JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 4187−4193 **4187**

Inhibitory Effect on Activator Protein-1, Nuclear Factor-KappaB, and Cell Transformation by Extracts of Strawberries (*Fragaria* × *ananassa* Duch.)

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The inhibitory effects of strawberry (*Fragaria* × *ananassa* Duch.) antioxidant enzymes on tetradecanoylphorbol-13-acetate (TPA) or ultraviolet-B (UVB) induced activator protein-1 (AP-1) and nuclear factor-kappaB (NF-κB) were studied. The inhibitory effects of strawberry extracts on the proliferation and transformation of human and mouse cancer cells were also evaluated. Strawberries had high activities of glutathione peroxidase, superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase, and glutathione reductase. Strawberry extracts inhibited the proliferation of human lung epithelial cancer cell line A549 and decreased TPA-induced neoplastic transformation of JB6 P^+ mouse epidermal cells. Pretreatment of JB6 P^+ mouse epidermal cells with strawberry extract resulted in the inhibition of both UVB- and TPA-induced AP-1 and NF-κB transactivation. Furthermore, strawberry extract also blocked TPA-induced phosphorylation of extracellular signal-regulated kinases (ERKs) and UVB-induced phosphorylation of ERKs and JNK kinase in JB6 P^+ mouse epidermal cell culture. These results suggest that the ability of strawberries to block UVB- and TPA-induced AP-1 and NF-κB activation may be due to their antioxidant properties and their ability to reduce oxidative stress. The oxidative events that regulate AP-1 and NF-κB transactivation can be important molecular targets for cancer prevention. The strawberries may be highly effective as a chemopreventive agent that acts by targeting the down-regulation of AP-1 and NF-κB activities, blocking MAPK signaling, and suppressing cancer cell proliferation and transformation.

KEYWORDS: Antioxidant enzymes; activator protein-1; nuclear factor-kappaB; mitogen-activated protein kinase; neoplastic transformation; *Fragaria* × *ananassa* Duch.

INTRODUCTION

Epidemiological studies have shown that diets rich in fruits and vegetables are associated with longer life expectancy, and this may be due to the beneficial effects of the antioxidants contained therein (*1*). Fruits and vegetables have shown a remarkably high scavenging activity toward chemically generated radicals (*2*). They are effective in inhibiting the oxidation of human low-density lipoproteins and thus have potential effects in preventing various human diseases (*3*).

Strawberries are good sources of natural antioxidants (*2*, *4*). In addition to the usual nutrients such as vitamins and minerals, strawberries are also rich in anthocyanins, flavonoids, and phenolic acids (*2*). Our previous study showed that strawberries have high oxygen radical absorbance activity (*5*, *6*). However, little information is available about the enzymatic system for active oxygen detoxification in strawberries. Modulation of antioxidant enzymes are hypothesized to affect protection against reactive oxygen species.

Reports focusing on the chemopreventive effects of strawberry fruit are limited. To elucidate the mechanism of the antitumorigenic effects of strawberries, we studied the suppression effects of strawberries on the activation of activator protein-1 (AP-1) and nuclear factor-kappaB (NF-κB) as well as cellular transformation in mouse epidermal JB6 P^+ cells. AP-1 and NF-κB are transcription factors associated with carcinogenesis (*7*). Inhibition of AP-1 activity has been shown to lead to the suppression of cell transformation (*8*). NF-κB is also an important regulator in deciding cell fate, such as programmed cell death and proliferation control, and is critical in tumorigenesis (*9*). 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) and ultraviolet-B (UVB) are well-known tumor promoters and can produce reactive oxygen species (ROS) and stimulate

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10.1021/jf0478049
Published on Web 04/09/2005

AP-1 and NF-κB activity by activating mitogen-activated protein kinases (MAPK) signaling pathways such as the extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun amino-terminal kinases (JNKs), and the p38 MAP kinases (*10*, *11*).

Little information is available on anti-inflammatory and chemopreventive effects of strawberries and their underlying mechanisms at the cellular and molecular levels. The purpose of this study was to evaluate the oxygen scavenging enzymes that might be actively involved in oxygen detoxification in strawberries and to study the potential inhibitory effects of strawberries on TPA- or UVB-induced AP-1 and NF-κB activities and the inhibitory effect on proliferation and transformation of cancer cells as well as the underlying signal kinase pathways.

