Review Article

Tea Polyphenols and Prevention of Epigenetic Aberrations in Cancer

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Abstract

Tea polyphenols are secondary metabolites of tea plants and are well known for beneficial health effects. They can protect from a variety of illnesses including cancers. Tea polyphenols can prevent cancer by modulating epigenetic aberrations taking place in DNA methylation, histone modifications, and micro-RNAs. By altering these epimutations, they regulate chromatin dynamics and expression of genes those induce or suppress cancer formation. However, majority of the studies in existing literature are carried out for green tea polyphenols rather than black tea polyphenols despite the fact that black tea is the most commonly consumed form of tea (78%) followed by green tea (20%) and other forms of tea. Research findings indicate that tea polyphenols may be potential source from which drugs with less side effects and affordable price can be developed.

Keywords: DNA methylation, epigenetics, histone acetylation, micro-RNA, tea polyphenols

Introduction

Tea is one of the most commonly consumed beverages in the world, second only to water. It is even more popular than coffee, wine, and carbonated soft drinks. Tea is made from leaves of tea plant *Camellia sinensis*. Most commonly used form of tea is black tea and it accounts for 78% of world's tea consumption,[1] followed by green tea (20%), oolong tea, and white tea. Tea contains a wide variety of chemical compounds, but the major ones associated with the taste, aroma, and health effects of tea are polyphenols. The chemical composition varies among different tea types. This difference is caused due to different agronomic practices such as plucking standard, pruning and manure application, and most importantly by oxidation during processing of freshly harvested tea leaves. Green tea is prepared from unoxidized leaves; thus, in green tea, natural polyphenols of tea are preserved. To prepare black tea, leaves are treated to be fully oxidized. In black tea, polyphenols are dimerized due to extended oxidation with enzymatic reactions, leading to leaf darkening and producing tea aroma.

Tea Polyphenols

Polyphenols are the main bioactive molecules in tea. Dried tea extract contains 25%–40% polyphenols.[2] The major polyphenolic compounds in tea are the flavan-3-ols called catechins. Catechins include epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate and epicatechin, gallocatechins and gallocatechin gallate. Among these, EGCG is the most prevalent one. Catechins are present in large amounts in green tea. Catechins account for 6%–16% of the dry green tea leaves with EGCG constituting 10%–50% of catechins. Complex polyphenols theaflavins (TFs) and thearubigins (TRs) are another group of polyphenolic compounds found in black teas. During preparation of black tea, extended oxidation causes most of the catechins to be oxidized to TF and TR, which give the extract its characteristic red-brown color.

Epigenetics of Normal Cells

Epigenetics is defined as the study of heritable changes in gene expression that occur independent of changes in the primary DNA sequence. Recent studies show other than genetic alterations epigenetic aberrations play significant role in

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producing cancer.[3] While there is no change in DNA sequence, epigenetic changes include methylation of DNA, histone tail modifications, and the effects of noncoding RNA. These modifications are required to regulate the normal functioning of genome in the cell primarily by altering its compactness and thus accessibility to different regulatory proteins.

DNA methylation

Among the epigenetic modifications, DNA methylation is probably the most commonly described epigenetic modification. Methylation occurs on cytosine residue of CpG dinucleotides, in which a cytosine (5') is followed by a guanine residue (3'). CpG regions are concentrated in certain portions of DNA, known as CpG islands. In humans, CpG islands occupy approximately 60% of the promoters.[4] CpG islands within promoter regions are highly susceptible to methylation.[5] DNA methylation is normally associated with gene inactivation. DNA methylation causes gene silencing by preventing or promoting the recruitment of regulatory proteins to DNA. For example, it may inhibit binding of certain transcription factors, or it may provide binding sites for proteins those mediate gene repression through interactions with histone deacetylases (HDACs).[3] Paradoxically, it is also reported that DNA methylation of transcription factor binding sites can inhibit binding of repressor proteins, which may lead to inducing gene activation.[6]

DNA methylation has important role in X chromosome inactivation and silencing of germ line-specific genes and repetitive elements.[5] Methylation of repetitive DNA sequences contributes in chromosomal stability. Tissue-specific DNA methylation has been reported in different somatic tissues.[3] Here, methylation plays an important role in the regulation of developmentally important genes.

DNA methylation is catalyzed by DNA methyltransferase (DNMTs) enzymes. The DNMT family has several enzymes. In humans, there are four DNMTs, DNMT 1, DNMT 2, DNMT 3a, and DNMT 3b. DNMT1 is a maintenance enzyme that maintains methylation following DNA replication and DNMT 2 has weak methylation activity. DNMT 3a and DNMT 3b are responsible for *de novo* methylation of CpG islands. Although DNMT3a and DNMT3b are important for *de novo* methylation during embryogenesis, recent studies suggest that these enzymes are also important in the maintenance of DNA methylation patterns.[5] DNMT 3a and DNMT 3b complete the methylation process following replication and proofread the DNMT 1 activity.[5]

Histone modifications

Histone proteins in eukaryotic nuclei play a central role in packaging of DNA to dense chromatin structure. Therefore, histone proteins play crucial role in gene expression by regulating accessibility of specific transcription factors to the DNA. Chromatin is made up of repeating units called nucleosomes. Each nucleosome has a core histone octamer made up of two each of H2A, H2B, H3, and H4. One histone octamer is wrapped around by DNA of 146 base pair units. A linker histone H1 binds to the nucleosome and the linker DNA between two nucleosomes and stabilizes the chromatin structure.

