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Apigenin: A Promising Molecule for Cancer Prevention

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Abstract

Apigenin, a naturally occurring plant flavone, abundantly present in common fruits and vegetables is recognized as a bioactive flavonoid shown to possess anti-inflammatory, antioxidant and anticancer properties. Epidemiologic studies suggest that a diet rich in flavones is related to a decreased risk of certain cancers, particularly cancers of the breast, digestive tract, skin, prostate and certain hematological malignancies. It has been suggested that apigenin may be protective in other diseases that are affected by oxidative process such as cardiovascular and neurological disorders, although more research needs to be conducted in this regard. Human clinical trials examining the effect of supplementation of apigenin on disease prevention have not been conducted although there is considerable potential for apigenin to be developed as a cancer chemopreventive agent.

Keywords

apigenin; chemoprevention; flavonoids; apoptosis; cell cycle

Introduction

Evidences suggest that carcinogenesis is a multi-step process of genetic and epigenetic abnormalities that drives the progressive transformation of normal cells towards malignancy (1). Cancer is a preventable disease, in fact, some common cancers are easier to treat and cure if they are detected early (2,3). Screening of various organs may result in removal of precancerous lesions before their progression to cancer. More than half of human cancers can be prevented by the avoidable causes such as, cigarette smoking, recurrent infections, protection from excessive sun exposure, and reductions in occupational and environmental toxins (3). The combination of healthy diet, regular physical activity, and weight control could also lead to prevention of various cancers. Nutritional modification has long been considered to be an effective regimen for cancer prevention (4). Plant-based diet, especially fruits and vegetables contain substantial quantities of molecules that have chemopreventive potential to fight against cancer development. Such compounds include vitamins, trace elements and a variety of other molecules with antioxidant and anti-inflammatory properties. Carotenoids, flavanoids, polyphenols, isoflavones, catechins, and several other components that are found in leafy and green vegetables are molecules that are known to reduce the risk from several forms of human cancers (5). In recent years, cancer chemoprevention has emerged as one of the major approaches for reducing cancer burden (6). Cancer chemoprevention aims to inhibit

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or delay the development of neoplasia by blocking neoplastic inception as well as reversing the progression of transformed cells before the appearance of malignant lesions (7). The most rational approach to cancer chemoprevention is to design and test new agents that act on specific molecular and cellular targets (6,7). There are at least two strategies for the development of chemopreventive protocols. One is to identify natural dietary agents through epidemiological studies demonstrating the effect of agent(s) in cancer incidence and mortality, geographic variations and migration associated changes in dietary and lifestyle practices. A second approach relies on designing and synthesizing of molecularly target-based agents. These approaches require isolation, characterization and preclinical evaluation of test agents for their development as chemopreventive agent (6,7).

Epidemiological, case-control and experimental studies have suggested that various bioactive agents present in healthy diet can reduce the risk of cancer. In the Zutphen study, where cohorts of 878 men were followed beginning in 1960 for 25 years (8). According to this study the incidence or mortality from all causes of cancer or with the mortality from alimentary or respiratory tract cancers were considered with the intake of 5 flavonoids: myricetin, quercetin, kaempferol, luteolin and apigenin. The results supported the findings that high intake of flavonoids from vegetables and fruits was inversely associated with risk of cancer. In a large cohort study the association between flavonoid intake and human cancers were investigated which followed 9,959 Finnish men from 1967 to 1991 (9). This study provides stronger evidence for a protective role of flavonoids against lung cancer and other malignant neoplasms. In another study, associations between dietary flavonoid intakes of five common dietary flavonoid of the Zutphen study were investigated (10). The results confirmed that dietary intake of these flavonoids reduced the risk of ovarian cancer. Similar findings were observed in a case control study of Italy (11). The study investigated the relation of 6 classes of flavonoids with ovarian cancer risk, using data from a multi-centric case-control study between 1992 and 1999 that included 1,031 cases with histologically, confirmed epithelial ovarian cancer and 2,411 hospital controls, essentially confirming the previous findings. Interestingly, another case control study on intake of flavonoids and breast cancer risk investigating six principal classes of flavonoids was conducted in Italy between 1991 and 1994 on 2,569 women with incident histologically confirmed breast cancer, and 2,588 hospital controls (12). This study documented an inverse association between flavones and breast cancer risk. Another recent study performed with flavonoids on the recurrence risk of neoplasia with resected colorectal cancer patients (13). Eighty seven patients, 36 with resected colon cancer and 51 patients after polypectomy were divided into 2 groups: 31 patients received flavonoid mixture (daily standard dose 20 mg apigenin and 20 mg epigallocatechin-gallate) and compared with a 56 matched control group. Fourteen patients receiving flavonoid had no cancer recurrence in this group and only one adenoma developed. Conversely, the cancer recurrence rate of the 15 matched untreated controls was 20% (3 of 15) and adenomas evolved in 4 of those patients (27%). The combined recurrence rate for neoplasia was 7% (1 of 14) in the treated patients and 47% (7 of 15) in the controls. This study demonstrated that flavonoid intervention is negatively associated with disease recurrence and its consumption can reduce the recurrence rate of neoplasia in patients with sporadic colorectal neoplasia.

Flavonoids are a family of polyphenolic compounds synthesized by plants with a similar structure, are divided into subclasses, including anthocyanidins, flavanols, flavanones, flavonols, flavones and isoflavones (14). Several beneficial properties have been attributed to these dietary compounds, including antioxidant, anti-inflammatory, and anti-carcinogenic effects. According to an estimate, average intakes of flavonoids as flavonols and flavones have ranged from 6 mg/day in Finland to 64 mg/day in Japan, with intermediate intakes in the United States (13 mg/day), Italy (27 mg/day) and the Netherlands (33 mg/day). These estimates were based on analysis of five plant flavonoids: quercetin, kaempferol, myricetin, luteolin and apigenin in composite food samples for the population analyzed in the Seven Countries Study

(15). In another study on the Hungarian population the intake of flavonoid was lower than in Dutch (23 mg/day), Danish (28 mg/day) and Finnish citizens (55 mg/day). The intake of five flavonoids in 17 different diets was estimated (16). Lowest intake (1-9 mg/day) was estimated in South African diets, whereas the highest flavonoid intake (75-81 mg/day) was from a Scandinavian diet. In addition to flavonoid intake, dietary sources of the flavonols and flavones varied between different countries, with major contributions from tea in Japan (95%) and the Netherlands (64%), red wine and beer in Italy (46%) and vegetables and fruits in Finland (100%) and the United States (80%). In Australia, tea remains the major dietary flavonoid with apparent dietary consumption up to 351 mg/person/day, of which 75% were flavan-3-ols (17). One of the molecule that has gained considerable interest as beneficial agent for human health with cancer preventive and/or therapeutic properties is plant flavone, apigenin.