MATERIALS AND METHODS

Chemicals. Hydrogen peroxide (30% w/w), *N*,*N*-dimethyl-*p*-nitrosoaniline, xanthine, xanthine oxide, ascorbate oxidase, dithiothreitol (DTT), glutathione (oxidized form), glutathione (GSH, reduced form), glutathione reductase, guaiacol, β-nicotinamide adenine dinucleotide (β-NADH, reduced form), β-nicotinamide adenine dinucleotide phosphate (β-NADPH, reduced form), and nitro blue tetrazolium (NBT) were purchased from Sigma Chemical Co. (St. Louis, MO). Eagle's EMEM and DMEM were obtained from Whittaker Biosciences (Walkersville, MD). EDTA (ethylenediaminetetracetic acid, disodium salt, dihydrate-Na_2 EDTA·$2H_2O$), FBS, L-glutamine, penicillin, and streptomycin were purchased from Life Technologies, Inc. (Gaithersburg, MD). Luciferase assay kits were obtained from Promega (Madison, WI). PhosphoPlus MAPK antibody kits were purchased from New England BioLabs (Beverly, MA).

Fruit Sample Preparation. Strawberry fruits (*Fragaria* × *ananassas* cv. Earliglow) used in this study were grown at Butler's Orchard in Germantown, MD, and were hand-harvested at a commercially mature stage, sorted to eliminate damaged, shriveled, and unripe fruit, and selected for uniform size and color. Undamaged berries were selected, randomized, frozen in liquid nitrogen, and then stored at −80 °C until they were assayed for antioxidant enzymes. For the assay of AP-1 and NF-κB activation, MAPKs phosphorylation, and anchorage-independent transformation, strawberry fruit extract was prepared using 1 mL of ddH_2O per gram of strawberry fruit and mixed and blended at high speed. The blended homogenate was strained, centrifuged, filter sterilized, and stored at −20 °C. All extracts were centrifuged at 6000*g* and 4 °C for 20 min, and the supernatant was filtered. The supernatants were transferred to vials, stored at −80 °C, and then used for analyses.

Antioxidant Enzyme Measurements. *Glutathione Peroxidase (GSH-POD, EC 1.11.1.9) and Glutathione Reductase (GR, EC 1.6.4.2).* Triplicate fruit tissue (10 g of fresh weight) was homogenized in 10 mL of 0.1 M Tris-HCl buffer (pH 7.8) containing 2 mM EDTA-Na and 2 mM DTT. The homogenate was centrifuged at 20000*g* for 30 min at 4 °C, and the supernatant was used for the GSH-POD and GR assays.

GSH-POD activity was determined using the method of Tappel (*12*) with a slight modification. The reaction mixture contained 0.1 M Tris-HCl buffer (pH 8.0), 0.4 mM EDTA, 1.0 mM NaN_3, 1.0 mM H_2O_2, 1.0 mM glutathione (GSH), 0.15 mM NADPH, 1 unit of glutathione reductase, and 100 μL of enzyme extract. The total reaction volume was 1.0 mL. The reaction was started by adding H_2O_2. GSH-POD activity was determined by the rate of NADPH oxidation at 340 nm via a spectrophotometer (Shimadzu UV-160A, Shimadzu Scientific Instruments, Columbia, MD). Enzyme activity was expressed as nanomoles of NADPH oxidized per milligram of protein per minute.

GR activity was assayed according to the method of Smith et al. (*13*). The activity of GR was determined by monitoring glutathione-dependent oxidation of NADPH at 340 nm. The reaction was started by adding GSSG, and the rate of oxidation was calculated using the extinction coefficient of NADPH (6.22 mM^{-1} cm^{-1}). GR activity was expressed as nanomoles of NADPH oxidized per milligram of protein per minute.

Superoxide Dismutase (SOD, EC 1.15.1.1). Triplicate fruit tissue (10 g) was pulverized in a cold mortar and pestle with 10 mL of potassium phosphate buffer (0.1 M, pH7.3) containing 1 mM EDTA and 2 mM DTT. The homogenate was strained through four layers of Miracloth and centrifuged at 12000*g* for 10 min at 4 °C. The supernatant was purified according to the method of Wang et al. (*14*) before the SOD enzyme activity was assayed.

