Targeting DNA Methylation with Green Tea Catechins

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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.

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 (EGG; 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 interactions 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 (p16INK4a 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 p16INK4a, 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 p15INK4b and p16INK4a 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 SsII 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 π1 (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 p16INK4A genes. The downregulation of UHRF1 was dependent upon the generation of reactive oxygen species by EGCG. Upregulation of p16INK4A was strongly correlated with decreased promoter binding by UHRF1. UHRF1 overexpression counteracted EGCG-induced G1-arrested cells, apoptosis and upregulation of p16INK4A 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...
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

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

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

References

4 Jones PA, Baylin SB: The fundamental role of DNA methylation in the maintenance of DNA methylation and that, 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?

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