Apigenin, chemically known as 4′, 5, 7,-trihydroxyflavone, with molecular formula $C_{15}H_{10}O_5$ whose molecular weight is MW 270.24. In nature apigenin also exists as a dimer, biapigenin, mainly isolated from the buds and flowers of *Hypericum perforatum* (Figure 1), which has neuroprotective effects (18). Apigenin is abundantly present in common fruits such as grapefruit, plant-derived beverages and vegetables such as parsley, onions, oranges, tea, chamomile, wheat sprouts and in some seasonings. One of the most common sources of apigenin consumed as single ingredient herbal tea is chamomile, prepared from the dried flowers from *Matricaria chamomilla* (19). This is an annual herbaceous plant indigenous to Europe and Western Asia that has been naturalized in Australia, Britain and the United States. Also known as German chamomile, Hungarian chamomile, mayweed, sweet false chamomile or wild chamomile, the plant is cultivated in Germany, Hungary, Russia and other Southern and Eastern European countries for the flower heads. Infusions of chamomile contain maximum concentrations of apigenin ranging from 0.8-1.2% and essential oils which have aromatic, flavoring and coloring properties. Chamomile is consumed in the form of tea at the rate of over 1 million cups per day. Other sources for apigenin include beverages such as wine and beer brewed from natural ingredients. Apigenin is commonly present as a constituent in red wine (20). Like red wine, beer also provides a good source of apigenin (21). In natural sources, apigenin is present as apigenin-7-*O*-glucoside and various acylated derivatives (22).

Apigenin and Human Health

Apigenin has gained particular interest in recent years as a beneficial and health promoting agent because of its low intrinsic toxicity and because of its striking effects on normal *versus* cancer cells, compared with other structurally-related flavonoids (23). There is very little evidence to date to suggest that apigenin promotes adverse metabolic reactions *in vivo* when consumed as part of a normal diet. In recent years, apigenin has been increasingly recognized as a cancer chemopreventive agent. Interest in the possible cancer preventive of apigenin has increased owing to reports of potent antioxidant and anti-inflammatory activities. Indirect support for this assumption is correlated with a study where consumption of flavonoid free diets by healthy human volunteers has been reported to lead to a decrease in markers of oxidative stress in blood *viz.* plasma antioxidant vitamins, erythrocyte superoxide dismutase (SOD) activity and lymphocyte DNA damage commonly associated with enhanced disease risk, suggesting the beneficial effects of flavonoids (24).

A number of the biological effects of apigenin in numerous mammalian systems *in vitro* as well as *in vivo* are related to its antioxidant effects and its role in scavenging free radicals. Furthermore, it exhibits anti-mutagenic, anti-inflammatory, antiviral, and purgative effects (25). The actions of apigenin in inhibiting the cell cycle, diminishing oxidative stress, improving the efficacy of detoxification enzymes, inducing apoptosis, and stimulating the immune system are quite limited (25-27). One human study demonstrated that apigenin was absorbed systemically by a subject fed a diet high in parsley; this subject was found to have

elevated levels of the antioxidant enzymes erythrocyte glutathione reductase and superoxide dismutase (28). Activities of erythrocyte catalase and glutathione peroxidase, however, were found to be unchanged. Other biological effects induced by flavonoids include reduction of plasma levels of low-density lipoproteins, inhibition of platelet aggregation, and reduction of cell proliferation (25-27,29). This is apparent from another cross-sectional study conducted in Japan in which total intake of flavonoids among women was found to be inversely correlated with plasma total cholesterol and low-density lipoprotein concentration, after adjustment for age, body mass index and total energy intake (30). The effects of flavonoids on the hematologic systems were performed, a 7 day study of 18 healthy men and women examining the effects of a daily dietary supplement providing quercetin (377 ± 10 mmol from onions) and apigenin (84 ± 6 mg from parsley) on platelet aggregation and other hemostatic variables. They observed no significant changes in collagen- or ADP- induced platelet number, factor VII, plasminogen, and PAI-1 activity or fibrinogen concentrations (31). These inherent properties of flavonoids categorize them as a class of beneficial compounds which possess health-promoting and disease-preventing dietary effects.

Apigenin-Targets of Action

Apigenin has been shown to possess anti-mutagenic properties in a setting of nitropyrene-induced genotoxicity in Chinese hamster ovary cells (32). Apigenin has also been shown to inhibit benzo[a]pyrene and 2-aminoanthracene-induced bacterial mutagenesis (32). Laboratory studies have demonstrated that apigenin promotes metal chelation, scavenges free radicals, and stimulates phase II detoxification enzymes in cell culture and in *in vivo* tumor models (33). Exposure to apigenin prior to a carcinogenic insult has been shown to afford a protective effect in murine skin and colon cancer models (34,35). Apigenin is a strong inhibitor of ornithine decarboxylase, an enzyme that plays a major role in tumor promotion (36). In addition, apigenin has been shown to increase the intracellular concentration of glutathione, enhancing the endogenous defense against oxidative stress (37).

The anti-carcinogenic effects of apigenin have been demonstrated in a skin carcinogenesis model. Topical application of apigenin inhibited dimethyl benzanthracene-induced skin tumors (36). In addition, apigenin administration diminished the incidence of UV light-induced cancers and increased tumor free survival in similar experiments (34).

The anti-inflammatory properties of apigenin are evident in studies that have shown suppression of LPS-induced cyclooxygenase-2 and nitric oxide synthase-2 activity and expression in mouse macrophages (38). Analyses of structure-activity relationships of 45 flavones, flavonols and their related compounds showed that luteolin, ayanin, apigenin and fisetin are the strongest inhibitors of IL-4 production (39). This work was further confirmed in ovalbumin immunized BALB/C mice where production of IL-4 was down-regulated by apigenin (40). In another study, apigenin treatment resulted in suppression of tumor necrosis factor (TNF) α-induced Nuclear Factor (NF)-κB activation in human umbilical vein endothelial cells (41).

Several studies have demonstrated that apigenin exerts a broad range of molecular signaling effects (42). Apigenin has been reported to inhibit protein kinase C activity, mitogen activated protein kinase (MAPK), transformation of C3HI mouse embryonic fibroblasts and the downstream oncogenes in v-Ha-ras-transformed NIH3T3 cells (43,44). Apigenin is a well known inhibitor of protein-tyrosine kinases and has been shown to block peroxisome proliferation regulated kinase (ERK), a MAPK in isolated hepatocytes (45). We have recently reported that apigenin-mediated inhibition of cell proliferation is due to modulations in MAPK, PI3K-Akt in human prostate cancer cells (46). Apigenin has further been shown to down regulate the expression of the Na^+/Ca^{2+}-exchanger, a protein important for calcium extrusion

in neonatal rat cardiac myocytes (47). Apigenin treatment has been shown to decrease the levels of phosphorylated EGFR tyrosine kinase and of other MAPK and their nuclear substrate c-myc, which causes apoptosis in anaplastic thyroid cancer cells (48). Furthermore, apigenin has been shown to inhibit the expression of casein kinase (CK)-2 in both human prostate and breast cancer cells (49,50).

It has been demonstrated that apigenin exerts its effects on the cell cycle. Exposure of a wide array of malignant cells, including epidermal cells and fibroblasts to apigenin induces a reversible G2/M and G0/G1 arrest by inhibiting p34 (cdc2) kinase activity, accompanied by increased p53 protein stability (51,52). Apigenin has also been shown to induce WAF1/p21 levels resulting in cell cycle arrest and apoptosis in androgen-responsive human prostate cancer, LNCaP cells and androgen-refractory DU145 cells, regardless of the Rb status and p53-dependence or p53 independence (53,54). In addition, apigenin has been shown to induce apoptosis in a wide range of malignant cells (55-57). Apigenin treatment has been shown to alter the Bax/Bcl-2 ratio in favor of apoptosis, associated with release of cytochrome c and induction of Apaf-1, which leads to caspase activation and PARP-cleavage (54).

