Inhibition of Adenoma Progression to Adenocarcinoma in a 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone–Induced Lung Tumorigenesis Model in A/J Mice by Tea Polyphenols and Caffeine

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Abstract

The present study investigated the inhibitory effects of Polyphenon E [a standardized green tea polyphenol preparation containing 65% (−)-epigallocatechin-3-gallate] and caffeine on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)–induced lung tumor progression from adenoma to adenocarcinoma. Female A/J mice were treated with a single dose of NNK (103 mg/kg body weight, i.p.) and kept for 20 weeks for the mice to develop lung adenomas. The mice were then given a solution of 0.5% Polyphenon E or 0.044% caffeine as the sole source of drinking fluid until week 52. Both treatments significantly decreased the number of visible lung tumors. Histopathologic analysis indicated that Polyphenon E administration significantly reduced the incidence (by 52%) and multiplicity (by 63%) of lung adenocarcinoma. Caffeine also showed marginal inhibitory effects in incidence and multiplicity of adenocarcinoma (by 48% and 49%, respectively). Markers of cell proliferation, apoptosis, and related cell signaling were studied by immunohistochemistry, and the labeling index and staining intensity were quantified by the Image-Pro system. Polyphenon E and caffeine treatment inhibited cell proliferation (by 57% and 50%, respectively) in adenocarcinomas, enhanced apoptosis in adenocarcinomas (by 2.6- and 4-fold, respectively) and adenomas (both by 2.5-fold), and lowered levels of c-Jun and extracellular signal-regulated kinase (Erk) 1/2 phosphorylation. In the normal lung tissues, neither agent had a significant effect on cell proliferation or apoptosis. The results show that tea polyphenols (and perhaps caffeine) inhibit the progression of NNK-induced lung adenoma to adenocarcinoma. This effect is closely associated with decreased cell proliferation, enhanced apoptosis, and lowered levels of c-Jun and Erk1/2 phosphorylation. (Cancer Res 2006; 66(23): 11494-501)

Introduction

Lung cancer is the leading cause of cancer-related deaths in the United States and one of the most common cancers worldwide (1). Although there have been new developments in lung cancer diagnosis and treatment, individuals at high risk have few options to reduce their risk apart from reducing their exposure to tobacco smoke. Chemoprevention, defined as the administration of natural or synthetic compounds to inhibit, retard, or reverse the process of carcinogenesis, could be an effective approach to reduce the risk for developing this disease (2, 3). Green tea, one of the most popular beverages in the world, is considered a promising cancer chemopreventive agent (4). Administration of green tea and its constituents to experimental animals has been shown to inhibit the formation and growth of several tumor types, including cancers of the lung, skin, oral cavity, esophagus, stomach, intestine, colon, and prostate (4, 5). However, the mechanisms of the inhibitory action of green tea are not fully understood.

In the 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)–induced lung tumor model in mice and rats, administration of green tea, black tea, or tea polyphenols significantly inhibited the development of tumors (4, 6–11). We also reported that black tea infusion inhibited the progression of pulmonary adenoma to adenocarcinoma (9). The effect of green tea constituents on the progression from lung adenoma to adenocarcinoma, however, has not been studied sufficiently. Studies *in vivo* and *in vitro* have shown the antiproliferative and proapoptotic activities of green tea and green tea polyphenols (4, 5, 12–14). These activities are proposed to be key to their tumor prevention effects. Studies on cell lines have indicated that the inhibition of tumor cell proliferation by green tea polyphenols, such as (−)-epigallocatechin-3-gallate (EGCG), may be mediated through alterations of growth-related signal transduction pathways or cell cycle arrest (14, 15). These proposed mechanisms are based on *in vitro* studies and have not been validated in a lung carcinogenesis model.

Chemopreventive activities of caffeine have been found in animal models and human studies (11, 16–21). Conney and colleagues showed that caffeine inhibited UVB-induced skin carcinogenesis primarily by selective stimulation of apoptosis (17, 22–24). Caffeine has also been shown to inhibit lung tumorigenesis (11, 20, 21), but the mechanism of the tumor-inhibitive effect of caffeine in the lung remains unclear.

Polyphenon E is a standardized green tea polyphenol preparation containing 65% EGCG. This preparation has been investigated extensively in terms of toxicology and pharmacokinetics, and is in phase I and phase II clinical chemoprevention trials at different institutions (25–28). In the present study, the effects of Polyphenon E and caffeine on the progression of NNK-induced lung adenoma to adenocarcinoma in A/J mice were examined. Cell proliferation, apoptosis, and related changes in the mitogen-activated protein kinase (MAPK) pathway were analyzed immunohistochemically.

