# *Flavonoids: Promising Anticancer Agents*

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***Abstract:*** Flavonoids are polyphenolic compounds that are ubiquitously in plants. They have been shown to possess a variety of biological activities at nontoxic concentrations in organisms. The role of dietary flavonoids in cancer prevention is widely discussed. Compelling data from laboratory studies, epidemiological investigations, and human clinical trials indicate that flavonoids have important effects on cancer chemoprevention and chemotherapy. Many mechanisms of action have been identified, including carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multidrug resistance or a combination of these mechanisms. Based on these results, flavonoids may be promising anticancer agents.  Med Res Rev, 23, No. 4, 519–534, 2003

**Key words:** flavonoids; anticancer; mechanisms

## *1. INTRODUCTION*

Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. These compounds possess a common phenylbenzopyrone structure (C6-C3-C6), and they are categorized according to the saturation level and opening of the central pyran ring, mainly into flavones, flavanols, isoflavones, flavonols, flavanones, and flavanonols (Fig. 1).[1,2]

Flavonoids have probably existed in the plant kingdom for over one billion years. They are present in practically all dietary plants, like fruits and vegetables (Table I). Therefore, they are consumed in considerable amounts and are also heat stable. It is estimated that the human intake of all flavonoids is a few hundreds of milligrams per day.[3] Additionally, flavonoids are found in several medical plants, and herbal remedies containing flavonoids have been used in folk medicine around the world, especially in China.[4–8] Licorice is the most used crude drug in Kampo medicines (traditional Chinese medicines modified in Japan). Flavonoids from licorice extract may be useful

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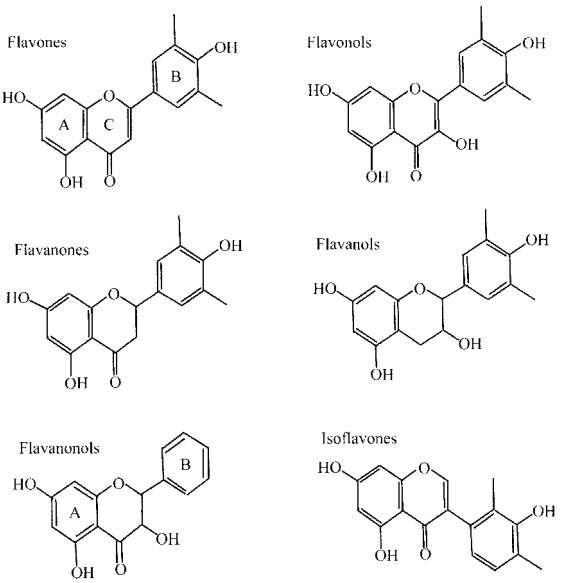


***Figure 1.*** Chemical structures of the flavonoid family.

chemopreventive agents for peptic ulcer or gastric cancer in H. pylori-infected individuals.[9] Sophoranone, extracted from a traditional Chinese medicine Shan Dou Gen, inhibited cell growth and induced apoptosis in various lines of cancer cells such as human stomach cancer MKN7 cells and human leukemia U937 cells.[10] It was validated *in vivo* and *in vitro* that the *Crescentia alata* (Bignoniaceae), mainly containing flavonols rutin, kaempferol 3-*O*-rutinoside and kaempferol, was used in the traditional medicine of Guatemala as an anti-inflammatory remedy.[11] In folk medicine, the use of B. ferruginea stem bark for the treatment of rheumatic pains might be attributed to five of its constituents (3-*O*-methylquercetin, myricetin, ferrugin, quercetin 3-*O*-glucoside, and a biflavanol gallocatechin-[4′-*O*-7]-epigallocatechin) with xanthine oxidase inhibiting and superoxide scavenging activity.[12] Cirsimarin and cirsimaritin, flavonoids of Microtea debilis exhibiting adenosine antagonistic properties in rats, may partly explain the effectiveness of Microtea debilis against proteinuria in traditional medicine.[13]

