Dietary Polyphenols May Affect DNA Methylation

Mingzhu Fang, Dapeng Chen, and Chung S. Yang*

Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854-8020

Abstract

Certain dietary polyphenols, such as (−)-epigallocatechin 3-gallate (EGCG) from green tea and genistein from soybean, have been demonstrated to inhibit DNA methyltransferases (DNMT) in vitro. This inhibitory activity is associated with the demethylation of the CpG islands in the promoters and the reactivation of methylation-silenced genes such as p16\(^{ink4a}\), retinoic acid receptor β, O\(^6\)-methylguanine methyltransferase, human mutL homolog 1, and glutathione S-transferase-π. These activities have been observed in human esophageal, colon, prostate, and mammary cancer cell lines, and the activity can be enhanced by the presence of histone deacetylase inhibitors or by a longer-term treatment. Many other polyphenolic compounds have lower activities in inhibiting DNMT. Catechol polyphenols may indirectly inhibit DNMT by generating S-adenosyl-L-homocysteine on their methylation by S-adenosyl-L-methionine. In theory, prevention or reversal of hypermethylation-induced inactivation of key tumor suppression genes or receptor genes by DNMT inhibitors could be an effective approach for cancer prevention. Because of the rather low bioavailability of most polyphenolic compounds, how much of an effect dietary polyphenols would have on DNA methylation in humans is not clear. The effect of normal dietary consumption of a single polyphenolic compound is probably insignificant. However, the combination of polyphenols with dietary histone deacetylase inhibitors and the additive effect of different dietary chemicals may produce some effects. On the other hand, the consumption of excessive amounts of polyphenols in dietary supplements may affect DNA methylation status. All these possibilities remain to be examined.


DNA methylation, primarily at the C5 position of cytosine, affects gene expression in many biological processes such as differentiation, genomic imprinting, DNA mutation, and DNA repair (1–3). DNA hypermethylation, usually occurring at promoter CpG islands, is a major epigenetic mechanism in silencing the expression of genes (4–7). The importance of promoter hypermethylation as well as global hypomethylation in carcinogenesis has been extensively discussed (5–9).

In the past several years, our laboratory has studied DNA hypermethylation during the course of human esophageal carcinogenesis using resected esophageal samples and esophageal biopsy samples from Linxian (now named Linzhou City), a high esophageal cancer incidence area in northern China (10–14). We found that genes such as O\(^6\)-methylguanine methyltransferase (MGMT), retinoic acid receptor β (RARβ), the tumor suppressor p16\(^{ink4a}\), and the DNA repair gene hMLH1 were frequently inactivated by hypermethylation in early lesions of esophageal basal cell hyperplasia. In samples with more advanced lesions, dysplasia, and squamous cell carcinoma, the frequencies of hypermethylation of these genes were even higher, and additional genes were inactivated by DNA hypermethylation. These events, as illustrated in Figure 1, together with p53 mutations and Rb aberrations, are believed to contribute to carcinogenesis (10–14). DNA hypermethylation and the inactivation of many of these genes also occurred in esophageal cancer cell lines such as KYSE 510 and KYSE 150; some of these events were reversed by treatment with 2′-deoxy-5-azacytidine (DAC), an inhibitor of 5-cytosine DNA methyltransferase (DNMT).

1 Published in a supplement to The Journal of Nutrition. Presented as part of the International Research Conference on Food, Nutrition, and Cancer held in Washington, DC, July 13–14, 2006. This conference was organized by the American Institute for Cancer Research and the World Cancer Research Fund International and sponsored by (in alphabetical order) the California Walnut Commission; Campbell Soup Company; Cranberry Institute; Hormel Institute; IP-6 International, Inc.; Kyushu University, Japan Graduate School of Agriculture; National Fisheries Institute; and United Soybean Board. Guest editors for this symposium were Vay Liang W. Go, Susan Higginbotham, and Ivana Vucenik. Guest Editor Disclosure: V.L.W. Go, no relationships to disclose; S. Higginbotham and I. Vucenik are employed by the conference sponsor, the American Institute for Cancer Research.