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doi:10.1158/0008-5472.CAN-06-1497

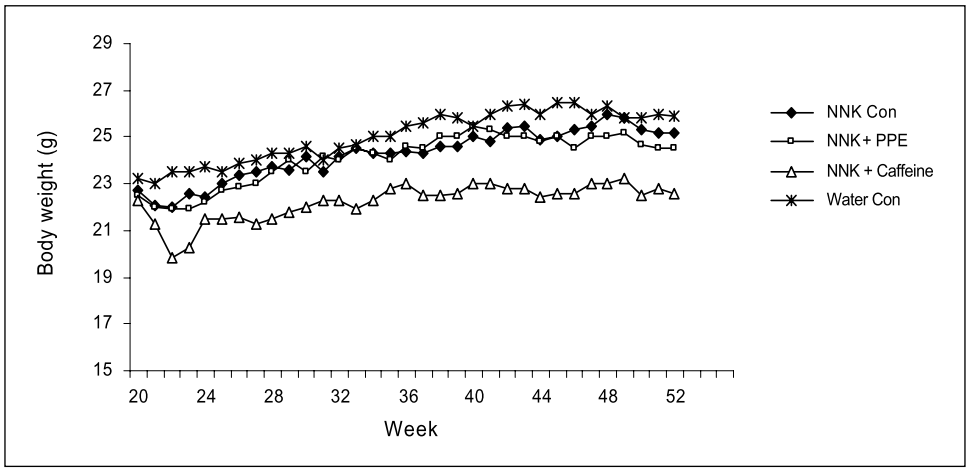

Figure 1. Effect of long-term Polyphenon E and caffeine administration on body weight. Polyphenon E (0.5%) or caffeine (0.044%) solution was given to the mice as the sole source of drinking fluid from weeks 20 to 52 after NNK injection (*Con,* control). The body weight was measured weekly. Values from the three cages per group were averaged for each time point. Significant reduction of body weight by caffeine was observed at weeks 22 and 23, and after week 27 ($P < 0.05$, Student's t test).

Materials and Methods

Reagents. NNK was obtained from Chemsyn Science Laboratories (Lenexa, KS). Polyphenon E was a gift from Dr. Yukihiko Hara (Mitsui Norin Co., Ltd., Tokyo, Japan). This is a standardized green tea polyphenol preparation, containing 65% EGCG, 7% epicatechin-3-gallate, 3% epigallocatechin, 9% epicatechin, 3% gallocatechin gallate, and >0.5% caffeine. Caffeine was obtained from Sigma-Aldrich (St. Louis, MO). AIN-93M rodent diet was purchased from Research Diets, Inc. (New Brunswick, NJ). Extracellular signal-regulated kinase (Erk) 1/2, phosphorylated (phospho)-Erk1/2, c-Jun, and phospho-c-Jun antibodies were purchased from Cell Signaling Technology (Beverly, MA). Antibody against cleaved caspase-3 was purchased from R&D Systems, Inc. (Minneapolis, MN). Anti–proliferating cell nuclear antigen (anti-PCNA) antibody was purchased from Oncogene Research Products (San Diego, CA).

Animal treatment. Female A/J mice (4-6 weeks old) were purchased from the Jackson Laboratory (Bar Harbor, ME). The animals were acclimated in our laboratory for 2 weeks and then treated with a single dose of NNK (103 mg/kg body weight, i.p.) or saline in the first experiment. They were fed an AIN-93M diet and maintained at room temperature (20 ± 2°C), at relative humidity of 50 ± 10% and with an alternating 12-hour light/dark cycle. Body weight and food consumption were measured weekly. At 20 weeks after NNK injection, by which point the mice were expected to have developed lung adenomas (29), 0.5% Polyphenon E or 0.044% caffeine solution was given to the mice as the sole source of drinking fluid until the termination of the experiment at week 52. The solution was freshly prepared and placed in the water bottle every Monday, Wednesday, and Friday. The concentration of caffeine was equivalent to its concentration in 0.6% green tea (30); 0.5% Polyphenon E was equivalent to its concentration in 1.5% tea solids. The control animals were given distilled water. The animals were sacrificed by cervical dislocation. The lungs of each animal were removed, inflated, and fixed with 10% buffered formalin. The livers and the perirenal fat pad were also removed, and their weights were measured. Visible tumors (>0.1 mm in diameter) on the surface of the lungs were counted and the sizes were measured.

Histopathologic analysis. The formalin-fixed lungs were embedded in paraffin with dorsal side facing down, so that most of the tumors would be present in 60 serial 5-μm sections. The sections were mounted on glass slides, with each slide containing two sections from all five lobes of the lung. Three slides (taken from serial sections; numbers 1, 15, and 30) from each

Table 1. The inhibitory effects of Polyphenon E and caffeine on tumor progression

Group	No.	Gross tumor data			Histopathologic tumor data			
		Incidence (%)	Multiplicity	Volume (mm^3/mouse)	Incidence (%)		Multiplicity	
					Adenoma	Adenocarcinoma	Adenoma	Adenocarcinoma
Control	11	36	0.55 ± 0.29	2.51 ± 1.71	36	0	0.44 ± 0.21	0
NNK	23	97	4.56 ± 1.25	4.39 ± 2.50	97	65 (15/23)	2.52 ± 0.78	0.91 ± 0.51
NNK + Polyphenon E	20	95	2.92 ± 1.23*	2.97 ± 2.27	95	30 (6/20)†	2.25 ± 0.65	0.35 ± 0.24*
NNK + Caffeine	24	100	2.55 ± 1.17*	2.54 ± 2.11	100	33 (8/24)	2.07 ± 0.46	0.46 ± 0.31