Total SOD activity was assayed photochemically; dicoumarol was included in the reaction mixture to inhibit reduction by pyridine nucleotide and to obtain a completely $O_2^{\bullet -}$- dependent reduction of NBT. One unit of SOD was defined as the amount of enzyme that produced a 50% inhibition of NBT reduction under assay conditions. Because inhibition is not linearly correlated with SOD concentration, a V/v transformation was used to obtain linearity (V = basic reaction rate without blackberry fruit extract, v = reaction rate with extract). Linear correlation gave the following equation: SOD units/mL = $(0.459V/v - 0.032) \times$ dilution factor. The correlation coefficient for this line was 0.985.

Ascorbate Peroxidase (AsA-POD, EC 1.11.1.11) and Guaiacol Peroxidase (G-POD, EC 1.11.1.7). Triplicate fruit tissue (10 g) was pulverized in a cold mortar and pestle with 10 mL of potassium phosphate buffer (0.1 M, pH7.3) containing 1 mM EDTA and 2 mM DTT. The homogenate was centrifuged at 12000*g* for 10 min at 4 °C. The supernatant was used for the AsA-POD, and G-POD assays.

AsA-POD activity was assayed according to the method of Amako et al. (*15*). The reaction was started by adding H_2O_2. Enzyme activity was expressed as nanomoles of ascorbate oxidized per milligram of protein per minute.

The G-POD assay mixture contained 0.1 M phosphate buffer (pH 6.1), 4 mM guaiacol as donor, 3 mM H_2O_2 as substrate, and 1.0 mL of crude enzyme extract. The total reaction volume was 3.0 mL. The rate of change in absorbance at 420 nm was measured, and the level of enzyme activity was expressed as the difference in absorbance (OD) per milligram of protein per minute.

Cell Culture and Assay of AP-1 and NF-κB Activity in Vitro. JB6 P^+ mouse epidermal cells that stably transfected with AP-1-luciferase or NF-κB-luciferase reporter plasmid (*16*, *17*) were cultured in Eagle's MEM (EMEM) containing 5% FBS, 2 mM L-glutamine, and 1% penicillin−streptomycin (10000 units/mL penicillin and 10 mg/mL streptomycin). A confluent monolayer of JB6 P^+ cells was trypsinized, and 5×10^4 viable cells (suspended in 1 mL of EMEM supplemented with 5% FBS) were added to each well of a 24-well plate. Plates were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Twelve hours later, cells were cultured in EMEM supplemented with 0.5% FBS for 12−24 h to minimize basal AP-1 activity or NF-κB activity before treatment for 1 h with or without strawberry extracts at the concentrations indicated. The cells were then exposed to TPA (20 ng/mL) or UVB (4 kJ/m^2) irradiation and cultured for an additional 48 h in the same medium to monitor the effects on AP-1 or NF-κB induction. The cells were extracted with 200 μL of lysis buffer provided in the luciferase assay kit by the manufacturer. Luciferase activity was measured using a Monolight luminometer, model 3010. The results were expressed as relative AP-1 or NF-κB activity compared with untreated controls.

Protein Kinase Phosphorylation Assay. Immunoblots for phosphorylation of ERKs, JNKs, and p38 kinase were carried out using PhosphoPlus MAPK antibody kits as described in the protocol of New England BioLabs. Phosphospecific antibodies were used to detect phosphorylated sites of ERKs, JNKs, and p38 kinase (*18*). Non-phosphospecific antibodies against ERKs, JNKs, and p38 kinase proteins provided in each assay kit were used to normalize the phosphorylation assay by using the same transferred membrane blot.

Inhibition of Proliferation of Cancer Cells by Strawberry Extract. The human lung epithelial cancer A549 cells (1×10^4) were cultured in 10% FBS DMEM medium for 72 h with or without the indicated doses of strawberry extract. The inhibition of A549 cell growth was indicated as the decrease of cell number. The cell number of A549 was determined using a Coulter Multisizer II cell counter at the end of experiment. Data are expressed as mean ± standard error (SE) of three samples.