Histone proteins of nucleosome core contain a globular C-terminal domain and an N-terminal tail. The N-terminal tails of histones are subjected to a variety of posttranslational covalent modifications including phosphorylation, methylation, acetylation, ubiquitylation, glycosylation, and sumoylation those regulate key cellular processes including gene expression. Acetylation is probably the most widely studied histone modification. Histones are acetylated at ε-amino nitrogen of lysine residues by a class of enzymes called histone acetyltransferases (HATs). Histone acetylation has been proposed to be directly related to transcription regulation. Acetylation partially neutralizes the positive charge of histones that weakens their interaction with the DNA, probably facilitating the access of transcription factors to their recognition elements leading to enhanced transcription. HDACs reverse back acetylated state of histones. Interplay between HATs and HDACs determines chromatin's activity states. Histones are methylated by the activities of histone methyltransferases and histone demethylases remove methyl groups from histones. These histone-modifying enzymes interact with each other as well as with the DNA-modifying enzymes to combine chromatin stability and gene expression.

Gene activation or repression depends upon the types of histone modifications, for example, while lysine acetylation is linked to transcriptional activation, methylation of this amino acid may induce or repress gene expression. For example, trimethylation of lysine 4 on histone H3 (H3K4me3) is related to gene activation, whereas the similar modification of H3K9 (H3K9me3) and H3K27 (H3K27me3) are related to transcription repression.[3] Histone phosphorylation is mostly associated with gene transcription.[7]

MicroRNA

Other than the above-stated modifications, an important epigenetic change includes microRNA (miRNA) that is involved in modifying genes posttranscriptionally. They constitute a class of small (approximately 22 nucleotides in length) noncoding RNA and act to downregulate the expression of genes. This occurs through base pairing between the miRNA and an mRNA and subsequent mRNA degradation or translational repression.[8] More than 1000 human miRNA sequences are known today.[9] It is suggested that more than one-third of human genes are regulated by miRNAs.[10] Noncoding RNA is essential to normal cell processes (e.g., introns and splicing); however, aberrant miRNA expression patterns are linked to chromosomal instability.[8]

Epigenetic Changes in Cancer

The classic view of cancer etiology states that genetic alterations or mutations leading to inappropriate gene expression lead to cancer development. Although this was thought as primary cause of the disease, recently, epigenetic

changes have been identified to play significant role in causing cancer. Studies reveal that epimutations or epigenetic changes in DNA methylation patterns at CpG sites contribute significantly to tumor progression. A cancer epigenome is marked by genome-wide hypomethylation and site-specific CpG island promoter hypermethylation that directly alters gene expression. Hypomethylation causes genomic instability, activation of protooncogenes. Hypermethylation-mediated silencing has been reported for some transcription factor genes GATA-4 and GATA-5 and potential downstream antitumor target genes in colorectal and gastric cancers.[11] Silencing of DNA repair genes leads to accumulation of mutations that can lead to the development of cancer. DNMTs play crucial role in these changes. In cancer cells along with maintaining DNA methylation, DNMT 1 also has the ability to *de novo* methylate the DNA of tumor suppressor genes.[7] Decreased activity of DNMT 1 can lead to a state of hypomethylation.

High-throughput sequencing has revealed loss of histone acetylation during tumorigenesis that results in gene repression. HDACs and HATs can also be altered in cancer. Other than histone acetylation, cancer cells can also undergo widespread changes in histone methylation patterns. Alterations in H3K9 and H3K27 methylation patterns have been found to be linked with aberrant gene silencing in various forms of cancer.[3]

miRNAs have been shown to affect the hallmarks of cancer, including sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis.[9] Significant changes take place in miRNA expression during tumorigenesis. Many tumor suppressor miRNAs that target growth-promoting genes are repressed in cancer. For example, miR-15 and miR-16 that target antiapoptotic gene Bcl-2 are downregulated in chronic lymphocytic leukemia, whereas let-7 that targets the oncogene, RAS, is found to be downregulated in lung cancer.[3]

Epigenetic Changes and Tea Polyphenols

Epigenetic changes, however, are reversible and may be targeted by dietary interventions. One major cause is that nutrients are the main source of methyl groups or act as coenzymes for the one-carbon metabolism that regulates methyl transfer and DNA synthesis.[12] There is a continuous search for affordable therapeutics with least side effects in cancer management, where phytochemicals and traditional medicines may play important role.

