Acta Med. Okayama, 2023
Vol. 77, No. 1, pp. 65–70

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Original Article

Effect of a Cyclooxygenase-2 Inhibitor in Combination with (−)-Epigallocatechin Gallate or Polyphenon E on Cisplatin-Induced Lung Tumorigenesis in A/J Mice

Ken Sato[a], Nagio Takigawa[b], Toshio Kubo[a], Hideki Katayama[c], Daizo Kishino[c], Toshiaki Okada[a], Akiko Hisamoto[a], Junko Mimoto[a], Nobuaki Ochi[b], Tadashi Yoshino[d], Hiroshi Ueoka[c], Mitsune Tanimoto[a], Yoshionobu Maeda[a], and Katsuyuki Kiura[a]*

Departments of [a]Hematology, Oncology, and Respiratory Medicine, [d]Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan, [b]Department of General Internal Medicine 4, Kawasaki Medical School, Okayama 700-8505, Japan, [c]Department of Medicine, Yamaguchi-Ube Medical Center, Ube, Yamaguchi 755-0241, Japan

We investigated the effects of celecoxib combined with (−)-epigallocatechin-3-gallate (EGCG) or polyphenon E in a cisplatin-induced lung tumorigenesis model. Four-week-old female A/J mice were divided into seven groups: (*i*) Control, (*ii*) 150 mg/kg celecoxib (150Cel), (*iii*) 1,500 mg/kg celecoxib (1500Cel), (*iv*) EGCG + 150 mg/kg celecoxib (EGCG + 150Cel), (*v*) EGCG + 1,500 mg/kg celecoxib (EGCG + 1500Cel), (*vi*) polyphenon E + 150 mg/kg celecoxib (PolyE + 150Cel), and (*vii*) polyphenon E + 1,500 mg/kg celecoxib (PolyE+1500Cel). All mice were administered cisplatin (1.62 mg/kg of body weight, i.p.) 1×/week for 10 weeks and sacrificed at week 30; the numbers of tumors on the lung surface were then determined. The tumor incidence and multiplicity (no. of tumors/mouse, mean ± SD) were respectively 95% and 2.15 ± 1.50 in Control, 95% and 2.10 ± 1.29 in 150Cel, 86% and 1.67 ± 1.20 in 1500Cel, 71% and 1.38 ± 1.24 in EGCG + 150Cel, 67% and 1.29 ± 1.38 in EGCG + 1500Cel, 80% and 1.95 ± 1.36 in PolyE + 150Cel, and 65% and 1.05 ± 0.10 in PolyE + 1500Cel. The combination of high-dose celecoxib with EGCG or polyphenon E significantly reduced multiplicity in cisplatin-induced lung tumors.

Key words: celecoxib, cisplatin, EGCG, lung tumor, polyphenon E

Cisplatin is widely used in the treatment of malignant diseases, but it has also been shown to be a carcinogen in experimental animals [1]. We reported the outcomes of 92 patients who underwent radiation and cisplatin-containing chemotherapy for unresectable stage III non-small-cell lung cancer, with a mean follow-up of 8.9 years. The incidence of second primary cancer was 2.4 per 100 patient-years [2]. More recently, the long-term results of the International Adjuvant Lung Cancer Trial evaluating adjuvant cisplatin-based chemotherapy in resected lung cancer were published [3], and they confirmed the significant efficacy of adjuvant chemotherapy at 5 years. However, second malignancies were observed in 9 of 932 patients in the chemotherapy arm and 2 of 935 patients in the control arm. Although there was no significant difference in incidence between the two arms, survivors should be fol-

Received April 29, 2022; accepted October 13, 2022.
*Corresponding author. Phone: +81-86-235-7227; Fax: +81-86-232-8226
E-mail: kkiura@md.okayama-u.ac.jp (K. Kiura)

Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

lowed carefully over a long term, and chemoprevention should be considered for patients who have received previous cisplatin treatment.

