Targeting DNA Methylation with Green Tea Catechins

Eugenia C. Yiannakopoulou

Department of Medical Laboratories, Faculty of Health and Caring Professions, Technological Educational Institute of Athens, Athens, Greece

Key Words

Green tea catechins · Epigallocatechin gallate · DNA methylation · DNA methyltransferase · Tumor suppressor gene activation

Abstract

Aberrant epigenetic alterations in the genome such as DNA methylation play a significant role in cancer development. Green tea catechins have been reported to modulate epigenetic processes. This review aims to synthesize evidence on the modulation of DNA methylation by green tea catechins. Green tea catechins have been reported to reverse DNA methylation of tumor suppressor genes and increase transcription of these genes. Green tea catechins and especially epigallocatechin gallate modulate DNA methylation by attenuating the effect of DNA methyltransferase 1 (DNMT1). However, the exact mechanism of DNMT1 inhibition is not delineated. Suggested mechanisms include direct enzymatic inhibition, indirect enzymatic inhibition, reduced DNMT1 expression and translation. The possible effect of green tea catechins on other pathways of DNA methylation, i.e. methyl-CpG binding domain proteins, has not been investigated. Furthermore, the link between redox properties and epigenetic modulation by green tea catechins has not been defined either. Since green tea catechins are natural compounds with a rather acceptable safety profile, further research on their action as inhibitors of DNA methylation seems worthwhile.

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Introduction

Classical knowledge indicates that mutation or deletion of coding regions is responsible for gene inactivation. However, since the early 1990s, a new molecular pathway of gene silencing has been discovered [1]. In this pathway, the DNA promoter hypermethylation is a key epigenetic mechanism for the silencing of many genes, including those for cell cycle regulation, receptors, DNA repair and apoptosis [2–4]. Hypermethylation of CpG islands may inhibit transcription by recruiting the methyl-CpG binding domain proteins or by interfering with the recruitment and function of basal transcription factors or transcriptional coactivators [5, 6]. Silencing of the expression of gatekeeper or mismatch genes promotes growth in the affected cells. DNA methylation of tumor suppressor genes is thought to be an early event in carcinogenesis [7]. DNA methylation is modulated by DNA methyltransferases (DNMT) [8, 9]. The human genome includes 4 genes that code DNMTs (DNMT1, DNMT2, DNMT3A and DNMT3B) with DNMT1 and DNMT3B being the more potent [8–10]. On the other hand, global DNA hypomethylation is associated with chromosomal instability and is an early event in the neoplastic progression of most human and animal cancers including colon cancer [6, 11].

Polyphenols, particularly flavonoids, constitute the most interesting component of green tea leaves. Catechins (flavan-3-ols) are the main flavonoids present in green tea. The four major catechins are: (–)-epigallocatechin-3-gallate (EGCG), which represents approximately 59% of the

total of catechins; (–)-epigallocatechin (EGC; approximately 19%); (–)-epicatechin-3-gallate (ECG; approximately 13.6%), and (–)-epicatechin (EC; approximately 6.4%) [12, 13]. The potential cancer chemopreventive and therapeutic properties of teas and tea polyphenols have been in the focus of research efforts in the last 2 decades. Recent data have shown strong chemopreventive and possibly cancer chemotherapeutic effects of green tea polyphenols and EGCG against cancers of the skin (UV radiation and chemically induced), lung, breast, colon, liver, stomach and prostate [14–19]. In vitro and in vivo data suggest that green tea catechins inhibit growth, proliferation, migration and invasion of estrogen receptor (ER)+ and ER– breast cancer cell lines [20–23]. In addition, it has been shown that green tea catechins block the steps of carcinogenesis [24]. Although the molecular mechanisms of the antiproliferative action of green tea catechins have not been delineated, green tea catechins seem to be multitarget agents, modulating multiple signaling pathways [25]. In addition, green tea catechins have been reported to modulate epigenetic processes. Aberrant epigenetic alterations in the genome such as DNA methylation and chromatin remodeling play a significant role in cancer development. Since epigenetic alterations are considered to be more easily reversible compared to genetic changes, epigenetic therapy is potentially very useful in reversing some of these defects. Indeed, epigenetic processes have been recognized as a new target for anticancer drug design. This review aims to synthesize evidence on the modulation of DNA methylation by green tea catechins.

