

**Special Article****Chemoprevention molecular and biochemical mechanisms involved in cancer control and management****George Albert Karikas**

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**Abstract**

**Background:** Cancer is a growing health problem around the world. Throughout history, natural products have been the most significant source of anticancer and chemopreventing agents. More than 1,000 different phytochemicals are already proved to possess interesting chemopreventing activities.

**Aim:** To present a number of recent biochemical and molecular mechanisms, in relation to natural and synthetic chemopreventing substances for cancer control and management. **Results:** Vegetables and fruit are excellent sources of cancer-preventive substances. Natural products consist of a wide variety of biologically active phytochemicals including phenolics, flavonoids, carotenoids, etc. have been shown to suppress early and late stages of carcinogenesis. Effectiveness of chemopreventive agents reflects their ability to counteract certain upstream signals, such as NF-kB,  $\beta$ -catenin, etc. Furthermore, epigenetic modified agents, alone or in combination with conventional anticancer drugs may prove to be a significant advance over the conventional anticancer control.

**Conclusion:** Chemoprevention by edible phytochemicals is nowadays considered to be an inexpensive, readily applicable, and accessible approach to cancer control and management. In addition, the promotion of awareness and consumption of phytochemicals as a cancer-preventive strategy would be cost-effective, within the health system.

**Key words:** cancer chemoprevention, epigenetic mechanisms, phytochemicals , polyphenols, drugs

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**Introduction**

Cancer is a growing health problem around the world, particularly with the steady rise in life expectancy. According to a recent report by the World Health Organization, there are now more than 10 million cases of cancer per year worldwide. Cancer results from a multistage, multi-mechanism carcinogenesis process that

involves mutagenic, cell death and epigenetic mechanisms, during the three distinguishable but closely allied stages: initiation, promotion, and progression. Since reducing the initiation phase to a zero level is impossible, the most effective intervention would be at the promotion phase to

eliminate premalignant cells before they become malignant<sup>1</sup>.

Natural products, in general, have been the most significant source of drugs in science. Throughout history, these products have afforded a rich source of compounds that have found many applications in the fields of medicine, pharmacy and biochemistry<sup>2</sup>.

The fact that about 7 million people die from various types of cancer every year, making this disease responsible for 12.5% of deaths worldwide, raises an overwhelming demand to develop new, more potent and effective anticancer, as well as chemopreventing agents<sup>3</sup>.

Therefore, the concept of delaying or preventing this transformation remains a viable and attainable goal for the future<sup>4</sup>.

Vegetables and fruit are excellent sources of cancer-preventive substances. Intervention to slow down, arrest or reverse the process of carcinogenesis by the use of either natural or synthetic substances individually or in combination therapy has emerged as a promising and pragmatic medical approach to reduce cancer risk. Epidemiological and experimental evidence emphasize that specific compounds may positively inhibit carcinogenesis at various sites, including the oral cavity, esophagus, stomach, colon/rectum, lung, breast, and prostate, but at the same time, another compelling body of evidence, together with the data from animal and *in vitro* studies, strongly supports the relationship between dietary constituents and the risk of cancer development<sup>5</sup>. The American National Cancer Institute has identified about 35 plant-based foods containing 1,000 different phytochemicals, that possess cancer-preventive properties. The most exciting findings have been achieved with antioxidant vitamins and their precursors, which are found in dark, leafy green vegetables and yellow/ orange fruit and vegetables. Recently, the focus and emphasis have shifted to the non-nutritive phytochemicals<sup>2</sup>. The present short review present a number of recent biochemical and molecular mechanisms, in relation to promising natural and synthetic chemopreventing substances for cancer control and management.

## Chemopreventing mechanisms

The mechanistic insight into chemoprevention includes induction of cell cycle arrest and apoptosis or inhibition of signal transduction pathways mainly the mitogen-activated protein kinases (MAPK), protein kinases C (PKC), phosphoinositide 3-kinase (PI3K), glycogen synthase kinase (GSK) which leads to abnormal cyclooxygenase-2 (COX-2), activator protein-1 (AP-1), and nuclear factor kappa-light chain-enhancer of activated B cells (NF- $\kappa$ B). Effectiveness of chemopreventive agents reflects their ability to counteract certain upstream signals that leads to genotoxic damage, redox imbalances and other forms of cellular stress. Targeting malfunctioning molecules along the disrupted signal transduction pathway in cancer represents a rational strategy in chemoprevention. NF- $\kappa$ B and AP-1 provide mechanistic links between inflammation and cancer. Thus cell signaling cascades and their interacting factors have become important targets of chemoprevention and phenolic phytochemicals and plant extracts seem to be promising in this endeavor<sup>6</sup>.

