EXPERT REVIEW

Apigenin: A Promising Molecule for Cancer Prevention

Sanjeev Shukla • Sanjay Gupta

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.

KEY WORDS apigenin · apoptosis · cell cycle · chemoprevention · flavonoids

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
MAPK	mitogen-activated protein kinase
MLL	mixed lineage leukemia
NF-κB	nuclear factor-kappaB
ODC	ornithine decarboxylase
PARP	poly (ADP-ribose) polymerase
PI3K	phosphatidylinositol 3-kinase
PMA	phorbol 12-myristate 13-acetate
Rb	retinoblastoma
SOD	superoxide dismutase
TNF	tumor necrosis factor
UV	ultraviolet
VEGF	vascular endothelial growth factor

S. Shukla · S. Gupta (✉)
Departments of Urology & Nutrition,
Case Western Reserve University,
10900 Euclid Avenue,
Cleveland, Ohio 44106, USA
e-mail: sanjay.gupta@case.edu

S. Shukla · S. Gupta
Department of Urology,
University Hospitals Case Medical Center,
Cleveland, Ohio 44106, USA

S. Gupta
Division of General Medical Sciences,
Case Comprehensive Cancer Center,
Cleveland, Ohio 44106, USA

INTRODUCTION

Evidence suggests 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 a 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). A plant-based diet, especially fruits and vegetables, contains 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 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 molecular-target-based agents. These approaches require isolation, characterization and preclinical evaluation of test agents for their development as chemopreventive agents (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), 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 five 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 was investigated; it 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 flavonoids from 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 six classes of flavonoids with ovarian cancer risk, using data from a multicentric 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 looked at flavonoids and the recurrence risk of neoplasia with resected colorectal cancer patients (13). Eighty-seven patients, 36 with resected colon cancer and 51 after polypectomy, were divided into 2 groups: 31 patients received flavonoid mixture (daily standard dose 20 mg apigenin and 20 mg epigallocatechin-gallate), 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 flavonoid consumption can reduce the recurrence rate of neoplasia in patients with sporadic colorectal neoplasia.

Flavonoids, 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). The 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 vary among 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 molecules that has gained considerable interest as beneficial agent for human health with cancer preventive and/or therapeutic properties is plant flavone apigenin.

Apigenin is chemically known as 4′, 5, 7,-trihydroxy-flavone, with molecular formula $C_{15}H_{10}O_5$ and molecular weight MW 270.24. In nature, apigenin also exists as a dimer, biapigenin, mainly isolated from the buds and flowers of *Hypericum perforatum* (Fig. 1), which has neuro-protective effects (18). Apigenin is abundantly present in common fruits, such as oranges and grapefruit; plant-derived beverages, such as tea; vegetables, such as parsley and onions; as well as in chamomile, wheat sprouts and some seasonings. One of the most common sources of apigenin consumed as a 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* cancerous 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 effects 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

Fig. 1 Chemical structure of apigenin and biapigenin.
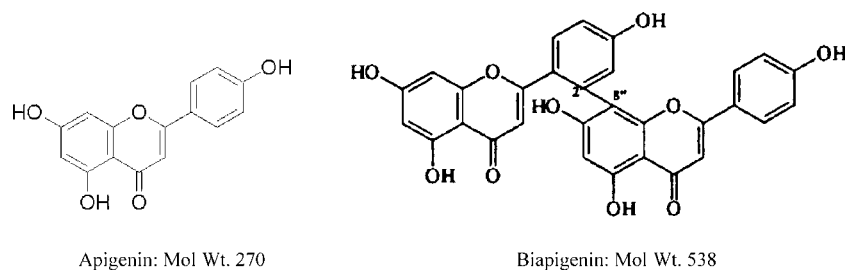
Apigenin: Mol Wt. 270   Biapigenin: Mol Wt. 538

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 investigated by 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.

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 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.

A 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 I.

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

Table I 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

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 investigated by a 7-day study of 18 healthy men and women, which examined 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.

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 increase 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 in 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, and 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, anti-estrogen-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 and 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 used 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 overexpressed 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 were 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 suggests 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 have shown 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 inhibits 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).

A flavonoid-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 (1) inhibiting chymotrypsin-like activity of purified 20S proteasome and of 26S proteasome, (2) accumulating putative ubiquitinated forms of two proteasome target proteins, Bax and IκBα, and (3) 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—with one common compound apigenin-7-O-glucoside in all the extract, have 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—quercetin, 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 and 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-hour 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/6 N 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) and 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]. A 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 and 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 and 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 show a direct growth-inhibitory effect on human prostate tumors implanted in athymic nude mice. Oral feeding of apigenin resulted in dose-dependent (1) increase in the protein expression of WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18, (2) down-modulation of the protein expression of cyclins D1, D2, and E and cyclin-dependent kinases (cdk), cdk2, cdk4, and cdk6, (3) decrease in retinoblastoma phosphorylation at serine 780, (4) increase in the binding of cyclin D1 toward WAF1/p21 and KIP1/p27, and (5) decrease in the binding of cyclin E toward cdk2 in both types of tumors (128). More recent studies show apigenin in LNCaP and PC-3 cells causes G0-G1 phase arrest and 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 and 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 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) used human prostate cancer PC-3 cells to demonstrate 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; its over-expression results in carcinogenesis) and mRNA in mouse and even in human keratinocyte cell lines (137,138). These studies make 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 phenol-sulfotransferase, 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 increased 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 down-regulation 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 aromatase, 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 chemo-preventive strategy for the high-risk individual that may have impact in the neoplastic transformation. Since apigenin is one of the most bioactive plant flavones 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 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 chemo-preventive and/or therapeutic agent.