Apigenin has shown promise in inhibiting tumor cell invasion and metastases by regulating protease production (58). Apigenin under *in vivo* conditions is also effective in inhibiting TNFα-induced intracellular adhesion molecule-1 upregulation in cultured human endothelial cells (59). *In vivo* studies have also shown that apigenin inhibits melanoma lung metastases by impairing interaction of tumor cells with endothelium (60). Furthermore, exposure of endothelial cells to apigenin results in suppression of the expression of VEGF, an important factor in angiogenesis via degradation of HIF-1α protein (61). Apigenin has also been shown to inhibit the expression of HIF-1α and VEGF *via* the PI3K/Akt/p70S6K1 and HDM2/p53 pathways in human ovarian cancer cells (62).

Studies demonstrated that apigenin is an effective inhibitor of aromatose and 17β-hydroxysteroid dehydrogenase activities in human placental microsomes, with resulting effects on steroid metabolism (63). Oral administration of apigenin was shown to cause a significant increase in uterine weight and overall uterine concentration of estrogen receptor (ER)-α in female mice (64), and also suppresses prostate and breast cancer cell growth through estrogen receptor β1 (65). Apigenin has been shown to decrease intracellular and secreted levels of PSA in androgen-responsive human prostate cancer LNCaP cells (53). It has also been shown that oral administration of apigenin suppresses the levels of IGF-I in prostate tumor xenografts and increases levels of IGFBP-3, a binding protein that sequesters IGF-I in vascular circulation (66). Further studies have demonstrated that apigenin exposure to human prostate carcinoma DU145 cells caused increase in protein levels of E-cadherin and inhibited nuclear translocation of β-catenin and its retention to the cytoplasm (67). These studies imply that apigenin may have the potential to inhibit hormone-related cancers as well.

Recent study demonstrated effects of apigenin on the immune system in C57BL/6 mice. Apigenin feeding for 2 weeks resulted in significant suppression of total immunoglobulin (Ig) E levels whereas levels of IgG, IgM and IgA were not affected (40). Apigenin feeding further resulted in decreased production of regulated-on-activation normal T cell expressed and secreted (RANTES) and soluble tumor necrosis factor receptor I in mouse serum.

Other important targets of apigenin include heat shock proteins (61), telomerase (68), fatty acid synthase (69), matrix metalloproteinases (70), and aryl hydrocarbon receptor activity (71) HER2/neu (72), casein kinase 2 alpha (73), all of which have relevance to the development of various human diseases. A list of potential targets of apigenin in various human cancers is shown in table 1.

Apigenin-Role in Cancer Prevention

Many of the biological effects of apigenin in numerous mammalian systems *in vitro* as well as *in vivo* are related to its antioxidant effects and its role in scavenging free radicals. Furthermore, it exhibits anti-mutagenic, anti-inflammatory, antiviral, and purgative effects (74). The actions of apigenin in inhibiting the cell cycle, diminishing oxidative stress, improving the efficacy of detoxification enzymes, inducing apoptosis, and stimulating the immune system are quite limited (74-76). One human study demonstrated that apigenin was absorbed systemically by a subject fed a diet high in parsley; this subject was found to have elevated levels of the antioxidant enzymes erythrocyte glutathione reductase and superoxide dismutase (77). Activities of erythrocyte catalase and glutathione peroxidase, however, were found to be unchanged. Other biological effects induced by flavonoids include reduction of plasma levels of low-density lipoproteins, inhibition of platelet aggregation, and reduction of cell proliferation (74-76,78). This is apparent from another cross-sectional study conducted in Japan in which total intake of flavonoids among women was found to be inversely correlated with plasma total cholesterol and low-density lipoprotein concentration, after adjustment for age, body mass index and total energy intake (79). The effects of flavonoids on the hematologic systems were performed, a 7 day study of 18 healthy men and women examining the effects of a daily dietary supplement providing quercetin (377 ± 10 mmol from onions) and apigenin (84 ± 6 mg from parsley) on platelet aggregation and other hemostatic variables. They observed no significant changes in collagen- or ADP induced platelet number, factor VII, plasminogen, PAI-1 activity or fibrinogen concentrations (80). These inherent properties of flavonoids categorize them as a class of beneficial compounds which possess health-promoting and disease-preventing dietary effects. Below we provide an update on the protective effects of apigenin in various human malignancies.

Apigenin-Role in Human Cancers

Breast Cancer

Studies have demonstrated anti-proliferative effects of apigenin on human breast cancer cell lines with different levels of HER2/neu expression. Apigenin exhibited potent growth inhibitory activity in HER2/neu over-expressing breast cancer cells but was much less effective in inhibiting growth of cells expressing basal levels of HER2/neu (72). Induction of apoptosis was also observed in HER2/neu over-expressing breast cancer cells in a dose- and time-dependent manner after apigenin treatment (81). The cell survival pathway involving phosphatidylinositol 3-kinase (PI3K) and Akt/PKB is known to play an important role in inhibiting apoptosis in HER2/neu expressing breast cancer cells. Apigenin has been shown to inhibit Akt function in tumor cells by directly inhibiting PI3K activity and consequently inhibiting Akt kinase activity (82). Additionally, inhibition of HER2/neu auto-phosphorylation and trans-phosphorylation resulting from depleting HER/neu protein *in vivo* was observed after apigenin treatment. Further studies from the same group showed that exposure of HER2/neu expressing breast cancer cells to apigenin resulted in induction of apoptosis by depleting HER2/neu protein and, in turn, suppressing the signaling of the HER2/HER3-PI3K/Akt pathway. Apoptosis in breast cancer cells exposed to apigenin was induced through cytochrome C release and rapid induction of DNA fragmentation factor 45. Another recent study demonstrated that apigenin induces apoptosis in MDA-MB-453 human breast cancer cells by involving both intrinsic and extrinsic apoptotic pathways (83). Additional synergistic effects were observed when combining 5-fluorouracil with apigenin which inhibited cell growth and induced apoptosis *via* down modulation of ErbB2 expression and Akt in MDA-MB-453 human breast cancer cells (84). Apigenin has also been shown to down-regulate the levels of cyclin D1, D3 and cdk4 and increases p27 protein levels in breast cancer cells (81).

It has been reported that peptide hormones and protein kinase C (PKC)-activating phorbol ester (PMA), protect cells from apoptosis through activation of cellular signaling pathways such as the MAPK and PI3K pathways (82). Additional studies have demonstrated suppression of TNFα-induced apoptosis by treatment with PMA in MCF-7 breast carcinoma cells (82). The ability of apigenin to block PMA-mediated cell survival was correlated with suppression of PMA-stimulated AP-1 activity, providing evidence of the ability of apigenin to affect cell survival pathways and offering an explanation for its anti-tumor activity.

The effect of apigenin on protease-mediated invasiveness was evaluated in estrogen-insensitive breast tumor cell line MDA-MB231, showing that apigenin strongly inhibited tumor cell invasion in a dose-dependent manner (58). Apigenin inhibits growth and induces G2/M arrest by modulating cyclin-CDK regulators and ERK MAP kinase activation in breast carcinoma cells (85). The growth inhibitory effects of apigenin were observed on MCF-7 cells that express two key cell cycle regulators, wild-type p53 and the retinoblastoma tumor suppressor protein (Rb), and in MDA-MB-468 cells which are mutant for p53 and Rb negative. Apigenin-mediated cell growth inhibition along with G2/M arrest was accompanied by significant decrease in cyclin B1 and CDK1 protein levels, resulting in a marked inhibition of CDK1 kinase activity. Furthermore, apigenin treatment reduced the protein levels of CDK4, cyclin D1 and A, inhibited Rb-phosphorylation but did not affect the protein levels of cyclin E, CDK2 or CDK6. Recently, studies have shown that apigenin induces G (2)/M phase cell cycle arrest in SK-BR-3 cells which is via regulation of CDK1 and p21 (Cip1) pathway. In addition, apigenin treatment resulted in ERK MAP kinase phosphorylation and activation in MDA-MB-468 cells (86).