Epigenetic mechanisms involved also in carcinogenesis. Carcinogenesis is a long-term process and both genetic and epigenetic factors contribute to cancer development. Epigenetic changes, such as DNA methylation, histone modifications and post transcriptional gene regulation by non-coding microRNAs (miRNAs) are easily influenced by dietary and environmental factors. These processes affect transcript stability, DNA folding, nucleosome positioning, chromatin compaction, and complete nuclear organization of the genetic material. Synergistically and cooperatively they determine whether a gene is silenced or expressed, as well as the timing and tissue-specificity of the expression of these genes. Disruption of the epigenome certainly underlies disease development<sup>7</sup>.

Dietary polyphenols can potentially impact all three epigenetic modifications, which in turn contribute towards their chemopreventive potential. Although epigenetic changes are heritable in somatic cells, these modifications are also potentially reversible, which makes them attractive and

promising avenues for cancer preventive and therapeutic strategies. Dietary polyphenols from green tea, turmeric, soybeans, broccoli and others have shown to possess multiple cell-regulatory activities within cancer cells<sup>8</sup>. From a clinical point of view, epigenetics seem to offer a very promising and attractive fact., in contrast to genetic changes such mutations, gene deletions, DNA binding<sup>9</sup>. Unlike mutations, which exist for the lifetime, epigenetically modified genes can be restored. Methylation silenced genes can be demethylated, and histone complexes can be rendered transcriptionally active by modification of acetylation and methylation of various histones via nutrients, drugs and other dietary interventions<sup>10</sup>.

An ideal chemopreventive agent should have: 1) little or no toxicity; 2) high efficacy in multiple sites; 3) capability of oral consumption; 4) known mechanisms of action; 5) low cost, and human acceptance. A variety of grains, cereals, nuts, soy products, olives, beverages confer a protective effect against cancer<sup>11</sup>. In particular, natural products consist of a wide variety of biologically active phytochemicals including phenolics, flavonoids, carotenoids, alkaloids and nitrogen containing as well as organosulfur compounds, which have been shown to suppress early and late stages of carcinogenesis<sup>12</sup>. Chemopreventing agents and their sources inducing epigenetic changes are given in Table 1.

**Table 1. Polyphenols acting as epigenetics via specific mechanisms (#)**

POLYPHENOLS	SOURCE	DNMTs inhibition	HDACs inhibition	Tumor suppressors
coffe polyphenols	coffe	#		
curcumin	turmeric	#	#	#
dihydrocoumarin	sweet glover		#	
epigallocatechin-3-gallate	green tea	#	#	#
garcinol	garcinia		#	
genistein	soya	#		
lycopene	tomatoes	#		
quercetin	plant food	#		
resveratrol	red wine	#	#	
rosmarinic acid	oregano	#		
sanguinarine	blood root		#	

Recently, the bioactive triterpene, **lupeol**, commonly found in fruits like fig, mango, etc, has attracted interest in the context of chemoprevention attributable in large part to its antioxidant<sup>13</sup>, apoptosis-inducing and antiproliferative anti-mutagenic, anti-inflammatory<sup>14</sup> properties as well as its efficacy in inhibition of *in vivo* and *in vitro* cancer growth. Triterpenes represent a

varied class of natural products, which occur commonly and are found in fruits, vegetables and other parts of several medicinal plants e.g. *Arbutus unedo*, *Tipuana tipu*, etc<sup>15</sup> have seen tremendous efforts by researchers worldwide to develop this wonderful molecule for its clinical use for the treatment of a variety of disorders. The last 15 years studies also provide insight into the

mechanism of action of lupeol and suggest that it is a multi-target agent with immense anti-inflammatory potential targeting key molecular pathways which involve NF- $\kappa$ B, cFLIP, Fas, Kras, phosphatidylinositol-3-kinase (PI3 K)/Akt and Wnt/ $\beta$ -catenin in a variety of cells. It is noteworthy that lupeol at its effective therapeutic doses exhibits no toxicity to normal cells and tissues<sup>16</sup>. NF- $\kappa$ B, a transcription factor, is now known to be closely connected to the process of tumorigenesis based on a multiplicity of evidence. NF- $\kappa$ B is activated in response to tobacco, stress, dietary agents, obesity, alcohol, infectious agents, irradiation, and environmental stimuli that account for as much as 95% of all cancers. NF- $\kappa$ B: a) : regulates the expression of most anti-apoptotic gene products associated with the survival of the tumor; b) : regulates the gene products linked with proliferation of tumors; c) : controls the expression of gene products linked with invasion, angiogenesis, and metastasis of cancer. While most carcinogens activate NF- $\kappa$ B, most chemopreventive agents suppress its activation. These observations suggest that NF- $\kappa$ B is intimately intertwined with cancer growth and metastasis. AP1 is another transcription factor that regulates expression of genes that are involved in cellular adaptation, differentiation and proliferation. Functional activation of AP1 is associated with malignant transformation as well as tumor promotion<sup>17</sup>.

