.

induced increase in the number of epidermal p21(WAF1/CIP1)-positive cells by 111%, and enhanced the UV-induced increase in the number of apoptotic sunburn cells by 204% at 10 h after exposure to UV (Fig. 1). In another experiment, we investigated the effect of oral administration of 0.6% green tea for 2 weeks on the time course for the effect of a 30-mJ/cm^2 dose of UV on the number of epidermal p53-positive cells, p21-positive cells, and apoptotic sunburn cells (Fig. 2). A stimulatory effect of p.o.-administered green tea on UV-induced increases in the number of epidermal p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells was observed (Fig. 2). Western blot analyses that were corrected for loading of the gels by α-tubulin measurements indicated a UV-induced increase in the level of p53 in the epidermis, at 2 and 6 h after UV irradiation, and prior treatment with 0.6% green tea for 2 weeks enhanced the UV-induced increase in the levels of epidermal p53 by severalfold at 2 h and by 87% at 6 h after UV exposure (Fig. 3).

Stimulatory Effect of Oral Administration of Caffeine on UV-induced Increases in Epidermal p53, p21(WAF1/CIP1), and Apoptotic Sunburn Cells in SKH-1 Mice. Oral administration of caffeine (0.44 mg/ml; the concentration of caffeine that is present in 0.6% green tea) to SKH-1 mice for 2 weeks before irradiation once with 30 mJ/cm^2 of UV enhanced the UV-induced increase in the

number of epidermal p53-positive cells by 227% (above that which occurred with UV alone), enhanced the UV-induced increase in the number of epidermal p21(WAF1/CIP1)-positive cells by 359%, and enhanced the UV-induced increase in the number of apoptotic sunburn cells by 191% at 10 h after irradiation (Fig. 4). In a second experiment, pretreatment of the mice with p.o.-administered caffeine for 2 weeks stimulated UV-induced increases in the number of epidermal p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells by 221, 104, and 92%, respectively, at 10 h after UV exposure.

In another study, we investigated the effect of oral administration of caffeine (0.44 mg/ml) on the time course for the effect of a 30-mJ/cm^2 dose of UV on the number of epidermal p53-positive cells, p21-positive cells, and apoptotic sunburn cells (Fig. 5). A stimulatory effect of p.o.-administered caffeine on UV-induced increases in the number of epidermal p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells was observed (Fig. 5).

Effect of Oral Administration of Green Tea or Caffeine on UV-induced Increases in BrdUrd Incorporation into Epidermal DNA in SKH-1 Mice. Treatment of the mice with UV (30 mJ/cm^2) increased BrdUrd incorporation into epidermal DNA by about 3-fold at 16 h after UV administration (time of maximum stimulation), and
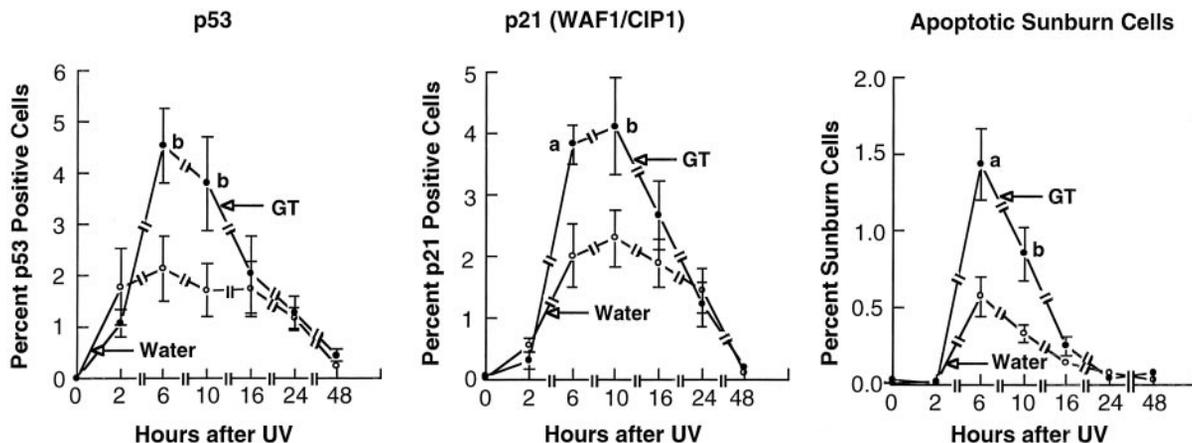

Fig. 2. Effect of oral administration of green tea on the time course for UV-induced increases in p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells. Female SKH-1 mice were treated with water or green tea (*GT*; 6 mg of tea solids/ml) as their sole source of drinking fluid for 2 weeks. The mice were then treated with UV (30 mJ/cm^2) and were killed at the indicated times after UV. Each value represents the mean from five mice; *bars,* SE. *a*, $P < 0.01$; *b*, $P < 0.05$.
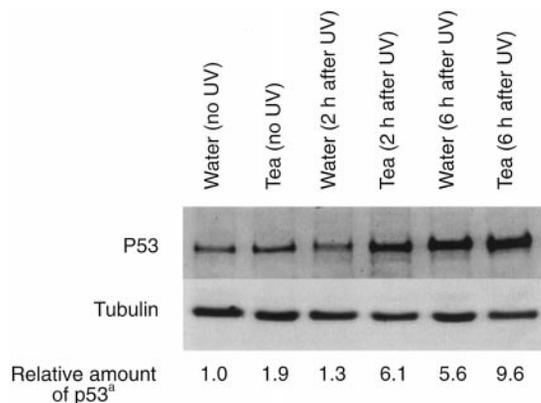
Fig. 3. Effect of oral administration of green tea on UV-induced increases in p53 protein levels by Western blot analysis. Female SKH-1 mice were treated with water or green tea (6 mg of tea solids/ml) as their sole source of drinking fluid for 2 weeks before irradiation with UV (30 mJ/cm^2). Forty μg of epidermal protein were electrophoresed on SDS-PAGE and electroblotted onto a poly(vinylidene difluoride) membrane. The samples were incubated with p53 primary antibody for 1 h at room temperature and then with a peroxidase-conjugated secondary antibody. The samples were developed by enhanced chemiluminescence. α-Tubulin was used as an indicator of equality of zone loading. [a] from densitometer measurements corrected for differences in loading of the different lanes.