Methods

PubMed, Scopus, Google Scholar and Science Citation Index were searched with the search terms ‘EGCG’, ‘green tea catechins’, ‘breast cancer’, ‘DNA methylation’, ‘promoter hypermethylation’, ‘global DNA hypomethylation’, ‘epigenomics’ and ‘epigenetics’. The search covered the period from 1966 up to and including February 2013. Trials that provided evidence on the effect of green tea catechins on the modulation of epigenomic processes met the inclusion criteria. Only full publications were considered. There was no language restriction. The reference list of all identified trials was checked for more relevant articles.

Results

In silico Data

Attempting to investigate the mechanism of DNMT1 inhibition at the molecular level, computational modeling analyses have been conducted to probe the interac-

tions between EGCG and human DNMT1 [26–28]. A homology model of the human DNMT1 has been used to model the three-dimensional structure of the DNMT1 catalytic domain. Based on molecular modelling, it has been suggested that EGCG shows competitive inhibition of DNMT1 by effectively forming at least four hydrogen bonds within the DNMT1 catalytic binding center, thus blocking the entry of the DNA nucleotide cytosine into its active site and preventing the methylation process [26–28].

Experimental Trials

Wong et al. [29] reported that EGCG, at physiologically relevant concentrations in vitro, inhibited DNMT and induced the transcription factor Foxp3. In addition, it was shown that in the in vitro culture system, EGCG also reduced the gene expression of all three DNMTs (DNMT 1, DNMT3a and DNMT3b), which correlated with reduced DNA methylation in the Jurkat T cells treated with EGCG [29].

Nandakumar et al. [30] showed that green tea catechins decreased global DNA methylation levels in human epidermoid carcinoma A431 cells in a dose-dependent manner. In addition, EGCG decreased DNMT activity, messenger RNA (mRNA) and protein levels of DNMT1, DNMT3a and DNMT3b and resulted in reexpression of messenger RNAs (mRNAs) and protein expressions of tumor suppressor genes (*p16^{INK4a}* and *Cip1/p21*) [30]. Treatment of human esophageal KYSE 510 cells with 5–50 µmol/l of EGCG for 12–144 h has been shown to lower DNMT1 activity leading to concentration- and time-dependent reversal of hypermethylation and re-expression of genes including the tumor suppressor *p16^{INK4a}*, *RARβ* (retinoic acid receptor-β), *MGMT* (O⁶-methylguanine methyltransferase) and the DNA mismatch repair gene *hMLH1* (human mutL homologue 1) [10]. Reactivation of some methylation-silenced genes by EGCG has also been demonstrated in human colon cancer HT-29 cells, esophageal cancer KYSE 150 cells and prostate cancer PC3 cells [26, 27]. Berner et al. [31] have shown that EGCG treatment suppressed promoter methylation of tumor suppressor genes *p15^{INK4b}* and *p16^{INK4a}* in Caco2 cells [31]. Gao et al. [32] have demonstrated that EGCG reverses promoter methylation of tumor suppressor WIF-1 and restores WIF-1 expression in H460 and A549 lung cancer cells [32]. Gu et al. [33] have shown that EGCG induces apoptosis in renal carcinoma cells possibly through promoter demethylation of tissue factor pathway inhibitor-2, a member of the Kunitz-type serine proteinase inhibitor family, which is inversely related to

an increasing degree of malignancy [33]. Kato et al. [34] have shown that EGCG partially reversed the hypermethylation status of the tumor suppressor gene RECK and significantly enhanced the expression level of RECK mRNA in oral squamous cell carcinoma cells [34].

Although hypermethylation of gene promoters is generally associated with gene silencing, there are exceptions to this rule such as the *hTERT* (human telomerase reverse transcriptase), a promoter that, paradoxically, is highly methylated in most tumor cell types, rendering *hTERT* active. Treatment with EGCG can also inhibit oncogene expression through influencing the DNA methylation status of these genes. Meeran et al. [35] have shown that treatment with EGCG inhibited the transcription of the tumor-promoting gene *hTERT*, the catalytic subunit of telomerase, through epigenetic mechanisms in ER+ MCF-7 and ER- MDA-MB-231 cells. The downregulation of *hTERT* expression was due partly to *hTERT* promoter hypomethylation mediated through inhibition of DNMT [35]. Mittal et al. [36] have demonstrated that green tea catechins induce apoptosis in MCF-7 breast cancer cells through downregulation of telomerase. They have shown that treatment of MCF-7 cells with EGCG dose-dependently inhibited telomerase activity, and also inhibited the mRNA expression of *hTERT*. The same investigators also demonstrated that EGCG also inhibited the protein expression of *hTERT*, which indicated that inhibition of telomerase was associated with downregulation of *hTERT* [36]. Belretch et al. [37] reported that treatment of MCF-7 cells with EGCG resulted in decreased *hTERT* mRNA expression. Furthermore, downregulation of *hTERT* gene expression in MCF-7 cells appeared to be largely due to epigenetic alterations, as evidenced by the time-dependent decrease in *hTERT* promoter methylation [37].