Further effects of apigenin and other phytoestrogens on DNA synthesis (estimated by thymidine incorporation analysis) were evaluated in estrogen-dependent MCF-7 cells in the presence of estradiol (E2), tamoxifen, insulin, or epidermal growth factor (87,88). The results show that apigenin was capable of inhibiting E2-induced DNA synthesis in these cells. Overall, the effects of apigenin and other phytoestrogens in the presence of E2 or growth factors were variable and concentration dependent.

A study to characterize the estrogenic and anti-estrogenic activities of flavonoids was performed in the ER-positive MCF-7 human breast cancer cell line using an ER-dependent reporter gene assay and an ER competition binding assay (89). In these studies apigenin was shown to possess anti-estrogenic activities which may be mediated through ER binding-dependent and independent mechanisms. Recently a study suggested that apigenin targets both ERalpha-dependent and ERalpha-independent pathways on estrogen-responsive, antiestrogen-sensitive MCF7 breast cancer cells and growth inhibitory effect on two MCF7 sublines with acquired resistance to antiestrogens *viz.* tamoxifen or fulvestrant (90). These anti-estrogenic activities were deemed to be biologically significant in the regulation of breast cancer cell proliferation.

The combined effects of multiple flavonoids on breast cancer resistance protein (BCRP) were demonstrated, several plant flavonoids including apigenin were used alone or in combinations to evaluate the potential interactions for BCRP inhibition (91). Apigenin and other flavonoids were shown to inhibit the BCRP protein, which was highly efficacious in combination at equimolar concentrations. Another study compared the endocrine disruption activities of compounds in materials used to package foods including bisphenol derivatives and plant flavonoids, including apigenin, on human breast cancer cell lines MCF-7 which is ER(+) and MDA-MB453 which is AR(+) and GR(+). These studies suggested that natural compounds had a biphasic effect: at high concentrations they act as GR agonists and in low concentrations they may act as partial androgen receptor (AR) agonists (92). In another study apigenin and genistein have been shown to stimulate the proliferation of MCF-7 and T47D cells [estrogen

receptor alpha (ER alpha-positive)], but do not stimulate the proliferation of an ER alpha-negative cell line (MDA-MB-435 cells) (93). These studies indicate that estrogenicity of the phytochemicals are quantitatively important in inducing cell proliferation or inhibiting aromatase, suggesting that perhaps a more cautionary approach should be taken before taken as food supplements (94).

Other study has shown that plant flavonoids can induce apoptosis in human breast and prostate cancer cells, an effect that is associated with their ability to inhibit the activity of fatty acid synthase, a key metabolic enzyme that catalyzes the synthesis of long chain fatty acids over-expressed in neoplastic and malignant cells (69). In this study at least six plant-derived flavonoids, including apigenin, had marked inhibitory effects on cancer cell growth and survival which appear to be related to their ability to inhibit fatty acid synthesis. More recent observation confirmed that extra virgin olive oil derived apigenin content was able to suppress the expression of lipogenic enzyme fatty acid synthase in SKBR and MCF-7/HER2 cells (95). The proteasomal chymotrypsin-like activity was inhibited by the apigenin and induces apoptosis by the activation of caspase 3, 7 and poly (ADP-ribose) polymerase cleavage, in cultured MDA-MB-231 cells and also in MDA-MB-231 xenografts (96). Further studies have shown that apigenin inhibits hepatocyte growth factor-induced MDA-MB-231 cells invasiveness and metastasis by blocking Akt, ERK, and JNK phosphorylation and also inhibits clustering of β-4-integrin function at actin rich adhesive site (97).

Cervical Cancer

The first report about apigenin in human cervical carcinoma HeLa cells demonstrated apigenin inhibited the growth through an apoptotic pathway. Apigenin inhibited cell growth, caused G1 phase growth arrest and induced apoptosis which was p53 dependent and associated with a marked increase in the expression of p21/WAF1 protein and with the induction of Fas/APO-1 and caspase-3 expression. Apigenin also decreased the expression of Bcl-2 protein, an anti-apoptotic factor (98).

Further studies demonstrated that apigenin can interfere with cell proliferation, cell survival, and gap junctional coupling. Exposure of non-invasive wild-type HeLa cells and their connexin43 (Cx43) transfected counterparts to apigenin resulted in a significant and reversible inhibition of translocation of both cell types. The effect of apigenin on cell proliferation was less pronounced especially at low apigenin concentration, whereas its influence on cell motility correlated with the reduction of the invasive potential of HeLa Cx43 cells (99). Another recent study with medicinal herb feverfew (*Tanacetum parthenium*) extract containing parthenolide, camphor, luteolin, and apigenin showed the anti-proliferative activity against SiHa human cervical cancer cells (100).

Colon Cancer

In various human colon carcinoma cell lines apigenin treatment resulted in cell growth inhibition and G2/M cell cycle arrest, which was associated with inhibition of p34 (cdc2) kinase, and with reduced accumulation of p34 (cdc2) and cyclin B1 proteins (101). Additional studies performed in individual and interactive influences of seven apigenin analogs on cell cycle, cell number, and cell viability in human colonic carcinoma cell lines (102). These findings indicate that the induction of cell-cycle arrest by five of seven tested apigenin analogs and the additive induction by the combination of flavonoids at low doses cooperatively protect against colorectal cancer through conjoint blocking of cell-cycle progression.

An important effect of apigenin is to increase the stability of the tumor suppressor p53 gene in normal cells (51). It is speculated that apigenin may play a significant role in cancer prevention by modifying the effects of p53 protein. Exposure of p53-mutant cancer cells to apigenin results

in inhibition of cell growth and alteration of the cell cycle as demonstrated in a study in which apigenin treatment resulted in growth-inhibition and G2/M phase arrest in two p53-mutant cancer cell lines, HT-29 and MG63 (103). These effects were associated with a marked increase in the protein expression of p21/WAF1 in a dose- and time-dependent manner. These results suggest that there is a p53-independent pathway for apigenin in p53-mutant cell lines, which induces p21/WAF1 expression and growth-inhibition. Further assessment that adenomatous polyposis coli (APC) dysfunction may be critical for apigenin to induce cell cycle arrest in human colon cancer HT29-APC cells (mutated APC), but apigenin enhances APC expression and apoptosis in cells (104). Synergistic studies on 5, 6-Dichloro-ribifuranosylbenzimidazole (DRB)- and apigenin- induced sensitization of colon cancer cells to TNFα-mediated apoptosis. Inhibition of CK2 in HCT-116 and HT-29 cells with the use of two specific CK2 inhibitors, DRB and apigenin, resulted in a synergistic reduction in cell survival when used in conjunction with TNFα (105). Chemopreventive activity of apigenin may be mediated by its ability to modulate the MAPK cascade. Apigenin induced a dose-dependent phosphorylation of both ERK and p38 kinase but had little effect on the phosphorylation of c-jun amino terminal kinase (JNK) (35). Further studies on apigenin suggest that it inhibits ornithine decarboxylase (ODC) activity and the formation of aberrant crypt foci in two different mouse models systems; azoxymethane (AOM)-induced CF-1 mice and Min mice with mutant adenomatous polyposis coli (APC) gene (106).