NOTE: After a single dose of NNK (103 mg/kg body weight, i.p.), Polyphenon E (0.5% solution) or caffeine (0.044% solution) was administered to mice as the sole source of drinking fluid from weeks 20 to 52. There were 25 mice in each NNK-treated group and 12 mice in the water control group at week 20. Two mice in the Polyphenon E treatment group and one control mouse died from spontaneous rhabdomyosarcoma within 1 week before the termination time point. Six other animals also died before the termination of the experiment, but did not seem to be related to their treatments. Values are the mean ± SE. Gross tumor data were analyzed under a dissection microscope. Tumors >0.1 mm were counted. Tumor volumes (mm^3) were measured using the formula $V = 4/3\pi r^3$, where r is the radius of the tumor determined by the mean values of the longest and shortest diameters.

*$P < 0.05$ by Student's t test compared with the NNK group.

†$P < 0.05$ by χ^2 test compared with the NNK group.

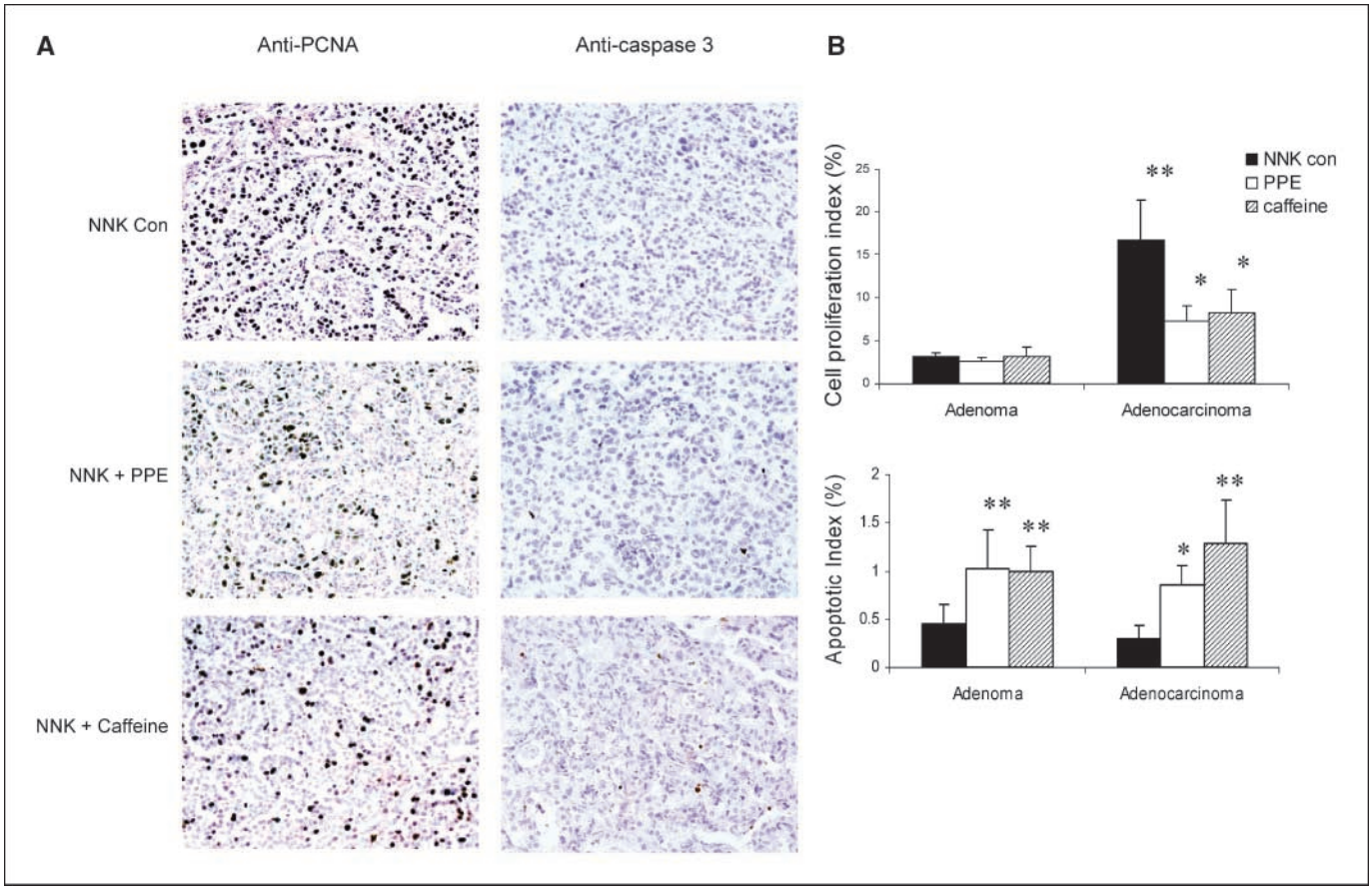

Figure 2. Inhibition of cell proliferation and induction of apoptosis by Polyphenon E and caffeine treatment. *A,* anti-PCNA and anti-cleaved-caspase 3 immunostaining of adenocarcinomas from samples of NNK control, Polyphenon E–treated, and caffeine-treated groups (×200). *B,* Polyphenon E and caffeine treatment significantly reduced PCNA labeling index in adenocarcinoma. Polyphenon E and caffeine treatment significantly induced apoptosis in both adenoma and adenocarcinoma. *Columns,* mean from 40 adenomas of each group and 21, 7, and 11 adenocarcinomas in NNK control, Polyphenon E–treated, and caffeine-treated groups, respectively; *bars,* SE. *, $P < 0.05$, Student's *t* test, compared with results of NNK control group; **, $P < 0.001$, Student's *t* test, compared with result of adenomas.

sample were stained with H&E for histopathologic assessment. The lung tumors were diagnosed based on previously established criteria and categorized as adenoma and adenocarcinoma (31).