In chemoprevention research, there is considerable evidence that (−)-epigallocatechin-3-gallate (EGCG) inhibits enzyme activities and signal transduction pathways, resulting in the suppression of cell proliferation and the enhancement of apoptosis, as well as the inhibition of cell invasion, angiogenesis, and metastasis [4]. We observed that EGCG partially prevented cisplatin-induced lung tumorigenesis in A/J mice [5], and the multiplicity of lung tumors in the mice treated with cisplatin + EGCG was reduced to 55% of that of the mice treated with cisplatin alone.

Polyphenon E, which is a standard green tea polyphenol containing EGCG, reduced the progression of lung adenomas to adenocarcinomas [6]. It was also reported that the synergistic inhibitory action of a combination of polyphenon E and atorvastatin (a lipid-lowering agent) against 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone induced lung carcinogenesis in A/J mice [7].

Cyclooxygenase (COX)-2 has a variety of roles in the pathogenesis and progression of cancer including differentiation, apoptosis, adhesion, the extracellular matrix, and angiogenesis [8, 9]. We observed COX-2 overexpression in cisplatin-induced lung tumors and subsequently investigated the efficacy of celecoxib for preventing cisplatin-induced tumorigenesis in an A/J mouse model [10]; the results revealed that high-dose celecoxib (1,500 mg/kg diet) inhibited cisplatin-induced lung tumorigenesis in A/J mice. In the present study, we investigated the effects of celecoxib combined with EGCG and those of celecoxib combined with polyphenon E.

Materials and Methods

Animals and chemicals. A total of 203 female A/J mice (Jackson Laboratories, Bar Harbor, ME, USA), 4-weeks-old and weighing approx. 15 g were used. They were housed five or six per plastic cage and were given free access to tap water and standard laboratory chow (MF, Oriental Yeast Co., Tokyo). The mice were kept in an air-conditioned room maintained at 55 ± 10% humidity with a 12-h light/dark cycle in the Animal Center for Medical Research, Okayama University Graduate School of Medicine. The mice were treated in accordance with the guidelines of the Okayama University Institutional Committee on the Treatment of Experimental Animals. Celecoxib and cisplatin were kindly provided by Pharmacia (Tokyo) and Nippon Kayaku Co. (Tokyo), respectively. Both EGCG (> 90% purity) and polyphenon E containing EGCG (60.0%) were kindly provided by Mitsui Norin Co. (Fujieda, Japan).

The EGCG (1 mg/ml in tap water) and polyphenon E (1.7 mg/ml in tap water) were provided to the mice as drinking water. The experimental diets that included celecoxib (150 mg/kg diet, 1,500 mg/kg diet) were prepared by mixing celecoxib into the standard chow.

Experimental design. In Experiment 1, sixty mice were divided into three groups of 20 mice each, who received the following: (*i*) no treatment (the Control group), (*ii*) treatment with celecoxib 150 mg/kg (the 150Cel group), and (*iii*) treatment with EGCG (1 mg/ml in tap water) + celecoxib 1,500 mg/kg (the 1500Cel group). Mice were administered cisplatin (1.62 mg/kg of body weight, i.p.) once a week for 10 weeks between 7 and 16 weeks of age. All mice were sacrificed at week 30 and the number of tumors on the lung surface of each mouse was determined.

In Experiment 2, 143 mice were divided into seven groups of 20 or 21 mice each, who received the following: (*i*) no treatment (Control group), (*ii*) celecoxib 150 mg/kg diet (150Cel group), (*iii*) celecoxib 1,500 mg/kg diet (1500Cel group), (*iv*) EGCG 0.1% in tap water + celecoxib 150 mg/kg diet (EGCG + 150Cel group), (*v*) EGCG 0.1% in tap water + celecoxib 1,500 mg/kg diet (EGCG + 1500Cel group), (*vi*) polyphenon E 0.17% in tap water + celecoxib 150 mg/kg diet (PolyE + 150Cel group), and (*vii*) polyphenon E 0.17% in tap water + celecoxib 1,500 mg/kg diet (PolyE + 1500Cel group). All mice were administered cisplatin (1.62 mg/kg of body weight, i.p.) once a week for 10 weeks between 7 and 16 weeks of age.