2 Author Disclosure: No relationships to disclose.

3 This work was supported by NIH grants CA105331 and CA88961.

4 To whom correspondence should be addressed. E-mail: csyang@rci.rutgers.edu.

5 Abbreviations used: COMT, catechol O-methyltransferase; DAC, 2′-deoxy-5-azacytidine; DAPK1, death-associated protein kinase 1; DMeEGCG, 4′,4′-dimethyl EGCG; DNMT, 5-cytosine DNA methyltransferase; EC, (−)-epicatechin; EGC, (−)-epigallocatechin-3-gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin-3-gallate; ERα, estrogen receptor α; GSTP1, glutathione S-transferase-π; HDAC, histone deacetylase; hMLH1, human mutL homolog 1; MeEGCG, 4′-methyl EGCG; MGMT, O\(^6\)-methylguanine methyltransferase; RARβ, retinoic acid receptor β; RASSF1A, Ras association domain family 1A; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosylmethionine; SFN, sulforaphane; TSA, trichostatin.
The development of DNMT inhibitors such as DAC and zebularine as cancer therapeutic agents is a very active research field (8,15,16). In theory, prevention or reversal of hypermethylation-induced inactivation of key tumor suppression genes or receptor genes by DNMT inhibitors could be an effective approach for cancer prevention. Strong inhibitors such as DAC and zebularine may not be suitable for this purpose because of their toxicity. Therefore, we looked into dietary constituents such as (−)-epigallocatechin-3-gallate (EGCG), the most abundant and active polyphenol in green tea, and genistein, a well-studied isoflavone from soy. Previously, Day et al. (17) reported that the consumption of a genistein diet by mice was positively correlated with changes in prostate DNA methylation at CpG islands as determined by differential methylation hybridization.

Inhibition of catechol O-methyltransferases and DNMT by EGCG and analogs

EGCG has been well studied for its anticancer activities but the mechanisms are not well understood (18–20). In our studies on the biotransformation of tea polyphenols, we found that EGCG is readily methylated by S-adenosylmethionine (SAM) at the 4′ and 4″ positions in the presence of catechol O-methyltransferase (COMT) (21–23). Demethylation of SAM results in the formation of S-adenosyl-L-homocysteine (SAH). Through the use of 3,4-dihydroxy-β-phenylalanine and catechol estrogens as substrates for COMT, EGCG was found to be a mixed-type (competitive and noncompetitive) inhibitor (23,24).

Because COMT and DNMT belong to the same superfamily of SAM-dependent methyltransferases with common core structures, we suspected that EGCG may also be an inhibitor of DNMT. With nuclear extracts from KYSE 510 cells as the source of DNMT and poly(dI-dC)-poly(dI-dC) as the substrate, EGCG was found to be a competitive inhibitor of DNMT with a K_i of 4.8 μM (Fig. 2). [This value is slightly lower than the value reported previously (25).] The present K_m of 8.79 nmol/mg, is more accurate than our previous value because of an error in our previous calculation.] EGCG structural analogs from green tea, (−)-epicatechin-3-gallate (EC), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC) as well as EGCG metabolites 4′-methyl EGCG (MeEGCG) and 4′,4″-dimethyl EGCG (DiMeEGCG), all inhibited DNMT dose-dependently: EGCG > ECG, MeEGCG > EGC, and DiMeEGCG > EC (25). Molecular modeling of the interaction between EGCG and DNMT revealed a substantial interactive region with hemimethylated DNA and a cytosine-active pocket for subsequent methylation (25). Docking of EGCG into this pocket indicated that the gallate moiety (D-ring) was oriented at approximately the same position as the pyrimidyl ring of cytosine, with possible hydrogen bond formation with Glu_1265 and Pro_1223, in the structural model of DNMT1. In addition, possible hydrogen bond formation between the hydroxyl groups of the EGCG A and B rings with Ser_1229 and Cys_1235, respectively, also may have contributed to the high-affinity binding. This model can also explain the lower inhibitory activities of EGCG analogs and metabolites.