Immunohistochemistry. Immunohistochemistry was done on lung tissue sections using phospho-Erk1/2 (1:100), phospho-c-Jun (1:100), PCNA (1:100), and cleaved-caspase 3 (1:200) antibodies to localize and to quantify the level of these markers. In brief, antigens were unmasked in 10 mmol/L sodium citrate buffer (pH 6.0) or antigen-unmasking solution (DAKO, Copenhagen, Denmark). Endogenous peroxidase was quenched using 3% H_2O_2 in distilled water. Sections were then blocked for 1 hour at room temperature in PBS containing 3% normal serum and incubated with primary antibody overnight at 4°C. Biotin-conjugated secondary antibody (1:200) and avidin-biotin peroxidase complex (1:100) were then applied to the sections. Diaminobenzidine (Sigma, St. Louis, MO) was used as a chromagen. Negative controls were processed in the absence of the primary antibody.

Image analysis. Slide images were analyzed using a Nikon research microscope linked to an Image-Pro Plus System (Media Cybernetics, Sliver Spring, MD.). In brief, each lesion was snapped in two to five images under ×20 lens (depending on the size of the lesion), and saved as a "tiff" file with 2,560 resolution. For each image, we used the "count/size" function command to perform a cell number counting operation. Basic steps were as follows: (*a*) define the area to be analyzed, (*b*) set the colors that identify the positive and counterstaining, and (*c*) automatically count the objects by computer. This generated a percentage of positive stained cells. To analyze staining intensity, we used the "histogram analysis" function to measure the area associated with a specific intensity value. This required no counterstaining (for each antibody, two sets of staining were done for staining index and intensity analysis). Basic steps were as follows: (*a*) convert the image into gray scale (black background), (*b*) define the area to be analyzed, and (*c*) analyze the area of interest. Intensity value was automatically expressed as mean ± SD. Background intensity was also analyzed by the same steps and then subtracted from the intensity of the area of interest.

Statistical analysis. The data on tumor multiplicity, labeling index, and intensity of staining were analyzed by Student's *t* test and ANOVA test by SPSS software. Effects on tumor incidence were analyzed by χ^2 test.

Results

General health status of experimental animals during long-term Polyphenon E and caffeine treatment. During the 32-week experimental period in this experiment starting from week 20, no significant differences were found in food consumption (average 2.5 g/mouse/d) among groups. A significant increase in fluid intake was found in the caffeine-treated group compared with other groups (3.5 versus 2.5 mL/mouse/d), probably due to caffeine addiction. The body weights of the mice in the caffeine treated group were ~10% lower than other groups ($P < 0.01$; Fig. 1). The lowered body weight is probably due to reduction of body fat rather than toxicity; the perirenal fat pad weights were lower in the caffeine-treated group (0.11 g/mouse) compared with the control groups (0.16 g/mouse). No signs of toxicity or differences in liver weight, expressed as a percentage of body weight, were found in any of the groups.

Inhibition of adenoma to adenocarcinoma progression by Polyphenon E and caffeine. Data on tumor incidence and histopathology are summarized in Table 1. The incidence of spontaneous tumors in the negative control group was 36%. Almost all the NNK-treated mice developed lung tumors. Treatment with Polyphenon E and caffeine had no effect on tumor incidence, but significantly reduced the number of visible lung tumors, showing 37% and 44% inhibition, respectively ($P < 0.05$). Tumor volume was also slightly reduced by Polyphenon E or caffeine treatment (32% and 42% reduction, respectively, $P < 0.1$).

Histologically, the lung tumors were categorized as adenoma or adenocarcinoma based on previously established criteria (31). Tumors containing areas of both adenoma and adenocarcinoma were categorized as adenocarcinoma, and only the adenocarcinoma field of the tumor was analyzed. Both adenomas and adenocarcinomas were observed in NNK-treated mice, whereas only adenomas were found in the mice without NNK treatment. Polyphenon E significantly reduced the incidence of adenocarcinoma (52% inhibition, $P < 0.05$) and the multiplicity of adenocarcinoma (63% reduction, $P < 0.05$) of the NNK-treated mice. Inhibition of adenocarcinoma development was also found in mice treated with caffeine, but the effect was only marginal statistically significant ($P = 0.09$ for both incidence and multiplicity). Treatment with Polyphenon E and caffeine did not affect adenoma multiplicity or incidence.