These polyphenolic compounds display a remarkable spectrum of biological activities including those that might be able to influence processes that are dysregulated during cancer development. These include, for example, antiallergic, anti-inflammatory, antioxidant, antimutagenic, anticarcinogenic, and modulation of enzymatic activities.[5,14–16] They may therefore have beneficial health

***Table I.*** Subclasses and Dietary Sources of Flavonoids

| *Flavonoid subgroup* | *Representative flavonoids* | *Major food sources* |
|---|---|---|
| Flavonols | Kaempherol, myricetin, quercetin, rutin | Onions, cherries, apples, broccoli, kale, tomato, berries, tea, red wine, tartary buckwheat |
| Flavones | Apigenin, chrysin, luteolin | Parsley, thyme |
| Isoflavones | Daidzein, genistein, glycitein, formononetin | Soya beans, legumes |
| Flavanols | Catechin, gallocatechin | Apples, tea |
| Flavanones | Eriodictyol, hesperitin, naringenin | Oranges, grapefruit |
| Flavanonols | Taxifolin | Limon, aurantium |

effects and can be considered possible chemopreventive or therapeutic agents against cancer.[17,18] This review article will focus on the anticancer activity of flavonoids as well as their molecular mechanisms, since they are among the most promising anticancer agents.

## 2. EVIDENCE OF ANTICANCER EFFECTS

### A. Epidemiological Data of Flavonoids

The weight of the epidemiological evidence for a protective effect of flavonoids against cancer is impressive. A growing number of epidemiological studies suggest that high flavonoid intake may be correlated with a decreased risk of cancer.[19]

More recently, in a population-based case-control study conducted in Shanghai from 1996–1998 which included 250 incident breast cancer cases and their individually matched controls, Dai et al.[20] reported that urinary excretion of total isoflavonoids and mammalian lignans was substantially lower in breast cancer cases than in controls (urine samples from breast cancer cases collected before cancer therapy). The median excretion rate of total isoflavonoids was 13.97 nmol/mg creatinine in cases and 23.09 in controls ($P = 0.01$), and that of total lignans was 1.77 in cases and 4.16 in controls ($P < 0.01$). This study strongly suggests a potential role of flavonoids in breast cancer preventing.

In a cohort study of 25-year follow-up on 9,959 Finnish men and women aged 15–99 years and initially cancer free, dietary intake of flavonoids was inversely associated with the incidence of cancer at all sites combined.[21] The association was primarily due to the lower rates of lung cancer, with relative risk of 0.54 (highest vs. lowest quartiles), and was not attributed to the intake of vitamin E, vitamin C, beta-carotene, or total calories. Knekt and co-workers[22] also estimated flavonoid intakes of 10,054 men and women mainly on the basis of the flavonoid concentrations in Finnish foods with a dietary history method. They found that men with higher quercetin intakes had a lower lung cancer incidence, and men with higher myricetin intakes had a lower prostate cancer risk. These data suggest a protective role of flavonoids against cancer.

A population-based case-control study in Hawaii further investigated the association between intake of flavonoids-powerful dietary and lung cancer risk. This study involved 582 patients with incident lung cancer and 582 age-, sex-, and ethnicity-matched control subjects. After adjusting for smoking and intake of saturated fat and beta-carotene, an inverse association was observed between lung cancer risk and the consumption of onions, apples, or white grapefruits as well as the calculated total intake of quercetin.[23] These results agree well with a former case-control study involving 541 cases of lung cancer and 540 hospitalized controls in Uruguay, but beta-carotene and vitamin E also associated with the reduction in risk of lung cancer.[24]

In addition, the research group in Uruguay conducted a case-control study in the period of January 1996–December 1997, and found that flavonoids displayed a marked reduction by 70% in the risks of cancer of oral cavity, pharynx, larynx, and esophagus.[25] Another case-control study in Spain, including 354 cases of gastric cancer and 354 hospitalized controls, suggests that flavonoids such as quercetin and kaempferol may have protective effects against gastric cancer while specific carotenoids (alpha-carotene, beta-carotene, lutein, and lycopene) not.[26] A cohort of 34,651 postmenopausal cancer-free women aged 55–69 years were followed from 1986 to 1998. After adjustment for potential confounders, catechin intake was inversely associated with rectal cancer incidence only.[27] All these studies provide evidence for a protective role of flavonoids against cancer.