this effect was not significantly influenced by pretreatment with green tea (Fig. 6). At 48 h after exposure to UV (30 mJ/cm^2), BrdUrd incorporation into epidermal DNA was inhibited by 28% in mice pretreated with 0.6% green tea for 2 weeks ($P < 0.05$; Fig. 6). The epidermal mitotic index in these mice increased from 2- to 3-fold at 16–24 h after UV irradiation and returned toward control values at 48 h. Pretreatment of the mice with green tea had no effect on these UV-induced increases in mitotic index. In a separate experiment, pretreatment of SKH-1 mice with 0.6% green or black tea for 12 weeks before irradiation with UV (90 mJ/cm^2) inhibited BrdUrd incorporation into epidermal DNA by 26 and 27%, respectively, at 48 h after UV irradiation ($P < 0.05$; data not shown). The results of our studies indicate that oral administration of 0.6% green tea has either no effect or only a small inhibitory effect on the incorporation of BrdUrd into DNA. Administration of green tea had no effect on UV-induced changes in the mitotic index.

In another study, treatment of the mice with UV (30 mJ/cm^2) increased BrdUrd incorporation into DNA by about 2.4-fold at 16 h after UV administration, and this effect was not influenced by pretreatment of the mice with caffeine (Fig. 6). At 48 h after exposure to

UV (30 mJ/cm^2), BrdUrd incorporation into epidermal DNA was inhibited by 30% in mice pretreated with caffeine (Fig. 6). In a separate experiment, pretreatment of SKH-1 mice with caffeine (0.44 mg/ml) for 12 weeks before irradiation with 90 mJ/cm^2 of UV inhibited BrdUrd incorporation into epidermal DNA by 58% at 48 h after UV administration.

DISCUSSION

A search for safe agents that enhance the levels of wild-type p53 and other tumor suppressor proteins is a worthwhile but relatively underexplored approach to cancer chemoprevention, and this concept is also discussed elsewhere (28, 52, 53). In the present study, we have shown that oral administration of green tea or caffeine as the sole source of drinking fluid for 2 weeks enhanced UV-induced increases in the number of p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells in the epidermis of SKH-1 mice. The concentrations of green tea (6 mg of lyophilized tea solids/ml) or caffeine (0.44 mg/ml) administered to the mice in the present study or in additional studies where these concentrations were given for several months had no effect on food consumption or body weight, but these concentrations were ~50% higher than those commonly ingested by humans. Additional studies are needed to determine whether lower dose levels of tea and caffeine are also effective enhancers of UV-induced increases in p53, p21(WAF1/CIP1), and apoptotic sunburn cells. It should be noted that the 30 mJ/cm^2 dose of UV used in our study is within the range of normal human exposure. An erythemic sunburn dose of UV in humans is ~40 mJ/cm^2 (54). Outdoor occupational exposure of humans to UV or a sunbathing exposure in the summer was reported to range from 50 to 100 mJ/cm^2 per day (55, 56).

It is of interest that the effect of a single 30 mJ/cm^2 dose of UV on epidermal BrdUrd incorporation into DNA of epidermal cells (BrdUrd-positive-staining cells) described in Fig. 6 differs markedly from what we observed earlier for a 180 mJ/cm^2 dose of UV. With the low dose of UV, there was a gradual 2- to 3-fold increase in BrdUrd incorporation into DNA that started at 10 h and peaked at 16 h after irradiation and then fell toward control values by 48 h (Fig. 6). When mice were exposed to 180 mJ/cm^2 of UV, there was a profound inhibition (>90%) in BrdUrd incorporation into DNA at 8–12 h after UV irradiation, followed by a dramatic increase in BrdUrd incorporation into DNA that was ~30-fold above the control value by 48 h after UV exposure (40). The marked inhibition in DNA synthesis that occurs shortly after the high dose of UV
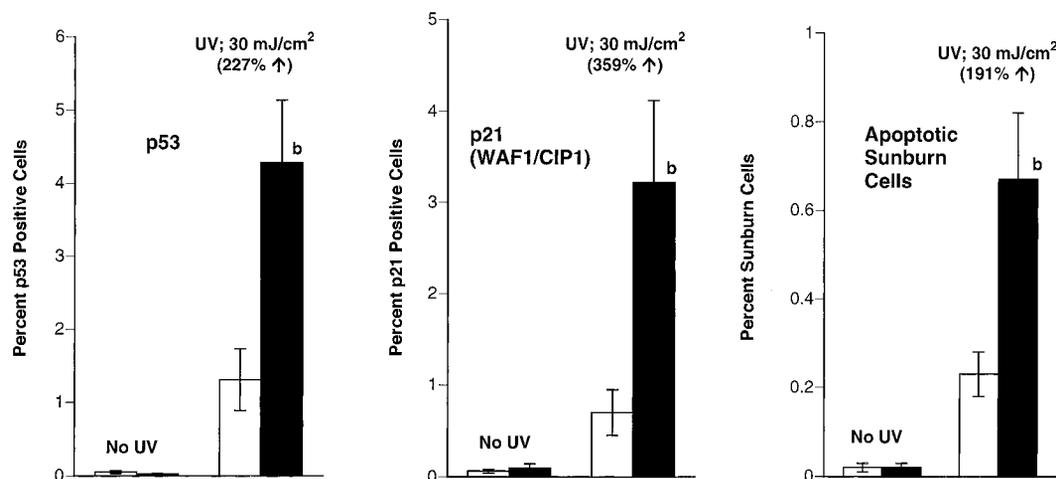