Mice in the corresponding group freely accessed tap water (control), EGCG (1 mg/ml in tap water) or polyphenon E (1.7 mg/ml in tap water), and the standard chow (control), low-dose celecoxib (150 mg/kg diet), or high-dose celecoxib (1,500 mg/kg diet). All mice were sacrificed at week 30, and the number of tumors on the lung surface of each mouse was determined.

Immunohistochemistry. The lungs were inflated with 10% formalin, embedded in paraffin, and cut into 4-μm-thick sections. Tissue sections were deparaffinized in xylene and washed in ethanol. Endogenous peroxidase activity was then inhibited using 0.3% H_2O_2

followed by antigen retrieval. Slides were blocked with rabbit serum antibody (Vector Laboratories, Burlingame, CA, USA). A polyclonal rabbit anti-murine COX-2 primary antibody (Cayman Chemical, Ann Arbor, MI, USA) was added at a 1:200 dilution. Biotin-conjugated secondary antibody (Vector Laboratories) was applied and incubated with avidin biotin enzyme reagent (Vector Laboratories). Primary Ki-67 antibody was added at a 1:5,000 dilution. Then, diaminobenzidine (DAB, Vector Laboratories) as a peroxidase substrate was applied. The sections were rinsed in H_2O, counterstained with methyl green, dehydrated, and covered with a xylene-based mounting medium. COX-2 and Ki-67 expressions were evaluated in >100 cells from each tumor.

Statistical analyses. The chi-square test and Mann-Whitney test were used to assess the tumor incidence and tumor multiplicity, respectively. The differences in weight loss among the groups were evaluated by the Cochran Cox test. The results are expressed as mean ± standard deviation (SD), and p-values <0.05 were deemed significant.

Results

Experiment 1. Table 1 shows the tumor incidence and multiplicity in each group. The tumor incidence was 20% (4/20) in the Control group, 85% (17/20) in the 150Cel group, and 75% (15/20) in the 1500Cel group. In the 150Cel group, 2.50 ± 1.96 lung tumors per mouse were observed, and in the 1500Cel group, there were 1.60 ± 1.54 tumors per mouse. However, there was no significant difference in multiplicity between the 150Cel and 1500Cel groups (p = 0.066). Treatment with cisplatin or cisplatin + EGCG did not lead to significant weight loss compared with the controls (Fig. 1).

Experiment 2. Table 2 shows the tumor incidence and multiplicity in each group. The tumor incidence was 95% (19/20) in Control group, 95% (19/20) in the 150Cel group, 86% (18/21) in the 1500Cel group, 71% (15/21) in the EGCG + 150Cel group, 67% (14/21) in the EGCG + 1500Cel group, 80% (16/20) in the PolyE + 150Cel group, and 65% (13/20) in the PolyE + 1500Cel group. Celecoxib (1,500 mg/kg) when combined with polyphenon E significantly reduced tumor incidence in mice treated by cisplatin (p < 0.05, Control vs. PolyE + 1500Cel group). The tumor multiplicity (number of tumors/mouse) values were 2.15 ± 1.50 (Control), 2.10 ± 1.29 (150Cel), 1.67 ± 1.20 (1500Cel), 1.38 ± 1.24 (EGCG + 150Cel), 1.29 ± 1.38 (EGCG + 1500Cel), 1.95 ± 1.35 (PolyE + 150Cel), and 1.05 ± 0.10 (PolyE + 1500Cel). Celecoxib (1,500 mg/kg)

Table 1 The inhibitory effects of EGCG on gross tumor incidence and multiplicity on cisplatin-induced lung tumorigenesis in A/J mice

Group	Incidence[a]	Multiplicity[b]	
1. Control	20% (4/20)	0.35 ± 0.75	
2. Cisplatin	85% (17/20)	2.50 ± 1.96	p = 0.066
3. Cisplatin + EGCG	75% (15/20)	1.60 ± 1.54	

[a], Number of tumorigenesis mouse/mouse; [b], Number of tumors per mouse, mean ± SD.