Lee et al. [28] have reported the in vitro inhibition of DNMT3a and DNMT3b by tea polyphenols using the prokaryotic SssI DNMT. The same investigators have demonstrated the enzymatic inhibition of human DNMT1 by catechin, epicatechin and EGCG [28]. In addition, in the same study, they investigated the effects of EGCG and catechin on the methylation status of the *RARβ* gene in the human breast cancer cell lines MCF-7 and MDA-MB-231. The *RARβ* gene promoter region is hypermethylated in these cells. MCF-7 and MDA-MB-231 cells were treated with 0, 0.2, 1, 5, 25, or 50 μmol/l EGCG for 3 or 6 days, respectively, depending on the rate of cell growth. It was shown that EGCG or catechin partially inhibited the methylation status of the promoter regions of the *RARβ* gene [28].

In a very interesting study, Meeran et al. [38] investigated the possibility of reactivation of ERs in ER- breast cancer cells through treatment with green tea polyphenols. The investigators observed that treatment of ERα-breast cancer cells with green tea polyphenols led to the reactivation of ERα expression. The reactivation of ERα expression was consistently correlated with ERα promoter hypomethylation and hyperacetylation [38].

Pandey et al. [39] have investigated the effects of green tea polyphenols on glutathione-S-transferase *pi* (GSTP1) reexpression in human prostate cells. Exposure of human prostate cancer LNCaP cells to 1–10 μg/ml of green tea polyphenols for 1–7 days caused a concentration- and time-dependent reexpression of GSTP1 which correlated with DNMT1 inhibition. Methyl-specific PCR and sequencing revealed extensive demethylation in the proximal GSTP1 promoter and regions distal to the transcription factor binding sites. In addition, green tea polyphenol exposure resulted in a decrease in DNMT1 protein expression. Cells treated with varying doses of green tea polyphenols for 3 days resulted in a dose-dependent 0.5- to 0.85-fold decrease in the levels of mRNA of DNMT1 [39].

A very recently published trial suggests that green tea catechins inhibit DNA methylation targeting ubiquitin-like containing PHD and Ring finger 1 (UHRF1) that contribute to silencing of tumor suppressor genes by recruiting DNMT1 to their hemi-methylated promoters. The investigators showed that EGCG downregulates UHRF1 and DNMT1 expression in Jurkat cells, with subsequent upregulation of the p73 and p16^{INK4A} genes. The downregulation of UHRF1 was dependent upon the generation of reactive oxygen species by EGCG. Upregulation of p16^{INK4A} was strongly correlated with decreased promoter binding by UHRF1. UHRF1 overexpression counteracted EGCG-induced G1-arrested cells, apoptosis and upregulation of p16^{INK4A} and p73. The investigators concluded that downregulation of UHRF1 is upstream to many cellular events, including G1 cell arrest, upregulation of tumor suppressor genes and apoptosis [40].

Discussion

Green tea catechins seem to be promising multitarget agents in cancer chemoprevention and adjuvant and metastatic treatment of cancer as they are natural compounds with a rather acceptable safety profile targeting multiple signaling pathways. Furthermore, green tea catechins

Table 1. Experimental studies suggesting modulation of DNA methylation by green tea catechins