The intake of flavonoids is inversely associated with subsequent cancer in most but not all prospective epidemiological studies. There are few contrary reports[28–30] that may be due to differences in bioavailability of the various flavonoids, and their effects on individual cancer sites cannot be excluded meriting further investigation.

### *B. In Vitro Studies of Flavonoids*

Many researchers have conducted *in vitro* studies on the potential anticancer activity of flavonoids in diverse cell systems. The collected reports on the inhibitory properties of flavonoids against carcinogenesis are summarized in Table II.

Hirano and co-workers examined anticancer efficacy of 28 flavonoids on human acute myeloid leukemia cell line HL-60, and compared differences between antiproliferative activity and cytotoxicity of these compounds with those of four clinical anticancer agents. Eight of the 28 flavonoids showed considerable suppressive effects on HL-60 cell growth with IC50s ranging from 10–940 ng/ml. The flavonoid genistein had the strongest effects almost equivalent to the effects of current anticancer agents with little cytotoxicity against HL-60 cells, whereas the regular anticancer agents had potent cytotoxicity.[56]

Kuntz et al.[48] screened more than 30 flavonoids for their effects on cell proliferation and potential cytotoxicity in human colon cancer cell lines Caco-2 and HT-29. Almost all compounds displayed antiproliferative activity without cytotoxicity. There was no obvious structure-activity relationship in the antiproliferative effects either on basis of the subclasses (i.e., isoflavones, flavones, flavonols, and flavonones) or with respect to kind or position of substituents within a class.[48]

An array of 55 flavones having a variety of substituents was evaluated by Cushman and Nagarathnam for cytotoxicity in five cancer cell cultures, A-549 lung carcinoma, MCF-7 breast carcinoma, HT-29 colon adenocarcinoma, SKMEL-5 melanoma, and MLM melanoma. Fifteen of the 55 flavone derivatives were significantly active against at least one of these cell cultures.[57] In addition, seven of the 27 examined Citrus flavonoids were observed to inhibit the proliferation of tumor cells, while less active against normal human cells.[58]

### *C. In Vivo Studies of Flavonoids*

Flavonoids have been demonstrated to inhibit carcinogenesis *in vitro* and substantial evidence indicates that they can also do so *in vivo*.[59–61] They may inhibit carcinogenesis by affecting the molecular events in the initiation, promotion, and progression stages. Animal studies and

***Table II.*** Anticancer Activities of Flavonoids in Various Cancer Cell Lines

| *Cancer* | *Cell* | *Flavonoid* | *References* |
|---|---|---|---|
| Human oral cancer | HSC-2, HSG, SCC-25 | Flavanones, isoflavans, EGC, chalcones, EGCG, curcumin, genistein, ECG, quercetin, cisplatin | Refs. [31–35] |
| Human breast cancer | MCF-7 | Flavanones, daidzein, genistein, quercetin, luteolin | Refs. [36,37] |
| Human thyroid cancer | ARO, NPA, WRO | Genistein, apigenin, kaempferol, chrysin, luteolin, biochanin A | Refs. [38,39] |
| Human lung cancer | SK-LU1, SW900, H441, H661, haGo-K-1, A549 | Flavone, quercetin | Refs. [40,41] |
| Human prostate cancer | LNCaP, PC3, DU145 | Catechin, epicatechin, quercetin, kaempferol, luteolin, genistein, apigenin, myricetin, silymarin | Refs. [42–45] |
| Human colon cancer | Caco-2, HT-29, IEC-6, HCT-15 | Flavone, quercetin, genistein, anthocyanin | Refs. [46–50] |
| Human leukaemia | HL-60, K562, Jurkat | Apigenin, quercetin, myricetin, chalcones | Refs. [51–54] |
| B16 mouse melanoma | 4A5 | Chalcones | Ref. [55] |

investigations using different cellular models suggested that certain flavonoids could inhibit tumor initiation as well as tumor progression.[62–68]