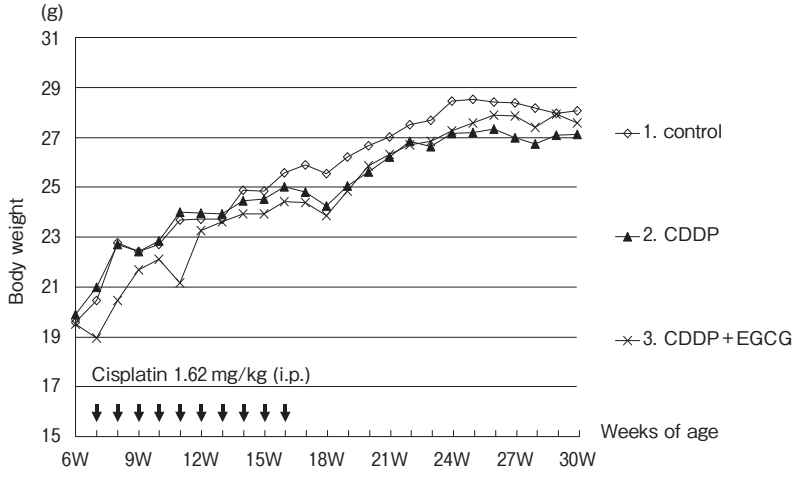

Fig. 1 Change in body weight of the three groups over the treatment period in Experiment 1. *Arrows*, the time points of the intraperitoneal (i.p._ administration of cisplatin (1.62 mg/kg body weight).

combined with EGCG or polyphenon E significantly reduced tumor multiplicity in mice treated with cisplatin ($p < 0.05$, Control vs. EGCG + 1500Cel, and Control vs. PolyE + 1500Cel). As illustrated in Figure 2, celecoxib (150 mg/kg diet) also significantly prevented cisplatin-induced weight loss ($p < 0.05$, Control vs. 150Cel).

Brown-stained tumor cells indicate an overexpression of COX-2 (Fig. 3). The numbers of COX-2-positive cells were not significantly different among the treatment arms (A, cisplatin; B, cisplatin + EGCG; C, cisplatin + celecoxib (1,500 mg/kg) + EGCG; D, cisplatin + celecoxib (1,500 mg/kg) + polyphenon E). Figure 4 shows cells stained with Ki-67 antibody; brown staining indicates an overexpression of Ki-67. There were no significant differences in the number of the cell numbers expressing Ki-67 among the groups (A, cisplatin; B, cisplatin + EGCG; C, cisplatin + celecoxib (1,500 mg/kg) + EGCG; D, cisplatin + celecoxib (1,500 mg/kg) + polyphenon E).

Table 2 Tumor Incidence and multiplicity

Group	Incidence[a]		Multiplicity[b]	
1	95% (19/20)	#	2.15 ± 1.50	*
2	95% (19/20)		2.10 ± 1.29	
3	86% (18/21)		1.67 ± 1.20	
4	71% (15/21)		1.38 ± 1.24	
5	67% (14/21)		1.29 ± 1.38	
6	80% (16/20)		1.95 ± 1.36	
7	65% (13/20)		1.05 ± 0.10	

a, Number of tumorigenesis mouse/mouse; b, Number of tumors per mouse, mean ± SD; 1, cisplatin; 2, cisplatin + celecoxib 150 mg/kg; 3, cisplatin + celecoxib 1,500 mg/kg; 4, cisplatin + EGCG + celecoxib 150 mg/kg; 5, cisplatin + EGCG + celecoxib 1,500 mg/kg; 6, cisplatin + polyphenon E + celecoxib 150 mg/kg; 7, cisplatin + polyphenon E + celecoxib 1,500 mg/kg; Statistical significance determined by χ^2 test, #, $p < 0.05$; statistical significance determined by Mann-Whitney, *, $p < 0.05$