Fig. 4. Effect of oral administration of caffeine on UV-induced increases in p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells. Female SKH-1 mice were treated with water (☐) or caffeine (0.44 mg/ml; ■) as their sole source of drinking fluid for 2 weeks. The mice were then treated with UV (30 mJ/cm^2) and were killed 10 h later. Each value represents the mean from five mice; *bars,* SE. *b, P < 0.05.*
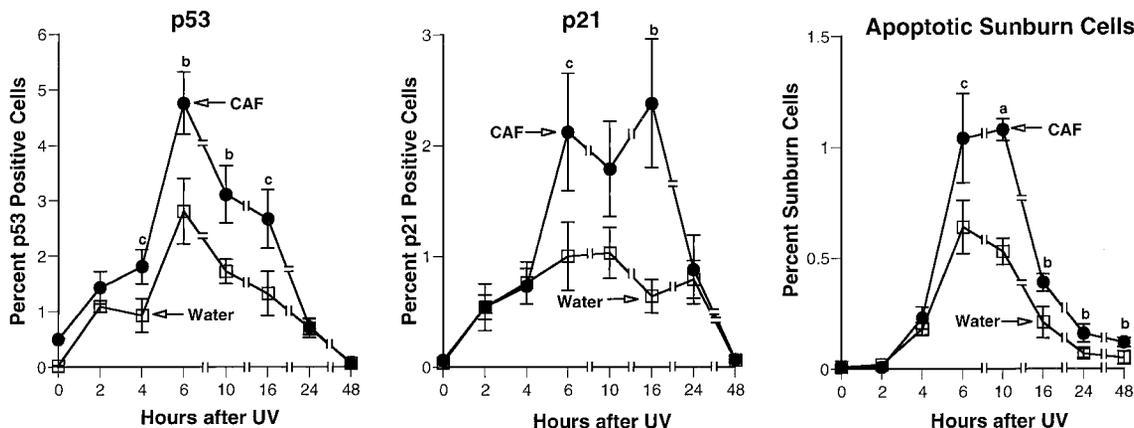
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Fig. 5. Effect of oral administration of caffeine on the time course for UV-induced increases in p53-positive cells, p21(WAF1/CIP1)-positive cells, and apoptotic sunburn cells. Female SKH-1 mice were treated with water or caffeine (*CAF*; 0.44 mg/ml) as their sole source of drinking fluid for 2 weeks, and the mice were killed at the indicated times after UV (30 mJ/cm^2). Each value represents the mean from five mice; *bars,* SE. *a, P* < 0.01; *b, P* < 0.05; *c, P* < 0.1.

allows extra time for the repair of damaged DNA before enhanced DNA synthesis and replication.

Although the present report describes the first *in vivo* example of a stimulatory effect of a chemopreventive agent on the level of a tumor suppressor protein and on downstream effectors, the effects of several chemopreventive agents on the level of p53 and/or downstream effectors *in vitro* in cell culture systems were reported recently (52, 53, 57–63). Treatment of cultured cells with the cancer chemopreventive agents *N*-acetylcysteine, phenethyl isothiocyanate, or resveratrol increased p53 levels and enhanced p53-dependent apoptosis (52, 53, 57, 58). Treatment of cultured cells with oltipraz also increased the level of p53 (59), and treatment of cultured human mammary epithelial cells with benzo(*a*)pyrene together with indole 3-carbinol, EGCG, or genestein increased the level of p53 compared with cells treated only with benzo(*a*)pyrene (60). Additional studies showed that treatment of cultured Hep G2 cells with the chemopreventive agents prolinedithiocarbamate and diethyldithiocarbamate or their reactive metabolites prolinethiuramdisulfide and tetraethylthiuramdisulfide (disulfiram) increased the level of p53, and the later two compounds also enhanced apoptosis (61). In another study, treatment of cultured cells with the plant flavonoid apigenin caused the accumulation of the hypophosphorylated form of the retinoblastoma (Rb) protein and an increased level of p21(WAF1/CIP1) (Ref. 62). The possible effect of apigenin on the level of p53 was not measured. A recent study indicated that treatment of cultured MCF-7 cells with EGCG increased the ratio of hypo- to hyperphosphorylated Rb, and this treatment also increased the levels of p53, p21(WAF1/CIP1), and p27 proteins (63). It should be noted that many of the cell culture studies with chemopreventive

agents described above used concentrations that were considerably higher than those expected to occur after the *in vivo* administration of these compounds. Accordingly, the relevance of these *in vitro* cell culture studies for animals and humans is uncertain.

The mechanism by which tea administration enhances UV-induced increases in the level of epidermal wild-type p53 protein is not known. Irradiation of cultured cells with UV increases the level of wild-type p53 by inhibiting breakdown of the protein. Tea administration could enhance UV-induced stabilization of p53, or it could also stimulate its synthesis. The effect of tea to stimulate UV-induced increases in the level of wild-type epidermal p53 protein could be mediated by: (*a*) an effect of tea to enhance UV-induced DNA strand breaks (or other DNA lesions) that result in a signal to increase the level of wild-type p53 (64); (*b*) an effect of tea to inhibit p53-induced increases in p300 and mdm-2 formation (p300 functions in mdm-2 formation; mdm-2 enhances p53 breakdown); (*c*) an effect of tea to up-regulate ARF (a protein that inhibits the action of mdm-2 on p53; Ref. 65); (*d*) an effect of tea to stimulate the synthesis of p53; and/or (*e*) an effect of tea to enhance the formation of functionally active phospho-p53 (Ser 15), which has a longer half-life than nonphosphorylated p53 because of impaired interaction with mdm-2. Enhanced synthesis of p53 has been observed after treatment of cultured papilloma cells with *N*-acetylcysteine (53). Studies on these and other potential mechanisms of tea-induced up-regulation of UV-induced increases in the level of p53 are needed.