The interactions between sulforaphane and apigenin resulted in the induction of UGT1A1 and GSTA1, the phase II detoxifying enzymes, in CaCo-2 cells (107). Apigenin was shown to induce UGT1A1 transcription but not GSTA1; sulforaphane induced both UGT1A1 and GSTA1 transcription in both dose- and time-dependent manner. The combination of sulforaphane and apigenin resulted in a synergistic induction of UGT1A1 mRNA expression, although this interaction was not seen for GSTA1, suggesting that different signal transduction pathways regulate the expression of detoxification enzymes. Additional studies suggest that apigenin is more potent than tricin or quercetin in down-regulating inducible COX-2 expression in HCEC cells (108).

Hematologic Cancer

Apigenin was also tested to ascertain its effects on human leukemia cells. Apigenin was shown to be markedly more effective than other tested flavonoids in inducing apoptosis in these cells (55). Further studies have shown that apigenin and quercetin both inhibit topoisomerase-catalyzed DNA irregularities that are involved in many aspects of leukemia cell DNA metabolism including replication and transcription reactions. Another recent study suggests that treatment with apigenin in different leukemia cell lines resulted in selective anti-proliferative and apoptotic effects in monocytic and lymphocytic leukemias; this selective apoptosis is mediated by induction of protein kinase C delta (109). Apigenin inhibit platelet function through several mechanisms including blockade of TxA(2) receptors (TPs). The inhibitory effect of apigenin in the presence of plasma, might in part rely on TxA(2) receptor antagonism. This was demonstrated through clear increase in the *ex vivo* anti-platelet effect of aspirin in the presence of apigenin, which encourages the idea of the combined use of aspirin and apigenin in patients in which aspirin fails to properly suppress the TxA(2) pathway (110).

Flavonoids based study was carried out mainly on apigenin, quercetin, kaempferol and myricetin for their proteasome-inhibitory and apoptosis-inducing abilities in human leukemia cells. They reported that apigenin and quercetin were much more potent than kaempferol and myricetin in (i) inhibiting chymotrypsin-like activity of purified 20S proteasome and of 26S proteasome, (ii) accumulating putative ubiquitinated forms of two proteasome target proteins, Bax and IκBα, and (iii) inducing activation of caspase-3 and cleavage of poly (ADP-ribose)

polymerase in Jurkat T cells (111). Furthermore, the proteasome-inhibitory abilities of these compounds correlated with their apoptosis-inducing potencies.

Further structurally related flavonoids, such as apigenin, quercetin, myricetin, and kaempferol were able to induce apoptosis in human promyelocytic leukemia HL-60 cells (55). Treatment of cells with flavonoids caused rapid induction of caspase-3 activity and stimulated proteolytic cleavage of poly-(ADP-ribose) polymerase. These flavonoids induced loss of mitochondrial transmembrane potential, elevation of reactive oxygen species production, release of mitochondrial cytochrome c into the cytosol, and subsequent induction of procaspase-9 with apigenin having the highest potency in inducing apoptotic effects. Olive leaves (*Olea europaea* L.) extract from seven principal Tunisian olive varieties; Chemchali, Chemlali, Chétoui, Gerboui, Sayali, Zalmati and Zarrazi having one common compound apigenin-7-*O*-glucoside in all the extract, has shown to reduce nitroblue tetrazolium (a differentiation marker) in HL-60 cells (112). Another study evaluated the potential of 22 flavonoids and related compounds by testing their apoptotic activity in leukemic U937 cells (113). In these studies, apigenin and several other flavones but not the isoflavones or flavanones tested were shown to induce apoptosis in U937 cells.

The protective effects of four flavonoids, querectin, rutin, luteolin and apigenin were evaluated by measuring the extent of H_2O_2-induced DNA damage in murine leukemia L1210 cells (114). The results show that apigenin, at low concentrations, was marginally effective in reducing the extent of DNA damage. However, at high concentrations apigenin induced DNA single strand breaks, indicating its ability to serve as a pro-oxidant. Another study evaluated the role of dietary bioflavonoids in inducing cleavage in the MLL gene, which may contribute to infant leukemia (115). Apigenin was shown to induce DNA cleavage in primary progenitor hematopoietic cells from healthy newborns and adults and in cell lines by targeting topoisomerase II, an enzyme that alters the DNA topology. It is not known whether this *in vitro* study can be extrapolated to human situations, because of the dose and bioavailability issue.

Lung Cancer

The effects of apigenin on lung cancer cells were evaluated, apigenin inhibited A549 lung cancer cell proliferation and vascular endothelial growth factor (VEGF) transcriptional activation in a dose-dependent manner (116). Apigenin inhibited VEGF transcriptional activation through the HIF-1 binding site, and specifically decreased HIF-1α, but not HIF-1β subunit expression in these cells. In a signaling pathway that mediates VEGF transcriptional activation, apigenin inhibited AKT and p70S6K1 activation. Lung cancer cells SQ-5 incubated with apigenin exhibited significantly greater radio-sensitivity and apoptosis than cells without apigenin (117). Another report demonstrates that apigenin exhibited inhibitory effect on hepatocyte growth factor -induced Akt phosphorylation in lung carcinoma A549 cells (118). In addition, the exposure of nude mice with lung cancer to apigenin inhibited HIF-1α and VEGF expression in the tumor tissues, suggesting an inhibitory effect of apigenin on angiogenesis. Another study demonstrated the efficacy of apigenin administration against experimental Lewis lung carcinomas (LLC), C-6 gliomas and DHDK 12 colonic cancers *in vivo* (119). Tumor bearing mice received 50 mg/kg/day of apigenin in three different galenical formulations during 12 days in 8-hourly intervals. No *in vivo* response was observed, in contrast to the high in vitro sensitivity of LLC, C-6, DHDK 12 and endothelial cells to apigenin; complete growth suppression occurs *in vitro* at concentrations beyond 30-μg/ml. Recent studies in B57BL/6N mice injected with B16-BL6 tumor cells demonstrated that the number of tumor cells adhering to lung vessels was significantly diminished in animals treated with a single dose of apigenin and quercetin (60).

Ovarian Cancer

In human ovarian cancer cells apigenin inhibited VEGF expression was observed at the transcriptional level through expression of HIF-1α *via* the PI3K/AKT/p70S6K1 and HDM2/p53 pathways. Apigenin has also been shown to inhibit tube formation by endothelial cells *in vitro* (62,120). Additionally, apigenin inhibited the activity of MAPK and PI3K in human ovarian carcinoma HO-8910PM cells (121). Apigenin inhibits expression of focal adhesion kinase (FAK), migration and invasion of human ovarian cancer A2780 cells. Further *in vivo* experiments also showed that apigenin inhibited spontaneous metastasis of A2780 cells implanted onto the ovary of nude mice (122). Recent study suggested that apigenin suppresses Id1 (Inhibitor of differentiation or DNA binding protein 1) protein in A2780 cells, which stimulates proliferation, inhibiting cell differentiation and facilitating tumor neo-angiogenesis (123). The suppression of Id1 protein after apigenin treatment was demonstrated through activating transcription factor 3 (ATF3).

Prostate Cancer

Knowles *et al* (124) compared the effects of selected bioflavonoids including apigenin on the proliferation of androgen-independent human prostate cancer PC-3 cells, which show complete growth retardation after apigenin exposure. The effects of bioflavonoids on the activity and phosphotyrosine content of oncogenic proline-directed protein kinase FA (PDPK FA) in human prostate carcinoma cells have also been studied. Long term treatment of human prostate carcinoma cells with low concentrations of quercetin, apigenin, and kaempferol potently induced tyrosine dephosphorylation and concurrently inactivated oncogenic PDPK FA in a concentration-dependent manner (125).