Discussion

Our present findings demonstrated that the combination of high-dose celecoxib with EGCG or polyphenon E reduced multiplicity in cisplatin-induced lung tumors, and the combination of high-dose celecoxib with polyphenon E decreased the tumor incidence. To our knowledge, this is the first report showing that the use of a COX-2 inhibitor in combination with EGCG or polyphenon E prevented cisplatin-induced tumorigenesis in a mouse model.

In patients with familial adenomatous polyposis (who have a nearly 100 percent risk of colorectal cancer), treatment with celecoxib significantly reduced the number of colorectal polyps [11]. Concerning chemoprevention for lung cancer, the intake of selective COX-2 inhibitors (celecoxib or rofecoxib) for ≥ 2 years produced a significant risk reduction (60%) of human lung cancer in a case control study [12]. Ki-67, which is a proliferation marker expressed in all phases of the cell cycle except in resting cells, has been studied as surrogate endpoint biomarker in many chemoprevention trials [13]. A placebo-controlled trial showed that an intervention with celecoxib (400 mg bid for 6 months) for the chemoprevention of lung cancer in former-smokers significantly reduced the Ki-67 labeling

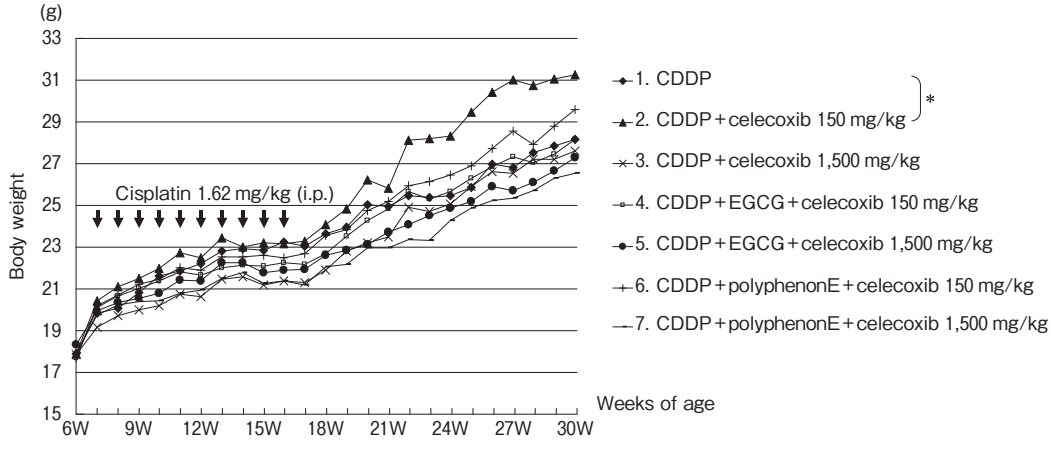

Fig. 2 Change in body weight of the seven groups over the treatment period in Experiment 2. *$p < 0.05$ by the Cochran Cox text. *Arrows*, the time points of the i.p. administration of cisplatin (1.62 mg/kg body weight).