An additional important question is whether the stimulatory effect of tea on UV-induced increases in apoptotic sunburn cells is mediated via p53. Examination of apoptotic sunburn cells from tea-treated animals failed to show elevated levels of p53-positive staining in these

Fig. 6. Effect of oral administration of green tea or caffeine on the time course for UV-induced increases in BrdUrd-positive cells. Female SKH-1 mice were treated with water, green tea (*GT*; 6.0 mg of tea solids/ml), or caffeine (*CAF*; 0.44 mg/ml) as their sole source of drinking fluid for 2 weeks, and the mice were killed at the indicated times after UV (30 mJ/cm^2). BrdUrd was injected i.p. 1 h before mice were killed. Each value represents the mean from five mice; *bars,* SE. *b, P* < 0.05.

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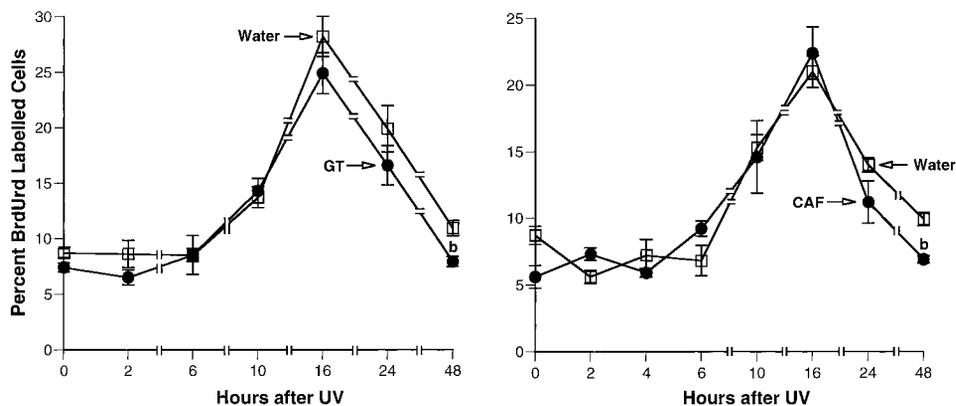
cells, which suggests that tea administration may stimulate UV-induced increases in the number of apoptotic sunburn cells by a mechanism that is independent of p53. Additional studies in p53 knockout mice are being pursued to evaluate the possible role of p53 for the stimulatory effect of tea administration on UV-induced increases in apoptotic sunburn cells. The possible effects of green tea administration on p53-mediated DNA repair are also being evaluated.

Caffeine, theophylline, and pentoxifylline are methylxanthines that sensitize cultured cancer cells to the toxic effects of radiation and/or to certain chemotherapeutic agents, but low millimolar concentrations are needed (66–69). The peak plasma concentrations of caffeine in humans after ingestion of a single 7.5-mg/kg dose of caffeine (equivalent to ∼7 cups of tea) was about 50 μM (70), and considerably lower steady-state plasma levels would be expected in moderate/heavy tea or coffee drinkers. It is believed that caffeine sensitizes cultured cancer cells to DNA-damaging agents by blocking the normal checkpoint control of the cell cycle, thereby allowing replication and apoptosis of the caffeine-treated cells [discussed in Sarkaria *et al.* (71)]. Recent studies suggest that caffeine-induced checkpoint defects are caused by an inhibitory effect of caffeine on kinase activities (ATM and ATR kinases) that are needed for the phosphorylation of p53 at Ser (15) and for the phosphorylation of other proteins that are important for checkpoint control of the cell cycle (71). The relationship between these *in vitro* studies on the inhibitory effects of high concentrations of caffeine on cell cycle checkpoint control and our *in vivo* studies indicating that p.o.-administered caffeine inhibits UV-induced carcinogenesis (15) and enhances UV-induced increases in p53, p21(WAF1/CIP1), and apoptotic sunburn cells without any effect (or a small inhibitory effect) on BrdUrd incorporation into DNA is not known.

Neoplastic epidermal JB6 RT101 cells were used recently as a model for studies on the reversion of the transformed phenotype. Elevated AP-1 activity was required for tumor promoter-induced forward progression of epidermal JB6 P$^+$ cells to a tumor phenotype (72). Three AP-1 inhibitors (retinoic acid, fluocinolone acetonide, and the cAMP elevator, forskolin) were active in inducing reversion of JB6 RT101 cells to a normal phenotype, and synergistic effects of the three AP-1 inhibitors were found (72). Because caffeine is a potent cAMP elevator because of its strong inhibitory effects on phosphodiesterase activity, it may have an effect similar to that of forskolin. The investigators suggested that inhibition of AP-1 activity may be one of the molecular mechanisms associated with reversion of the transformed phenotype. Tumor promoters such as 12-*O*-tetradecanoylphorbol-13-acetate and epidermal growth factor induce AP-1 activity in several cell types (73–75), and expression of AP-1 activity and transformation is dependent on upstream mitogen-activated protein kinase (76). It was found that blocking AP-1 activity in two radiation-induced, malignant mouse epidermal cell lines induced the reversion of these cell lines to a nontumorigenic phenotype (77). Treatment of a human keratinocyte cell line, HaCaT, with UV increased the levels of phospho-Erk, phospho-JNK, phospho-p38, and c-fos (78), and these effects were associated with activation of AP-1 (78, 79). EGCG inhibited UV-induced activation of AP-1 in HaCaT cells (79), and this effect was associated with an inhibitory effect of EGCG on UV-induced increases in c-fos and phospho-p38 MAP kinase (78). EGCG was also shown to inhibit AP-1 activity by inhibiting the formation of phospho-ERK and phospho-c-jun in *ras*-transformed JB6 mouse epidermal cells, but the levels of phospho-JNK and phospho-p38 were not reduced (80). High concentrations of EGCG were used in many of the cell culture studies described above, and the relevance of these studies for the *in vivo* effects of tea and tea constituents is unknown.