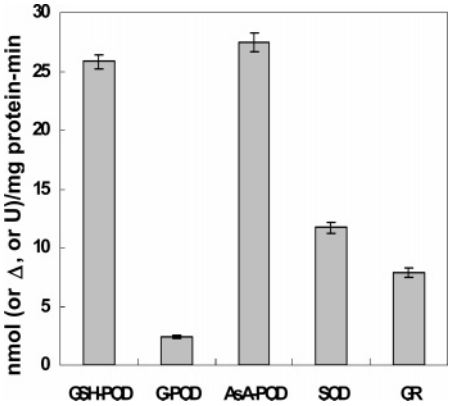

Figure 1. Activities of glutathione peroxidase (GSH-POD; nmol of NADPH/mg of protein·min), guaiacol peroxidase (G-POD; A/mg of protein·min), ascorbate peroxidase (AsA-POD; nmol of ascorbate/mg of protein·min), superoxide dismutase (SOD; units of SOD/mg of protein), and glutathione reductase (GR; nmol of NADPH/mg of protein·min) in strawberries. Data are expressed as mean ± SEM ($n = 3$).

Anchorage-Independent Transformation Assay. JB6 P^+ mouse epidermal cells (10^4) were exposed to TPA (20 ng/mL) in the presence or absence of strawberry extract, in 0.33% Bacto-agar containing 20% FBS over 0.5% agar medium containing 15% FBS EMEM. The cultures were maintained in a 37 °C, 5% CO_2 incubator for 2 weeks, and then the colonies were counted as described by Colburn et al. (*19*).

Protein Determination. Protein was determined according to Bradford (*20*) method, using bovine serum albumin (BSA) as a standard.

Statistical Analysis. Data presented are the means ± SE of values compared and analyzed using a one-way ANOVA test to assess the statistical significance between treatments. Statistical significance was set at $p \leq 0.05$.

RESULTS

The activities of the oxygen scavenging enzymes are shown in **Figure 1**. Strawberries had 11.7 units/mg of protein SOD, 2.9 A/mg of protein·min G-POD, 25.8 nmol/mg of protein·min GSH-POD, 27.5 nmol/mg of protein·min AsA-POD, and 7.9 nmol/mg of protein·min GR (**Figure 1**).

Pretreatment of JB6 P^+ cells with strawberry extracts produced a dose-dependent inhibition of AP-1 and NF-κB activity induced by either UVB or TPA (**Figures 2** and **3**). The AP-1 activity induced by TPA was inhibited 47−96% by strawberry extracts diluted 1:20 to 1:160 (**Figure 2**). Similar strawberry extracts inhibited UVB-induced AP-1 activity by 25−95%. The NF-κB activity induced by TPA or UVB was inhibited by 59−91 or 91−97%, respectively, with the tested dilution dose range from 1:20 to 1:160 (**Figure 3**).

Human lung cancer A549 cell proliferation was inhibited in a dose-dependent fashion after exposure to the strawberry extracts (1:250 to 1:500 dilution) (**Figure 4**). As shown in **Figure 5**, TPA efficiently induced JB6 P^+ cell transformation after 2 weeks of culture. The addition of strawberry extract significantly suppressed TPA-induced cell transformation in a concentration-dependent manner ($p < 0.05$). Strawberry extract alone had no effect on spontaneous cell transformation (data not shown).

Pretreatment of cells with strawberry extracts blocked UVB-induced phosphorylation of ERKs and JNKs in a dose-dependent manner (**Figure 6**). However, there was no effect on p38 kinase activation (**Figure 6**). Strawberry extracts also showed inhibition on TPA-induced phosphorylation of ERKs (**Figure 7**).