Apigenin has the capability to significantly reduce cell number and induce apoptosis in PWR-1E, LNCaP, PC-3, and DU145 cells (126). The PC-3 and DU145 cells were less susceptible to apigenin induced apoptosis than LNCaP and PWR-1E cells. The induction of apoptosis by apigenin is caspase-dependent. Apigenin generates reactive oxygen species, causes loss of mitochondrial Bcl-2 expression, increases mitochondrial permeability, causes cytochrome C release, and induces cleavage of caspase 3, 7, 8, and 9 and the concomitant cleavage of the inhibitor of apoptosis protein, cIAP-2. The over-expression of Bcl-2 in LNCaP B10 cells reduces the apoptotic effects of apigenin. Hessenauer *et al* (49) demonstrated a correlation between the activity of casein kinase (CK) 2 and certain growth properties of prostate cancer cells. Apigenin exposure led to inhibition of CK2 activity in both hormone-sensitive LNCaP cells and hormone-refractory PC-3 cells but only the hormone-sensitive LNCaP cells responded with apoptosis. These studies suggest that a high CK2 activity is not essential for growth or protection against apoptosis in hormone-refractory prostate cancer cells.

Gupta *et al* (23) evaluated the growth-inhibitory effects of apigenin on normal human prostate epithelial cells (NHPE), virally transformed normal human prostate epithelial PZ-HPV-7 cells, and human prostate adenocarcinoma CA-HPV-10 cells. Apigenin treatment to NHPE and PZ-HPV-7 resulted in almost identical growth inhibitory responses of low magnitude whereas significant decrease in cell viability was observed in CA-HPV-10 cells. Gupta *et al* (53) reported that apigenin inhibits the growth of androgen-responsive human prostate carcinoma LNCaP cells and described the molecular basis for this observation. The cell growth inhibition achieved by apigenin treatment resulted in a significant decrease in AR protein expression along with a decrease in intracellular and secreted forms of PSA. Apigenin treatment of LNCaP cells resulted in G1 arrest in cell cycle progression which was associated with a marked decrease in the protein expression of cyclin D1, D2 and E and their activating partner cdk2, 4 and 6 with concomitant induction of WAF1/p21 and KIP1/p27. The induction of WAF1/p21 appears to be transcriptionally upregulated and is p53 dependent. In addition, apigenin inhibited hyperphosphorylation of the pRb protein in these cells. Shukla & Gupta (54) studied apigenin-

mediated inhibitory effects in androgen-refractory human prostate carcinoma DU145 cells which have mutations in the tumor suppressor gene p53 and pRb. Exposure of DU145 cells to apigenin resulted in a dose and time dependent inhibition of growth, colony formation, and G1 phase arrest of the cell cycle. Apigenin exposure also resulted in alteration in Bax/Bcl2 ratio in favor of apoptosis, which was associated with the release of cytochrome c and induction of apoptotic protease-activating factor-1 (Apaf-1). This effect was found to result in a significant increase in cleaved fragments of caspase-9, -3, and poly (ADP-ribose) polymerase (PARP). Apigenin exposure also resulted in down modulation of the constitutive expression of NF-κB/p65 and NF-κB/p50 in the nuclear fraction which correlated with an increase in the expression of IκBα in the cytosol. In another study, Shukla & Gupta (127) examined whether apigenin was effective in inhibiting the expression of NF-κB, a gene that regulates several cell survival and anti-apoptotic genes. Exposure of PC-3 cells to apigenin inhibited DNA binding and reduced nuclear levels of the p65 and p50 subunits of NF-κB with concomitant decrease in IκBα degradation, IκB-α phosphorylation and IKKα kinase activity. In addition, apigenin exposure inhibited TNFα-induced activation of NF-κB via the IκBα pathway, thereby sensitizing the cells to TNFα-induced apoptosis. The inhibition of NF-κB activation correlated with a decreased expression of NF-κB-dependent reporter gene and suppressed expression of NF-κB-regulated genes, specifically, Bcl2, cyclin D1, cyclooxygenase-2, matrix metalloproteinase 9, nitric oxide synthase-2, and VEGF. Furthermore, Shukla *et al* (66) investigated the *in vivo* growth inhibitory effects of apigenin on androgen-sensitive human prostate carcinoma 22Rv1 tumor xenografts subcutaneously implanted in athymic male nude mice. Apigenin feeding resulted in dose-dependent inhibition of tumor growth which was associated with increased accumulation of human IGFBP-3 in mouse serum. Apigenin consumption by these mice also resulted in simultaneous decrease in serum IGF-I levels and induction of apoptosis in tumor xenografts, evidence favoring the concept that the growth inhibitory effects of apigenin involve modulation of IGF-axis signaling in prostate cancer. Further studies with pharmacologic intervention of apigenin have a direct growth inhibitory effect on human prostate tumors implanted in athymic nude mice. Oral feeding of apigenin resulted in dose-dependent (i) increase in the protein expression of WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18; (ii) down-modulation of the protein expression of cyclins D1, D2, and E; and cyclin-dependent kinases (cdk), cdk2, cdk4, and cdk6; (iii) decrease in retinoblastoma phosphorylation at serine 780; (iv) increase in the binding of cyclin D1 toward WAF1/p21 and KIP1/p27; and (v) decrease in the binding of cyclin E toward cdk2 in both types of tumors (128). More recent studies with apigenin in LNCaP and PC-3 cells causes G0-G1 phase arrest, decrease in total retinoblastoma (Rb) protein and its phosphorylation at Ser780 and Ser807/811 in dose- and time-dependent fashion. Apigenin treatment caused increased phosphorylation of ERK1/2 and JNK1/2 and this sustained activation resulted in decreased ELK-1 phosphorylation and c-FOS expression thereby inhibiting cell survival. Interestingly, apigenin caused a marked reduction in cyclin D1, D2 and E and their regulatory partners CDK 2, 4 and 6, operative in G0-G1 phase of the cell cycle. This was accompanied by a loss of RNA polymerase II phosphorylation, suggesting the effectiveness of apigenin in inhibiting transcription of these proteins (46). In another study using TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model, Shukla *et al* (67) demonstrated that oral administration of apigenin at doses of 20 and 50 μg/mouse/day, 6 days per week for 20 weeks, significantly decreased tumor volumes of the prostate as well as completely abolished distant-site metastases to lymph nodes, lungs, and liver. Administration of apigenin resulted in increased levels of E-cadherin and decreased levels of nuclear β-catenin, c-Myc, and cyclin D1 in the dorso-lateral prostates of TRAMP mice. These studies indicate that apigenin is effective in suppressing the prostate carcinogenesis in *in vivo* model, at least in part, by blocking β-catenin signaling. Furthermore, Shukla & Gupta, (129) demonstrated that apigenin at different doses resulted in ROS generation, which was accompanied by rapid glutathione depletion, disruption of mitochondrial membrane potential, cytosolic release of cytochrome c, and apoptosis in human prostate cancer 22Rv1 cells. There was accumulation of a p53 fraction to the mitochondria,

which was rapid and occurred between 1 and 3 h after apigenin treatment. *In vivo*, 22Rv1 xenograft studies confirmed that apigenin administration resulted in p53-mediated induction of apoptosis in 22Rv1 tumors. These results indicated that apigenin-induced apoptosis in 22Rv1 cells is initiated by a ROS-dependent disruption of the mitochondrial membrane potential through transcriptional-dependent and -independent p53 pathways.