ACKNOWLEDGEMENTS

REFERENCES

1. Hahn WC, Weinberg RA. Modelling the molecular circuitry of cancer. Nat Rev Cancer. 2002;2:331–41.
2. Austoker J. Cancer prevention in primary care. Current trends and some prospects for the future. BMJ. 1994;309:517–20.
3. Hajjar RR. Cancer in the elderly: is it preventable? Clin Geriatr Med. 2004;20:293–316.
4. Mackey S. Promoting lifestyle modification for cancer prevention. J Am Diet Assoc. 2004;104:1568–9.
5. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med. 2002;113:71S–88.
6. Lippman SM, Hong WK. Cancer prevention science and practice. Cancer Res. 2002;62:5119–25.
7. Gupta S. Prostate cancer chemoprevention: current status and future prospects. Toxicol Appl Pharmacol. 2007;224:369–76.
8. Heederik D, Kromhout H, Burema J, Biersteker K, Kromhout D. Occupational exposure and 25-year incidence rate of non-specific lung disease: the Zutphen Study. Int J Epidemiol. 1990;19:945–52.
9. Knekt P, Järvinen R, Seppänen R, Hellövaara M, Teppo L, Pukkala E, et al. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Am J Epidemiol. 1997;146:223–30.
10. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary flavonoids and cancer risk in the Zutphen Elderly Study. Nutr Cancer. 1994;22:175–84.
11. Rossi M, Negri E, Lagiou P, Talamini R, Dal Maso L, Montella M, et al. Flavonoids and ovarian cancer risk: a case-control study in Italy. Int J Cancer. 2008;123:895–8.
12. Bosetti C, Spertini L, Parpinel M, Gnagnarella P, Lagiou P, Negri E, et al. Flavonoids and breast cancer risk in Italy. Cancer Epidemiol Biomarkers Prev. 2005;14:805–8.
13. Hoensch H, Groh B, Edler L, Kirch W. Prospective cohort comparison of flavonoid treatment in patients with resected colorectal cancer to prevent recurrence. World J Gastroenterol. 2008;14:2187–93.
14. Patel D, Shukla S, Gupta S. Apigenin and cancer chemoprevention: progress, potential and promise. Int J Oncol. 2007;30:233–45.
15. Hertog MG, Kromhout D, Aravanis C, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med. 1995;155:381–6.
16. Aherne SA, O'Brien NM. Dietary flavonols: chemistry, food content, and metabolism. Nutrition. 2002;18:75–81.
17. Johannot L, Somerset SM. Age-related variations in flavonoid intake and sources in the Australian population. Public Health Nutr. 2006;9:1045–54.
18. Cheung ZH, Leung MC, Yip HK, Wu W, Siu FK, So KF. A neuroprotective herbal mixture inhibits caspase-3-independent apoptosis in retinal ganglion cells. Cell Mol Neurobiol. 2008;28:137–55.
19. McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of chamomile tea (Matricaria recutita L.). Phytother Res. 2006;20:519–30.
20. Bevilacqua L, Buiarelli F, Coccioli F, Jasionowska R. Identification of compounds in wine by HPLC-tandem mass spectrometry. Ann Chim. 2004;94:679–89.
21. Gerhauser C. Beer constituents as potential cancer chemopreventive agents. European J Cancer. 2005;41:1941–54.
22. Svehlikova V, Bennett RN, Mellon FA, Needs PW, Piacente S, Kroon PA, et al. Isolation, identification and stability of acylated derivatives of apigenin 7-O-glucoside from chamomile (Chamomilla recutita [L.] Rauschert). Phytochemistry. 2004;65:2323–32.
23. Gupta S, Afaq F, Mukhtar H. Selective growth-inhibitory, cell-cycle deregulatory and apoptotic response of apigenin in normal versus human prostate carcinoma cells. Biochem Biophys Res Commun. 2001;287:914–20.
24. Kim HY, Kim OH, Sung MK. Effects of phenol-depleted and phenol-rich diets on blood markers of oxidative stress, and urinary excretion of quercetin and kaempferol in healthy volunteers. J Am Coll Nutr. 2003;22:217–23.
25. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr. 2001;21:381–406.
26. O'Prey J, Brown J, Fleming J, Harrison PR. Effects of dietary flavonoids on major signal transduction pathways in human epithelial cells. Biochem Pharmacol. 2003;66:2075–88.
27. Thiery-Vuillemin A, Nguyen T, Pivot X, Spano JP, Dufresnne A, Soria JC. Molecularly targeted agents: their promise as cancer chemopreventive interventions. Eur J Cancer. 2005;41:2003–15.
28. Nielsen SE, Young JF, Daneshvar B, Lauridsen ST, Knuthsen P, Sandstrom B, et al. Effect of parsley (Petroselinum crispum) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. Br J Nutr. 1999;81:447–55.

29. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer. 2003;3:768–80.

30. Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R, Kinae N. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. J Nutr. 2000;130:2243–50.

31. Janssen K, Mensink RP, Cox FJ, Harryvan JL, Hovenier R, Hollman PC, et al. Effects of the flavonoids quercetin and apigenin on hemostasis in healthy volunteers: results from an in vitro and a dietary supplement study. Am J Clin Nutr. 1998;67:255–62.

32. Kuo ML, Lin JK. Genotoxicities of nitropyrenes and their modulation by apigenin, tannic acid, ellagic acid and indole-3-carbinol in the Salmonella and CHO systems. Mutat Res. 1992;270:87–95.

33. Middleton JR E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev. 2000;52:673–751.

34. Birt DF, Mitchell D, Gold B, Pour P, Pinch HC. Inhibition of ultraviolet light induced skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid. Anticancer Res. 1997;17:85–91.

35. Van Dross R, Xue Y, Knudson A, Pelling JC. The chemopreventive bioflavonoid apigenin modulates signal transduction pathways in keratinocyte and colon carcinoma cell lines. J Nutr. 2003;133:3800S–4.

36. Wei H, Tye L, Bresnick E, Birt DF. Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice. Cancer Res. 1990;50:499–502.

37. Myhrstad MC, Carlsen H, Nordstrom O, Blomhoff R, Moskaug JO. Flavonoids increase the intracellular glutathione level by transactivation of the gamma-glutamylcysteine synthetase catalytic subunit promoter. Free Radic Biol Med. 2002;32:386–93.

38. Liang YC, Huang YT, Tsai SH, Lin-Shiau SY, Chen CF, Lin JK. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. Carcinogenesis. 1999;20:1945–52.

39. Kawai M, Hirano T, Higa S, Arimitsu J, Maruta M, Kuwahara Y, et al. Favonoids and related compounds as anti-allergic substances. Allergol Int. 2007;56:113–23.

40. Yano S, Umeda D, Yamashita T, Ninomiya Y, Sumida M, Fujimura Y, et al. Dietary flavones suppress IgE and Th2 cytokines in OVA-immunized BALB/c mice. Eur J Nutr. 2007;46:257–63.

41. Choi JS, Choi YJ, Park SH, Kang JS, Kang YH. Flavones mitigate tumor necrosis factor-alpha-induced adhesion molecule upregulation in cultured human endothelial cells: role of nuclear factor-kappa B. J Nutr. 2004;4:1013–9.

42. Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: antioxidants or signalling molecules? Free Radic Biol Med. 2004;36:838–49.

43. Lee SF, Lin JK. Inhibitory effects of phytopolyphenols on TPA-induced transformation, PKC activation, and c-jun expression in mouse fibroblast cells. Nutr Cancer. 1997;28:177–83.

44. Lin JK, Chen YC, Huang YT, Lin-Shiau SY. Suppression of protein kinase C and nuclear oncogene expression as possible molecular mechanisms of cancer chemoprevention by apigenin and curcumin. J Cell Biochem Suppl. 1997;28–29:39–48.

45. Mounho BJ, Thrall BD. The extracellular signal-regulated kinase pathway contributes to mitogenic and antiapoptotic effects of peroxisome proliferators in vitro. Toxicol Appl Pharmacol. 1999;159:125–33.

46. Shukla S, Gupta S. Apigenin-induced cell cycle arrest is mediated by modulation of MAPK, PI3K-Akt, and loss of cyclin D1 associated retinoblastoma dephosphorylation in human prostate cancer cells. Cell Cycle. 2007;6:1102–14.

47. Carrillo C, Cafferatam EG, Genovese J, O'Reilly M, Roberts AB, Santa-Coloma TA. TGF-beta1 up-regulates the mRNA for the Na+/Ca2+ exchanger in neonatal rat cardiac myocytes. Cell Mol Biol. 1998;44:543–51.

48. Yin F, Giuliano AE, Van Herle AJ. Signal pathways involved in apigenin inhibition of growth and induction of apoptosis of human anaplastic thyroid cancer cells (ARO). Anticancer Res. 1999;19:4297–303.

49. Hessenauer A, Montenarh M, Gotz C. Inhibition of CK2 activity provokes different responses in hormone-sensitive and hormone-refractory prostate cancer cells. Int J Oncol. 2003;22:1263–70.