A recent study showed that fermented soy milk containing larger amounts of genistein and daidzein than unfermented one and isoflavone mixtures, given to rats starting at 7 weeks of age, inhibited mammary tumorigenesis induced by 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP).[69]

Dietary quercetin inhibited DMBA-induced carcinogenesis in hamster buccal pouch[70] and in rat mammary gland.[71] When given during the initiation stage, quercetin and ellagic acid, also inhibited DEN-induced lung tumorigenesis in mice.[72] In a medium-term multiorgan carcinogenesis model in rats, quercetin (1% in the diet) inhibited tumor promotion in the small intestine.[73] Feeding rats with quercetin or chalcone and 2-hydroxychalcone (0.05% in the diet), during either the initiation or promotion stage, inhibited 4-NQO-induced carcinoma formation in the tongue. These compounds also decreased cell proliferation and polyamine levels.[67]

Siess and co-workers investigated the effects of feeding rats with flavone, flavanone, tangeretin, and quercetin on two steps of aflatoxin B1 (AFB1)-induced hepatocarcinogenesis (initiation and promotion) and found that flavone, flavanone and tangeretin administered through the initiation period decreased the number of gamma-glutamyl transpeptidase-preneoplastic foci. Furthermore, feeding rats with flavanone during the phenobarbital-induced promotion step significantly reduced the areas of placental glutathione S-transferase preneoplastic foci. Therefore flavanone acts as an anti-initiator as well as an antipromotor.[74]

Inhibition of lung tumorigenesis by tea preparations rich in catechin has been demonstrated in A/J mice.[75] Administration of decaffeinated green or black tea to mice (as the sole source of drinking fluid) for 3 weeks starting 2 weeks before the 4-(methylnitrosamine)-1-(3-pyridyl)-1 butanone (NNK) treatment, or for 15 weeks starting 1 week after the NNK treatment, markedly reduced the number of tumors formed in the mice. In mice that had already developed adenomas at 16 weeks after the NNK injection, the progression of adenomas to adenocarcinomas was significantly inhibited by the administration of black tea from weeks 16–52. These experiments indicate that tea has broad inhibitory activity against lung carcinogenesis, and it is effective when administered during the initiation, promotion, or progression stages of carcinogenesis. Moreover, there is evidence for the suppression of tumor invasion and metastasis by flavonoids.

Catechins, a group of flavonoid molecules, inhibit invasion of mouse MO4 cells into embryonic chick heart fragments *in vitro*.[76] A polymethoxy flavonoid, nobiletin, from Citrus depressa inhibited the tumor-invasive activity of human fibrosarcoma HT-1080 cells in the Matrigel model, which was likely through suppressing the expression of matrix metalloproteases (MMPs) and augmenting of tissue inhibitors of metalloproteinases (TIMPs) production in tumor cells.[77]

When given i.p., quercetin and apigenin inhibited melanoma cell (B16-BL6) growth and metastatic potential in syngenetic mice, and interestingly, they significantly decreased the invasion of B16-BL6 cells *in vitro*.[61] When given s.c., apigenin (0.75 or 1.5 mg/kg body weight) significantly decreased the incidence of lymphatic vessel invasion of intestinal adenocarcinomas induced by azoxymethane, and that of cancer peritoneal metastasis enhanced by bombesin in male Wistar rats. The inhibitory effect of apigenin on cancer metastasis may be through inhibition of phosphorylation of mitogen-activated protein kinase (MAPK).[78]

### *D. Human Clinical Trials With Flavonoids*

The encouraging results of anticancer effects in preclinical studies have stimulated the clinical trials of flavonoids in human.