In summary, we have shown that oral administration of green tea or caffeine to SKH-1 mice for 2 weeks before irradiation with a low 30 mJ/cm^2 dose of UV greatly enhances UV-induced increases in the number of wild-type p53-positive cells, p21(WAF1/CIP1)-positive

cells, and apoptotic sunburn cells. These effects of green tea and caffeine may play a role in the inhibitory effects of these agents on UV-induced carcinogenesis. Additional studies are needed to determine whether the effects of green tea and caffeine described here for mice also occur in humans.

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REFERENCES

1. Friedman, R. J., Rigel, D. S., Berson, D. S., and Rivers, J. Skin cancer: basal cell and squamous cell carcinoma. *In:* A. I. Holleb, D. J. Fink, and G. P. Murphy (eds.), American Cancer Society Textbook of Clinical Oncology, pp. 290–305. Atlanta: The American Cancer Society, 1991.

2. Scotto, J., Fears, T. R., and Fraumeni, J. F., Jr. Solar radiation. *In:* D. Schottenfeld and J. F. Fraumeni, Jr. (eds.), Cancer Epidemiology and Prevention, Ed. 2, pp. 355–372. New York: Oxford University Press, 1996.

3. Singletary, S. E., and Balch, C. Malignant melanoma. *In:* A. I. Holleb, D. J. Fink, and G. P. Murphy (eds.), American Cancer Society Textbook of Clinical Oncology, pp. 263–270. Atlanta: The American Cancer Society, 1991.

4. Cole, C. A., Forbes, P. D., and Davies, R. E. An action spectrum for UV photocarcinogenesis. Photochem. Photobiol., *43:* 275–284, 1986.

5. De Gruijl, F. R., Sterenborg, H. J. C. M., Forbes, P. D., Davies, R. E., Cole, C., Kelfkens, G., van Weelden, H., Slaper, H., and van der Leun, J. C. Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. Cancer Res., *53:* 53–60, 1993.

6. Ziegler, A., Jonason, A. S., Leffell, D. J., Simon, J. A., Sharma, H. W., Kimmelman, J., Remington, L., Jacks, T., and Brash, D. E. Sunburn and p53 in the onset of skin cancer. Nature (Lond.), *372:* 773–776, 1994.

7. Nataraj, A. J., Trent, J. C., II, and Ananthaswamy, H. N. *p53* gene mutations and photocarcinogenesis. Photochem. Photobiol., *62:* 218–230, 1995.

8. Brash, D. E. Sunlight and the onset of skin cancer. Trends Genet., *13:* 410–414, 1997.

9. Leffel, D. J., and Brash, D. E. Sunlight and skin cancer. Sci. Am., *275:* 52–53, 56–59, 1996.

10. McKenzie, R., Connor, B., and Bodeker, G. Increased summertime UV radiation in New Zealand in response to ozone loss. Science (Washington DC), *285:* 1709–1711, 1999.

11. Gloeckler-Ries, L. A., Hankey, B. F., Harras, A., and Devesa, S. S. Cancer incidence, mortality, and patient survival in the United States. *In:* D. Schottenfeld and J. F. Fraumeni, Jr. (eds.), Cancer Epidemiology and Prevention, Ed. 2, pp. 168–191. New York: Oxford University Press, 1996.

12. Wang, Z. Y., Agarwal, R., Bickers, D. R., and Mukhtar, H. Protection against ultraviolet B radiation-induced photocarcinogenesis in hairless mice by green tea polyphenols. Carcinogenesis (Lond.), *12:* 1527–1530, 1991.

13. Wang, Z. Y., Huang, M-T., Ferraro, T., Wong, C-Q., Lou, Y-R., Reuhl, K., Iatropoulous, M., Yang, C. S., and Conney, A. H. Inhibitory effect of green tea in the drinking water on tumorigenesis by ultraviolet light and 12-*O*-tetradecanoylphorbol-13-acetate in the skin of SKH-1 mice. Cancer Res., *52:* 1162–1170, 1992.

14. Wang, Z. Y., Huang, M-T., Lou, Y-R., Xie, J-G., Reuhl, K., Newmark, H. L., Ho, C-T., Yang, C. S., and Conney, A. H. Inhibitory effects of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz[*a*]anthracene-initiated SKH-1 mice. Cancer Res., *54:* 3428–3435, 1994.

15. Huang, M-T., Xie, J-G., Wang, Z-Y., Ho, C-T., Lou, Y-R., Wang, C-X., Hard, G. C., and Conney, A. H. Effects of tea, decaffeinated tea, and caffeine on UVB light-induced complete carcinogenesis in SKH-1 mice: demonstration of caffeine as a biologically important constituent of tea. Cancer Res., *57:* 2623–2629, 1997.

