Apigenin and cancer chemoprevention: Progress, potential and promise (Review)

DEENDAYAL PATEL[1,2], SANJEEV SHUKLA[1,2] and SANJAY GUPTA[1-3]

Department of Urology, [1]Case Western Reserve University, [2]University Hospitals of Cleveland, [3]Case Comprehensive Cancer Center, Cleveland, OH 44106, USA

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Abstract. Cancer is one of the major public health burdens in the United States and in other developed countries, causing approximately 7 million deaths every year worldwide. Cancer rates vary dramatically in different regions and populations around the globe, especially between developing and developed nations. Changes in cancer prevalence patterns occur within regions as their populations age or become progressively urbanized. Migration has also contributed to such variations as changes in dietary habits influence cancer rates. These epidemiologic findings strongly suggest that cancer rates are influenced by environmental factors including diet, which is largely preventable. Approaches to prevent cancer include overlapping strategies viz. chemoprevention or dietary cancer prevention. Chemoprevention aims at prevention or reversal of the initiation phase of carcinogenesis or arrest at progression of carcinogenesis through the administration of naturally occurring constituents or pharmacological agents. Cancer prevention through diet may be largely achievable by increased consumption of fruits and vegetables. Considerable attention has been devoted to identifying plant-derived dietary agents which could be developed as promising chemopreventives. One such agent is apigenin. A naturally occurring plant flavone (4', 5, 7,-trihydroxyflavone) abundantly present in common fruits and vegetables including parsley, onions, oranges, tea, chamomile, wheat sprouts and some seasonings. Apigenin has been shown to possess remarkable anti-inflammatory, anti-oxidant and anti-carcinogenic properties. In the last few years, significant progress has been made in studying the biological effects of apigenin at cellular and molecular levels. This review examines the cancer chemopreventive effects of apigenin in an organ-specificity format, evaluating its limitations and its considerable potential for development as a cancer chemo-preventive agent.

Correspondence to: Dr Sanjay Gupta, Department of Urology, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106, USA
E-mail: sanjay.gupta@case.edu

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; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; LPS, lipopolysaccharide; MLL, mixed lineage leukemia; ODC, ornithine decarboxylase; PARP, poly (ADP-ribose) polymerase; PI3K, phosphatidylinositol 3-kinase; PMA, phorbol 12-myristate 13-acetate; NF-κB, nuclear factor-κB; MAPK, mitogen-activated protein kinase; Rb, retino-blastoma; SOD, superoxide dismutase; TNF, tumor necrosis factor; UV, ultraviolet; VEGF, vascular endothelial growth factor

Key words: cancer chemoprevention, apigenin, dietary agents, flavonoids, polyphenols

Contents

1. Introduction

Cancer is a major public health burden in the United States and in other developed countries causing approximately 7 million deaths every year worldwide (1). More than 11 million people are diagnosed with cancer every year and it is estimated that there will be 16 million new cases per year by 2020 (1). In the United States alone, a total of 1,399,790 new cancer cases and 564,830 deaths are expected to be reported in the year 2006 (2). Approximately, 1 in every 2 men and 1 in every 3 American women will have some type of cancer at some point during their lifetime. Cancer incidence rates stabilized among men from 1995 through 2000 but continued to increase among females by 0.4% per year from 1987 through 2000 (2,3). Mortality rates have decreased by 1.5% per year since 1992 among men, but stabilized from 1998 through 2000 among women. Cancer death rates have continued to decrease from the three major cancer types in men (lungs, colorectal, and prostate) and from breast and colorectal cancers in women.

In analyses by race and ethnicity, African-American men and women have 40% and 20% higher death rates from all cancers combined compared with white men and women, respectively (2,3). Cancer incidence and mortality rates are lower in other racial and ethnic groups than in whites and African-Americans for all types combined and for the four major cancer types. However, these other groups generally have higher rates for stomach, liver, and cervical cancers. Furthermore, minority populations are more likely to be diagnosed with advanced stage disease than are whites (2,3). By definition, cancer is the uncontrolled growth and spread of malignant cells that may affect almost any tissue of the body and have many different forms in each body area. Most cancers are named for the type of cell or organ in which they initiate. If a cancer spreads (metastasizes), the new tumor bears the same name as the original (primary) tumor. The most common cancers on a global scale for both men and women are cancers of the lung, colon/rectum and stomach. Among men, lung, colorectal and prostate cancer are the most common cancers worldwide. For women, the most common cancers besides lung and colorectal are breast and cervical cancer (2,3).