### Chemopreventing phytochemicals

**Curcumin**, a spice widely used in Indian cuisine, has been identified to show considerable anti-tumor effects. It is a yellow pigment that is present in the rhizome of turmeric (*Curcuma longa* L.) and related species and is one of the most extensively investigated phytochemicals, with regard to chemopreventive potential. The mechanisms implicated in the inhibition of tumorigenesis by curcumin are diverse and appear to involve a combination of anti-inflammatory, antioxidant, immunomodulatory, proapoptotic, and antiangiogenic properties via pleiotropic effects on genes and cell-signaling pathways

at multiple levels. When curcumin is combined with some cytotoxic drugs or certain other diet-derived polyphenols, synergistic effects have been demonstrated<sup>18</sup>.

A late finding is that curcumin binds directly to and activates VDR (the nuclear vitamin D receptor), inducing the VDR target genes CYP3A4, CYP24, p21 and TRPV6<sup>19</sup>. Despite our increasing knowledge on this substance there still remain many unknown effects that deserve intense investigation<sup>20</sup>.

**Gingerol**, a phenolic substance that is responsible for the spicy taste of ginger (*Zingiber officinale*) was reported to inhibit tumor promotion and PMA-induced ornithine decarboxylase (ODC) activity and TNF-production in mouse skin<sup>21</sup>.

**Capsaicin**, a pungent component of hot chilli pepper (*Capsicum annum* L.) has been suspected to act as a carcinogen or a co-carcinogen in experimental animals because of its irritant properties, but other studies indicate that this compound has chemopreventive and chemoprotective effects<sup>22</sup>.

**Epigallocatechin gallate** (EGCG) is an antioxidant and chemopreventive polyphenol that is found in green tea. It has been shown to suppress malignant transformation in a PMA-stimulated mouse epidermal JB6 cell line, which seemed to be mediated by blocking activation of AP1<sup>23</sup>.

**Genistein**, a soy-derived isoflavone, is believed to contribute to the putative breast- and prostate cancer preventive activity of soya. Genistein inhibited PMA-induced AP1 activity, expression of c-FOS and ERK activity in certain human mammary cell lines. Genistein treatment abrogated NF- $\kappa$ B DNA binding in human hepatocarcinoma cells stimulated with hepatocyte growth factor<sup>24</sup>.

**Resveratrol** (3,4',5-trihydroxy-*trans*stilbene) is a phytoalexin that is present in grapes (*Vitis vinifera*) and a key antioxidant ingredient of red wine. It is believed to be responsible for the so-called 'French

paradox', in which consumption of red wine has been shown to reduce the mortality rates from cardiovascular diseases and certain cancers. Resveratrol treatment inhibited PMA-induced COX2 expression and catalytic activity, via the cyclic-AMP response element (CRE) in human mammary epithelial cells<sup>25</sup>. It also inhibited PKC activation, AP1 transcriptional activity and the induction of COX2-promoter activity in PMA-treated cells. Resveratrol induced apoptosis and reduced the constitutive activation of NF- $\kappa$ B in both rat and human pancreatic carcinoma cell lines<sup>26</sup>. Of particular interest is that resveratrol is capable of causing DNA breakage in cells such as human lymphocytes. Such cellular DNA breakage is inhibited by copper specific chelators but not by iron and zinc chelating agents<sup>27</sup>.

In addition to the above mentioned phytochemicals, **quercetin**, a flavonoid which is ubiquitously distributed in edible plant foods, **caffeic acid phenethyl ester**, **sulphoraphane**, **silymarin**, **apigenin**, **emodin** and **anethole** have also been reported to suppress the activation of NF- $\kappa$ B and AP1, which might contribute to their chemopreventive and/or cytostatic effects<sup>28</sup>. Several dietary phytochemicals have been shown to downregulate the  $\beta$ -catenin-mediated signaling pathway as part of their molecular mechanism of chemoprevention. Curcumin and **caffeic acid phenethyl ester** inhibited tumorigenesis and decreased  $\beta$ -catenin expression in the multiple intestinal neoplasia (Min/+) mouse model<sup>29</sup>. Moreover, curcumin reduced the cellular levels of  $\beta$ -catenin through caspase-mediated cleavage of the protein<sup>30</sup>. Downregulation of  $\beta$ -catenin expression by resveratrol was observed in a human colon cancer cell line<sup>31</sup>.

