Reversal of hypermethylation and reactivation of genes by dietary polyphenolic compounds

Chung S Yang,
Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey, USA

Mingzhu Fang,
Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey, USA

Joshua D Lambert,
Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey, USA

Pearly Yan, and
Human Cancer Genetics Program, Department of Molecular Virology, Immunology and Medical Genetics, Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio, USA

Tim H-M Huang
Human Cancer Genetics Program, Department of Molecular Virology, Immunology and Medical Genetics, Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio, USA

Dietary polyphenolic compounds have been reported to have many interesting biological activities, including the induction of epigenetic changes and cancer prevention. In searching for the mechanisms of the anti-cancer action of (–)–epigallocatechin-3-gallate (EGCG), the major polyphenolic compound in green tea, we observed the inhibition of DNA methyltransferase (DNMT) activity by EGCG.[1] Treatment of cancer cells with EGCG caused the demethylation of the CpG islands in the promoter regions and the reactivation of methylation-silenced genes such as *p16^{INK4a}*, retinoic acid receptor-β (RAR-β), O^6-methylguanine methyltransferase (MGMT), human mutL homolog 1, and glutathione *S*-transferase-π. These activities have been observed in human esophageal, colon, prostate, or mammary cancer cell lines.[2] With nuclear extracts from KYSE 510 human esophageal cancer 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 Ki of 4.8 μM.[2] EGCG structural analogs from green tea, (–)–epicatechin-3-gallate (ECG), (–)–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. Their activities were lower, following the order EGCG > ECG, MeEGCG > EGC, DiMeEGCG > EC.

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. 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 in the structural

Correspondence: CS Yang, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854, USA. csyang@rci.rutgers.edu or csyang164@yahoo.com, Phone: +1-732-445-3400 ext. 248, Fax: +1-732-445-0687.

model of DNMT1, with possible hydrogen bond formation with Glu1265 and Pro1223. In addition, possible hydrogen bond formation between the hydroxyl groups of the EGCG A and B rings with Ser1229 and Cys1225, respectively, may also have contributed to the high-affinity binding. This model can also explain the lower inhibitory activities of EGCG analogs and metabolites.

The inhibition of DNMT and reversal of hypermethylation by EGCG and related compounds were also observed by Lee et al.[3,4] They observed that EGCG directly inhibited DNMT activity and partially reversed RAR-β methylation status. Other catechol polyphenols inhibited DNMT indirectly by being methylated and converting S-adenosyl-L-methionine (SAM) to S-adenosly-L-homocysteine (SAH), which is a strong inhibitor of DNMT. Caffeic acid and chlorogenic acid also partially inhibited methylation of the promoter region of the RAR-β gene in breast cancer cell lines.[4] However, the effect of EGCG may be gene specific or cell line specific and was not as robust as 5-aza-2′-deoxycytidine (DAC).[5] Significant demethylation and reactivation of several genes by EGCG were not observed by Chuang et al.[5] and Stresemann et al.[6] Mittal et al.[7] 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, this observation is not necessarily contradictory to the concept that EGCG can prevent or reverse the hypermethylation of certain specific genes.

In order to study the effect of long-term EGCG treatment, KYSE 510 cells were treated with 10 μM EGCG for 10–20 days. Freshly made EGCG solution was added to the culture medium every other day in the presence or absence of 5 U/ml superoxide dismutase (SOD) and 30 U/ml catalase (to prevent EGCG autooxidation) and the cells were subcultured every 5 days. The results showed that treatment of cells for 10, 15, and 20 days progressively increased the mRNA levels of p16 and MGMT, as determined by RT-PCR, and the presence of SOD and catalase enhanced the band intesity of p16 mRNA. The treatment also progressively enhanced the unmethylation-specific bands of the p16 and MGMT promoters, as determined by methylation-specific PCR. The changes in methylation status of other genes were determined by "differential methylation hybridization." In this case, amplified products from the untreated KYSE 510 cells were labeled with Cy5 and the EGCG-treated cells were labeled with Cy3 and both were co-hybridized to long-oligonucleotide CpG island array. In this scheme, hypomethylation events will manifest as array probes having higher intensity in the untreated cell line than in the treated cell line, thereby having a high ratio of Cy5:Cy3. MA plots [$M = \log_2(Cy5/Cy3)$ is the y-axis while $A = \frac{1}{2} \log_2 Cy5.Cy3$ is the x-axis] were used to visualize the intensity-dependent ratio. A twofold cut-off was used to select for hypomethylated and hypermethylated CpG island (CGI probes). In the EGCG-treated (20 days) cells, 22 genes were found to be hypomethylated in the gene promoter-first exon region. Experiments are ongoing to validate the results. Of these genes, molybdenum cofactor sulfurase, smothelin isoforma, and zinc finger protein 467 have been shown to be expressed in normal esophagus and osteoprotegerin has been implicated in Barrett's metaplasia.