Early in 1988, Weiss et al. conducted a Phase I and pharmacological study of flavone acetic acid (FAA), one of a series of novel flavonoids.[79]

A phase I, dose-escalation trial of quercetin (3,3′,4′,5,7-pentahydroxy-flavone), a naturally occurring flavonoid with many biological activities including inhibition of a number of tyrosine kinases, was performed by Ferry et al. Intravenous quercetin was found to inhibit lymphocyte tyrosine kinase in nine of 11 patients assayed. One hepatocellular carcinoma patient had a sustained (150 days) fall in serum alpha-fetoprotein and alkaline phosphatase during and after four low-dose, intravenous quercetin treatments (60 mg/m$^2$) on a 3-week schedule. Another patient with stage four ovarian cancer who had not responded to six courses of cyclophosphamide/cisplatin chemotherapy had a fall in the CA125 tumor marker from 295 to 55 units/ml following two treatments of intravenous quercetin (420 mg/m$^2$) 3 weeks apart. The authors recommend 1400 mg/m$^2$ as the bolus dose, which may be given either in 3-week or weekly intervals, for Phase II trials. They defined the maximum tolerated dose (MTD) as 1700 mg/m$^2$ three weekly, but the vehicle, dimethyl sulphoxide (DMSO) is unsuitable for further clinical development of quercetin.[80] After that, a synthetic water-soluble, pro-drug of quercetin (3′(*N*-carboxymethyl) carbomyl-3,4′,5,7-tetrahydroxyflavone), QC12 was studied in an initial phase I trial by this research group. The authors suggested this water-soluble pro-drug warrant further clinical investigation, starting with a formal phase I, IV, dose-escalation study.[81]

Flavopiridol is a novel semisynthetic flavone analogue of rohitukine, a leading anticancer compound from an Indian tree. Flavopiridol inhibits most cyclin-dependent kinases (CDKs) and displays unique anticancer properties. It is the first CDKs inhibitor to be tested in human clinical trials by Aventis Pharma (formerly Hoechst Marion Roussel) and the National Cancer Institute (NCI) for the potential treatment of cancer and proliferative disorders. Initial human clinical trials with infusional flavopiridol demonstrated activity in some patients with non-Hodgkin's lymphoma, renal, prostate, colon, and gastric carcinomas. By July 1999, the compound had entered phase II trials for gastric cancer and leukemia (CLL), and phase I/II trials for esophageal tumor and non-small cell lung cancer (NSCLC). Phase II trials for colon and renal cancer were also reported.[82,83]

Wang[18] extensively reviewed the therapeutic potential in human of four most widely investigated flavonoids: flavopiridol, catechins, genistein, and quercetin. According to his another report,[84] by May 2001, flavopiridol was in phase IIa trials and had achieved proof-of-concept in phase I/IIa trials as a monotherapy. Moreover, it was expected that the product be launched by 2003.

## 3. MAJOR MOLECULAR MECHANISMS OF ACTION

### A. Preventing Carcinogen Metabolic Activation

Studies *in vitro* and *in vivo* have shown that some flavonoids modulate the metabolism and disposition of carcinogens and can contribute to cancer prevention.[85–89]

One important mechanism by which flavonoids may exert their effects is through their interaction with phase I metabolizing enzymes (e.g., cytochrome P450), which metabolically activate a large number of procarcinogens to reactive intermediates that can interact with cellular nucleophiles and ultimately trigger carcinogenesis. Flavonoids are demonstrated to inhibit the activities of certain P450 isozymes such as CYP1A1 and CYP1A2.[23,90,91] Thus, they are likely to have a protective role against the induction of cellular damage by the activation of carcinogens.

Another mechanism of action is the induction of phase II metabolizing enzymes such as glutathione-S-transferase, quinone reductase, and UDP-glucuronyl transferase,[92,93] by which carcinogens are detoxified and thus more readily eliminated from the body. This would also help explain the chemopreventive effects of flavonoids against carcinogenesis.