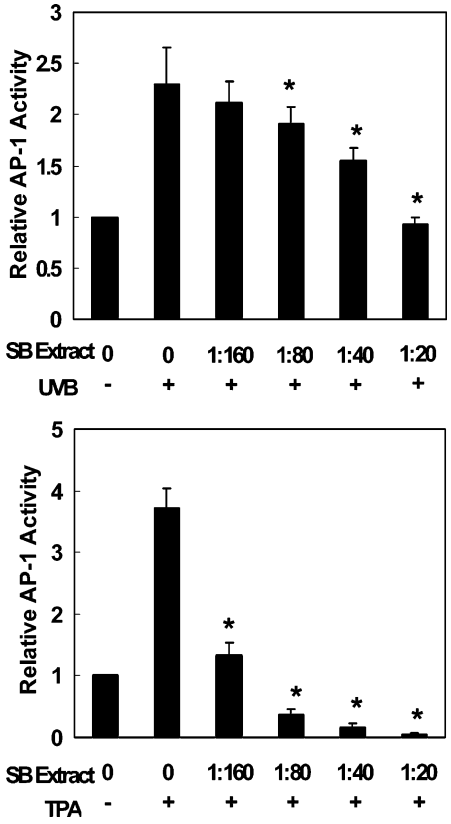

Figure 2. Strawberry suppresses UVB- and TPA-induced AP-1 activity. JB6 P^+ mouse epidermal cells that stably transfected with AP-1 luciferase reporter plasmid were cultured as described under Materials and Methods. The cells were pretreated with or without various diluted strawberry fruit extract as indicated for 1 h and then were exposed to TPA (20 ng/mL) or UVB (4 kJ/m^2) and cultured for an additional 48 h. AP-1 activity was determined by luciferase assay. Results, presented as relative AP-1 induction compared to untreated control cells, are expressed as means and standard errors from three wells. The experiment was repeated three times. Asterisks (∗) indicate a significant inhibition of TPA-induced AP-1 activation by strawberry fruit extract ($p \leq 0.05$).

DISCUSSION

In aerobic cells, ROS are formed during the normal aerobic metabolic process (*21*, *22*). It has been estimated that 1−5% of oxygen consumed by cells can form ROS (*21*). In addition to endogenous production, ROS can be produced from exogenous factors such as ionizing radiation, diet, and xenobiotics (*23*). Oxidative stress arises either from the overproduction of ROS or from the deficiency of antioxidant defense or repair mechanisms, resulting in reversible or irreversible damage to critical cellular macromolecules such as lipids, proteins, and DNA (*23*). The resulting unrepaired oxidative damage has been suggested to play a role in several chronic diseases, including cancer (*3*). Cells contain several mechanisms to inactivate ROS and repair or replace damaged cellular molecules to maintain cellular homeostasis (*24*). Strawberries had high antioxidant capacity against $ROO^•$, $O_2^{•-}$, H_2O_2, $OH^•$, and $^1O_2$ (*5*, *6*).

The activities of SOD, G-POD, GSH-POD, AsA-POD, and GR detected in strawberries in our study are comparable to those found in blackberries (*25*). These enzyme activities were correlated to the antioxidant activity in strawberries (data not shown). The activities of antioxidant enzymes in blackberry fruit have also been shown to be positively correlated to the antioxidant capacity (*25*).

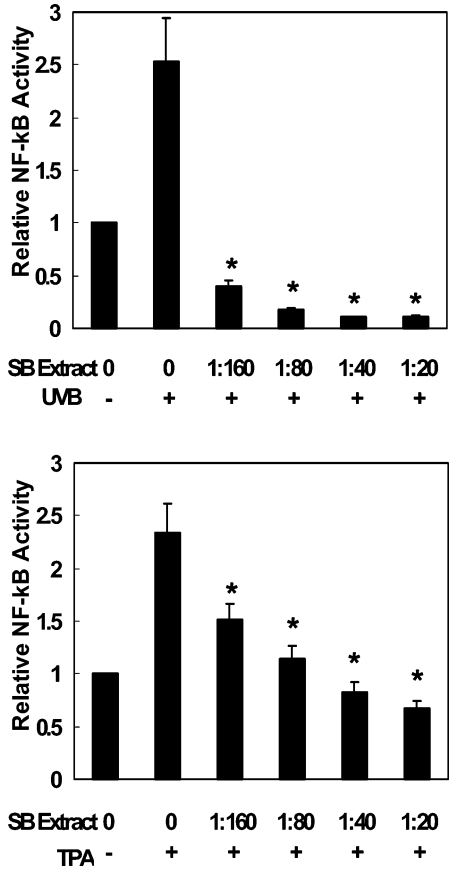

Figure 3. Strawberry extract suppresses UVB- and TPA-induced NF-κB activity. JB6 P^+ mouse epidermal cells that stably transinfected with NF-κB luciferase reporter plasmid were cultured as described under Materials and Methods. The cells were pretreated with or without various diluted strawberry fruit extract as indicated for 1 h and then were exposed to TPA (20 ng/mL) or UVB (4 kJ/m^2) and cultured for an additional 48 h. NF-κB activity was determined by luciferase assay. Results, presented as relative NF-κB induction compared to untreated control cells, are expressed as means and standard errors from three wells. The experiment was repeated three times. Asterisks (*) indicate a significant inhibition of TPA-induced AP-1 activation by strawberry fruit extract ($p \leq 0.05$).