Green tea polyphenols

Evidence in cell lines

Green tea polyphenols (GTPs) have been shown to inhibit DNA methylation and activation of epigenetically silenced genes in cell line studies. Bioactive compounds from green tea including EGCG have been found to alter DNMT activity in studies of esophageal, oral, skin, regulatory T-cells, lung, breast, and prostate cancer cells.[12] Fang *et al*.[13] suggested that tea polyphenol EGCG was found to inhibit DNMT activity and reactivate methylation-silenced genes in cancer cell lines. Reactivation of some methylation-silenced genes by EGCG was also demonstrated in human colon, esophageal, and prostate cancer cells.[13] Nandakumar *et al*.[14] showed that EGCG reduced DNA methylation levels in human epidermoid carcinoma cells in a dose-dependent manner. EGCG decreased the expression of DNMT 1, DNMT 3a, and DNMT 3b. EGCG also increased histone acetylation. In addition, EGCG treatment resulted in reactivation of silenced tumor suppressor genes, $p16^{INK4\alpha}$, and Cip1/p21. EGCG treatment was shown to restore Wnt inhibitory factor-1, an antagonist of Wnt proto-oncogene in nonsmall-cell lung cancer.[15] EGCG was also found to increase expression of RECK tumor suppressor gene in oral squamous cell carcinoma cell lines.[16] They suggested that EGCG played a key role in suppressing cell invasion through multiple mechanisms, possibly by demethylation effect on matrix metalloproteinase inhibitors such as RECK.

HDACs are broadly classified into four classes based on their sequence homology, I, II, III, and IV, among which Class I HDACs are overexpressed in the majority of human cancers, including prostate cancer. It was demonstrated that GTPs and their major constituent EGCG caused inhibition of Class I HDACs, in LNCaP human prostate cancer cells.[17] The decrease in HDAC activity was associated with increased global acetylation of histone proteins as well as local hyperacetylation of histone H3 on the p21/waf1 (a cell cycle kinase inhibitor) and bax (a proapoptotic protein) promoters. Thus, it can be said that GTPs may be safe and potential inhibitors of Class I HDACs. EGCG was shown to diminish the activity of HDAC including HDAC 1 in a human colon adenocarcinoma cell line also.[18] These findings are important as the inhibition of HDAC activity has been recognized as a potent strategy for cancer therapy and chemoprevention. EGCG has been reported to inhibit HAT also.[8]

EGCG influence on the expressions of miRNAs in human cancer cells was investigated and it was found that EGCG treatment up- or down-regulates some miRNAs in human hepatocellular carcinoma HepG2 cells.[19] One of the up-regulated miRNAs was miR-16 whose one target is antiapoptotic protein Bcl-2. EGCG treatment caused downregulation of Bcl-2 and induced apoptosis. It has been suggested by Daniel and Tollefsbol[20] that supplementation with polyphenols at nutritionally achievable levels could modulate miRNA expression.

In vivo studies

Inhibitory effect of EGCG on DNA methylation has been confirmed in mouse model also. Brewed green tea was administered instead of drinking water to male severe combined immunodeficiency (SCID) mice with human LAPC 4 prostate cancer cell xenografts.[21] LAPC 4 prostate cancer cells were implanted subcutaneously into the flank of SCID mice to generate tumour. A significant decrease in tumor volume was observed, and tumor size was found significantly correlated with GTP content in tumor tissue. An inhibition of DNMT 1 protein and gene expression in prostate xenograft

LAPC 4 tumor tissue was also found. Tumor volume and weight were also decreased significantly in these mice.

EGCG has been tested *in vivo* for its influence on miRNA profile changes in the tobacco-carcinogen-induced mouse lung tumor. In their study, 21 miRNAs were identified whose expressions are regulated by EGCG.[22] Target genes of these miRNAs include the key components in the signaling regulation pathways centralized with AKT, MAP kinase, and cell cycle regulators. The evidence from human subjects demonstrating significant dietary effects of tea polyphenols on DNA methylation is very limited in comparison to *in vitro* and animal studies. In a human study from Japan, a decreased methylation of CDX2 gene, the gene frequently hypermethylated in gastric carcinoma, was found to be associated with high green tea consumption (seven cups or more per day).[23]

Black tea polyphenols

It is noticeable that all the above-stated studies involved GTP-EGCG. Despite the fact that black tea is the most commonly consumed form of tea, only a scanty number of studies are found in the literature regarding the effect of black tea polyphenols TF and TR on the epigenetic changes in cancer. One study by Rajavelu *et al*.[24] depicted inhibition of mouse DNMT 3a by black tea polyphenol. Since epigenetic alterations can be reversed by tea polyphenols, epigenetic therapy using tea polyphenols can open new direction in cancer prevention and management.

Conclusion

GTPs, especially its major constituent EGCG, are found to have the ability to modulate epimutations. Therefore, they can be potential source of safe, cost-effective cancer drug. However, this property of tea polyphenols has been evidenced mostly from cell line studies, more animal or human studies are required to establish its *in vivo* activity before considering it for cancer drug development studies.

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Conflicts of interest

There are no conflicts of interest.

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