Reversal of hypermethylation and reactivation of RARβ, MGMT, p16INK4a, and hMLH1 genes by EGCG

In KYSE 510 cells, p16INK4a, RARβ, MGMT, and hMLH1 genes are hypermethylated at the CpG islands in the promoter regions, and the genes are inactivated. The unmethylation-specific bands of these 4 genes appeared after treatment of the cells with 20 or 50 μM EGCG for 6 d (25). Corresponding to the appearance of the unmethylation-specific bands was the reexpression of mRNA of these genes. The reversal of hypermethylation and reactivation of these genes by EGCG were similar to those produced by the classical DNMT inhibitor DAC. After treatment with 20 μM EGCG for 48 h, unmethylation-specific bands for these genes began to appear. The mRNA expression of these genes was also observed at 48 h; higher levels were observed at either 72 or 144 h depending on the genes. The reactivation of RARβ and hMLH1 was also demonstrated at the protein level by Western blots. The abilities of EGCG analogs and metabolites to reactivate RARβ mRNA roughly correlated to their inhibitory activities against DNMT (25). We also demonstrated the reactivation of RARβ in esophageal cancer cell line KYSE 150 and prostate cancer cell line PC3 as well as the activation of p16INK4a in colon cancer cell line HT-29 after treatment with 20 μM EGCG for 6 d (25).

The inhibition of DNMT and the partial demethylation of hypermethylated RARβ by EGCG were subsequently demonstrated in breast cancer cell lines MCF-7 and MDA-MB-231 cells (26). Similarly, EGCG or green tea polyphenols treatment was shown to cause the activation of glutathione S-transferase-π gene (GSTP1) in prostate cancer LNCaP cells (27,28). However, the effect of EGCG may be gene specific or cell line specific and
was not as robust as DAC (29). Significant demethylation and activation of several genes by EGCG were not observed by Chuang et al. (29) and Stresemann et al. (30). Mittal et al. (31) reported that topical applications of EGCG to the mouse skin inhibited UVB-induced global DNA hypomethylation. Because global DNA hypomethylation has been reported to be associated with hypermethylation and inactivation of specific genes during carcinogenesis (9), this observation is not necessarily contradictory to the concept that EGCG can prevent or reverse the hypermethylation of certain specific genes.

Reactivation of \( p16^{NK4a} \) and MGMT by long-term treatment with EGCG and by its combination with other agents

To determine whether long-term treatment of cells with EGCG can increase the extent of reactivation of methylation-silenced genes, we treated KYSE 510 cells with EGCG for different lengths of time. EGCG (20 \( \mu \text{mol/L} \)) was administered to the cells in fresh cell culture medium every other day. As shown in Figure 3, the \( p16^{NK4a} \) mRNA level increased with time; on days 25 and 40 the levels were \( \sim 3 \) times that of day 5. Withdrawing EGCG at day 10 did not seem to affect the subsequent increase of \( p16^{NK4a} \) mRNA. The pattern of changes of the MGMT mRNA levels was not as clear even though a time-dependent increase was observed on continuous treatment with EGCG. After EGCG treatment was withdrawn on day 10, the MGMT mRNA levels seemed to increase on days 15 and 20 but to decrease on days 25 and 40. In these long-term treatment studies, the unmethylation-specific bands of \( p16^{NK4a} \) and MGMT genes also appeared to increase with time (data not shown). These results are preliminary; additional studies are needed to confirm these results.

Treatment of KYSE 510 cells with 10 \( \mu \text{mol/L} \) of EGCG only weakly activated \( RAR\beta \) and \( p16^{NK4a} \), showing faint mRNA bands as determined by RT-PCR. Treatment with 0.5 \( \mu \text{mol/L} \) of trichostatin (TSA), a histone deacetylase (HDAC) inhibitor, also slightly activated these genes (Fig. 4 A). The combination of EGCG with TSA appeared to synergistically increase mRNA levels. The combination of DAC (2 \( \mu \text{mol/L} \)) with EGCG (10 \( \mu \text{mol/L} \)) or TSA (0.5 \( \mu \text{mol/L} \)) also produced a synergistic or additive effect. Figure 4B shows the apparent synergistic action between EGCG (10 \( \mu \text{mol/L} \)) and TSA (0.5 \( \mu \text{mol/L} \)) or butyric acid (2.5 mmol/L) in the reactivation of \( RAR\beta \) and the gene for death-associated protein kinase 1 (DAPK1) in mouse lung cancer cell line CL13. The nature of these actions remains to be characterized further.