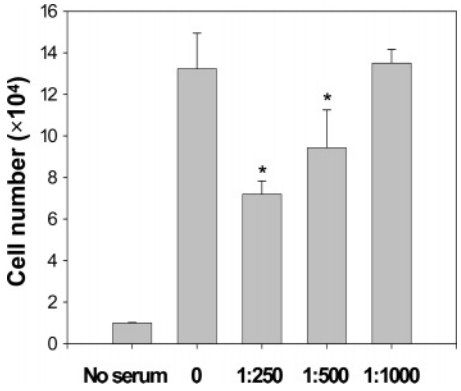

Figure 4. Inhibition of proliferation of cancer cells by strawberry extract. Human lung epithelial cancer A549 cells (1×10^4) suspended in 400 μL of 10% FBS DMEM medium with or without the indicated doses of strawberry extract were cultured for 72 h. The cell number of A549 was determined using a Coulter Multisizer II cell counter at the end of the experiment. Data are expressed as mean ± SE of three samples. Asterisks (*) indicate a significant inhibition of proliferation of cancer cells by strawberry extract ($p < 0.05$).

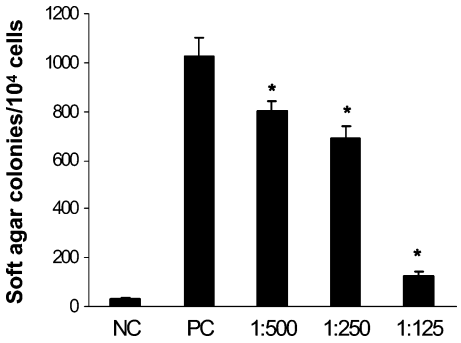

Figure 5. Inhibition of TPA-induced transformation by strawberry extract. JB6 P^+ mouse epidermal cells (1×10^4) were exposed to TPA (20 ng/mL) with or without strawberry extract in 0.33% agar for 14 days and scored for colonies at the end of the experiments. Results, presented as the number of colonies per 10^4 cells, are the mean and standard errors from two independent assays. Asterisks (*) indicate a significant difference from TPA-treated positive control ($p \leq 0.05$).

SOD catalyzes the breakdown of $O_2^{\bullet-}$ to O_2 and H_2O_2, removes singlet oxygen as well as $O_2^{\bullet-}$, prevents the formation of OH^-, and has been implicated as an essential defense against the potential toxicity of oxygen (*26*). G-POD is involved in a large number of biochemical and physiological processes. The mode of action of G-POD on the H_2O_2 substrate is to liberate free radicals. These free radicals are highly phytotoxic. The accumulation of H_2O_2 may cause changes in plant metabolism. The H_2O_2 formed by G-POD may be scavenged by catalase and GSH-POD. However, the catalase activity in ripe strawberries was not detectable. GSH-POD may be responsible for scavenging H_2O_2, catalyzing the peroxidation of reduced glutathione (GSH), and forming the oxidized disulfide form of glutathione (GSSG) as a product.

AsA-POD is a heme-containing protein and is highly specific for ascorbate as the electron donor. AsA-POD uses ascorbate as an electron donor, but the basic properties of AsA-POD are very different from those of G-POD with regard to their amino sequences and other molecular properties (*27*). Ascorbic acid is an essential compound in plant tissues and has been the focus of numerous studies in relation to the enzymatic or nonenzymatic oxidation reactions in the biological system (*28*, *29*). Ascorbic acid serves as an excellent antioxidant and plays a fundamental role in removal of hydrogen peroxide via the ascorbate−glutathione cycle and produces DHAsA. DHAsA is reduced to ascorbic acid by monodehydroascorbate reductase (MDAR) or dehydroascorbate reductase (DHAR) at the expense of NADH and glutathione (GSH) (*30*).

GR is a ubiquitous NADPH-dependent enzyme present in cells of both plants and animals (*31*). It has been suggested that in higher plants, GR may be a rate-limiting enzyme for defense against active O_2 toxicity (*32*). GR seems to be mainly located in the cytosol and catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to the reduced form (GSH) (*31*) and may play an important role in regulating fruit ripening. High activity of GR and high levels of GSH found in strawberries may allow for the removal of free radicals, which is associated with increased antioxidant capacity.

