Functional diversity of DNA methyltransferase inhibitors in human cancer cell lines.

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DNA methyltransferase inhibitors represent promising new drugs for cancer therapies. The first of these compounds (5-azacytidine, Vidaza) has recently been approved as an antitumor agent, and others are presently in various stages of their preclinical or clinical development. Most of the archetypal inhibitors have been established and characterized in different experimental systems, which has thus far precluded their direct comparison. We have now established defined experimental conditions that allowed a comparative analysis of the six most widely known DNA methyltransferase inhibitors: 5-azacytidine (5-aza-CR), 5-aza-2'-deoxycytidine (5-aza-CdR), zebularine, procaine, (-)-epigallocatechin-3-gallate (EGCG), and RG108. Of these, 5-aza-CR, 5-aza-CdR, zebularine, and EGCG were found to exhibit significant cytotoxicity in human cancer cell lines. 5-aza-CdR and EGCG were also found to be genotoxic, as evidenced by the induction of micronuclei. In addition, 5-aza-CR, 5-aza-CdR, and EGCG caused concentration-dependent demethylation of genomic DNA, whereas procaine and EGCG failed to induce significant effects. Finally, the experiments in cancer cell lines were complemented by a cell-free in vitro assay with purified recombinant DNA methyltransferase, which indicated that RG108 is the only drug capable of direct enzyme inhibition. These results show a substantial diversity in the molecular activities of DNA methyltransferase inhibitors and provide valuable insights into the developmental potential of individual drugs.

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Procaínamida is a specific inhibitor of DNA methyltransferase 1.

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CpG island hypermethylation occurs in most cases of cancer, typically resulting in the transcriptional silencing of critical cancer genes. Procaínamida has been shown to inhibit DNA methyltransferase activity and reactivate silenced gene expression in cancer cells by reversing CpG island hypermethylation. We report here that procaínamida specifically inhibits the hemimethylase activity of DNA methyltransferase 1 (DNMT1), the mammalian enzyme thought to be responsible for maintaining DNA methylation patterns during replication. At micromolar concentrations, procaínamida was found to be a partial competitive inhibitor of DNMT1, reducing the affinity of the enzyme for its two substrates, hemimethylated DNA and S-adenosyl-l-methionine. By doing so, procaínamida significantly decreased the processivity of DNMT1 on hemimethylated DNA. Procaínamida was not a potent inhibitor of the de novo methyltransferases DNMT3a and DNMT3b. As further evidence of the specificity of procaínamida for DNMT1, procaínamida failed to lower genomic 5-methyl-2'-deoxycytidine levels in HCT116 colorectal cancer cells when DNMT1 was genetically deleted but significantly reduced genomic 5-methyl-2'-deoxycytidine content in parental HCT116 cells and in HCT116 cells where DNMT3b was genetically deleted. Because many reports have strongly linked DNMT1 with epigenetic alterations in carcinogenesis, procaínamida may be a useful drug in the prevention of cancer.

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Reversal of GSTP1 CpG island hypermethylation and reactivation of pi-class glutathione S-transferase (GSTP1) expression in human prostate cancer cells by treatment with procaínamida.


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Among the many somatic alterations present in cancer cells, changes in DNA methylation may represent reversible "epigenetic" lesions, rather than irreversible "genetic" alterations. Cancer cell DNA is typically characterized by increases in the methylation of CpG dinucleotides clustered into CpG islands, near the transcriptional regulatory regions of critical genes, and by an overall reduction in CpG dinucleotide methylation. The transcriptional "silencing" of gene expression associated with such CpG island DNA hypermethylation presents an attractive therapeutic target: restoration of "silenced" gene expression may be possible via therapeutic reversal of CpG island hypermethylation. The most common somatic genome alteration yet reported for human PCAs, occurs early during human prostatic carcinogenesis and results in a loss of GSTP1 "caretaker" function, leaving prostate cells with inadequate defenses against oxidant and electrophile carcinogens. We report here that the drug procaínamida, a nonnucleoside inhibitor of DNA methyltransferases, reversed GSTP1 CpG island hypermethylation and restored GSTP1 expression in LNCaP human PCA cells propagated in vitro or in vivo as xenograft tumors in athymic nude mice.

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DNA methylation inhibitor, procaínamida, may decrease the tamoxifen resistance by inducing overexpression of the estrogen receptor.
beta in breast cancer patients.