Moreover, some flavonoids have been reported as potent aromatase inhibitors.[94–97] Substantial evidence supports the concept that estrogens be involved in mammary carcinomas. Estradiol, the most potent endogenous estrogen, is biosynthesized from androgens by the cytochrome P450 enzyme complex called aromatase. Inhibition of aromatase is an important approach for reducing growth

stimulatory effects of estrogens in hormone-dependent breast cancer.[94] Therefore, flavonoids could be considered potential agents against breast cancer through the inhibition of aromatase activity.

### *B. Antiproliferation*

Dysregulated proliferation appears to be a hallmark of susceptibility to neoplasia. Cancer prevention is generally associated with inhibition, reversion or retardation of cellular hyperproliferation. Most flavonoids have been demonstrated to inhibit proliferation in many kinds of cultured human cancer cell lines, whereas less or no toxic to human normal cells.[36,46,48,56,58]

The molecular mechanism of antiproliferation may involve the inhibition of the prooxidant process that causes tumor promotion. It is generally believed that the formation of growth promoting oxidants (reactive oxygen species, ROS) is a major ''catalyst'' of the tumor promotion and progression stages, which follow the initiation stage (carcinogen metabolic activation to mutagens). The prooxidant enzymes induced or activated by various tumor promoters, for example, phorbol esters, include the arachidonate metabolizing enzymes, cyclooxygenases (COX), and lipoxygenases (LOX). Flavonoids are particularly effective at inhibiting xanthine oxidase,[98,99] COX or LOX[55,100,101] and therefore inhibit tumor cell proliferation.

In addition, inhibition of polyamine biosynthesis could be a contributing mechanism to the antiproliferative activities of flavonoids. Ornithine decarboxylase is a rate-limiting enzyme in polyamine biosynthesis, which has been correlated with the rate of DNA synthesis and cell proliferation in several tissues. Several experiments show that flavonoids can inhibit ornithine decarboxylase induced by tumor promoters, and thus cause a subsequent decrease in polyamine and inhibition of DNA/protein synthesis.[63,66,67]

Furthermore, flavonoids are also effective at inhibiting signal transduction enzymes, for example, protein tyrosine kinase (PTK),[80,101] protein kinase C (PKC),[102] and phosphoinositide 3-kinases ($PIP_3$),[77,103] which are involved in the regulation of cell proliferation.

### *C. Cell Cycle Arrest*

Perturbations in cell cycle progression may account for the anticarcinogenic effects of flavonoids. Mitogenic signals commit cells to entry into a series of regulated steps allowing traverse of the cell cycle. Synthesis of DNA (S phase) and separation of two daughter cells (M phase) are the main features of cell cycle progression. The time between the S and M phases is known as G2 phase. This phase is important to allow cells to repair errors that occur during DNA duplication, preventing the propagation of these errors to daughter cells. In contrast, the G1 phase represents the period of commitment to cell cycle progression that separates M and S phases as cells prepare for DNA duplication upon mitogenic signals.

CDKs have been recognized as key regulators of cell cycle progression. Alteration and deregulation of CDK activity are pathogenic hallmarks of neoplasia. A number of cancers are associated with hyperactivation of CDKs as a result of mutation of the CDK genes or CDK inhibitor genes. Therefore, inhibitors or modulators would be of interest to explore as novel therapeutic agents in cancer.[82,83]

Checkpoints at both G1/S and G2/M of the cell cycle in cultured cancer cell lines have been found to be perturbed by flavonoids such as silymarin, genistein, quercetin, daidzein, luteolin, kaempferol, apigenin, and epigallocatechin 3-gallate.[104–106] Studies from different laboratories revealed that flavopiridol could induce cell cycle arrest during either G1 or G2/M by the inhibition of all CDKs thus far examined.[18,82]

### *D. Induction of Apoptosis*

The significant anticancer properties observed of flavonoids may be due to frank apoptosis.[33,38,46,48,51,54,55] Apoptosis is an active form of cell death that plays an essential role in the

development and survival by eliminating damaged or otherwise unwanted cell. It is tightly regulated by a set of genes that either promote apoptosis or promote cell survival, and is mediated through a highly organized network of interacting protease and their inhibitors in response to noxious stimuli from either inside or outside of the cell. Dysregulation of apoptosis could play a critical role in oncogenesis. A series of recent studies have demonstrated that most, if not all, chemotherapeutic agents exert their tumoricidal effects by inducing apoptosis in target cells and tissues.