Aerobic organisms constantly battle the adverse effects of ROS by increasing the production of biochemical antioxidants (such as glutathione, ascorbate, and β-carotene) or by inducing endogenous antioxidant enzymes including SOD, catalase, AsA-POD, GSH-POD, and GR. These scavenging antioxidant molecules and the endogenous antioxidant enzymes attenuate the ROS concentration to maintain an intracellular reduction and oxidation (redox) balance. Oxidative stress has been

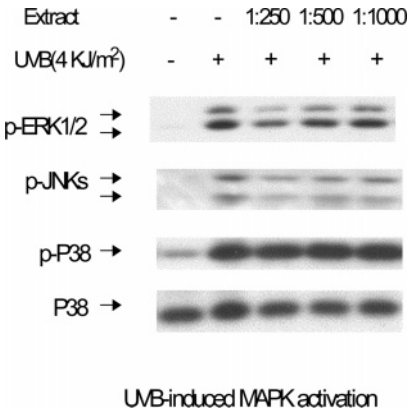

Figure 6. Inhibition of UVB-induced activation of MAPKs by strawberry extract. JB6 P^+ mouse epidermal cells were pretreated with strawberry fruit extract for 1 h and then exposed to UVB radiation (4 kJ/m^2) for 30 min. Proteins (20 μg) in the JB6 P^+ cell lysate were separated by SDS-PAGE and analyzed by Western blot with phosphospecific antibody against phosphorylated sites of ERKs, JUNKs, and p-38. Phosphorylated and nonphosphorylated proteins were detected by using the same transferred membrane blot following a stripping procedure. One representative of three similar results is shown.

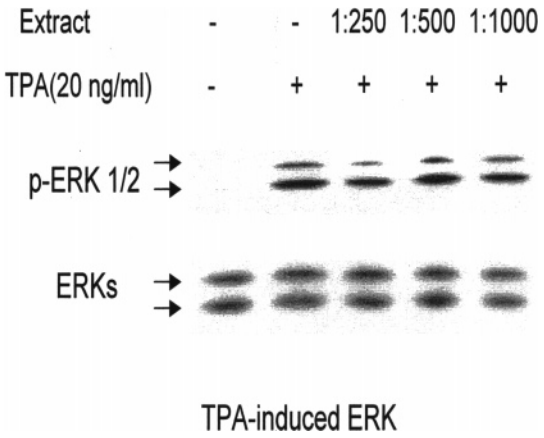

Figure 7. Inhibition of TPA-induced activation of ERKs by strawberry extract. JB6 P^+ mouse epidermal cells were pretreated with strawberry extract for 1 h and then exposed to TPA (20 ng/mL) for 30 min. Proteins (20 μg) in the cell extracts were separated by SDS-PAGE and analyzed by Western blot with phosphospecific antibody against phosphorylated sites of ERKs. Phosphorylated and nonphosphorylated proteins were detected by using the same transferred membrane blot following a stripping procedure. One representative of three similar results is shown.

implicated in both initiation and promotion/progression phases of carcinogenesis (*33*). Reduced levels of antioxidant enzymes, including manganese SOD (MnSOD) or, in some cases, CuZnSOD, were implicated in the transformation of cancer cells (*33*). In addition, the levels of small molecule antioxidants (such as glutathione and ascorbate), acting to antagonize oxygen free radicals, were lowered in many transformed cell types (*33*). Overexpression of manganese SOD (MnSOD), a natural antioxidant, was shown to revert transformation or tumor promotion response in human breast cancer MCF-7 cells and mouse epidermal JB6 P^+ cells (*34*, *35*).