The mechanism(s) of apigenin action on the IGF/IGF-IR (insulin-like growth factor receptor 1 protein) signaling pathway in human prostate cancer DU145 cells markedly reduced IGF-I-stimulated cell proliferation and induced apoptosis (130). This effect of apigenin was might be partially due to reduced auto-phosphorylation of IGF-IR. Inhibition of p-Akt by apigenin resulted in decreased phosphorylation of GSK-3beta. In another study, Kaur *et al* (131) using human prostate cancer PC-3 cells demonstrated that apigenin-mediated dephosphorylation of Akt resulted in inhibition of its kinase activity, which was confirmed by reduced phosphorylation of pro-apoptotic proteins BAD and glycogen synthase kinase-3, essential downstream targets of Akt. These results suggest that Akt inactivation and dephosphorylation of BAD is a critical event, at least in part, in apigenin-induced decreased cell survival and apoptosis. Mirzoeva *et al* (132) reported that hypoxia induced a time-dependent increase in the level of HIF-1alpha subunit protein in PC3-M cells, and treatment with apigenin markedly decreased HIF-1alpha expression under both normoxic and hypoxic conditions. Apigenin prevented the activation of the HIF-1 and its downstream target gene vascular endothelial growth factor (VEGF). Recent studies from the same group observed that apigenin inhibited the focal adhesion kinase (FAK)/Src, motility and invasion in the metastatic prostate carcinoma PC-3M cells (133).

Skin Cancer

Studies have shown that apigenin is effective in the prevention of UVA/B-induced skin carcinogenesis in SKH-1 mice (34). Topical application of apigenin has been shown to inhibit UV-mediated induction of ornithine decarboxylase activity, reduce tumor incidence and increase tumor free survival in mice. Several other studies have provided evidence that apigenin prevents UV-induced skin tumorigenesis by inhibiting the cell cycle and cyclin-dependent kinases (52). Exposure of mouse keratinocytes to apigenin induced G2/M cell cycle arrest and accumulation of the p53 tumor suppressor protein with increased expression of p21/WAF1. This arrest was accompanied by inhibition of p34 (cdk2) kinase protein level and activity, which was found to be independent of p21/WAF1 (134). In human diploid fibroblasts apigenin produced G1 cell-cycle arrest by inhibiting cdk2 kinase activity and inducing p21/WAF1. Li *et al* (135) established a short-term *in vivo* system to evaluate topical formulations of apigenin and to determine whether apigenin is effective when delivered as a topical preparation to the local skin lesions. It was observed that topical application of apigenin was capable of targeting local tissue. Another study by Li *et al* (136) demonstrated the *in vivo* and *in vitro* percutaneous absorption of apigenin using different vehicles. Recent observation suggests that apigenin suppresses the UVB-induced increase in COX-2 expression (a key enzyme which converts the arachidonic acid to prostaglandins, and its over-expression results in carcinogenesis) and mRNA in mouse and even in human keratinocyte cell lines (137,138). Through these studies it was apparent that delivery of apigenin into viable epidermis appears to be a necessary property for an apigenin formulation to be effective in skin cancer prevention.

Caltagirone *et al* (139) evaluated the combined effects of quercetin and apigenin on inhibition of melanoma growth, invasiveness and metastatic potential, and demonstrated that *in vivo* administration of apigenin and quercetin was effective in inhibiting melanoma lung tumor metastasis in B16-BL6 murine melanoma metastasis model, an effect that was postulated to be due to the impairment of endothelial interactions in malignant cells.

Thyroid Cancer

Yin *et al* (48) investigated the effects of some selected flavonoids including apigenin on human thyroid carcinoma cell lines, UCLA NPA-87-1 (NPA) (papillary carcinoma), UCLA RO-82W-1 (WRO) (follicular carcinoma), and UCLA RO-81A-1 (ARO) (anaplastic carcinoma). Among the flavonoids tested, apigenin was the most potent inhibitor of the proliferation of these cell lines. In another study, Yin *et al* (48) demonstrated that the inhibitory effect of apigenin on ARO cell proliferation was associated with inhibition of both EGFR tyrosine autophosphorylation and phosphorylation of its downstream effector MAPK. Subsequently, Schroder-van der Elst *et al* (140) evaluated the effects of flavonoids on iodide transport and growth of the human follicular thyroid cancer cell line (FTC133) which was stably transfected with the human Na (+)/I (-) symporter (hNIS). It was observed that apigenin inhibited NIS mRNA expression, a finding that may have therapeutic implications in the radioiodide treatment of thyroid carcinoma.

Endometrial cancer

O'Toole *et al* (141) identified genomic aberrations in endometrial cancer cells which were treated with the phyto-estrogenic compounds including apigenin using array-based comparative genomic hybridization. Over 20% of the array genes involving insulin metabolism were modulated in the cancer cells treated with beta-estradiol, compared to those treated with the same concentration of apigenin, suggesting that it may play a role in the treatment of endometrial cancer and in the treatment of postmenopausal women.

Gastric Cancer

Wu *et al* (142) recently evaluated the growth inhibition and apoptosis-inducing effect of apigenin on human gastric carcinoma SGC-7901 cells. Exposure of these cells to apigenin resulted in dose-dependent inhibition of the growth and clone formation of SGC-7901 cells by inducing apoptosis.

Liver Cancer

Initial studies on plant flavonoids have shown that structural analogs designated the flavonoid 7-hydroxyl group are potent inhibitors of the human P-form phenolsulfotransferase, which is of major importance in the metabolism of many drugs, resulting in either inactivation and rapid renal elimination of the highly ionized sulfuric acid ester conjugates formed or, in some instances, formation of conjugates with increased pharmacological activity (143). Introduction of a prenyl group into the molecule increase the hydrophobicity which would be expected to improve their biochemical and pharmacological properties through enhanced affinity for the lipophilic membrane, C8-prenylation of apigenin enhances the cytotoxicity and induces apoptotic cell death in H4IIE hepatoma cells without affecting anti-oxidative properties (144). Yee *et al* (145) investigated the inhibitory effects of luteolin and apigenin on human hepatocellular carcinoma HepG2 cells. Their results indicate that both flavonoids exhibited cell growth inhibitory effects which were due to cell cycle arrest and downregulation of the expression of CDK4 with induction of p53 and p21, respectively. Apigenin reduced cell viability, and induced apoptotic cell death in HepG2 cells. Additionally, it evoked a dose-related elevation of intracellular ROS level. Treatment with various inhibitors of the NADPH oxidase significantly blunted both the generation of ROS and induction of apoptosis by apigenin. These results suggest that ROS generated through the activation of the NADPH oxidase may play an essential role in the apoptosis induced by apigenin in HepG2 cells (86). In addition, Jeyabal *et al* (146) have shown the in vivo protective effects of apigenin against N-nitroso-diethylamine-induced and phenobarbitol promoted hepatocarcinogenesis in Wistar albino rats. Apigenin treatment of these rats at 25 mg/kg body weight for two weeks provided protection against the oxidative stress and DNA damage caused by the carcinogen.

Combination therapy of gemcitabine and apigenin enhanced anti-tumor efficacy in pancreatic cancer cells (MiaPaca-2, AsPC-1). In vitro, the combination treatment resulted in growth inhibition and apoptosis through the down-regulation of NF-κB activity with suppression of Akt activation. Further *in vivo*, combination therapy augmented tumor growth inhibition through the down-regulation of NF-kappa B activity with the suppression of Akt in tumor tissue. The combination of gemcitabine and apigenin enhanced anti-tumor efficacy and apoptosis induction (118).