50. Landesman-Bollag E, Song DH, Romieu-Mourez R, Sussman DJ, Cardiff RD, Sonenshein GE, et al. Protein kinase CK2: signaling and tumorigenesis in the mammary gland. Mol Cell Biochem. 2001;227:153–65.

51. Plaumann B, Fritsche M, Rimpler H, Brandner G, Hess RD. Flavonoids activate wild-type p53. Oncogene. 1996;13:1605–14.

52. Lepley DM, Pelling JC. Induction of p21/WAF1 and G1 cell-cycle arrest by the chemopreventive agent apigenin. Mol Carcinog. 1997;19:74–82.

53. Gupta S, Afaq F, Mukhtar H. Involvement of nuclear factor-kappa B, Bax and Bcl-2 in induction of cell cycle arrest and apoptosis by apigenin in human prostate carcinoma cells. Oncogene. 2002;21:3727–38.

54. Shukla S, Gupta S. Molecular mechanisms for apigenin-induced cell-cycle arrest and apoptosis of hormone refractory human prostate carcinoma DU145 cells. Mol Carcinog. 2004;39:114–26.

55. Wang IK, Lin-Shiau SY, Lin JK. Induction of apoptosis by apigenin and related flavonoids through cytochrome c release and activation of caspase-9 and caspase-3 in leukaemia HL-60 cells. Eur J Cancer. 1999;35:1517–25.

56. Iwashita K, Kobori M, Yamaki K, Tsushida T. Flavonoids inhibit cell growth and induce apoptosis in B16 melanoma 4A5 cells. Biosci Biotechnol Biochem. 2000;64:1813–20.

57. Hirano T, Oka K, Akiba M. Antiproliferative effects of synthetic and naturally occurring flavonoids on tumor cells of the human breast carcinoma cell line, ZR-75-1. Res Commun Chem Pathol Pharmacol. 1989;64:69–78.

58. Lindenmeyer F, Li H, Menashi S, Soria C, Lu H. Apigenin acts on the tumor cell invasion process and regulates protease production. Nutr Cancer. 2001;39:139–47.

59. Panes J, Gerritsen ME, Anderson DC, Miyasaka M, Granger DN. Apigenin inhibits tumor necrosis factor-induced intercellular adhesion molecule-1 upregulation in vivo. Microcirculation. 1996;3:279–86.

60. Piantelli M, Rossi C, Iezzi M, La Sorda R, Iacobelli S, Alberti S, et al. Flavonoids inhibit melanoma lung metastasis by impairing tumor cells endothelium interactions. J Cell Physiol. 2006;207:23–9.

61. Osada M, Imaoka S, Funae Y. Apigenin suppresses the expression of VEGF, an important factor for angiogenesis, in endothelial cells via degradation of HIF-1alpha protein. FEBS Lett. 2004;575:59–63.

62. Fang J, Xia C, Cao Z, Zheng JZ, Reed E, Jiang BH. Apigenin inhibits VEGF and HIF-1 expression via PI3K/AKT/p70S6K1 and HDM2/p53 pathways. FASEB J. 2005;19:342–53.

63. Le Bail JC, Laroche T, Marre-Fournier F, Habrioux G. Aromatase and 17 beta-hydroxysteroid dehydrogenase inhibition by flavonoids. Cancer Lett. 1998;133:101–6.

64. Hiremath SP, Badami S, Hunasagatta SK, Patil SB. Antifertility and hormonal properties of flavones of Striga orobanchioides. Eur J Pharmacol. 2000;391:193–7.

65. Mak P, Leung YK, Tang WY, Harwood C, Ho SM. Apigenin suppresses cancer cell growth through ERbeta. Neoplasia. 2006;8:896–904.

66. Shukla S, Mishra A, Fu P, MacLennan GT, Resnick MI, Gupta S. Up-regulation of insulin-like growth factor binding

protein-3 by apigenin leads to growth inhibition and apoptosis of 22Rv1 xenograft in athymic nude mice. FASEB J. 2005;19:2042–4.

67. Shukla S, MacLennan GT, Flask CA, Fu P, Mishra A, Resnick MI, et al. Blockade of beta-catenin signaling by plant flavonoid apigenin suppresses prostate carcinogenesis in TRAMP mice. Cancer Research. 2007;67:6925–35.

68. Menichincheri M, Ballinari D, Bargiotti A, Bonomini L, Ceccarelli W, D'Alessio R, et al. Catecholic flavonoids acting as telomerase inhibitors. J Med Chem. 2004;47:6466–75.

69. Brusselmans K, Vrolix R, Verhoeven G, Swinnen JV. Induction of cancer cell apoptosis by flavonoids is associated with their ability to inhibit fatty acid synthase activity. J Biol Chem. 2005;280:5636–45.

70. Kim MH. Flavonoids inhibit VEGF/bFGF-induced angiogenesis in vitro by inhibiting the matrix-degrading proteases. J Cell Biochem. 2003;89:529–38.

71. Reiners Jr JJ, Clift R, Mathieu P. Suppression of cell cycle progression by flavonoids: dependence on the aryl hydrocarbon receptor. Carcinogenesis. 1999;20:1561–6.