Expression of a  $\beta$ -catenin- TCF4-binding reporter construct was reduced in HEK293 cells by epigallocatechin-3-gallate<sup>32</sup>. Indole-3-carbinol altered the pattern of  $\beta$ -catenin mutation in chemically- induced rat colon tumors<sup>33</sup>, inhibited adhesion, migration and invasion of cultured human breast carcinoma cells, and upregulated E-cadherin and  $\beta$ -catenin<sup>34</sup>. A similar effect was observed with **tangeretin** from citrus<sup>35</sup>. COX inhibitors have also been found to suppress  $\beta$ -catenin

signalling and  $\beta$ -catenin-TCF/LEF transcriptional activity<sup>36</sup>.

### Epigenetic drugs

Epigenetic therapy, the use of drugs to correct epigenetic defects, is currently a new and fascinating area for drug development in the field of cancer prevention. Besides their promise as therapeutic agents, epigenetic drugs may also be used for prevention of various diseases, including cancer chemoprevention. Epigenetic therapy is a potentially very useful form of therapy because epigenetic defects, in contrast to genetic defects, are reversible<sup>37-38</sup>.

Additionally, there is growing trend, that epigenetic drugs alone or in combination with conventional anticancer drugs may prove to be a significant advance over the conventional anticancer drugs, which inherently tend to be very toxic by themselves<sup>39</sup>.

The current generation of epigenetic drugs primarily target to inhibit the activity and expression of DNMTs and HDACs. Among the DNMT inhibitors, nucleic acid inhibitors, such as **5-azacytidine** and **5-aza-2-deoxycytidine**, are the most important and widely studied epigenetic drugs. In addition certain non-nucleoside inhibitors such as **procainamide**, **procaine** and **EGCG** have also shown potent inhibitors of DNMT activity in various experimental and clinical studies<sup>40-43</sup>. Concerning HDAC inhibitors, **trichostatin A**, **suberoylanilide hydroxamic acid**, **valproic acid** and **phenyl butyrate**, have been widely used with some success in various studies. **Vorinostat** (suberoylanilide hydroxamic acid), a highly potent histone deacetylase inhibitor, was recently approved by the Food and Drug Administration for the treatment of cutaneous T-cell lymphoma<sup>44-46</sup>.

Several of these potentially useful epigenetic drugs are undergoing preclinical and clinical drug trials. Although the current generation of epigenetic drugs have provided the proof of principle in its favor, epigenetic therapy has its limitations. Some of these shortcomings include that both DNMT and HDAC inhibitors may activate oncogenes due

to lack of specificity, resulting in accelerated tumor progression<sup>47</sup>. New findings on hypermethylation in cancer led to a reevaluation of hypomethylating drugs *in vitro* and *in vivo*, resulting in Food and Drug Administration-approved drugs that are helping patients live longer with fewer side effects than conventional cytotoxic therapy<sup>48</sup>.

## Conclusions

Natural products consist of a wide variety of biologically active phytochemicals from green tea, turmeric, soybeans, broccoli, grains, cereals, nuts, soy products, olives and beverages, including phenolics, flavonoids, carotenoids, alkaloids and nitrogen containing as well as organosulfur compounds, which have been shown to suppress early and late stages of carcinogenesis. Effectiveness of natural chemopreventive agents reflects their ability to counteract certain upstream signals, such as NF- $\kappa$ B, AP1, TNF,  $\beta$ -catenin, etc. Interestingly, dietary polyphenols can also potentially impact epigenetic modifications, such as DNA methylation, histone modifications and post transcriptional gene regulation by non-coding microRNAs (miRNAs). In addition epigenetic drugs acting via similar mechanisms, alone or in combination with conventional anticancer drugs may prove to be a significant advance over the conventional anticancer drugs. It has become obvious, that chemoprevention in close relation to chemotherapy, enforced by edible phytochemicals is now considered to be an inexpensive, readily applicable, acceptable and accessible approach to cancer control and management. In conclusion, with healthcare costs being an international key issue today, it would be cost-effective to promote the awareness and consumption of phytochemicals as a cancer-preventive and therapeutic strategy, within the health system.

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