Reversal of hypermethylation and reactivation of \( p16^{NK4a} \), \( RAR\beta \), and MGMT by genistein and other compounds

Treatment of KYSE 510 cells with genistein (2–20 \( \mu \text{mol/L} \)) partially reversed DNA hypermethylation and reactivated \( p16^{NK4a} \), \( RAR\beta \), and MGMT (32). This was indicated by the appearance of unmethylation-specific bands by methylation-specific PCR as well as by the increased mRNA levels as determined by RT-PCR and real-time PCR. Partial reversal of DNA hypermethylation and reactivation of \( RAR\beta \) by genistein were also observed in KYSE 150 cells and prostate cancer LNCaP and PC3 cells. Genistein (20–50 \( \mu \text{mol/L} \)) dose-dependently inhibited DNMT activity, showing competitive and noncompetitive inhibition with respect to the substrate poly(dI-dC)-poly(dI-dC) and noncompetitive inhibition with respect to SAM (32). Two other isoflavones, biochanin A and daidzein, were less effective in inhibiting UVB-induced global DNA hypomethylation. Because global DNA hypomethylation has been reported to be associated with hypermethylation and inactivation of specific genes during carcinogenesis (9), this observation is not necessarily contradictory to the concept that EGCG can prevent or reverse the hypermethylation of certain specific genes.

Reactivation of \( p16^{NK4a} \) and MGMT by long-term treatment with EGCG and by its combination with other agents

To determine whether long-term treatment of cells with EGCG can increase the extent of reactivation of methylation-silenced genes, we treated KYSE 510 cells with EGCG for different lengths of time. EGCG (20 \( \mu \text{mol/L} \)) was administered to the cells in fresh cell culture medium every other day. As shown in Figure 3, the \( p16^{NK4a} \) mRNA level increased with time; on days 25 and 40 the levels were \( \sim 3 \) times that of day 5. Withdrawing EGCG at day 10 did not seem to affect the subsequent increase of \( p16^{NK4a} \) mRNA. The pattern of changes of the MGMT mRNA levels was not as clear even though a time-dependent increase was observed on continuous treatment with EGCG. After EGCG treatment was withdrawn on day 10, the MGMT mRNA levels seemed to increase on days 15 and 20 but to decrease on days 25 and 40. In these long-term treatment studies, the unmethylation-
inhibiting DNMT activity, reactivating RARβ, and inhibiting cancer cell growth. Genistein was a weaker DNMT inhibitor than EGCG, yet it was just as active or more active in demethylating hypermethylated genes and reactivating their expression. One possible reason for this is that genistein is more stable than EGCG in the cell culture medium and reaches higher intracellular concentrations than EGCG (data not shown). Another possibility is that genistein is also an (albeit weak) inhibitor of HDAC (32).

Treatment of KYSE 510 cells with a combination of genistein (5 μmol/L) with TSA (0.5 μmol/L) synergistically increased the mRNA levels of p16\(^{INK4a}\), RARβ, and MGMT. The level of unmethylated-specific DNA band of RARβ was increased by genistein but not by TSA (Fig. 5). The level of acetylated H3 was increased by TSA but not by genistein. In LNCaP cells, TSA significantly enhanced the effectiveness of genistein in reactivating RARβ, GSTP1, the gene for the Ras association domain family 1A (RASSF1A), and the gene for estrogen receptor α (ERα), whereas sulforaphane (SFN, 15 μmol/L) only slightly enhanced the reactivation of GSTP1 (Fig. 6).

**Inhibition of DNMT activity by other polyphenols**

In addition to the aforementioned polyphenols and isoflavones, we also studied some other commonly used phenolic compounds to determine their effects on the DNMT activity in nuclear extracts of KYSE 510 cells (Fig. 7). These include myricetin and quercetin (flavonols), hesperetin and naringenin (flavonols), apigenin and luteolin (flavonols), garcinol, curcumin, and hydroxycinnamic acid. All these compounds inhibited DNMT activities at 20 and 50 μmol/L, but their activities were lower than that of EGCG. At 50 μmol/L, hydroxycinnamic acid, garcinol, and luteolin inhibited DNMT activity by >50%.

Compounds possessing catechol structures are readily methylated by SAM in the presence of COMT, resulting in the conversion of SAM to SAH. The SAM:SAH ratio could affect DNMT activity. Lee et al. (26,33) demonstrated that many catechol polyphenols, such as quercetin, fisetin, and myricetin, inhibited DNMT by converting SAM to SAH and that myricetin is a strong inhibitor of DNMT. This mechanism was also proposed for the partial reversal of the promoter hypermethylation of the RARβ in breast cancer cell lines by caffeic acid and chlorogenic acid (33).