72. Way TD, Kao MC, Lin JK. Apigenin induces apoptosis through proteasomal degradation of HER2/neu in HER2/neu-over-expressing breast cancer cells via the phosphatidylinositol 3-kinase/Akt-dependent pathway. J Biol Chem. 2004;279:4479–89.

73. Kim JS, Eom JI, Cheong JW, Choi AJ, Lee JK, Yang WI, et al. Protein kinase CK2alpha as an unfavorable prognostic marker and novel therapeutic target in acute myeloid leukemia. Clin Cancer Res. 2007;13:1019–28.

74. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr. 2001;21:381–406.

75. O'Prey J, Brown J, Fleming J, Harrison PR. Effects of dietary flavonoids on major signal transduction pathways in human epithelial cells. Biochem Pharmacol. 2003;66:2075–88.

76. Thiery-Vuillemin A, Nguyen T, Pivot X, Spano JP, Dufresnne A, Soria JC. Molecularly targeted agents: Their promise as cancer chemopreventive interventions. Eur J Cancer. 2005;41:2003–15.

77. Nielsen SE, Young JF, Daneshvar B, Lauridsen ST, Knuthsen P, Sandstrom B, et al. Effect of parsley (Petroselinum crispum) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. Br J Nutr. 1999;81:447–55.

78. Scott EN, Gescher AJ, Steward WP, Brown K. Development of dietary phytochemical chemopreventive agents: biomarkers and choice of dose for early clinical trials. Cancer Prev Res (Phila Pa). 2009;2:525–30.

79. Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R, Kinae N. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. J Nutr. 2000;130:2243–50.

80. Janssen K, Mensink RP, Cox FJ, Harryvan JL, Hovenier R, Hollman PC, et al. Effects of the flavonoids quercetin and apigenin on hemostasis in healthy volunteers: results from an in vitro and a dietary supplement study. Am J Clin Nutr. 1998;67:255–62.

81. Way TD, Kao MC, Lin JK. Degradation of HER2/neu by apigenin induces apoptosis through cytochrome c release and caspase-3 activation in HER2/neu-overexpressing breast cancer cells. FEBS Lett. 2005;579:145–52.

82. Weldon CB, McKee A, Collins-Burow BM, Melnik LI, Scanduro AB, McLachlan JA, et al. PKC-mediated survival signaling in breast carcinoma cells: a role for MEK1-AP1 signaling. Int J Oncol. 2005;26:763–8.

83. Choi EJ, Kim GH. Apigenin Induces Apoptosis through a Mitochondria/Caspase-Pathway in Human Breast Cancer MDA-MB-453 Cells. J Clin Biochem Nutr. 2009;44:260–5.

84. Choi EJ, Kim GH. 5-Fluorouracil combined with apigenin enhances anticancer activity through induction of apoptosis in human breast cancer MDA-MB-453 cells. Oncol Rep. 2009;22:1533–7.

85. Yin F, Giuliano AE, Law RE, Van Herle AJ. Apigenin inhibits growth and induces G2/M arrest by modulating cyclin-CDK regulators and ERK MAP kinase activation in breast carcinoma cells. Anticancer Res. 2001;21:413–20.

86. Choi EJ, Kim GH. Apigenin causes G (2)/M arrest associated with the modulation of p21 (Cip1) and Cdc2 and activates p53-dependent apoptosis pathway in human breast cancer SK-BR-3 cells. J Nutr Biochem. 2009;20:285–90.

87. Wang C, Kurzer MS. Phytoestrogen concentration determines effects on DNA synthesis in human breast cancer cells. Nutr Cancer. 1997;28:236–47.

88. Wang C, Kurzer MS. Effects of phytoestrogens on DNA synthesis in MCF-7 cells in the presence of estradiol or growth factors. Nutr Cancer. 1998;31:90–100.

89. Collins-Burow BM, Burow ME, Duong BN, McLachlan JA. Estrogenic and antiestrogenic activities of flavonoid phytochemicals through estrogen receptor binding-dependent and-independent mechanisms. Nutr Cancer. 2000;38:229–44.

90. Long X, Fan M, Bigsby RM, Nephew KP. Apigenin inhibits antiestrogen-resistant breast cancer cell growth through estrogen receptor-alpha-dependent and estrogen receptor-alpha-independent mechanisms. Mol Cancer Ther. 2008;7:2096–108.

91. Zhang S, Yang X, Morris ME. Combined effects of multiple flavonoids on breast cancer resistance protein (ABCG2)-mediated transport. Pharm Res. 2004;21:1263–73.

92. Stroheker T, Picard K, Lhuguenot JC, Canivenc-Lavier MC, Chagnon MC. Steroid activities comparison of natural and food wrap compounds in human breast cancer cell lines. Food Chem Toxicol. 2004;42:887–97.

93. Seo HS, DeNardo DG, Jacquot Y, Laïos I, Vidal DS, Zambrana CR, et al. Stimulatory effect of genistein and apigenin on the growth of breast cancer cells correlates with their ability to activate ER alpha. Breast Cancer Res Treat. 2006;99:121–34.