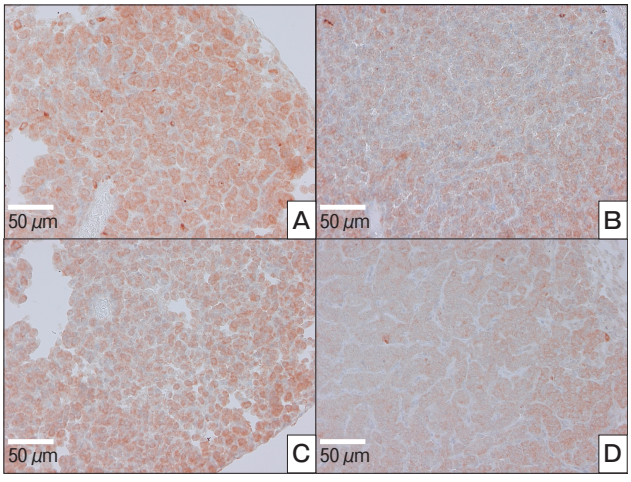

Fig. 3 Cisplatin-induced tumors were stained with COX-2 antibody. Brown-stained tumor cells overexpress COX-2. No significant difference was produced by the treatment. **A**, cisplatin; **B**, cisplatin + EGCG (0.1% in tap water); **C**, cisplatin + EGCG (0.1% in tap water) + celecoxib (1,500 mg/kg diet); **D**, cisplatin + polyphenon E (0.17% in tap water) + celecoxib (1,500 mg/kg diet).

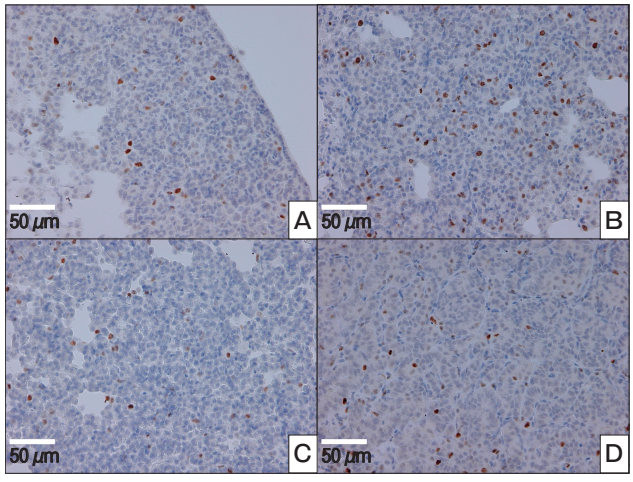

Fig. 4 Cisplatin-induced tumors were stained with Ki-67 antibody. The brown-stained tumor cells overexpress Ki-67. No significant difference was produced by the treatment. **A**, cisplatin; **B**, cisplatin + EGCG (0.1% in tap water); **C**, cisplatin + EGCG (0.1% in tap water) + celecoxib (1,500 mg/kg diet); **D**, cisplatin + polyphenon E (0.17% in tap water) + celecoxib (1,500 mg/kg diet).

index in the specimens of bronchial biopsies [14]. The Ki-67 response was associated with the computed tomography-measured response of lung nodules, which were defined as non-calcified pulmonary nodules ≥4-mm-diameter of solid, ground-glass, or part-solid attenuation. The Ki-67 responders, who were defined as showing a significant reduction of bronchial epithelial Ki-67 by celecoxib, had a significantly higher ratio of COX-2 to 15-hydroxyprostaglandin dehydrogenase mRNA compared to the non-responders. Ki-67 and COX-2 were thus considered to be important markers in the chemoprevention trials for lung cancer.

Kim *et al.* showed that celecoxib could reduce the Ki-67 expression at a high dose (400 mg bid) but not at a lower dose (200 mg bid) in the bronchial epithelium of current and former smokers [15]. In human colorectal cancer cell lines, EGCG significantly inhibited constitutive COX-2 mRNA and protein overexpression [16, 17]. In our present study's A/J mice, the administration of celecoxib could not reduce the Ki-67 expression or the COX-2 expression when used in combination with EGCG or polyphenon E. The mechanisms underlying the inhibition of tumorigenesis in our cisplatin-induced murine lung tumor model by the combination treatment in terms of angiogenesis [18], apoptosis [19], and inflammation [20] remain to be elucidated.