Flavonoids have been shown to induce apoptosis in some cancer cell lines, while sparing normal cells. The molecular mechanisms by which flavonoids induce apoptosis have not yet been clarified. Several mechanisms may be involved, including inhibition of DNA topoisomerase I/II activity,[51,101,107,108] decrease of reactive oxygen species (ROS),[109] regulation of heat shock proteins expression,[110] modulation of signaling pathways,[38] release of cytochrome c with a subsequent activation of caspase-9 and caspase-3,[51] downregulation of Bcl-2 and Bcl-X(L) expression but promotion of Bax and Bak expression, nuclear transcription factor kappaB (NF-kappaB), activation of endonuclease, and suppression of Mcl-1 protein.[46,55,109,111]

Preliminary evidence from our laboratory that apoptosis induced by tartary buckwheat flavonoid in HL-60 cells may be associated with early activation of caspase-3,[112] likely mediated through Fas and cytochrome c pathways, as well as regulated through the inactivation of NF-kappaB (manuscript submitted).

### *E. Promotion of Differentiation*

In addition to the anticancer properties mentioned above, it is of interest that certain flavonoids cause undifferentiated cancer cell lines to differentiate into cells exhibiting mature phenotypic characteristics.[113,114]

The flavones genistein, apigenin, luteolin, quercetin, and phloretin were found to induce differentiation of human acute myelogenous leukemia HL-60 cells into granulocytes and monocytes.[115] The isoflavone daidzein was also capable of doing so.[116] Erythroid differentiation of the human myelogenous leukemia K562 cell line and a multidrug-resistant subline (K562R) could also be induced by genistein.[117,118] Moreover, flavone was shown to induce differentiation in HT-29 human colon cancer cells.[46]

Cancers arise from cells harboring mutations that relinquish the need for exogenous growth factors. Deregulation of growth control ultimately leads to selection of clonal lines of cells that replicate at embryonic pace and yet fail to respond to differentiation and maturation signals. Nonphysiological inducers of terminal differentiation have been used as novel therapies for the prevention and therapy of cancer. Induction of terminal differentiation by flavonoids may lead to the eventual elimination of tumorigenic cells and rebalance of normal cellular homeostasis. Thus, these compounds could be developed into promising anticancer agents.

### *F. Antioxidative Activity*

Dietary flavonoids are natural antioxidants.[119] They may be against cancer through limit of damaging oxidative reactions in cells, which may predispose to the development of cancer. Oxygen-derived free radicals appear to possess the propensity to initiate as well as to promote carcinogenesis. Lipid peroxidation products originating from dying cells could also exert a cancer promotional effect.[120,121] Oxidation of DNA is likely to be an important cause of mutation that potentially can be reduced by antioxidants.[122]

Flavonoids are chemically one-electron donors. They serve as derivatives of conjugated ring structures and hydroxyl groups that have the potential to function as antioxidants in *in vitro* cell culture or cell free systems by scavenging superoxide anion, singlet oxygen, lipid peroxy-radicals, and/or stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species. *In vitro* studies are able to demonstrate for flavonols, flavones, and most recently

also for anthocyanins a considerable antioxidative activity, mainly based on scavenging of oxygen radicals.[122] Theoretical underpinnings for the efficacy of flavonoids as antioxidants *in vivo* come from the inhibition of low-density lipoprotein (LDL) oxidation, likely due to their reductive capacity and protein-binding properties.[123]

### *G. Inhibition of Angiogenic Process*

Flavonoids are known as angiogenesis inhibitors derived from natural sources.[124] The abilities of particular flavonoids to block solid tumor growth may be due to their inhibition of the neoangiogenic process.