Inhibition of cell proliferation and induction of cell apoptosis by Polyphenon E and caffeine. Cell proliferation was determined by immunohistochemistry with anti-PCNA antibody, and the results were quantified by semiautomatic image analysis (Fig. 2). As the tumor progressed from adenoma to adenocarcinoma, the PCNA labeling index increased from 4.7% to 16.8% (3.6-fold increase, $P < 0.001$). Polyphenon E and caffeine treatment significantly reduced the cell proliferation index in adenocarcinomas (57% and 50% reduction, respectively; $P < 0.05$; Fig. 2), but the differences in adenomas were not significant.

Cell apoptosis was determined by immunohistochemistry with anticleaved caspase-3 antibody (Fig. 2). About 0.3% to 0.5% tumor cells were positively stained by anticleaved caspase-3 antibody, whereas in normal lung tissues, the apoptotic index was $\sim$0.1%. The apoptotic indices in adenoma and adenocarcinoma were not different. Polyphenon E and caffeine treatment significantly increased the cell apoptotic index in both adenomas (both by 2.5-fold) and adenocarcinomas (by 4- and 2.6-fold, respectively; Fig. 2). There was no induction of apoptosis in nontumor regions.

Increased c-Jun and Erk1/2 protein phosphorylation during tumor progression. The staining patterns with anti-phospho-c-Jun and anti-phospho-Erk1/2 antibodies in normal lung tissue, adenoma, and adenocarcinoma are shown in Fig. 3. Compared with adenoma cells, adenocarcinoma cells had a more densely packed architecture. They had higher nuclear/cytoplasm ratios, more mitosis, cytologic atypia, and often invaded large airways. In normal tissues, very few cells were positively stained with phospho-c-Jun antibody. In adenomas, phospho-c-Jun–positive cells were

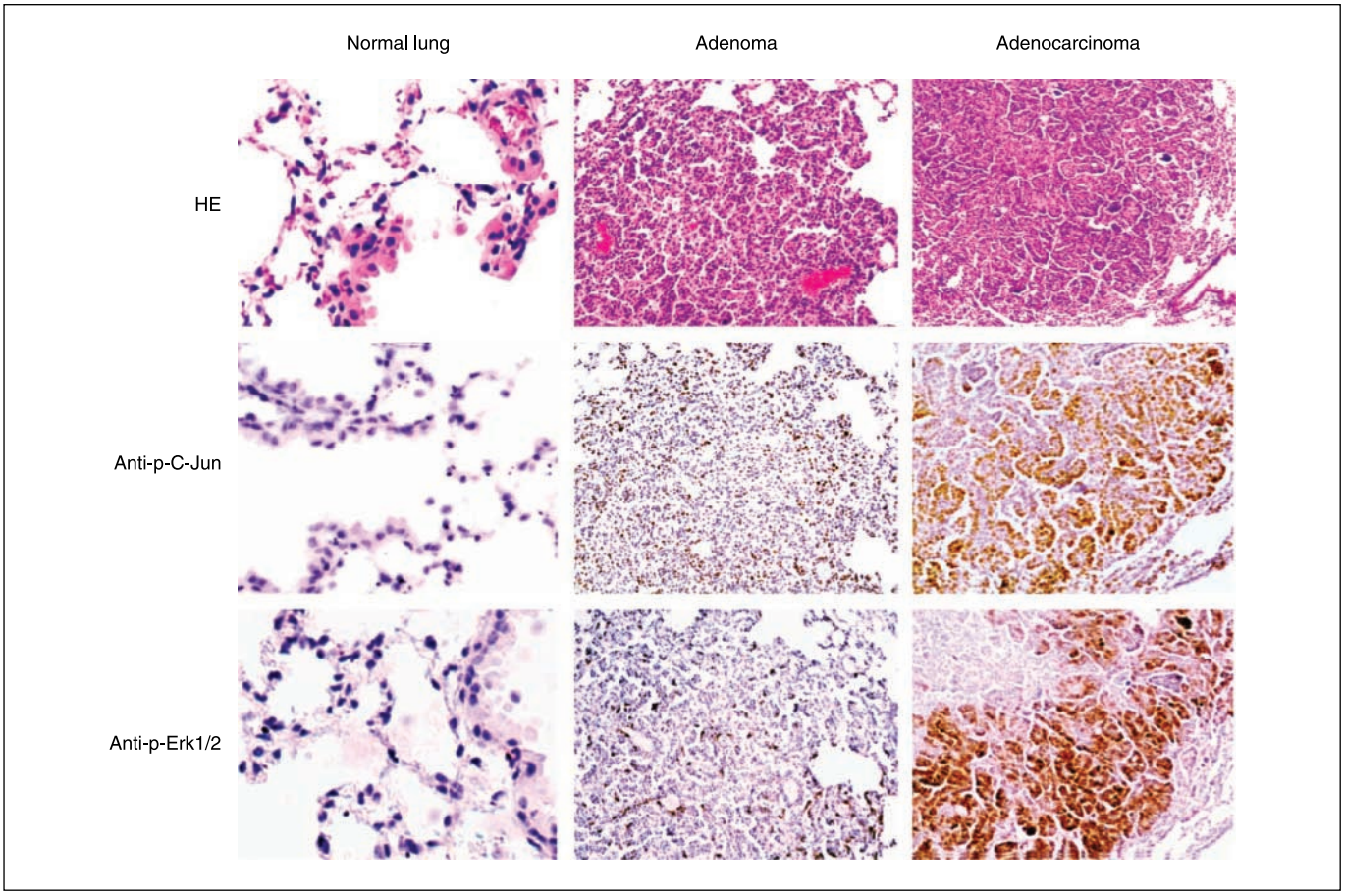

Figure 3. Phospho-c-Jun and phospho-Erk1/2 protein staining pattern in tumor progression. H&E staining of normal lung tissue (×400), adenoma (×100), and adenocarcinoma (×200); and their corresponding immunostaining with anti-p-c-Jun or anti-p-Erk1/2 antibodies.