Developments in the treatment of cancer have led to significantly improved survival and quality of life for cancer patients in the past three decades. The present major treatment modalities for cancer include chemotherapy, surgery and radiation therapy (4-6). In recent years immunotherapy has also been pursued in the management of cancer (7). Chemotherapy is one of the most effective treatments available for cancer (4-6). New drugs and therapeutic strategies are continuously being researched and developed, but the current status of chemotherapy is far from satisfactory (8). With some notable exceptions, the efficacy of chemotherapy is limited and severe drug-related side effects are common. It is believed that prolonged chemotherapy treatment weakens the immunological defense system of the body and leaves patients susceptible to other diseases and infections. Surgery is the least harmful conventional form of cancer treatment but not all cancers are surgically curable (9). Radiation therapy is another treatment option for cancer, but has a number of potentially harmful side-effects including weakened resistance to other diseases and the potential to be carcinogenic in itself (10). Therefore, there is an urgent need to develop mechanism-based approaches for the management of cancer. The practical goals of this approach should be to decrease the incidence of invasive cancer and deaths from cancer at an early age through pharmacological interventions relying on prevention rather than cure. Such an intervention is termed as 'Chemoprevention' (11-14).

Chemoprevention is a rapidly growing area of oncology which focuses on the prevention of cancer using naturally occurring or synthetic agents (11-14). This pharmacological approach relies on the identification of healthy individuals who are deemed to be at a higher risk of developing cancer, and for whom a pharmacological agent can effectively inhibit the onset of cancer. In addition to inhibiting or delaying the onset of neoplasia by blocking neoplastic inception, chemoprevention plays a role in preventing the development of invasive and metastatic properties in established neoplasms (11-15). Chemoprevention of cancer thus differs from cancer treatment in that the goal of this approach is to lower the rate of cancer incidence. The cancer inhibitory effects of a variety of nutrients derived from plants as well as of non-nutritive plant-derived constituents (phytochemicals) have been confirmed in a variety of cell culture systems and animal tumor models (15,16). This has led to an increased emphasis on cancer prevention strategies in which these dietary factors are utilized. Two major diet-related cancer prevention strategies have evolved viz. cancer chemoprevention and dietary cancer prevention, with appreciable overlap existing between them. Generally, cancer chemoprevention involves pharmacologic intervention with synthetic or naturally occurring compounds to prevent, inhibit or reverse carcinogenesis or prevent the development of invasive cancer (11-14). In contrast, diets containing an abundance of fruits and vegetables and other plant-derived agents are protective against a variety of diseases including epithelial cancers (17,18). Epidemiological studies, including a number of case-control and cohort studies, have provided data that overwhelmingly support an inverse association between intake of fruits and vegetables and cancer risk (19-21). These nutritional practices are effective types of dietary cancer prevention. Numerous components found in fruit and vegetables may contribute to their effectiveness in reducing the risk of cancer, including micronutrients, dietary fiber and various polyphenolic agents (15-17).

The principle plant-derived agents thought to provide protection against cancer are flavonoids and dietary fiber (22,23). Flavonoids are the most common and widely distributed polyphenolic compounds, ubiquitously present in foods of plant origin (24,25). Flavonoids comprise approximately 5,000 compounds that are defined chemically as substances composed of a common phenylchromanone structure (C6-C3-C6), with one or more hydroxyl substitutes (24,25). These

Table I. Chemical structure and source of some commonly occurring plant flavonoids.