**Alteration of levels of SAM, SAH, and homocysteine after the administration of EGCG to mice**

Consumption of polyphenols was reported to reduce SAM levels and increase homocysteine levels in humans (34). A severe decrease in SAM or the accumulation of SAH could reduce DNMT activity. We have examined this issue in our ongoing experiments on bioavailability, toxicity, and cancer-preventive activities of EGCG. The results are summarized as follows: 1) Administration of 0.16% or 0.32% EGCG in drinking fluid to CF-1 mice for 7 d had no significant effect on plasma homocysteine or methionine levels. 2) Administration of 0.5% Polyphenon E (a standardized green tea polyphenol preparation containing 65% EGCG and 15% other catechins) in drinking fluid to A/J mice for 7 d...
decreased the small intestinal level of SAM (34.5 vs. 45.0 nmol/g in the control group) without altering SAH level (16–19 nmol/g). The treatment did not affect the hepatic SAM and SAH levels. 3) Administration of 0.16% EGCG in drinking fluid to Apc\(^{-}\)/+ mice for 8 or 9 wk significantly decreased the small intestinal SAM levels (40.5 vs. 58.2 nmol/g in the control group) without affecting the SAH level. The treatment did not affect SAM and SAH levels in liver. EGCG (0.08%), administered similarly in drinking fluid, had no effect on small intestinal or hepatic levels of SAM and SAH. 4) Treatment of male CF-1 mice with a single i.g. dose of EGCG at 2000 mg/kg significantly increased plasma levels of homocysteine at 50 min and 3 h after treatment (both with <6–7 \(\mu\)mol/L vs. 3.9 \(\mu\)mol/L in the control group). At 8 h after treatment, the small intestine levels of homocysteine and methionine partially returned to but still significantly differed from the basal level. The hepatic SAM level decreased from a basal level of 60.9 nmol/g to 10.4, 11.4, and 41.2 nmol/g at 30 min, 3 h, and 8 h, respectively. The corresponding levels of SAH also decreased from 44.0 nmol/g to 32.9, 34.6, and 36.1 nmol/g, respectively. Apparently the SAH was converted to homocysteine. 5) Results from a dose-response study with i.g. administration of EGCG at 50–2000 mg/kg to male CF-1 mice are summarized in Table 1. At 3 h after the treatment, the hepatic SAM and SAH levels as well as the SAM:SAH ratio significantly decreased with higher concentrations of EGCG. With EGCG at 50 mg/kg, the SAH levels appeared to be higher than the basal level, but the difference was not statistically significant.

These studies indicate that the administration of EGCG (or Polyphenon E) through drinking fluid can moderately decrease the level of SAM (without increasing the level of SAH) in the intestine but have no significant effect on hepatic SAM or SAH levels. Acute i.g. treatment with high doses of EGCG significantly elevated plasma levels of homocysteine, decreased the levels of plasma methionine, and decreased the levels of intestinal SAM and SAH. A calculation based on caloric consumption indicates that a dose of 1000 mg/kg is equivalent to the consumption of ~4200 mg of EGCG or 20–35 cups of green tea by an individual, and it may produce toxicity.

Discussion and conclusions
The discussed studies demonstrate the inhibition of DNMT and promote cytosine hypermethylation as well as the reactivation of some methylation-silenced genes by EGCG, genistein, and related compounds. Although the activation of methylation-silenced genes in cell lines by the compounds appears to correlate with the inhibition of DNMT enzyme activity in nuclear extracts, other mechanisms of actions, such as the inhibition of HDAC, may also play a role. The effective concentrations of EGCG (10–50 \(\mu\)mol/L) observed in studies with cell lines are ~50 times higher than the plasma and tissue levels of EGCG generally observed after ingestion of tea (35,36). The oral digestive tract has direct contact and may be exposed to higher levels of EGCG. The effective genistein concentrations observed (5–20 \(\mu\)mol/L) for DNA demethylation are close to but still higher than the plasma level of genistein (0.7–6.0 \(\mu\)mol/L) reported after consumption of soy products by women (37,38). Whether EGCG, genistein, and other dietary polyphenols can reverse DNA hypermethylation and reactivate methylation-silenced genes in vivo still remain to be determined. A key issue is whether long-term treatment of cells or animals with these agents can increase their efficiencies; this subject deserves further investigation.