Angiogenesis is a strictly controlled process in the healthy adult human body, which is regulated by a variety of endogenous angiogenic and angiostatic factors. However, pathological angiogenesis can occur in cancer. When deprived of proper vascularization, the high proliferation rate in the tumor would be balanced by cell death due to the lack of diffusion of nutrients and oxygen. Angiogenesis inhibitors such as flavonoids, are able to interfere with various steps of angiogenesis, like basement destruction of blood vessels, proliferation and migration of endothelial cells, or the lumen formation. Therefore, these compounds may have potential for the treatment of solid tumors.[125,126]

### *H. Modulation of Multidrug Resistance*

Multidrug resistance due to P-glycoprotein (Pgp) or multidrug resistance associated protein (MRP) is a serious impediment to successful chemotherapy of cancer. Much effort has been spent to modulate multidrug resistance in the different species by using specific inhibitors, but generally with little success due to additional cellular targets and/or extrusion of the potential inhibitors.

Certain flavonoids have been reported to possess potent inhibitory activity against the drug-exporting function of Pgp, a plasma membrane ATP-binding cassette (ABC) transporter that extrudes cytotoxic drugs at the expense of ATP hydrolysis. Pgp consists of two homologous halves each containing a transmembrane domain (TMD) involved in drug binding and efflux, and a cytosolic nucleotide-binding domain (NBD) involved in ATP binding and hydrolysis, with an overall (TMD-NBD)2 domain topology. Modulation by flavonoids of cell multidrug resistance mediated by Pgp may be through (i) inhibiting the overexpression of multidrug resistance gene-1 (MDR1),[127] (ii) direct binding to NBDs with high affinity,[128] (iii) inhibiting ATPase activity, nucleotide hydrolysis and energy-dependent drug interaction with transporter-enriched membranes.[129,130] Acting through Pgp as a possible target, flavonoids are found to enhance doxorubicin (DOX) induced antitumor activity and increase the DOX concentrations in tumors. Thus, the unique property of reversal of multidrug resistance of these compounds might help protect against multidrug-resistant tumors.[54,131–133]

## *4. CONCLUSIONS*

Flavonoids are generally nontoxic and manifest a diverse range of beneficial biological activities. The role of dietary flavonoids in cancer prevention is widely discussed. There is much evidence that flavonoids have important effects on inhibiting carcinogenesis.

Epidemiological studies have provided data that high dietary intake of flavonoids with fruits and vegetables could be associated with a low cancer prevalence in humans. This is supported by a multitude of *in vitro* and *in vivo* studies, which show that flavonoids may inhibit various stages in the carcinogenesis process, namely tumor initiation, promotion, and progression. Based on the studies *in vivo* and *in vitro*, many mechanisms of action may be involved. These include carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multidrug resistance or a combination of these

mechanisms. Furthermore, the intriguing results from laboratory and epidemiological studies have stimulated the development of flavonoids in human clinical trials.

While these experiences strengthen the notion that flavonoids could be useful anticancer agents, to date few clinical studies have demonstrated that these bioflavonoids retain anticancer properties in humans *in vivo*. In addition, clinical trials available have required intravenously administered flavonoids at concentrations around 1400 mg/m$^2$ before effects are seen. These plasma concentrations are unlikely to be achieved using the dietary supplements currently available. Therefore, more focused clinical studies are required to establish whether such dietary effects of these compounds can be exploited to achieve cancer preventive or therapeutic effects in human.

In conclusion, considering that many chemotherapeutic agents against tumor cells without sparing normal cells remain a major obstacle and development of multidrug resistance further limits chemotherapy in cancer, the promising results will stimulate the development of flavonoids for cancer chemoprevention and chemotherapy.

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