Our present results demonstrated that low-dose celecoxib significantly prevented cisplatin-induced weight loss. The COX-2 inhibitor JTE-522 has been reported to prevent body weight loss in rats with N-nitrosomethylbenzylamine-induced tumors [21], choline-deficient rats, and rats receiving L-amino acid [22]. A phase II clinical trial showed that celecoxib significantly increased the lean body mass, and a significant decrease in tumor necrosis factor-α was demonstrated [23]. Low-dose celecoxib (150 mg/kg) but not high-dose celecoxib (1,500 mg/kg) significantly prevented cisplatin-induced weight loss (Control vs. 150Cel groups in Fig. 2). High-dose celecoxib seemed to reduce weight compared with low-dose celecoxib (150Cel vs. 1500Cel, EGCG + 1500Cel vs. EGCG + 150Cel, PolyE + 150Cel vs. PolyE + 1500Cel in Fig. 2) although there were no significant differences. We suspect that high-dose celecoxib may be toxic in mice. The mechanism by which low-dose celecoxib prevented the loss of body weight in our mouse model remains to be clarified.

In conclusion, the COX-2 inhibitor in combination with EGCG or polyphenon E prevented cisplatin-induced tumorigenesis in our mouse model. The mechanisms of the effect should be further pursued. Notably, the combination of celecoxib with EGCG strongly induced the expression of *growth arrest and DNA damage-inducible 153* (*GADD153*) and apoptosis in lung cancer cell lines [24]. We plan to examine the signaling

to apoptosis in our model.

Acknowledgments. This study was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (no. 21590995 to N. Takigawa and no. 21659209 to K. Kiura).