16. Lou, Y-R., Lu, Y-P., Xie, J-G., Huang, M-T., and Conney, A. H. Effects of oral administration of tea, decaffeinated tea, and caffeine on the formation and growth of tumors in high-risk SKH-1 mice previously treated with ultraviolet-B light. Nutr. Cancer, *33:* 146–153, 1999.

17. Hart, R. W., Setlow, R. B., and Woodhead, A. D. Evidence that pyrimidine dimers in DNA can give rise to tumors. Proc. Natl. Acad. Sci. USA, *74:* 5574–5578, 1977.

18. Cadet, J., Berger, M., Decarroz, C., Wagner, J. R., Van Lier, J. E., Ginot, Y. M., and Vigny, P. Photosensitized reactions of nucleic acids. Biochimie (Paris), *68:* 813–834, 1986.

19. Peak, M. J., Ito, A., Foote, C. S., and Peak, J. G. Photosensitized inactivation of DNA by monochromatic 334-nm radiation in the presence of 2-thiouracil: genetic activity and backbone breaks. Photochem. Photobiol., *47:* 809–813, 1988.

20. Beehler, B. C., Przybyszewski, J., Box, H. B., and Kulesz-Martin, M. F. Formation of 8-hydroxydeoxyguanosine within DNA of mouse keratinocytes exposed in culture to UVB and H$_2$O$_2$. Carcinogenesis (Lond.), *13:* 2003–2007, 1992.

21. Berton, T. R., Mitchell, D. L., Fischer, S. M., and Locniskar, M. F. Epidermal proliferation but not the quantity of DNA photodamage is correlated with UV-induced mouse skin carcinogenesis. J. Investig. Dermatol., *109:* 340–347, 1997.

22. Li, G., Mitchell, D. L., Ho, V. C., Reed, J. C., and Tron, V. A. Decreased DNA repair but normal apoptosis in ultraviolet-irradiated skin of p53-transgenic mice. Am. J. Pathol., *148:* 1113–1123, 1996.

23. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. Participation of p53 protein in the cellular response to DNA damage. Cancer Res., *51:* 6304–6311, 1991.

24. Kuerbitz, S. J., Plunkett, B. S., Walsh, W. V., and Kastan, M. B. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc. Natl. Acad. Sci. USA, *89:* 7491–7495, 1992.

25. Maltzman, W., and Czyzyk, L. UV irradiation stimulates levels of p53 cellular tumor antigen in nontransformed mouse cells. Mol. Cell. Biol., *4:* 1689–1694, 1984.

26. Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, A. J., Jr. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell, *71:* 587–597, 1992.

27. Lane, D. P. p53, guardian of the genome. Nature (Lond.), *358:* 15–16, 1992.

28. Smith, M. L., and Fornace, A. J., Jr. p53-mediated protective responses to UV irradiation. Proc. Natl. Acad. Sci. USA, *94:* 12255–12257, 1997.

29. Yang, J., and Duerksen-Hughes, P. A new approach to identifying genotoxic carcinogens: p53 induction as an indicator of genotoxic damage. Carcinogenesis (Lond.), *19:* 1117–1125, 1998.

30. Prives, C. Doing the right thing: feedback control and p53. Curr. Opin. Cell Biol., *5:* 214–218, 1993.

31. Levine, A. J. The tumor suppressor genes. Annu. Rev. Biochem., *62:* 623–651, 1993.

32. White, E. Death-defying acts: a meeting review on apoptosis. Genes Dev., *7:* 2277–2284, 1993.

33. Zambetti, G. P., and Levine, A. J. A comparison of the biological activities of wild-type and mutant *p53.* FASEB J., *7:* 855–865, 1993.

34. White, E. Life, death, and the pursuit of apoptosis. Genes Dev., *10:* 1–15, 1996.

35. Hall, P. A., McKee, P. H., Menage, H. P., Dover, R., and Lane, D. P. High levels of p53 protein in UV-irradiated normal human skin. Oncogene, *8:* 203–207, 1993.

36. Healy, E., Reynolds, N. J., Smith, M. D., Campbell, C., Farr, P. M., and Rees, J. L. Dissociation of erythema and p53 protein expression in human skin following UVB irradiation, and induction of p53 protein and mRNA following application of skin irritants. J. Investig. Dermatol., *103:* 493–499, 1994.

37. de Laat, A., Kroon, E. D., and de Gruijl, F. R. Cell cycle effects and concomitant p53 expression in hairless murine skin after longwave UVA (365 nm) irradiation: a comparison with UVB irradiation. Photochem. Photobiol., *65:* 730–735, 1997.

38. Saucedo, L. J., Carstens, B. P., Seavey, S. E., Albee, L. D., II, and Perry, M. E. Regulation of transcriptional activation of *mdm2* gene by p53 in response to UV radiation. Cell Growth Differ., *9:* 119–130, 1998.

39. Berg, R. J. W., van Kranen, H. J., Rebel, H. G., de Vries, A., van Vloten, W. A., van Kreul, C. F., van der Leun, J. C., and de Gruijl, F. R. Early p53 alterations in mouse skin carcinogenesis by UVB radiation: immunohistochemical detection of mutant p53 protein in clusters of preneoplastic epidermal cells. Proc. Natl. Acad. Sci. USA, *93:* 274–278, 1996.

40. Lu, Y-P., Lou, Y-R., Yen, P., Mitchell, D., Huang, M-T., and Conney, A. H. Time course for early adaptive responses to ultraviolet B light in the epidermis of SKH-1 mice. Cancer Res., *59:* 4591–4602, 1999.

41. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Prives, C., and Vogelstein, B. Identification of p53 as a sequence-specific DNA-binding protein. Science (Washington DC), *252:* 1708–1711, 1991.