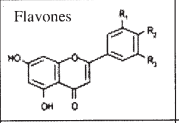
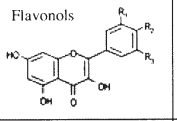
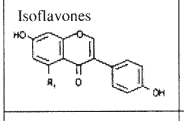
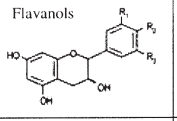
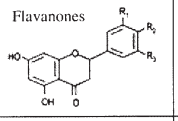
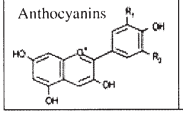
Structure	Representative flavonoids	Source
Flavones 	R1=H, R2=OH: Apigenin R1=R2=OH: Luteolin	Celery, parsley, thyme, onions etc. Red pepper, onions, lettuce, berries etc.
Flavonols 	R2=OH, R1=R3=H: Kaempferol R1=R2=OH, R3=H: Quercetin R1=R2=R3=OH: Myricetin	Black tea etc. Olive oil, apple peels, kale etc.
Isoflavones 	R1=H: Daidzein R1=OH: Genistein	Soybeans, legumes etc. Soybeans, legumes etc.
Flavanols 	R1=R2=OH, R3=H: Catechins R1=R2=R3=OH: Gallocatechin	Green tea etc. Green tea etc.
Flavanones 	R1=H, R2=OH: Naringenin R1=R2=OH: Eriodictyol R1=OH, R2=OCH3: Hesperetin	Citrus fruits, grape fruits etc. Tomatoes, mint, citrus fruits etc. Citrus fruits, grape fruits etc.
Anthocyanins 	R1=H, R2=H: Pelargonidin R1=OH, R2=H: Cyanidin R1=R2=OH: Delphinidin R1=OCH3, R2=OH: Petunidin R1=R2=OCH3: Malvidin	Aubergines, radishes etc. Red wine, beans, berries etc. Pomegranate, cherries, berries etc. Cherries, strawberries etc. Cherries, berries, blackcurrants etc.

Table II. Plant species and parts with the highest amounts of apigenin.

Achillea millefolium	Yarrow (plant)
Apium graveolens	Celery (plant)
Artemisia dracunculus	Tarragon (plant)
Camellia sinensis	Tea (leaf)
Chamaemelum nobile	Perennial chammomile (plant)
Coriandrum sativum	Cilantro (fruit)
Digitalis purpurea	Purple foxglove (flower)
Echinacea spp	Coneflower (leaf)
Gingko biloba	Biloba (leaf)
Glycyrrhiza glabra	Licorice (root)
Linum usitatissimum	Flax (plant)
Marrubium vulgare	Horehound (plant)
Matricaria retcutita	Annual chamomile (plant)
Mentha spicata	Spearmint (leaf)
Ocimum basilicum	Basil (plant)
Origanum vulgare	Oregano (plant)

are generally classified into flavones, flavanols (catechins), isoflavones, flavonols, flavanones and anthocyanins (Table I). Flavones and flavonols are structurally similar compounds, with flavonols having an extra hydroxyl substitution at the carbon 3-position.

Flavonoids have been demonstrated to exert a variety of biological effects in numerous mammalian systems *in vitro* as well as *in vivo*, acting as free radical scavengers and anti-oxidants, and exhibiting anti-mutagenic, anti-inflammatory, antiviral, and purgative effects (25). In addition, other biological effects induced by flavonoids include lowering plasma levels of low-density lipoproteins, inhibiting platelet aggregation, and reducing cell proliferation (23,25-27). These effects are related to their actions in inhibiting the cell cycle, diminishing oxidative stress, improving the efficacy of detoxification enzymes, inducing apoptosis, and stimulating the immune system (25-27). These inherent properties of flavonoids categorize them as a class of beneficial compounds which possess health-promoting and disease-preventing dietary effects, including efficacy in cancer prevention. An added benefit is that these compounds are associated with very little toxicity, making them excellent choices for chemoprevention protocols.

2. Apigenin - a natural plant flavone

Apigenin is abundantly present in common fruits and vegetables such as parsley, onions, oranges, tea, chamomile, wheat sprouts and in some seasonings (17,23-25). Common plants containing the most apigenin are shown in Table II. The cancer chemopreventive properties of apigenin were first demonstrated by Birt *et al* (28) who described the anti-mutagenic and anti-promotion properties of apigenin through inhibition of TPA-induced ornithine decarboxylase activity in mouse skin. These initial studies with apigenin generated further interest in the development of apigenin as a chemo-preventive and/or chemotherapeutic agent.

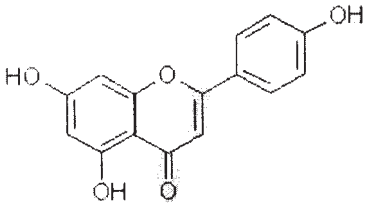


Figure 1. Chemical structure of apigenin.

3. Chemical structure and properties of apigenin

Apigenin is a flavonoid belonging to the flavone structural class and chemically known as 4', 5, 7,-trihydroxyflavone (Fig. 1). Apigenin is a low molecular weight flavonoid (MW 270.24) structurally forming yellow needles in pure form. The melting point of apigenin is 347.5; it is practically insoluble in water, moderately soluble in hot alcohol, and is soluble in dilute KOH and DMSO (29). It is incompatible with strong oxidizing agents. Pure apigenin is highly unstable and storage at -20˚C is recommended (30).