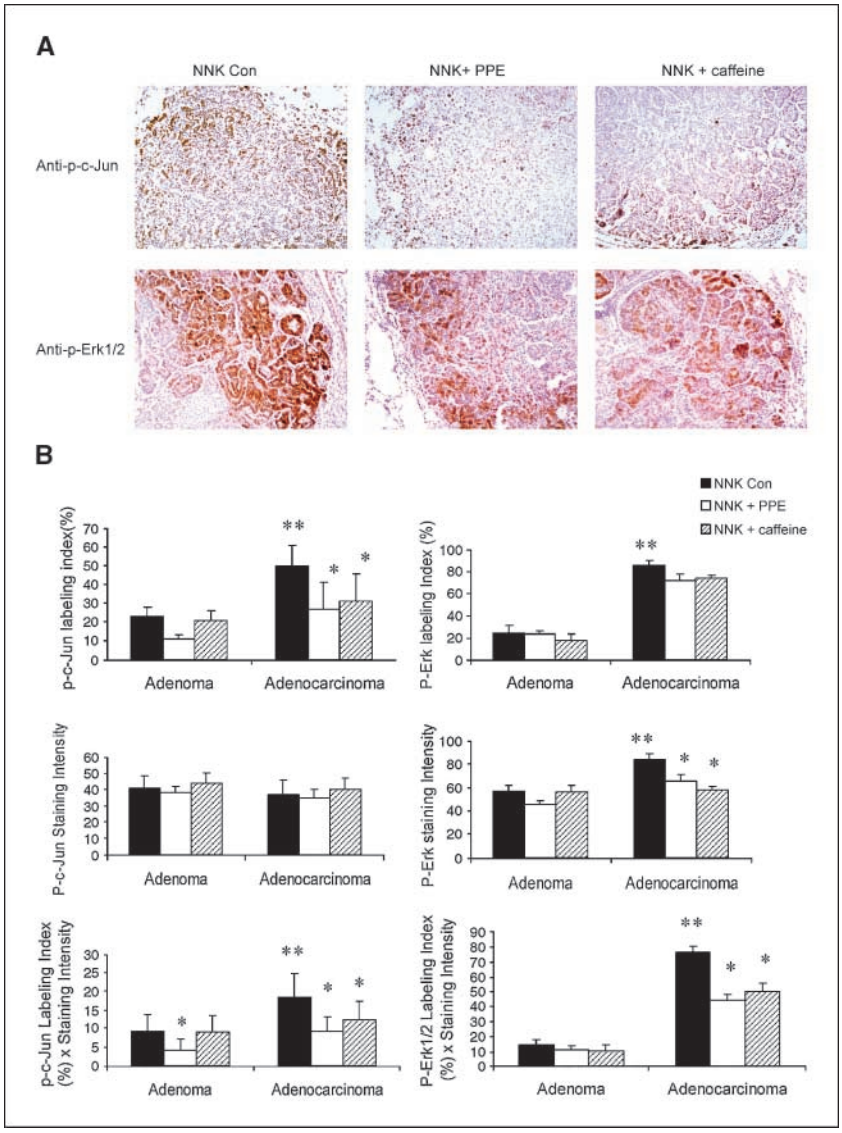

Figure 4. Inhibition of c-Jun and Erk1/2 phosphorylation by Polyphenon E and caffeine. c-Jun and Erk1/2 phosphorylation were detected by immunohistochemistry with anti-p-c-Jun and anti-p-Erk1/2 antibodies and analyzed using the Image-Pro system. *A,* phospho-c-Jun and phospho-Erk1/2 immunohistochemistry in adenocarcinomas of NNK control, Polyphenon E–treated, and caffeine-treated groups (×200). *B,* the labeling indices, staining intensity, and their multiplication for phospho-c-Jun and phospho-Erk1/2. *Columns,* mean from 40 adenomas of each group and 21, 7, and 11 adenocarcinomas in NNK control, Polyphenon E, and caffeine treatment groups, respectively; *bars,* SD. *, $P < 0.05$, Student's *t* test, compared with results of NNK control group; **, $P < 0.05$, Student's *t* test, compared with result of adenomas.

~23%. Most of them were randomly scattered around the tumor, mostly at the peripheral regions, and displayed nuclear labeling. In adenocarcinomas, over 50% of the tumor cells were positively stained for phospho-c-Jun, predominantly showing nuclear staining. The labeling index in adenocarcinomas was about twice than that in adenomas ($P < 0.05$; Figs. 3 and 4), but there was no difference in staining intensity between these two types of tumors.