94. Van Meeuwen JA, Korthagen N, de Jong PC, Piersma AH, Van den Berg M. (Anti) estrogenic effects of phytochemicals on human primary mammary fibroblasts, MCF-7 cells and their co-culture. Toxicol Appl Pharmacol. 2007;221:372–83.

95. Menendez JA, Vazquez-Martin A, Oliveras-Ferraros C, Garcia-Villalba R, Carrasco-Pancorbo A, Fernandez-Gutierrez A, et al. Analyzing effects of extra-virgin olive oil polyphenols on breast cancer-associated fatty acid synthase protein expression using reverse-phase protein microarrays. Int J Mol Med. 2008;22:433–9.

96. Chen T, Li LP, Lu XY, Jiang HD, Zeng S. Absorption and excretion of luteolin and apigenin in rats after oral administration of Chrysanthemum morifolium extract. J Agric Food Chem. 2007;55:273–7.

97. Lee SH, Ryu JK, Lee KY, Woo SM, Park JK, Yoo JW, et al. Enhanced anti-tumor effect of combination therapy with gemcitabine and apigenin in pancreatic cancer. Cancer Lett. 2008;259:39–49.

98. Zheng PW, Chiang LC, Lin CC. Apigenin induced apoptosis through p53-dependent pathway in human cervical carcinoma cells. Life Sci. 2005;76:1367–79.

99. Czyz J, Madeja Z, Irmer U, Korohoda W, Hulser DF. Flavonoid apigenin inhibits motility and invasiveness of carcinoma cells in vitro. Int J Cancer. 2005;114:12–8.

100. Wu C, Chen F, Rushing JW, Wang X, Kim HJ, Huang G, et al. Antiproliferative activities of parthenolide and golden feverfew

extract against three human cancer cell lines. J Med Food. 2006;9:55–61.

101. Wang W, Heideman L, Chung CS, Pelling JC, Koehler KJ, Birt DF. Cell-cycle arrest at G2/M and growth inhibition by apigenin in human colon carcinoma cell lines. Mol Carcinog. 2000;28:102–10.

102. Wang W, VanAlstyne PC, Irons KA, Chen S, Stewart JW, Birt DF. Individual and interactive effects of apigenin analogs on G2/M cell-cycle arrest in human colon carcinoma cell lines. Nutr Cancer. 2004;48:106–14.

103. Takagaki N, Sowa Y, Oki T, Nakanishi R, Yogosawa S, Sakai T. Apigenin induces cell cycle arrest and p21/WAF1 expression in a p53-independent pathway. Int J Oncol. 2005;26:185–9.

104. Chung CS, Jiang Y, Cheng D, Birt DF. Impact of adenomatous polyposis coli (APC) tumor supressor gene in human colon cancer cell lines on cell cycle arrest by apigenin. Mol Carcinog. 2007;46:773–82.

105. Farah M, Parhar K, Moussavi M, Eivemark S, Salh B. 5, 6-Dichloro-ribifuranosylbenzimidazole- and apigenin-induced sensitization of colon cancer cells to TNF-alpha-mediated apoptosis. Am J Physiol Gastrointest Liver Physiol. 2003;285:919–28.

106. Au A, Li B, Wang W, Roy H, Koehler K, Birt D. Effect of dietary apigenin on colonic ornithine decarboxylase activity, aberrant crypt foci formation, and tumorigenesis in different experimental models. Nutr Cancer. 2006;54:243–51.

107. Svehlikova V, Bennett RN, Mellon FA, Needs PW, Piacente S, Kroon PA, et al. Isolation, identification and stability of acylated derivatives of apigenin 7-O-glucoside from chamomile (Chamomilla recutita [L.] Rauschert). Phytochemistry. 2004;65:2323–32.

108. Al-Fayez M, Cai H, Tunstall R, Steward WP, Gescher AJ. Differential modulation of cyclooxygenase-mediated prostaglandin production by the putative cancer chemopreventive flavonoids tricin, apigenin and quercetin. Cancer Chemother Pharmacol. 2006;58:816–25.

109. Vargo MA, Voss OH, Poustka F, Cardounel AJ, Grotewold E, Doseff AI. Apigenin-induced-apoptosis is mediated by the activation of PKCdelta and caspases in leukemia cells. Biochem Pharmacol. 2006;72:681–92.

110. Navarro-Núñez L, Lozano ML, Palomo M, Martínez C, Vicente V, Castillo J, et al. Apigenin inhibits platelet adhesion and thrombus formation and synergizes with aspirin in the suppression of the arachidonic acid pathway. J Agric Food Chem. 2008;56:2970–6.

111. Chen D, Daniel KG, Chen MS, Kuhn DJ, Landis-Piwowar KR, Dou QP. Dietary flavonoids as proteasome inhibitors and apoptosis inducers in human leukemia cells. Biochem Pharmacol. 2005;69:1421–32.