The possibility that catechol polyphenols may inhibit DNMT indirectly by decreasing SAM and increasing SAH is very interesting. The key element of this hypothesis is that SAH is a potent inhibitor of DNMT (33). Our studies, however, suggest that this type of inhibition may not occur in vivo because elevated tissue levels of SAH were not observed and only a modest decrease in SAM levels was observed after oral administration of EGCG. Only high acute doses of EGCG cause a marked decrease of SAM (and SAH) levels in liver; this may be a situation associated with toxicity.

Consumption of polyphenols from diet and beverages will not have a major effect on DNA methylation in animals and humans with a normal diet. The effect may be more significant, however, if the diet has limited amounts of methyl donors. With the consumption of large quantities of polyphenols through dietary supplements, the high tissue level of polyphenols may affect DNA methylation. This possibility remains to be demonstrated. This may also be a situation where caution needs to be applied to avoid possible toxicity from the oxidation of polyphenols. For the prevention of cancer, the combination of polyphenol DNMT inhibitors together with HDAC inhibitors, such as butyric acid and sulforaphane, or other inhibitors are promising approaches.

### Table 1: Effects of (-)-epigallocatechin 3-gallate (EGCG) treatment on tissue levels of S-adenosylmethionine (SAM) and S-adenosyl-L-homocysteine (SAH)<sup>1</sup>

| Dose, mg/kg | Liver | | | Small Intestine | | |
|---|---|---|---|---|---|
| | SAH, nmol/g | SAM, nmol/g | SAM/SAH | SAH, nmol/g | SAM, nmol/g | SAM/SAH |
| 0 | 44.1 ± 3.3 | 58.2 ± 1.1 | 1.3 ± 0.1 | 4.1 ± 0.4 | 71.4 ± 3.9 | 17.8 ± 1.6 |
| 50 | 43.2 ± 6.7 | 57.0 ± 6.2 | 1.3 ± 0.2 | 3.9 ± 0.8 | 73.8 ± 6.0 | 18.6 ± 2.5 |
| 100 | 44.1 ± 3.9 | 44.9 ± 1.7 | 1.0 ± 0.2 | 2.6 ± 0.2* | 70.7 ± 5.2 | 26.9 ± 1.5* |
| 200 | 32.3 ± 2.3* | 27.9 ± 0.8* | 0.9 ± 0.1 | 2.2 ± 0.2* | 72.1 ± 6.7 | 33.5 ± 1.9* |
| 500 | 33.0 ± 2.6* | 22.2 ± 2.3* | 0.6 ± 0.1* | 3.8 ± 0.4 | 68.3 ± 6.0 | 17.9 ± 2.0 |
| 1000 | 29.2 ± 1.8* | 16.2 ± 0.9* | 0.6 ± 0.1* | 3.7 ± 0.3 | 67.0 ± 6.3 | 18.0 ± 1.6 |
| 2000 | 31.4 ± 2.4* | 9.4 ± 1.0* | 0.3 ± 0.1* | 3.6 ± 0.4 | 58.6 ± 6.2 | 16.2 ± 1.5 |

<sup>1</sup> CF1 mice were administered i.g. a single dose of EGCG (50–2000 mg/kg). After 3 h, liver and small intestine were removed. The levels of S-adenosylmethionine (SAM) and S-adenosyl-L-homocysteine (SAH) were analyzed by HPLC. Data are presented as means ± SE (n = 6).

* P < 0.05.