Adrenal Cortical Cancer

Laboratory studies of adrenocortical cancers have revealed aberrations in a wide variety of signaling pathways and enzymes including aromatose, a key enzyme in the synthesis of estrogen from androgens. Sanderson *et al* (147) investigated the effects of various flavonoids on the catalytic and promoter specific expression of aromatose in H295R human adenocortical cancer cells. Plant-flavonoids were shown to be potent aromatose inhibitors, a finding associated with increased intracellular cAMP concentrations. Ohno *et al* (148) further investigated the effects of plant flavonoids on cortisol production in H295R cells. Their results indicate that cells exposed to apigenin demonstrate decreased cortisol production and 3β-HSD II and P450c21 activity.

Neuroblastoma

Torkin *et al* (149) investigated the effect of apigenin on various human neuroblastoma cell lines. Apigenin treatment has been shown to result in inhibition of colony-forming ability and survival, and induction of apoptosis in human neuroblastoma cells. The mechanism of action of apigenin seems to involve p53, as it increased the levels of p53 and the p53-induced gene products p21WAF1/CIP1 and Bax. Furthermore, apigenin induced cell death and apoptosis of neuroblastoma cells expressing wild-type but not mutant p53. Apigenin was shown to increase caspase-3 activity and PARP cleavage in these cells. Further studies with apigenin in neuroblastoma SH-SY5Y cells resulted in increased apoptosis, which was associated with increases in intracellular free [Ca(2+)] and Bax:Bcl-2 ratio, mitochondrial release of cytochrome c and activation of caspase-9, calpain, caspase-3 and caspase-12 as well (150).

Conclusions and Future Directions

Epidemiologic studies considerably support the notion that diets rich in plant flavones are associated with a number of health benefits, including a reduction of the risk of developing certain cancers. Integration of dietary modification rich in flavones might be a comprehensive chemopreventive strategy for the high-risk individual that may have impact in the neoplastic transformation. Since apigenin is one of the most bioactive plant flavone and is widely distributed in common fruits, beverages and vegetables, its consumption through diet is highly recommended. Based on the studies provided apigenin affects several critical pathways and/or targets which are associated with several health disorders including cancer. Further research is required before apigenin could be brought to the clinical trials. In addition, apigenin has been demonstrated to help in improving cardiovascular conditions, stimulate immune system and provide some protection against cancer. Establishing whether or not therapeutic effects of apigenin are beneficial to patients will require research and generation of scientific evidence. However, based on the above highlighted findings apigenin has potential for further investigation and development as a cancer chemopreventive and/or therapeutic agent.

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Abbreviations

Apaf-1	apoptotic protease activating factor 1
AR	androgen receptor
CK	casein kinase
DFF	DNA fragmentation factor
EGFR	epidermal growth factor receptor
ER	estrogen receptor
ERK	extracellular signal-activated kinase
HIF	hypoxia-inducible factor
Id	Inhibitor of differentiation or DNA binding protein
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
LPS	lipopolysaccharide
MLL	mixed lineage leukemia
ODC	ornithine decarboxylase
PARP	poly (ADP-ribose) polymerase
PI3K	phosphatidylinositol 3-kinase
PMA	phorbol 12-myristate 13-acetate
NF-κB	nuclear factor-kappaB
MAPK	mitogen-activated protein kinase
Rb	retinoblastoma
SOD	superoxide dismutase
TNF	tumor necrosis factor
UV	ultraviolet
VEGF	vascular endothelial growth factor

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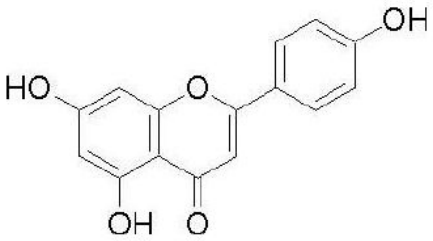
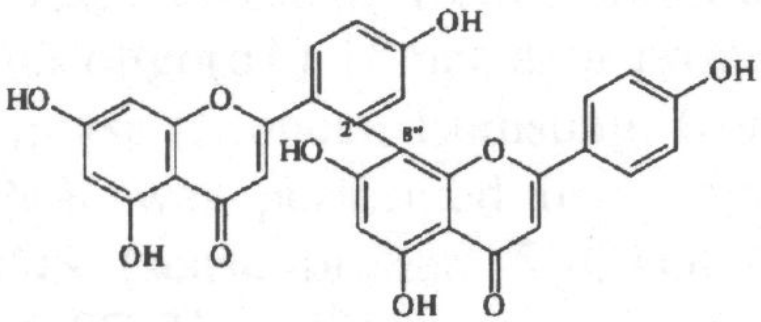


Apigenin: Mol Wt. 270



Biapigenin: Mol Wt. 538

Figure 1.
Chemical structure of apigenin and biapigenin

Table 1

Molecular targets of apigenin in various human cancers

Cancers	Targets
Breast cancer	ErbB2, Bax, Bcl2, p450 CYP1, Caspases, p21, p53, Aromatase, VEGF, ERK, JNK, PI3-Akt COX-2, ERβ, CK2, PKC, MAPK, Cyclin D1 and HER2/neu
Cervical cancer	Bax, Bcl2, p53, p21, Fas/Apo-1 and Caspase3
Colon cancer	APC, ODC, TRAIL, COX-2, PGE-2, MMP, GST, UDP-glucuronosyltransferases (UGT), p34 (cdc2) and p21
Hematologic cancer	Platelet aggregation, 20S, 26S, Bax and IkBα
Lung cancer	VEGF, Akt and p70S6K1
Ovarian cancer	Id1, VEGF, p70S6K1, HIF1 and FAK
Prostate cancer	FAK, Src, PTEN, IGF1R, IGFBP-3, IGF-1, GSK-3β, Akt, p21, p27, VEGF, NF-κB, CK2, IAPs, TRAIL, p53, Caspases, HIF1, FAK, β-Catenin, c-Myc, Cyclins, Cyclin-dependent kinases, MAPK, PI3-Akt, ERβ, AP-1, Bax, Bcl2 and 17β-hydroxysteroid oxidoreductase
Skin cancer	p21, COX-2 and PKC
Thyroid cancer	ERK, MAPK
Liver cancer	NF-κB, Akt and cyclin-dependent kinases (cdk)
Adrenal cancer	Aromatase and cytochrome p450
Neuroblstoma	Bcl2, p53 and caspases

AP-1, Activator protein-1; APC, Adenomatous polyposis coli; COX-2, Cyclooxygenase-2; ErbB2, Erythroblastic leukemia viral oncogene homolog 2; ERβ, Estrogen receptor beta; ERK, Extracellular Signal-Regulated Kinase; FAK, Focal Adhesion Kinase; GST, Glutathione S-transferases; Id1, Inhibitor of differentiation or DNA binding protein 1; IGF-1, Insulin-like growth factor 1; IGF1R, Insulin-like growth factor 1 receptor; IGFBP-3, Insulin-like growth factor binding protein 3; JNK, c-Jun N-terminal kinase; MMP, Matrix metalloproteinase; MAPK, Mitogen-activated protein kinase; ODC, Ornithine decarboxylase; PKC, Protein Kinase C; PTEN, Phosphatase and tensin homolog; Src, v-src sarcoma; TRAIL, TNF-related apoptosis-inducing ligand; UDP-glucuronosyltransferases VEGF, Vascular endothelial growth factor