42. Chen, X., Bargonetti, J., and Prives, C. p53, through p21(WAF1/CIP1), induces cyclin D1 synthesis. Cancer Res., *55:* 4257–4263, 1995.

43. Zhan, Q., Fan, S., Bae, I., Guillouf, C., Liebermann, D. A., O'Connor, P. M., and Fornace, A. J., Jr. Induction of *bax* by genotoxic stress in human cells correlates with normal p53 status and apoptosis. Oncogene, *9:* 3743–3751, 1994.

44. El-Deiry, W. S., Harper, J. W., O'Connor, P. M., Velculescu, V. E., Canman, C. E., Jackman, J., Pietenpol, J. A., Burrell, M., Hill, D. E., Wang, Y., Wiman, K. G., Mercer, W. E., Kastan, M. B., Kohn, K. W., Elledge, S. J., Kinzler, K. W., and Vogelstein, B. WAF1/CIP1 is induced in p53-mediated G_1 arrest and apoptosis. Cancer Res., *54:* 1169–1174, 1994.

45. Lu, Y-P., Lou, Y-R., Xie, J-G., Yen, P., Huang, M-T., and Conney, A. H. Inhibitory effect of black tea on the growth of established skin tumors in mice: effects on tumor size, apoptosis, mitosis and bromodeoxyuridine incorporation into DNA. Carcinogenesis (Lond.), *18:* 2163–2169, 1997.

46. Lu, Y-P., Lou, Y-R., Wang, H., Xie, J-G., Brash, D., Huang, M. T., and Conney, A. H. Stimulatory effect of oral administration of green tea or caffeine on UVB-induced increases in epidermal wild-type p53, p21(WAF1/CIP1) and apoptotic sunburn cells in SKH-1 mice. Proc. Am. Assoc. Cancer Res., *40:* 532, 1999.

47. Young, A. R. The sunburn cell. Photodermatology, *4:* 127–134, 1987.

48. Lu, Y-P., Lou, Y-R., Yen, P., Newmark, H. L., Mirochnitchenko, O. I., Inouye, M., and Huang, M-T. Enhanced skin carcinogenesis in transgenic mice with high expression of glutathione peroxidase or both glutathione peroxidase and superoxide dismutase. Cancer Res., *57:* 1468–1474, 1997.

49. Midgley, C. A., Owens, B., Briscoe, C. V., Thomas, D. B., Land, D. P., and Hall, P. A. Coupling between gamma irradiation, p53 induction and the apoptotic response depends upon cell type *in vivo.* J. Cell Sci., *108:* 1843–1848, 1995.

50. Liu, M., Dhanwada, K. R., Birt, D. F., Hecht, S., and Pelling, J. C. Increase in p53 protein half-life in mouse keratinocytes following UV-B irradiation. Carcinogenesis (Lond.), *15:* 1089–1092, 1994.

51. Fukuda, K., Iwasaka, T., Hachisuga, T., Sugimori, H., Tsugitomi, H., and Mutoh, F. Immunocytochemical detection of S-phase cells in normal and neoplastic cervical epithelium by anti-BrdU monoclonal antibody. Anal. Quant. Cytol. Histol., *12:* 135–138, 1990.

52. Huang, C., Ma, W-Y., Li, J., Hecht, S. S., and Dong, Z. Essential role of p53 in phenethyl isothiocyanate-induced apoptosis. Cancer Res., *58:* 4102–4106, 1998.

53. Liu, M., Pelling, J. C., Ju, J., Chu, E., and Brash, D. E. Antioxidant action via p53-mediated apoptosis. Cancer Res., *58:* 1723–1729, 1998.

54. Johnson, B. E. Reactions of normal skin to solar radiation. *In:* A. Jarret (ed.), Physiology and Pathophysiology of Skin, Vol. 8, pp. 2413–2492. London: Academic Press, 1984.

55. Larko, O., and Diffey, B. L. Natural UV-B radiation received by people with outdoor, indoor, and mixed occupations and UV-B treatment of psoriasis. Clin. Exp. Dermatol., *8:* 279–285, 1983.

56. Diffey, B. L. Solar ultraviolet radiation effects on biological systems. Phys. Med. Biol., *36:* 299–328, 1991.

57. Huang, C., Ma, W-Y., Goranson, A., and Dong, Z. Resveratrol suppresses cell transformation and induces apoptosis through a p53-dependent pathway. Carcinogenesis (Lond.), *20:* 237–242, 1999.

58. Hsieh, T-C., Juan, G., Darzynkiewicz, Z., and Wu, J. M. Resveratrol increases nitric oxide synthase, induces accumulation of p53 and p21(WAF1/ClP1), and suppresses cultured bovine pulmonary artery endothelial cell proliferation by perturbing progression through S and G_2. Cancer Res., *59:* 2596–2601, 1999.

59. Chi, W-J., Dong, S-L., Lin-Shiau, S-Y., Boone, C. W., Kelloff, G. J., and Lin, J-K. Oltipraz, a novel inhibitor of hepatitis B virus transcription through elevation of p53 protein. Carcinogenesis (Lond.), *19:* 2133–2138, 1998.

60. Katdare, M., Osborne, M. P., and Telang, N. T. Inhibition of aberrant proliferation and induction of apoptosis in pre-neoplastic human mammary epithelial cells by natural phytochemicals. Oncol. Rep., *5:* 311–315, 1998.

61. Liu, G-Y., Frank, N., Bartsch, H., and Lin, J-K. Induction of apoptosis by thiuramdisulfides, the reactive metabolites of dithiocarbamates, through coordinative modulation of NFκB, c-fos/c-jun, and p53 proteins. Mol. Carcinog., *22:* 235–246, 1998.