Similar to the staining pattern of phospho-c-Jun, very few normal pulmonary cells were positively stained with phospho-Erk antibody. In lung adenomas, the phospho-Erk1/2 labeling index was ~25%. The phospho-Erk1/2–positive cells were randomly scattered around the tumor, mostly at the peripheral region, and showed nuclear staining. In adenocarcinomas, however, both the nuclei and cytoplasm were stained intensely. The phospho-Erk1/2 labeling index in adenocarcinomas was ~3.5-fold higher than in adenomas ($P < 0.001$; Figs. 3 and 4). The staining intensity of the phospho-Erk1/2 positively stained adenocarcinoma cells was significantly higher than that in adenoma (32% greater, $P < 0.005$; Fig. 4). The "Erk1/2 protein phosphorylation level" (labeling index × staining intensity), was ~5-fold higher in adenocarcinomas than that in adenomas in NNK-treated mice ($P < 0.001$; Figs. 3 and 4).

Adenomas within adenocarcinomas were observed, suggesting that adenocarcinomas derive from adenomas in this carcinogenesis model. As the tumor progresses, a few cells within the adenoma transformed from oval to cuboidal or columnar shape and these cells seemed to be more aggressive, and to form tubular or papillary architectures (Fig. 5). When these cells become dysplastic, the tumor would progress toward malignancy (9, 31). By immunostaining, we found that once the tumor cells became cuboidal or columnar, their phospho-Erk1/2 levels increased, especially in the cytoplasm, suggesting that Erk1/2 phosphorylation levels were closely correlated with tumor progression. This associations, however, were not found for phospho-c-Jun.

Inhibition of c-Jun and Erk1/2 phosphorylation by Polyphenon E and caffeine. As shown in Fig. 4*A*, Polyphenon E and caffeine treatment significantly reduced the phospho-c-Jun labeling index in adenocarcinoma (46% and 38% reduction, respectively, $P < 0.05$), but had little effect on the staining intensity of phospho-c-Jun–positive cells (Fig. 4). Polyphenon E

treatment also significantly reduced the phospho-c-Jun index in adenoma (by 52%, $P < 0.05$; Fig. 4*B*). The reduction found in the "c-Jun protein phosphorylation level" (labeling index × staining intensity) was mostly due to reduced labeling index (Fig. 4*B*). The total c-Jun levels were also examined by immunohistochemistry with anti-c-Jun antibody. However, either Polyphenon E or caffeine treatment reduced the total c-Jun level (data not shown), suggesting that down-regulation of phospho-c-Jun may be a posttranslational event.

Polyphenon E and caffeine treatment significantly reduced the phospho-Erk1/2 staining intensity (22% and 31% reduction, respectively, $P < 0.05$; Fig. 4), but had little effect on the percentage of phospho-Erk1/2–positive cells (Fig. 4). The "Erk1/2 protein phosphorylation level (labeling index × staining intensity) was significantly reduced by Polyphenon E and caffeine treatment ($P < 0.05$; Fig. 4). On the other hand, the treatments had little effect on either the phospho-Erk1/2 labeling index or intensity in adenomas. The total Erk1/2 levels were also examined by immunohistochemistry with anti-Erk1/2 antibody. No difference was found in Polyphenon E and caffeine-treated adenomas and adenocarcinomas compared with nontreated tumors (data not shown), suggesting the inhibition of phospho-Erk may also have occurred at posttranslational levels.

Discussion

Previous studies conducted by our laboratory and by others have indicated that the administration of tea catechins and caffeine inhibited NNK-induced lung adenoma formation (4, 6–8). The present study showed that even after lung adenomas had formed, administration of tea polyphenols inhibited the progression of adenoma to adenocarcinoma. The effect of caffeine in this study was marginal, possibly due to the large SD observed among the mice.

The administration of caffeine resulted in ~10% body weight loss, which is likely to be due to decreased body fat stores. This was consistent with our previous result (32, 33) and the observation by Lu et al. (34), and might be related to increased locomotor activity as observed by Michna et al. (35). There was no significant difference in survival rate among different treatment groups. The comparable survival rates, body weights, and the ratio of liver weight to body weight among the differently treated groups suggest that there was no toxicity associated with Polyphenon E and caffeine at the doses used.

Compared with adenomas, adenocarcinoma cells form a more tightly packed architecture, and express features typical of polymorphic malignant cells. Higher levels of phospho-c-Jun, phospho-Erk1/2, and PCNA were observed in adenocarcinomas, suggesting that MAPK-induced cell proliferation is important for tumor malignant transformation. c-Jun and Erk activation also has been consistently identified in non–small cell carcinomas in humans (36–39), suggesting molecular pathologic similarities between NNK-induced lung tumors and human non–small cell carcinomas. Thus, the NNK-induced lung tumor model seems to be a suitable model for preclinical study of cancer chemoprevention. In the NNK-induced tumorigenesis model we used, the lung tumors developed were small, usually around 0.5 mm in diameter. We used immunohistochemistry combined with semiautomated image analysis to study the level of specific markers. Both labeling index and staining intensity were analyzed, providing a practical and precise method of image analysis.