For centuries, apigenin has been utilized as a traditional or alternative medicine. For example, passion flower, which contains high levels of apigenin, has been used effectively to treat asthma, intransigent insomnia, Parkinson's disease, neuralgia, and shingles (31). Apigenin is a major constituent of chamomile, which is recognized for its antiphogistic, anti-spasmodic and antibacterial effects. Chamomile tea consumed 3-4 cups a day has been used for centuries as a folk medicine remedy for relieving indigestion or calming gastritis (31). In addition, chamomile preparations are widely used in skin care products to reduce cutaneous inflammation and other dermatological diseases (32). Alcoholic tincture of the whole flowering plant of chamomile has been used topically as a rinse, gargle, cream, ointment or bath additive. It has also been used as a vapor inhalant (31). In recent years, apigenin has been increasingly recognized as a cancer chemopreventive agent.

4. Biological properties of apigenin

Apigenin has gained particular interest in recent years as a beneficial and health promoting agent because of its low intrinsic toxicity and differential effects in normal versus cancer cells compared with other structurally related flavonoids (33). There is very little evidence to date to suggest that apigenin promotes adverse metabolic reactions *in vivo* when consumed in nutritionally relevant quantities. Following the observations by Birt *et al* (28) several studies have confirmed that apigenin possesses: i) anti-oxidant, ii) anti-mutagenic, iii) anti-carcinogenic, iv) anti-inflammatory, v) anti-proliferative, and vi) anti-progression properties.

Apigenin has been shown to posses anti-mutagenic properties against nitropyrene-induced genotoxicity in Chinese hamster ovary cells (34). Apigenin has also been shown to inhibit benzo[a]pyrene and 2-aminoanthracene-induced bacterial mutagenesis (34). 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 (35). Exposure to

apigenin prior to a carcinogenic insult has been shown to afford a protective effect in murine skin and colon cancer models (36,37). Apigenin is a strong inhibitor of ornithine decarboxylase, an enzyme that plays a major role in tumor promotion (38). Further, apigenin has been shown to increase the intracellular concentration of glutathione, enhancing the endogenous defense against oxidative stress (39).

The anti-carcinogenic effect of apigenin has been demonstrated in a skin carcinogenesis model. Topical application of apigenin inhibited dimethyl benzanthracene-induced skin tumors (38). Apigenin also diminished UV-induced cancer incidence and increased tumor free survival in similar experiments (36).

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 (40). In a separate study, apigenin treatment resulted in suppression of the constitutive and tumor necrosis factor (TNF) α-induced nuclear factor (NF)-κB activation in human umbilical vein endothelial cells (41).

Several studies have demonstrated that apigenin exerts a broad range of molecular signaling effects (42). Apigenin has been reported to inhibit protein kinase C activity, mitogen activated protein kinase (MAPK), transformation of C3HI mouse embryonic fibroblasts and the downstream oncogenes in v-Ha-*ras*-transformed NIH3T3 cells (43,44). Apigenin is a well-known inhibitor of protein-tyrosine kinases and has been shown to block peroxisome proliferation regulated kinase (ERK), a MAPK in isolated hepatocytes (45). 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 (46). 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 (47). Furthermore, apigenin has been shown to inhibit the expression of casein kinase (CK)-2 in both human prostate and breast cancer cells (48,49).

It has been demonstrated that apigenin exerts its effects on the cell cycle. It has been shown that 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 (50,51). 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 (52,53). In addition, apigenin has been shown to induce apoptosis in a wide range of malignant cells (54-56). 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 (53).

Apigenin has shown promise in inhibiting tumor cell invasion and metastases by regulating protease production (57). Apigenin under *in vivo* conditions is also effective in inhibiting TNFα-induced intracellular adhesion molecule-1 up-regulation in cultured human endothelial cells (58). *In vivo* studies have also shown that apigenin inhibits melanoma lung metastases by impairing interaction of tumor cells with endothelium (59). 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 (60). 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 (61).