Isoflavones were also found to demethylate and reactivate some of the methylation-silenced genes described above, and genistein was the most potent isoflavone from soy.[8] Genistein (20–50 mmol/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.[8] Two other isoflavones, biochanin A and daidzein, were less effective in inhibiting DNMT activity, reactivating RAR-β, 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 it reaches higher intracellular concentrations than EGCG. Another

possibility is that genistein is also an inhibitor of histone deacetylase (HDAC).8 The extent of demethylation was enhanced by extending the treatment period with EGCG or genistein or by its combination with a HDAC inhibitor.

Combination treatment of KYSE 510 cells with 2 μM genistein and 5 μM EGCG, or with 5 μM genistein and 10 μM EGCG, apparently enhanced the expression of p16 compared to the treatment with genistein or EGCG alone. When KYSE 510 cells were treated with genistein (5 μM) for 5 days and then with the HDAC inhibitor trichostatin (0.5 μM) for 3 hours, the levels of acetylated histone H3 and H4 were increased by the synergistic action of these two agents. Chromatin immunoprecipitation (ChIP) assay indicated that genistein and trichostatin synergistically increase the binding of acetylated H3 and H4 to the promoter region of RAR-β and MGMT. Other dietary constituents such as quercetin, luteolin, and hydroxycinnamic acid were also found to inhibit DNMT, but their activities were lower than that of EGCG. Whether these compounds cause demethylation and reactivation of methylation-silenced genes in cells still remains to be investigated.

Catechol polyphenols can affect DNA methylation by 1) directly inhibiting DNMT activity and 2) being methylated and decreasing the level of SAM, the methyl donor, and producing SAH, which is an inhibitor of DNMT. Consumption of high doses of chlorogenic acid was reported to reduce SAM levels and increase homocysteine levels in humans.[9] In several sets of studies in mice, we observed that chronic administration of EGCG and tea polyphenol mixtures (0.16–0.5%) in drinking fluid moderately decreased the levels of SAM without increasing the levels of SAH in the intestine; the treatments had no effects on hepatic and plasma levels of SAM and SAH. However, acute i.g. treatment with high doses (100–2000 mg/kg) of EGCG significantly decreased the intestinal levels of SAM and SAH, decreased the plasma levels of methionine, and elevated the plasma levels of homocysteine. Because of the rather low bioavailability of most dietary polyphenols and their rapid glucuronidation and sulfation, normal dietary consumption of polyphenols does not appear to cause significant epigenetic changes in humans. Nevertheless, dietary polyphenols in combination with other agents, such as histone deacetylase inhibitors, may cause significant epigenetic changes. Moreover, consumption of excessive amounts of polyphenols may deliver concentrations in the intestine and liver high enough to change DNA methylation. Future research is needed to further evaluate the effects of dietary polyphenols on DNA methylation and their impact on human health.

Acknowledgments

References

1. Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, Lu H, Welsh W, Yang CS. Tea polyphenol (–)–epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. Cancer Res 2003;63:7563–7570. [PubMed: 14633667]

2. Fang MZ, Chen D, Yang CS. Dietary polyphenols may affect DNA methylation. J Nutr 2007;137 (Suppl):S223–S228.

3. Lee WJ, Shim JY, Zhu BT. Mechanisms for the inhibition of DNA methyltransferases by tea catechins and bioflavonoids. Mol Pharmacol 2005;68:1018–1030. [PubMed: 16037419]

4. Lee WJ, Zhu BT. Inhibition of DNA methylation by caffeic acid and chlorogenic acid, two common catechol-containing coffee polyphenols. Carcinogenesis 2006;27:269–277. [PubMed: 16081510]

5. Chuang JC, Yoo CB, Kwan JM, et al. Comparison of biological effects of non-nucleoside DNA methylation inhibitors versus 5-aza-2′-deoxycytidine. Mol Cancer Ther 2005;4:1515–1520. [PubMed: 16227400]

6. Stresemann C, Brueckner B, Musch T, Stopper H, Lyko F. Functional diversity of DNA methyltransferase inhibitors in human cancer cell lines. Cancer Res 2006;66:2794–2800. [PubMed: 16510601]

7. Mittal A, Piyathilake 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 hypomethylation. Neoplasia 2003;5:555–565. [PubMed: 14965448]

8. Fang MZ, Chen D, Sun Y, Jin Z, Christman JK, Yang CS. Reversal of hypermethylation and reactivation of p16INK4a, RARbeta, and MGMT genes by genistein and other isoflavones from soy. Clin Cancer Res 2005;11:7033–7041. [PubMed: 16203797]

9. Olthof MR, Hollman PC, Zock PL, Katan MB. Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. Am J Clin Nutr 2001;73:532–538. [PubMed: 11237928]