112. Abaza L, Talorete TP, Yamada P, Kurita Y, Zarrouk M, Isoda H. Induction of growth inhibition and differentiation of human leukemia HL-60 cells by a Tunisian gerboui olive leaf extract. Biosci Biotechnol Biochem. 2007;71:1306–12.

113. Monasterio A, Urdaci MC, Pinchuk IV, Lopez-Moratalla N, Martinez-Irujo J. Flavonoids induce apoptosis in human leukemia U937 cells through caspase- and caspase-calpain-dependent pathways. J Nutr Cancer. 2004;50:90–100.

114. Horvathova K, Novotny L, Vachalkova A. The free radical scavenging activity of four flavonoids determined by the comet assay. Neoplasma. 2003;50:291–5.

115. Strick R, Strissel PL, Borgers S, Smith SL, Rowley JD. Dietary bioflavonoids induce cleavage in the MLL gene and may contribute to infant leukemia. Proc Natl Acad Sci USA. 2000;97:4790–5.

116. Li ZD, Liu LZ, Shi X, Fang J, Jiang BH. Benzo[a]pyrene-3, 6-dione inhibited VEGF expression through inducing HIF-1alpha degradation. Biochem Biophys Res Commun. 2007;357:517–23.

117. Watanabe N, Hirayama R, Kubota N. The chemopreventive flavonoid apigenin confers radiosensitizing effect in human tumor cells grown as monolayers and spheroids. J Radiat Res (Tokyo). 2007;48:45–50.

118. Lee WJ, Chen WK, Wang CJ, Lin WL, Tseng TH. Apigenin inhibits HGF-promoted invasive growth and metastasis involving blocking PI3K/Akt pathway and beta 4 integrin function in MDA-MB-231 breast cancer cells. Toxicol Appl Pharmacol. 2008;226:178–91.

119. Engelmann C, Blot E, Panis Y, Bauer S, Trochon V, Nagy HJ, et al. Apigenin–strong cytostatic and anti-angiogenic action in vitro contrasted by lack of efficacy in vivo. Phytomedicine. 2002;9:489–95.

120. Fang J, Zhou Q, Liu LZ, Xia C, Hu X, Shi X, et al. Apigenin inhibits tumor angiogenesis through decreasing HIF-1alpha and VEGF expression. Carcinogenesis. 2007;28:858–64.

121. Zhu F, Liu XG, Liang NC. Effect of emodin and apigenin on invasion of human ovarian carcinoma HO-8910PM cells in vitro. Ai Zheng. 2003;22:358–62.

122. Hu XW, Meng D, Fang J. Apigenin inhibited migration and invasion of human ovarian cancer A2780 cells through focal adhesion kinase. Carcinogenesis. 2008;29:2369–76.

123. Li ZD, Hu XW, Wang YT, Fang J. Apigenin inhibits proliferation of ovarian cancer A2780 cells through Id1. FEBS Lett. 2009;583:1999–2003.

124. Knowles LM, Zigrossi DA, Tauber RA, Hightower C, Milner JA. Flavonoids suppress androgen-independent human prostate tumor proliferation. Nutr Cancer. 2000;38:116–22.

125. Lee SC, Kuan CY, Yang CC, Yang SD. Bioflavonoids commonly and potently induce tyrosine dephosphorylation/inactivation of oncogenic proline-directed protein kinase FA in human prostate carcinoma cells. Anticancer Res. 1998;18:1117–21.

126. Morrissey C, O'Neill A, Spengler B, Christoffel V, Fitzpatrick JM, Watson RW. Apigenin drives the production of reactive oxygen species and initiates a mitochondrial mediated cell death pathway in prostate epithelial cells. Prostate. 2005;63:131–42.

127. Shukla S, Gupta S. Suppression of constitutive and tumor necrosis factor alpha-induced nuclear factor (NF)-kappaB activation and induction of apoptosis by apigenin in human prostate carcinoma PC-3 cells: correlation with down-regulation of NF-kappaB-responsive genes. Clin Cancer Res. 2004;10:3169–78.

128. Shukla S, Gupta S. Molecular targets for apigenin-induced cell cycle arrest and apoptosis in prostate cancer cell xenograft. Mol Cancer Ther. 2006;5:843–52.

129. Shukla S, Gupta S. Apigenin-induced prostate cancer cell death is initiated by reactive oxygen species and p53 activation. Free Radic Biol Med. 2008;44:1833–45.

130. Shukla S, Gupta S. Apigenin suppresses insulin-like growth factor I receptor signaling in human prostate cancer: an in vitro and in vivo study. Mol Carcinog. 2009;48:243–52.

131. Kaur P, Shukla S, Gupta S. Plant flavonoid apigenin inactivates Akt to trigger apoptosis in human prostate cancer: an in vitro and in vivo study. Carcinogenesis. 2008;29:2210–7.