Studies by Le Bail *et al* (62) demonstrated that apigenin is an effective inhibitor of aromatose and 17ß-hydroxysteroid dehydrogenase activities in human placental microsomes, with resulting effects on steroid metabolism. 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 (63). Apigenin has been shown to decrease intracellular and secreted levels of PSA in androgen-responsive human prostate cancer LNCaP cells (52). 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 (64). These studies imply that apigenin may have the potential to inhibit hormone-related cancers as well.

Other important targets of apigenin include heat shock proteins (60), telomerase (65), fatty acid synthase (66), matrix metalloproteinases (67), and aryl hydrocarbon receptor activity (68) HER2/neu (69), all of which have relevance to cancer development and progression.

5. Apigenin and cancer

Interest in the possible health benefits of apigenin has increased owing to its potent antioxidant and anti-inflammatory activities observed *in vitro*. There is growing evidence from epidemiological and case-control studies that higher intake of plant flavonoids reduces the risk of chronic diseases including cancer (70,71). In contrast, 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 anti-oxidant vitamins, erythrocyte superoxide dismutase (SOD) activity and lymphocyte DNA damage commonly associated with enhanced disease risk, suggesting the beneficial effects of flavonoids on human health (72). A study of total dietary intake of 10,054 men and women that was conducted to establish a dietary baseline and dietary history showed that men with higher myricetin intakes had a lower risk of prostate cancer (73). In the Zutphen elderly study, which was based on the analyses of five flavonoids (quercetin, kaempferol, myricetin, apigenin and luteolin) in composite food samples for 738 median aged (65-84 years) individuals without a history of cancer and followed for 5 years, high intake of flavonoids from vegetables and fruits was inversely associated with the risk of cancer (74). A recent case-control study conducted in Greece on 820 women with breast cancer and 1,548 control patients without breast cancer demonstrated a strong significant inverse association between flavone intake in leafy vegetables and the development of breast cancer (75). More recent case-control studies of breast cancer conducted in Italy which included 2,569 women with histologically confirmed breast cancer and 2,588 hospital controls have found an inverse

association between flavone consumption and breast cancer risk, essentially confirming the results of the Greek study (76). These observational and case-control reports on plant flavone intake encourage further studies on types of human cancer. Below we provide an update on the protective effects of apigenin on various human cancers.

Apigenin and breast cancer. Breast cancer is the most common cancer among women in the Western world and the second leading cause of cancer-related deaths in women (2,3). Several cancers, including breast cancer, have a lower incidence in Asia than in Western countries (77). This may be attributed to the typical Asian dietary regimens which are rich in flavonoid-containing plants, and which are thought to be anti-tumorigenic. Furthermore, apigenin has been shown to have anti-proliferative effects 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 (69). Induction of apoptosis was also observed in HER2/neu-overexpressing breast cancer cells in a dose- and time-dependent manner after apigenin treatment (78). 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 (79). 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 (78). 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 (78).

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 (79). Recent studies have demonstrated suppression of TNFα-induced apoptosis by treatment with PMA in MCF-7 breast carcinoma cells (79). 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.

Lindenmeyer *et al* (57) demonstrated the effect of apigenin on protease-mediated invasiveness in estrogen-insensitive breast tumor cell line MDA-MB231, showing that apigenin strongly inhibited tumor cell invasion in a dose-dependent manner. Apigenin inhibits growth and induces G2/M arrest by modulating cyclin-CDK regulators and ERK MAP kinase activation in breast carcinoma cells (80). The growth inhibitory effects of apigenin were observed on MCF-7 cells that express two key cell cycle regulators, wild-type p53 and the retinoblastoma tumor suppressor protein (Rb), and in MDA-MB-468 cells which are mutant for p53 and Rb negative. Apigenin-mediated cell growth inhibition along with G2/M arrest was accompanied by significant decrease in cyclin B1 and CDK1 protein levels, resulting in a marked inhibition of CDK1 kinase activity. Furthermore, apigenin treatment reduced the protein levels of CDK4, cyclin D1 and A, inhibited Rb-phosphorylation but did not affect the protein levels of cyclin E, CDK2 or CDK6. In addition, apigenin treatment resulted in ERK MAP kinase phosphorylation and activation in MDA-MB-468 cells.

Wang and Kurzer (81,82) evaluated the effect of apigenin and other phytoestrogens on DNA synthesis (estimated by thymidine incorporation analysis) in estrogen-dependent MCF-7 cells in the presence of estradiol (E2), tamoxifen, insulin, or epidermal growth factor. The results show that apigenin was capable of inhibiting E2-induced DNA synthesis in these cells. Overall, the effect of apigenin and other phyto-estrogens in the presence of E2 or growth factors was found to be variable and concentration-dependent.