The process of multistep carcinogenesis consists of initiation, promotion, and progression (*36*). AP-1 and NF-κB are two eukaryotic transcription factors that regulate genes implicated in ROS-induced responses, and both factors are targets of oxidative stimuli (*37*). AP-1 has been identified as a target of the MAPK family, including ERKs, JNKs, and p38 kinase (*38*). AP-1 and its regulated gene expression have been shown to play an important role in cell proliferation and tumor promotion (*38*). Activation of AP-1 appears to be required for the preneoplastic to neoplastic progression in JB6 P^+ cells (*17*). NF-κB is a transcription factor activated in response to both inflammatory and non-inflammatory exogenous stimuli (*9*). NF-κB exists in cells in an inactive state by forming a complex with IκBα, an inhibitor of NF-κB (*9*). Signals leading to IκBα phosphorylation trigger the release of NF-κB from IκBα, resulting in an activation and translocation of NF-κB from the cytoplasm to the nucleus, where it binds to the promoters of its target genes (*9*). AP-1 and NF-κB were both considered to be the mediators of tumor promotion because of their ability to alter gene expression in response to tumor promoters and oncogenes, including TPA, UV radiation, and ROS (*8*). It was also demonstrated that activation of AP-1 and NF-κB is required for cell proliferation, transformation, and tumor development (*39*, *40*). Therefore, we tested the effects of strawberry extracts on TPA- or UVB-induced AP-1 and NF-κB activity by using a reporter gene assay and found that pretreatment of JB6 P^+ cells with strawberry extracts produced a dose-dependent inhibition of AP-1 and NF-κB activity induced by either UVB or TPA. These results suggest that strawberry extracts are effective inhibitors of TPA- and UVB-induced signal transduction pathways.

Cell proliferation is one of the important parts in the progression of a cancer tumor. Aberrations in the regulation of a number of key pathways controlling cell proliferation are necessary for the establishment of all tumors. Deregulation of cell proliferation together with suppressed apoptosis is the minimal common platform for all cancer evolution and progression (*41*). We found that human lung cancer A549 cell proliferation was inhibited in a dose-dependent after exposure to the strawberry extracts. Dietary freeze-dried strawberries were shown to effectively inhibit *N*-nitrosomethylbenzylamine-induced tumorigensis in the rat esophagus (*41*). Several other fruits have also shown inhibition of $HepG_2$ human liver cancer cell proliferation (*42*−*44*).

JB6 P^+ cells respond to TPA treatment by cell transformation (*7*). We found that TPA efficiently induced JB6 P^+ cell transformation after 2 weeks of culture and that the addition of strawberry extracts on suppressed TPA-induced cell transformation was in a concentration-dependent manner. Strawberry extracts have also been shown effectively to inhibit benzo[*a*]-pyrene (B[a]P)-induced Syrian hamster embryo (SHE) cellular transformation (*45*).

It was suggested that transcription factors of AP-1 and NF-κB might play important roles in cell transformation (*46*). Inhibition of AP-1 and NF-κB activation by a variety of agents has also been shown to reduce neoplastic transformation (*7*, *8*, *16*, *34*). Our data show that strawberry extracts inhibit TPA- or UVB-induced AP-1 and NF-κB activation, suggesting that the inhibition of TPA-induced cell transformation by strawberry extracts may be a result of inhibition of AP-1 and/or NF-κB activation.

MAPKs are components of kinase cascades that connect extracellular stimuli to specific transcription factors, thereby converting these signals into cellular responses (*7*). In mammalian systems, there are three subgroups of MAPKs: ERKs, JNKs, and p38 MAPKs. Strawberry extracts blocked UVB-induced phosphorylation of ERKs and JNKs in a dose-dependent manner. Strawberry extracts also showed inhibition on TPA-induced phosphorylation of ERKs (**Figure 7**). Modulation of ERK response coordinately altered the activation of AP-1 and

NF-κB, and ERK is required for neoplastic transformation of JB6 P^+ cells. The results from our study indicate that ERKs may be the primary targets of strawberry extracts that lead to suppression of AP-1 and NF-κB activation, cell proliferation, and cell transformation in JB6 P^+ cells.

In conclusion, these data suggest that strawberries have high activities of SOD, G-POD, GSH-POD, and AsA-POD. These oxygen enzymes could increase the capacity of free radical scavenging. The high activity of GR may play an important role as antioxidant and could prevent the formation of S−S bonds, also providing high antioxidant activity in strawberries. Strawberry extracts inhibited TPA- and UVB-induced AP-1 and NF-κB activities, significantly suppressed TPA-induced cell transformation, and blocked TPA-induced phosphorylation of ERKs and UVB-induced phosphorylation of ERKs and JNK kinase in cell culture. Thus, strawberries may be highly effective as a chemopreventive agent that acts by targeting the down-regulation of AP-1 and NF-κB activities, blocking MAPK signaling, and suppressing cell proliferation and transformation.

ACKNOWLEDGMENT

We thank Sue Kim for technical assistance.

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Received for review December 28, 2004. Revised manuscript received March 9, 2005. Accepted March 15, 2005.

JF0478049