132. Mirzoeva S, Kim ND, Chiu K, Franzen CA, Bergan RC, Pelling JC. Inhibition of HIF-1 alpha and VEGF expression by the chemopreventive bioflavonoid apigenin is accompanied by Akt inhibition in human prostate carcinoma PC3-M cells. Mol Carcinog. 2008;47:686–700.

133. Franzen CA, Amargo E, Todorović V, Desai BV, Huda S, Mirzoeva S, et al. The chemopreventive bioflavonoid apigenin inhibits prostate cancer cell motility through the focal adhesion kinase/Src signaling mechanism. Cancer Prev Res (Phila Pa). 2009;2(9):830–41.

134. McVean M, Xiao H, Isobe K, Pelling JC. Increase in wild-type p53 stability and transactivational activity by the chemopreventive agent apigenin in keratinocytes. Carcinogenesis. 2000;21:633–9.

135. Li B, Birt DF. *In vivo* and *in vitro* percutaneous absorption of cancer preventive flavonoid apigenin in different vehicles in mouse skin. Pharm Res. 1996;13:1710–5.

136. Li B, Pinch H, Birt DF. Influence of vehicle, distant topical delivery, and biotransformation on the chemopreventive activity of apigenin, a plant flavonoid, in mouse skin. Pharm Res. 1996;13:1530–4.

137. Tong X, Van Dross RT, Abu-Yousif A, Morrison AR, Pelling JC. Apigenin prevents UVB-induced cyclooxygenase 2 expression: coupled mRNA stabilization and translational inhibition. Mol Cell Biol. 2007;27:283–96.

138. Van Dross RT, Hong X, Essengue S, Fischer SM, Pelling JC. Modulation of UVB-induced and basal cyclooxygenase-2 (COX-2) expression by apigenin in mouse keratinocytes: role of USF transcription factors. Mol Carcinog. 2007;46:303–14.

139. Caltagirone S, Rossi C, Poggi A, Ranelletti FO, Natali PG, Brunetti M, *et al*. Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential. Int J Cancer. 2000;87:595–600.

140. Schroder-van der Elst JP, van der Heide D, Romijn JA, Smit JW. Differential effects of natural flavonoids on growth and iodide content in a human Na+/I-symporter-transfected follicular thyroid carcinoma cell line. Eur J Endocrinol. 2004;150:557–64.

141. O'Toole SA, Sheppard BL, Sheils O, O'Leary JJ, Spengler B, Christoffel V. Analysis of DNA in endometrial cancer cells treated with phyto-estrogenic compounds using comparative genomic hybridisation microarrays. Planta Med. 2005;71:435–9.

142. Wu K, Yuan LH, Xia W. Inhibitory effects of apigenin on the growth of gastric carcinoma SGC-7901 cells. World J Gastroenterol. 2005;11:4461–4.

143. Eaton EA, Walle UK, Lewis AJ, Hudson T, Wilson AA, Walle T. Flavonoids, potent inhibitors of the human P-form phenolsulfotrans-ferase: Potential role in drug metabolism and chemoprevention. Drug Metab Dispos. 1996;24:232–7.

144. Watjen W, Weber N, Lou YJ, Wang ZQ, Chovolou Y, Kampkotter A, *et al*. Prenylation enhances cytotoxicity of apigenin and liquiritigenin in rat H4IIE hepatoma and C6 glioma cells. Food Chem Toxicol. 2007;45:119–24.

145. Yee SB, Lee JH, Chung HY, Im KS, Bae SJ, Choi JS, *et al*. Inhibitory effects of luteolin isolated from Ixeris sonchifolia Hance on the proliferation of HepG2 human hepatocellular carcinoma cells. Arch Pharm Res. 2003;26:151–6.

146. Jeyabal PV, Syed MB, Venkataraman M, Sambandham JK, Sakthisekaran D. Apigenin inhibits oxidative stress-induced macromolecular damage in N-nitrosodiethylamine (NDEA)-induced hepatocellular carcinogenesis in Wistar albino rats. Mol Carcinog. 2005;44:11–20.

147. Sanderson JT, Hordijk J, Denison MS, Springsteel MF, Nantz MH, Van den Berg M. Induction and inhibition of aromatase (CYP19) activity by natural and synthetic flavonoid compounds in H295R human adrenocortical carcinoma cells. Toxicol Sci. 2004;82:70–9.

148. Ohno S, Shinoda S, Toyoshima S, Nakazawa H, Makino T, Nakajin S. Effects of flavonoid phytochemicals on cortisol production and on activities of steroidogenic enzymes in human adrenocortical H295R cells. J Steroid Biochem Mol Biol. 1999;80:355–63.

149. Torkin R, Lavoie JF, Kaplan DR, Yeger H. Induction of caspase-dependent, p53-mediated apoptosis by apigenin in human neuroblastoma. Mol Cancer Ther. 2005;4:1–11.

150. Das A, Banik NL, Ray SK. Mechanism of apoptosis with the involvement of calpain and caspase cascades in human malignant neuroblastoma SH-SY5Y cells exposed to flavonoids. Int J Cancer. 2006;119:2575–85.