Collins-Burow *et al* (83) performed a study to characterize the estrogenic and anti-estrogenic activity of flavonoids in the ER-positive MCF-7 human breast cancer cell line using an ER-dependent reporter gene assay and an ER competition binding assay. In these studies apigenin was shown to possess anti-estrogenic activities which may be mediated through ER binding-dependent and independent mechanisms. These anti-estrogenic activities were deemed to be biologically significant in the regulation of breast cancer cell proliferation.

Zhang *et al* (84) demonstrated the combined effects of multiple flavonoids on breast cancer resistance protein (BCRP). Several plant flavonoids including apigenin were used alone or in combinations to evaluate the potential interactions for BCRP inhibition. Apigenin and other flavonoids were shown to inhibit the BCRP protein, which was highly efficacious in combination at equimolar concentrations. In another study, Stroheker *et al* (85) tested and 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.

Brusselmans *et al* (66) have shown that plant flavonoids can induce apoptosis in human breast and prostate cancer cells, an effect that is associated with their ability to inhibit the activity of fatty acid synthase, a key metabolic enzyme that catalyzes the synthesis of long chain fatty acids over-expressed in neoplastic and malignant cells. In these studies, at least 6 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.

Apigenin and cervical cancer. Cervical cancer is the second most common type of cancer in women worldwide, after breast cancer. Statistically, over 10,500 women in the United States

develop cervical cancer, and approximately 3,900 women die from this disease (2,3). Cervical cancer develops in the lining of the cervix, the lower part of the uterus (womb) that enters the vagina (birth canal). This condition usually develops over a prolonged period of time. Normal cervical cells may gradually undergo changes to become precancerous and then cancerous. Cervical intraepithelial neoplasia (CIN) is the term used to describe these abnormal changes. A preponderance of evidence supports a causal link between human papillomavirus infection and cervical neoplasia (86). The presence of high-risk human papillomavirus genital subtypes increases the risk of malignant transformation.

Zheng *et al* (87) reported for the first time that apigenin inhibited the growth of human cervical carcinoma HeLa cells 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.

Czyz *et al* (88) 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 concentrations, whereas its influence on cell motility correlated with the reduction of the invasive potential of HeLa Cx43 cells.

Apigenin and colon cancer. More than 130,000 cases of colon cancer are diagnosed in the United States each year, and >50,000 people die of colon cancer each year. According to the American Cancer Society, it is the third most common type of cancer in both men and women in the United States (2,3). Incidence is slightly higher in men than in women, and is highest in African-American men. Most (over 95%) colorectal cancers are adenocarcinomas that develop when mutation occurs in epithelial cells lining the colon or rectum (3). The disease often begins as a neoplastic intestinal polyp, also called an adenoma, which is considered precancerous. As neoplastic epithelial cells attain the ability to invade and metastasize, the lesion becomes malignant. Wang e*t al* (89) studied the effects of apigenin on cell growth and the cell cycle in various human colon carcinoma cell lines. Exposure of colon cancer cells to apigenin resulted in cell growth inhibition, followed by reversible G2/M arrest associated with inhibition of p34 (cdc2) kinase, reduced accumulation of p34 (cdc2) and cyclin B1 proteins. In addition, Wang *et al* (90) studied the individual and interactive influences of seven apigenin analogs on cell cycle, cell number, and cell viability in human colonic carcinoma cell lines. These findings indicate that the induction of cell-cycle arrest by five of even 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 (50). 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 (91). 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. Farah *et al* (92) reported on 5, 6-dichloro-ribifurano-sylbenzimidazole (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, affected a synergistic reduction in cell survival when used in conjunction with TNFα. Van Dross *et al* (37) demonstrated that the 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).

Svehlikova *et al* (93) demonstrated the interactions between sulforaphane and apigenin in the induction of UGT1A1 and GSTA1, the phase II detoxifying enzymes, in CaCo-2 cells. Apigenin was shown to induce UGT1A1 transcription but not GSTA1; sulforaphane induced both UGT1A1 and GSTA1 transcription in both a 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.