Estrogen is the main stimulant in the development and growth of breast cancer. The estrogen receptor antagonist tamoxifen has been most common in adjuvant hormonal therapy. Although tamoxifen has been an effective adjuvant therapy, approximately 30% of patients treated with this agent still die within 10 years of follow-up treatment, and relapses can occur for > or = 20 years following therapy. However, the underlying cause of treatment failure in many breast cancer patients receiving tamoxifen is resistance to tamoxifen. ERbeta may influence estrogen action through the ERalpha pathway and the hormone refractoriness of breast cancer. ERbeta, the carboxy terminal splicing variant of ERbeta, has been considered a dominant repressor of ERalpha function, because ERbeta inhibits transcriptional activity of ERalpha rather than ERbeta wild type (wt). Tamoxifen responders tended to exhibit a lower ratio of ERbeta to ERbeta T than non-responders. Induction of ERbeta reduces growth of exponentially proliferating cells. Since the promoter region of ERbeta is rich in CpG dinucleotides, loss of expression of ERbeta observed in some tumours could be due to aberrant methylation of CpG islands. Treatment of ERbeta-negative cell lines with DNA methyl transferase inhibitors restored ERbeta expression, providing experimental evidence that silencing of ERbeta in breast carcinomas could be due to promoter hypermethylation. Procainamide, used for cardiac arrhythmias, has been proposed as being a non-nucleoside inhibitor of DNA methylation and also demethylates and reactivates tumor suppressor genes in breast cancer cell lines. Therefore, concomitant use of procainamide with tamoxifen in ERalpha-positive and ERbeta-negative breast cancers may increase the tamoxifen response. Procainamide, given orally may also be used in breast cancer patients who developed resistance during the tamoxifen treatment. In vivo and in vitro studies evaluating effectiveness of concomitant use of procainamide and tamoxifen in tamoxifen resistant and ERbeta-negative breast cancer may further support our hypothesis. Copyright 2004 Elsevier Ltd.

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Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines.


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Hypermethylation of CpG islands in the promoter regions is an important mechanism to silence the expression of many important genes in cancer. The hypermethylation status is passed to the daughter cells through the methylation of the newly synthesized DNA strand by 5-cytosine DNA methyltransferase (DNMT). We report herein that (-)epigallocatechin-3-gallate (EGCG), the major polyphenol from green tea, can inhibit DNMT activity and reactivate methylation-silenced genes in cancer cells. With nuclear extracts as the enzyme source and polydeoxyinosine-deoxycytosine as the substrate, EGCG dose-dependently inhibited DNMT activity, showing competitive inhibition with a K(i) of 6.89 microM. Studies with structural analogues of EGCG suggest the importance of D and B ring structures in the inhibitory activity. Molecular modeling studies also support this conclusion, and suggest that EGCG can form hydrogen bonds with Pro1223, Glu1265, Cys1225, Ser1229, and Arg1309 in the catalytic pocket of DNMT. Treatment of human esophageal cancer KYSE 510 cells with 5-50 microM of EGCG for 12-144 h caused a concentration- and time-dependent reversal of hypermethylation of p16(INK4a), retinoic acid receptor beta (RARbeta), O(6)-methylguanine methyltransferase (MGMT), and human mutL homolog 1 (hMLH1) genes as determined by the appearance of the unmethylation-specific bands in PCR. This was accompanied by the expression of mRNA of these genes as determined by reverse transcription-PCR. The re-expression of RARbeta and MGMT by EGCG was demonstrated by Western blot. Reactivation of some methylation-silenced genes by EGCG was also demonstrated in human colon cancer HT-29 cells, esophageal cancer KYSE 150 cells, and prostate cancer PC3 cells. The results demonstrate for the first time the inhibition of DNA methylation by a commonly consumed dietary constituent and suggest the potential use of EGCG for the prevention or reversal of related gene-silencing in the prevention of carcinogenesis.

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Dietary polyphenols may affect DNA methylation.

Fang M, Chen D, Yang CS. J Nutr. 2007 Jan;137(1 Suppl):222S-228S. Citation.

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 p16INK4a, retinoic acid receptor beta, O(6)-methylguanine methyltransferase, human mutL homolog 1, and glutathione S-transferase-pi. These activities have been observed in human esophageal, colon, prostate, breast, and cervical cancers. Polyphenols inhibit DNMT by generating S-adenosyl-L-homocysteine on their methylation by S-adenosyl-L-methionine. In theory, prevention or reversal of DNA methylation-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.

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Mechanisms of cancer prevention by green and black tea polyphenols.


Drinking green tea is associated with decreased frequency of cancer development. This review outlines the wide range of mechanisms by which epigallocatechin gallate (EGCG) and other green and black tea polyphenols inhibit cancer cell survival. EGCG suppressed androgen receptor expression and signalling via several growth factor receptors. Cell cycle arrest or apoptosis involved caspase activation, nuclear factor-kappaB (NF-kappaB) translocation of the transcription factor nuclear factor-kappaB as a result of decreased IkappaB kinase activity. Polyphenols up- or down-regulated activity of a number of key enzymes, including mitogen-activated protein kinases and protein kinase C, and increased or decreased protein/mRNA levels, including that of cyclins, oncoproteins, and tumor suppressor genes. Metastasis was inhibited via effects on urokinase and matril metalloproteinases. Polyphenols reduced angiogenesis, in part by decreasing vascular endothelial growth factor production and receptor phosphorylation. Recent work demonstrated that EGCG reduced dihydrofolate reductase activity, which would affect nucleic acid and protein synthesis. It also acted as an aryl hydrocarbon receptor antagonist by directly binding the receptor's molecular chaperone, heat shock protein 90. In conclusion, green and black tea polyphenols act at numerous points regulating cancer cell growth, survival, and metastasis, including effects at the DNA, RNA, and protein levels.

http://www.medicinacomplementar.com.br/conversao/36-0539.html
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