---

**Literature Cited**

3. Zingg JM, Jones PA. Genetic and epigenetic aspects of DNA methyl-
    ylation on genome expression, evolution, mutation and carcinogenesis.
4. Herman JG, Baylin SB. Gene silencing in cancer in association with
10. Xing EP, Nie Y, Wang LD, Yang GY, Yang CS. Aberrant methylation of
    p16INK4a and deletion of p15INK4b are frequent events in human
11. Xing EP, Yang GY, Wang LD, Shi ST, Yang CS. Loss of heterozygosity of
    the Rb gene correlates with pRb protein expression and associates with
    p53 alteration in human esophageal cancer. Clin Cancer Res. 1999;5:
    1231–40.
    multiple gene hypermethylation in the development of esophageal
    LD, Yang CS. Hypermethylation-associated inactivation of retinoic acid
    receptor beta in human esophageal squamous cell carcinoma. Clin
    hypermethylation and inactivation of O(6)-methylguanine-
    DNA methyltransferase in esophageal squamous cell carcinomas and its
15. Issa JP. Optimizing therapy with methylation inhibitors in myelodys-
16. Silverman LR, Mufti GJ. Methylation inhibitor therapy in the treatment
    S12–23.
    V, Forsee KM, MacDonald RS, et al. Genistein alters methylation
18. Yang CS, Maliaikal P, Meng X. Inhibition of carcinogenesis by tea. Annu
19. Lambert JD, Hong J, Yang GY, Liao J, Yang CS. Inhibition of carcinogenesis by
    2005;81:1 Suppl:284S–95S.
20. Yang CS, Lambert JD, Hou Z, Ju J, Lu G, Hao X. Molecular targets for
    the cancer preventive activity of tea polyphenols. Mol Carcinog. 2006;
    and identification of 4’-O-methyl-(–)-epigallocatechin in humans.
    and characterization of methylated and ring-fission metabolites of tea
23. Lu H, Meng X, Yang CS. Enzymology of methylation of tea catechins
    and inhibition of catechol-O-methyltransferase by (–)-epigallocatechin
    liver catechol-O-methyltransferase by tea catechins and their
    metabolites: structure-activity relationship and molecular-modeling
    polyphenol (–)-epigallocatechin-3-gallate inhibits DNA methyltrans-
    ferase and reactivates methylation-silenced genes in cancer cell lines.
    Cancer Res. 2003;63:7563–70.
    methyltransferases by tea catechins and bioflavonoids. Mol Pharmacol.
27. Shukla S, Trohan S, Resnick MI, Gupta S. Epigallocatechin-3-gallate
    causes demethylation and activation of GSTP1 gene expression in
    human prostate cancer LNCaP cells. Proc Amer Assoc Cancer Res.
28. Pandey M, Gupta S. Green tea polyphenols (GPT) cause activation of
    the GSTP1 gene in prostate cancer LNCaP cells by promoter demeth-
29. Chuang JC, Yoo CB, Kwan JM, Li TW, Liang G, Yang AS, Jones PA.
    Comparison of biological effects of non-nucleoside DNA methyla-
    tion inhibitors versus 5-aza-2′-deoxycytidine. Mol Cancer Ther.
    diversity of DNA methyltransferase inhibitors in human cancer cell
31. Mittal A, Pyathulake C, Hara Y, Katiyar SK. Exceptionally high
    protection of photocarcinogenesis by topical application of (–)-
    epigallocatechin-3-gallate in hydrophilic cream in SKH-1 hairless mouse
    model: relationship to inhibition of UVB-induced global DNA hypo-
32. Fang MZ, Chen D, Sun Y, Jin Z, Christina JK, Yang CS. Reversal of hyper-
    methylation and reactivation of p16INK4a, RARbeta, and MGMT
    genes by genistein and other isoflavones from soy. Clin Cancer Res.
33. Lee WJ, Zhu BT. Inhibition of DNA methylation by caffeic acid and
    chlorogenic acid, two common catechol-containing coffee polyphenols.
34. Othof MR, Hollman PC, Zock PL, Katan MB. Consumption of high
    doses of chlorogenic acid, present in coffee, or of black tea increases
35. Lee MJ, Maliaikal P, Chen L, Meng X, Bondoc FY, Prabhu S, Lambert
    G, Mohr S, Yang CS. Pharmacokinetics of tea catechins after ingestion
    of green tea and (–)-epigallocatechin-3-gallate by humans: formation of
    different metabolites and individual variability. Cancer Epidemiol
36. Lambert JD, Lee MJ, Diamond L, Ju J, Hong J, Bose M, Newmark HL,
    Yang CS. Dose-dependent levels of epigallocatechin-3-gallate in human
    colon cancer cells and mouse plasma and tissues. Drug Metab Dispos.
    2006;34:8–11.
37. Adlercreutz CH, Goldin BR, Gorbach SL, Hockerstedt KA, Watanabe S,
    1999;125:3 Suppl:757S–70S.
38. Xu X, Harris KS, Wang HJ, Murphy PA, Hendrich S. Bioavailability
39. Wardlaw SA, Zhang N, Belinsky SA. Transcriptional regulation of basal
    cAMP response element-binding protein. Mol Pharmacol. 2002;62:
    326–33.