Authors	Experimental model	Main finding	Suggested mechanism
Meeran et al. [38]	MDA-MB-453 (ER-) and MDA-MB-231 (ER-) cells	green tea polyphenols led to the reactivation of ER α expression partly via ER α promoter hypomethylation	inhibition of DNMTs activity and expression
Wong et al. [29]	Jurkat T cells	EGCG at physiologically relevant concentrations inhibited DNMT	reduction of the gene expression of all three DNMTs
Nandakumar et al. [30]	human epidermoid carcinoma cell line A431	EGCG reduced the levels of DNA methylation and DNMT activity	reduction of the gene expression of all three DNMTs
Meeran et al. [35]	ER+ MCF-7 and ER- MDA-MB-231 cells	EGCG inhibited the transcription of the tumor-promoting gene <i>hTERT</i>	downregulation of hTERT expression partly due to hTERT promoter hypomethylation
Berner et al. [31]	Caco2 cells	EGCG treatment suppressed promoter methylation of tumor suppressor genes	not investigated
Pandey et al. [39]	human prostate cancer LNCaP cells	treatment with green tea polyphenols caused a reexpression of GSTP1	inhibition of DNMT1 protein expression
Kato et al. [34]	oral squamous cell carcinoma cells	EGCG partially reversed the hypermethylation status of the tumor suppressor gene RECK	not investigated
Berletch et al. [37]	MCF-7 cells	treatment of MCF-7 cells with EGCG resulted in decreased hTERT mRNA expression	not further investigated
Gao et al. [32]	H460 and A549 lung cancer cells	EGCG reversed promoter methylation of tumor suppressor WIF-1	not investigated
Gu et al. [33]	renal carcinoma cells	EGCG induces apoptosis possibly via promoter demethylation	not investigated
Lee et al. [28]	in vitro enzymatic inhibition of DNMT using recombinant prokaryotic SssI DNMT and human DNMT1 as model enzymes	EGCG inhibited SssI DNMT- and DNMT1-mediated DNA methylation	direct inhibition of the DNMTs and indirect inhibition of the enzyme
Mittal et al. [36]	MCF-7 cells	treatment of MCF-7 cells with EGCG inhibited telomerase activity and mRNA expression of hTERT	epigenetic regulation of hTERT
Fang et al. [27]	esophageal KYSE 510 cells, human colon cancer HT-29 cells, esophageal cancer KYSE 150 cells, and prostate cancer PC3 cells	reversal of hypermethylation of p16(INK4a), RAR β , MGMT, and hMLH1 genes; reactivation of some methylation-silenced genes by EGCG	enzymatic inhibition of DNMT by EGCG

have been shown to reduce cell proliferation through modulation of cell cycle progression and through induction of apoptosis of cancer cells. Epigenetic modulation seems to be upstream of many cellular events including G1 cell arrest, upregulation of tumor suppressor genes

and apoptosis [40]. Finally, redox regulation might be upstream of epigenetic modulation. Green tea catechins also modulate redox regulation. Thus, green tea catechins could be promising multitarget chemopreventive and chemotherapeutic agents. Especially modulation of DNA

methylation by green tea catechins could lead to the up-regulation of tumor suppressor genes and the downregulation of oncogenes.

Experimental evidence suggests that green tea catechins and especially EGCG modulate DNA methylation by attenuating the effect of DNMT1 (table 1). However, the exact mechanism of DNMT1 inhibition is not delineated. Suggested mechanisms include direct enzymatic inhibition, indirect enzymatic inhibition, reduced DNMT1 expression and translation. Data based on in vitro assays suggest enzymatic inhibition of DNMT3a and DNMT3b by green tea catechins. However, the biological effect of this inhibition has not been investigated. In addition, the possible effect of green tea catechins on other pathways of DNA methylation, i.e. methyl-CpG binding domain proteins has not been investigated. Furthermore, the link between redox properties and epigenetic modulation by green tea catechins has not been defined either; is modulation of redox properties upstream of modulation of DNA methylation?

In addition, an interesting question is if there are any possible negative effects of modulation of DNA methylation by green tea catechins. Although inhibition of DNMT

is expected to prevent hypermethylation, severe inhibition of DNMT activity, as suggested by recent genetic studies, may cause DNA hypomethylation, genomic instability and early development of cancers such as T-cell lymphomas and sarcomas. Recently, several inactivating mutations of de novo DNMTs in various types of tumor have been identified. In addition, it has been shown that loss of de novo DNA methylation activity at advanced tumor stages leads to the promoter DNA demethylation-dependent expression of specific oncogenes. These data suggest that de novo DNMTs also have an important role in the maintenance of DNA methylation and that, in addition to acting as oncogenes, they also behave as tumor suppressors [41, 42].

In conclusion, experimental evidence indicates that green tea catechins modulate DNA methylation. However, the exact mechanisms that underlie the inhibition of DNA methylation by green tea catechins are not clearly defined. Since green tea catechins are natural compounds with a rather acceptable safety profile, further research on their action as inhibitors of DNA methylation seems worthwhile.

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