62. Lepley, D. M., and Pelling, J. C. Induction of p21(WAF1) and G_1 cell-cycle arrest by the chemopreventive agent apigenin. Mol. Carcinog., *19:* 74–82, 1997.

63. Liang, Y. C., Lin-shiau, S. Y., Chen, C. F., and Lin, J. K. Inhibition of cyclin-dependent kinases 2 and 4 activities as well as induction of Cdk inhibitors p2l and p27 during growth arrest of human breast carcinoma cells by (−)-epigallocatechin-3-gallate. J. Cell. Biochem., *75:* 1–12, 1999.

64. Nelson, W. G., and Kastan, M. B. DNA strand breaks: the DNA template alterations that trigger p53-dependent DNA damage response pathways. Mol. Cell. Biol., *14:* 1815–1823, 1994.

65. Kamijo, T., Weber, J. D., Zambetti, G., Zindy, F., Roussel, M. F., and Sherr, C. J. Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. Proc. Natl. Acad. Sci. USA, *95:* 8292–8297, 1998.

66. Yao, S. L., Akhtar, A. J., McKenna, K. A., Bedi, G. C., Sidransky, D., Mabry, M., Ravi, R., Collector, M. I., Jones, R. J., Sharkis, S. J., Fuchs, E. J., and Bedi, A. Selective radiosensitization of p53-deficient cells by caffeine-mediated activation of p34^{cdc2} kinase. Nat. Med., *2:* 1140–1143, 1996.

67. Russell, K. J., Wiens, L. W., Demers, G. W., Galloway, D. A., Plon, S. E., and Groudine, M. Abrogation of the G_2 checkpoint results in differential radiosensitization of G_1 checkpoint-deficient and G_1 checkpoint-competent cells. Cancer Res., *55:* 1639–1642, 1995.

68. Powell, S. N., DeFrank, J. S., Connell, P., Eogan, M., Preffer, F., Dombkowski, D., Tang, W., and Friend, S. Differential sensitivity of p53$^{(−)}$ and p53$^{(+)}$ cells to caffeine-induced radiosensitization and override of G_2 delay. Cancer Res., *55:* 1643–1648, 1995.

69. Musk, S. R., and Steel, G. G. Override of the radiation-induced mitotic block in human tumour cells by methylxanthines and its relationship to the potentiation of cytotoxicity. Int. J. Radiat. Biol., *57:* 1105–1112, 1990.

70. Tang-Liu, D. D., Williams, R. L., and Riegelman, S. Disposition of caffeine and its metabolites in man. J. Pharmacol. Exp. Ther., *224:* 180–185, 1983.

71. Sarkaria, J. N., Busby, E. C., Tibbetts, R. S., Roos, P., Taya, Y., Karnitz, L. M., and Abraham, R. T. Inhibition of ATM and ATR kinase activities by the radiosensitizing agent, caffeine. Cancer Res., *59:* 4375–4382, 1999.

72. Dong, Z., Lavrovsky, V., and Colburn, N. H. Transformation reversion induced in JB6 RT101 cells by AP-1 inhibitors. Carcinogenesis (Lond.), *16:* 749–756, 1995.

73. Angel, P., Baumann, I., Stein, B., Delius, H., Rahmsdorf, H. J., and Herrlich, P. 12-*O*-Tetradecanoyl-phorbol-13-acetate induction of the human collagenase gene is mediated by inducible enhancer element located in the 5′-flanking region. Mol. Cell. Biol., *7:* 2256–2266, 1987.

74. Risse-Hackl, G., Adamkiewicz, J., Wimmel, A., and Schuermann, M. Transition from SCLC to NSCLC phenotype is accompanied by an increased TRE-binding activity and recruitment of specific AP-1 proteins. Oncogene, *16:* 3057–3068, 1998.

75. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P., and Karin, M. Phorbol ester-inducible genes contain a common *cis* element recognized by a TPA-modulated *trans*-acting factor. Cell, *49:* 729–739, 1987.

76. Huang, C., Ma, W-Y., Young, M. R., Colburn, N., and Dong, Z. Shortage of mitogen-activated protein kinase is responsible for resistance to AP-1 transactivation and transformation in mouse JB6 cells. Proc. Natl. Acad. Sci. USA, *95:* 156–161, 1998.

77. Domann, F. E., Levy, J. P., Birrer, M. J., and Bowden, G. T. Stable expression of a c-*jun* deletion mutant in two malignant mouse epidermal cell lines blocks tumor formation in nude mice. Cell Growth Differ., *5:* 9–16, 1994.

78. Chen, W., Dong, Z., Valcic, S., Timmermann, B. N., and Bowden, G. T. Inhibition of ultraviolet B-induced c-*fos* gene expression and p38 mitogen-activated protein kinase activation by (−)-epigallocatechin gallate in a human keratinocyte cell line. Mol. Carcinog., *24:* 79–84, 1999.

79. Barthelman, M., Bair, W. B., III, Stickland, K. K., Chen, W., Timmermann, B. N., Valcic, S., Dong, Z., and Bowden, G. T. (−)-Epigallocatechin-3-gallate inhibition of ultraviolet B-induced AP-1 activity. Carcinogenesis (Lond.), *19*: 2201–2204, 1998.

80. Chung, J. Y., Huang, C., Meng, X., Dong, Z., and Yang, C. S. Inhibition of activator protein 1 activity and cell growth by purified green tea and black tea polyphenols in H-*ras*-transformed cells: structure-activity relationship and mechanisms involved. Cancer Res., *59:* 4610–4617, 1999.

Stimulatory Effect of Oral Administration of Green Tea or Caffeine on Ultraviolet Light-induced Increases in Epidermal Wild-Type p53, p21(WAF1/CIP1), and Apoptotic Sunburn Cells in SKH-1 Mice

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