References

1. Leopold WR, Batzinger RP, Miller EC, Miller JA and Earhart RH: Mutagenicity, tumorigenicity, and electrophilic reactivity of the stereoisomeric platinum(II) complexes of 1, 2-diaminocyclohexane. Cancer Res (1981) 41: 4368–4377.
2. Takigawa N, Kiura K, Segawa Y, Watanabe Y, Kamei H, Moritaka T, Shibayama T, Ueoka H, Gemba K, Yonei T, Tabata M, Shinkai T, Hiraki S, Takemoto M, Kanazawa S, Matsuo K and Tanimoto M: Second primary cancer in survivors following concurrent chemoradiation for locally advanced non-small-cell lung cancer. Br J Cancer (2006) 95: 1142–1144.
3. Arriagada R, Dunant A, Pignon JP, Bergman B, Chabowski M, Grunenwald D, Kozlowski M, Le Pechoux C, Pirker R, Pinel MI, Tarayre M and Le Chevalier T: Long-term results of the international adjuvant lung cancer trial evaluating adjuvant Cisplatin-based chemotherapy in resected lung cancer. J Clin Oncol (2010) 28: 35–42.
4. Yang CS, Wang X, Lu G and Picinich SC: Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. Nat Rev Cancer (2009) 9: 429–439.
5. Mimoto J, Kiura K, Matsuo K, Yoshino T, Takata I, Ueoka H, Kataoka M and Harada M: (−)-Epigallocatechin gallate can prevent cisplatin-induced lung tumorigenesis in A/J mice. Carcinogenesis (2000) 21: 915–919.
6. Lu G, Liao J, Yang G, Reuhl KR, Hao X and Yang CS: 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. Cancer Res (2006) 66: 11494–11501.
7. Lu G, Xiao H, You H, Lin Y, Jin H, Snagaski B and Yang CS: Synergistic inhibition of lung tumorigenesis by a combination of green tea polyphenols and atorvastatin. Clin Cancer Res (2008) 14: 4981–4988.
8. Taketo MM: Cyclooxygenase-2 inhibitors in tumorigenesis (part I). J Natl Cancer Inst (1998) 90: 1529–1536.
9. Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M and DuBois RN: Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell (1998) 93: 705–716.
10. Okada T, Takigawa N, Kishino D, Katayama H, Kuyama S, Sato K, Mimoto J, Ueoka H, Tanimoto M and Kiura K: Selective cyclooxygenase-2 inhibitor prevents cisplatin-induced tumorigenesis in A/J mice. Acta Med Okayama (2012) 66: 245–251.
11. Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK and Levin B: The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med (2000) 342: 1946–1952.
12. Harris RE, Beebe-Donk J and Alshafie GA: Reduced risk of human lung cancer by selective cyclooxygenase 2 (COX-2) blockade: results of a case control study. Int J Biol Sci (2007) 3: 328–334.
13. Lee JJ, Liu D, Lee JS, Kurie JM, Khuri FR, Ibarguen H, Morice RC, Walsh G, Ro JY, Broxson A, Hong WK and Hittelman WN: Long-term impact of smoking on lung epithelial proliferation in current and former smokers. J Natl Cancer Inst (2001) 93: 1081–1088.
14. Mao JT, Roth MD, Fishbein MC, Aberle DR, Zhang ZF, Rao JY, Tashkin DP, Goodglick L, Holmes EC, Cameron RB, Dubinett SM, Elashoff R, Szabo E and Elashoff D: Lung cancer chemoprevention with celecoxib in former smokers. Cancer Prev Res (Phila) (2011) 4: 984–993.
15. Kim ES, Hong WK, Lee JJ, Mao L, Morice RC, Liu DD, Jimenez CA, Eapen GA, Lotan R, Tang X, Newman RA, Wistuba II and Kurie JM: Biological activity of celecoxib in the bronchial epithelium of current and former smokers. Cancer Prev Res (Phila) (2010) 3: 148–159.
16. Shimizu M, Deguchi A, Joe AK, McKoy JF, Moriwaki H and Weinstein IB: EGCG inhibits activation of HER3 and expression of cyclooxygenase-2 in human colon cancer cells. J Exp Ther Oncol (2005) 5: 69–78.
17. Peng G, Dixon DA, Muga SJ, Smith TJ and Wargovich MJ: Green tea polyphenol (−)-epigallocatechin-3-gallate inhibits cyclooxygenase-2 expression in colon carcinogenesis. Mol Carcinog (2006) 45: 309–319.
18. Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM, Edwards DA, Flickinger AG, Moore RJ and Seibert K: Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res (2000) 60: 1306–1311.
19. Liu XH, Yao S, Kirschenbaum A and Levine AC: NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-regulates bcl-2 expression in LNCaP cells. Cancer Res (1998) 58: 4245–4249.
20. Kisley LR, Barrett BS, Dwyer-Nield LD, Bauer AK, Thompson DC and Malkinson AM: Celecoxib reduces pulmonary inflammation but not lung tumorigenesis in mice. Carcinogenesis (2002) 23: 1653–1660.
21. El-Bayoumy K, Iatropoulos M, Amin S, Hoffmann D and Wynder EL: Increased expression of cyclooxygenase-2 in rat lung tumors induced by the tobacco-specific nitrosamine 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanone: the impact of a high-fat diet. Cancer Res (1999) 59: 1400–1403.
22. Yamamoto H, Kondo M, Nakamori S, Nagano H, Wakasa K, Sugita Y, Chang-De J, Kobayashi S, Damdinsuren B, Dono K, Umeshita K, Sekimoto M, Sakon M, Matsuura N, Monden M: JTE-522, a cyclooxygenase-2 inhibitor, is an effective chemopreventive agent against rat experimental liver fibrosis1. Gastroenterology (2003) 125: 556–571.
23. Mantovani G, Maccio A, Madeddu C, Serpe R, Antoni G, Massa E, Dessi M, Panzone F: Phase II nonrandomized study of the efficacy and safety of COX-2 inhibitor celecoxib on patients with cancer cachexia. J Mol Med (Berl) (2010) 88: 85–92.
24. Suganuma M, Kurusu M, Suzuki K, Tasaki E, Fujiki H: Green tea polyphenol stimulates cancer preventive effects of celecoxib in human lung cancer cells by upregulation of GADD153 gene. Int J Cancer (2006) 119: 33–40.