Polyphenon E (0.5%) and caffeine (0.044%) reduced adenocarcinoma multiplicity and incidence. These inhibitory effects were at least in part due to induction of apoptosis and inhibition of cell proliferation during tumor progression. Inhibition of c-Jun phosphorylation, MAPK, and activator protein-1 pathways by tea catechins has been reported in many *in vitro* studies (40–45). In the

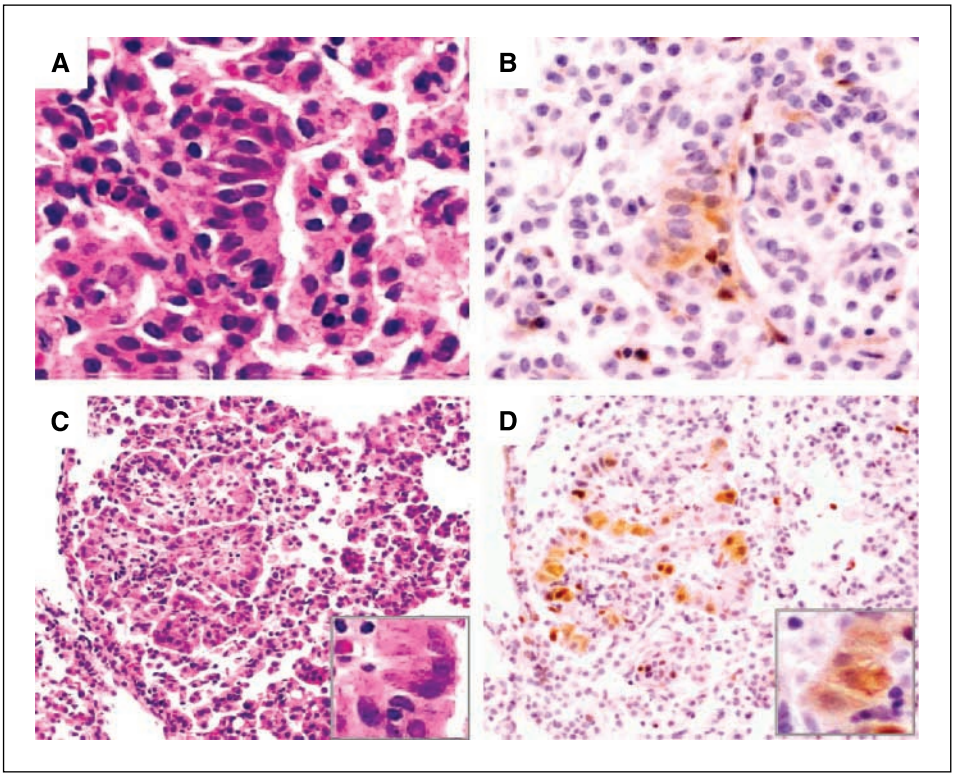

Figure 5. Erk phosphorylation correlates with tumor cell progression. *A,* once tumor cell turned into columnar shape, Erk phosphorylation began to increase in the cytoplasm. *B,* higher Erk phosphorylation level was found in dysplasia within adenoma.

present study, the observed inhibition of phospho-c-Jun and phospho-Erk activities was probably due to inhibition of phosphorylation, because total c-Jun and total Erk levels were not changed after treatments. This activity is likely to contribute to the inhibition of the progression of lung adenoma to adenocarcinoma by Polyphenon E and caffeine.

The inhibition of c-Jun and Erk1/2 phosphorylation found in this study might be the direct effects of EGCG and caffeine or the downstream events of their inhibitory activities. The genes that were affected by treatment with green tea and Polyphenon E in a similar carcinogenesis model have been reported by Lu et al. (46). Direct targets of EGCG action identified by different methods in other systems have also been reported (47, 48). The relations between these observations and the presently observed inhibition of c-Jun and Erk1/2 phosphorylation remain to be investigated.

We previously showed that black tea, given in drinking fluid, inhibited the progression of adenoma to adenocarcinoma in both malignant tumor incidence and multiplicity in a similar NNK-induced lung carcinogenesis model in A/J mice (9). Because the level of EGCG in black tea is only ~3% and the bioavailability of black tea polyphenols (theaflavins and thearubigens) is very low (4), we suggest that caffeine was a major active constituent in black tea infusion for inhibiting the progression of adenoma to adenocarcinoma in this previous study. This suggestion is consistent with the reported inhibitory effect of caffeine on NNK-induced lung tumorigenesis in rats (11). Conaway et al. (49) recently reported that phenethyl isothiocyanate and sulforaphane inhibited lung tumor progression induced by tobacco carcinogens in A/J mice. These studies suggest that tumor progression from adenoma to adenocarcinoma could be inhibited by commonly used dietary constituents, such as tea polyphenols, caffeine, and isothiocyanate. The effective use of these dietary agents for the prevention of lung tumor progression, for example, in ex-smokers, demands more attention.

Acknowledgments

Received 4/27/2006; revised 7/28/2006; accepted 9/29/2006.

Grant support: NIH grant CA88961 and facilities funded by NIH grants ES50522 and CA 72720.

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