Apigenin and leukemia. Leukemia is a malignancy characterized by the production of large numbers of abnormal white cells in the bone marrow, resulting in replacement of normal blood cell precursors by malignant cells. As a result, fewer normal white cells are produced. In humans, there are three types of blood cells found in the blood stream: leukocytes (white blood cells), erythrocytes (red blood cells), and thrombocytes (blood platelets). It is in these blood cell lines that leukemia develops. Leukemia literally means 'many white cells in the blood'. It is one of the commonest and most lethal types of cancer on a global scale, and is especially prevalent in the United States (2,3). Apigenin was tested to ascertain its effect on human leukemia cells. Apigenin was shown to be markedly more effective than other tested flavonoids in inducing apoptosis in these cells, which was due to cell differentiation (54). Further studies have shown that apigenin and quercetin both inhibit topoisomerase-catalysed DNA irregularities that are involved in many aspects of leukemia cell DNA metabolism including replication and transcription reactions.

Chen *et al* (94) examined the flavonoids viz. apigenin, quercetin, kaempferol and myricetin for their proteasome-inhibitory and apoptosis-inducing abilities in human leukemia cells. They reported that apigenin and quercetin were much more potent than kaempferol and myricetin in: i) inhibiting the chymotrypsin-like activity of purified 20S proteasome and

of 26S proteasome, ii) accumulating putative ubiquitinated forms of two proteasome target proteins, Bax and IκBα, and iii) inducing activation of caspase-3 and cleavage of poly (ADP-ribose) polymerase in Jurkat T cells. Furthermore, the proteasome-inhibitory abilities of these compounds correlated with their apoptosis-inducing potencies.

Wang *et al* (54) demonstrated that structurally related flavonoids, such as apigenin, quercetin, myricetin, and kaempferol were able to induce apoptosis in human leukemia HL-60 cells. 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. In another study, Monasterio *et al* (95) evaluated the potential of 22 flavonoids and related compounds by testing the apoptotic activity in leukemic U937 cells. In these studies, apigenin and several other flavones but not the isoflavones or flavanones tested were shown to induce apoptosis in U937 cells.

Horvathova *et al* (96) evaluated the protective effects of four flavonoids, querectin, rutin, luteolin and apigenin on the extent of H_2O_2-induced DNA damage in murine leukemia L1210 cells. 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. In another study, Strick *et al* (97) evaluated the role of dietary bioflavonoids in inducing cleavage in the MLL gene, which may contribute to infant leukemia. 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.

Apigenin and lung cancer. Lung cancer is the leading cause of cancer-related deaths in the world, with increasing incidence in many developed countries. There is a close relationship between the number of lung cancer cases and lung cancer deaths in the United States. This is because of the low 5-year survival rate for this disease. Although lung cancer survival rates have improved over the last 40 years, the overall survival rate (~13%) remains low in comparison to other cancers (2,3). Epidemiological data suggest that consumption of plant flavonoids may be associated with a decreased risk of lung cancer. Liu *et al* (98) have shown that apigenin inhibited A549 lung cancer cell proliferation and vascular endothelial growth factor (VEGF) transcriptional activation in a dose-dependent manner. Apigenin inhibited VEGF transcriptional activation through the HIF-1 binding site, and specifically decreased HIF-1α, but not HIF-1ß subunit expression in these cells. In a signaling pathway that mediates VEGF transcriptional activation, apigenin inhibited AKT and p70S6K1 activation. 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 by Engelmann *et al* (99) demonstrated the efficacy of apigenin administration against experimental Lewis lung carcinomas (LLC), C-6 gliomas and DHDK 12 colonic cancers *in vivo*. Tumor bearing mice received 50 mg/kg/day of apigenin in three different galenical formulations for 12 days in 8-h intervals. The results did not exhibit an *in vivo* response as observed with the high *in vitro* sensitivity of LLC, C-6, DHDK 12 and endothelial cells to apigenin where complete growth suppression occurs at concentrations >30 μg/ml.

Apigenin and ovarian cancer. Ovarian cancer is a disease produced by the rapid unregulated growth and division of malignant surface epithelial cells within one or both ovaries. Ovarian cancer is one of the most common causes of cancer death among women (2,3). It is a disease that principally affects middle and upper-class women in industrialized nations. It is uncommon in underdeveloped countries, perhaps because of different dietary factors in these regions. Fang *et al* (61) demonstrated that apigenin inhibits expression of VEGF in human ovarian cancer cells. Apigenin inhibited VEGF expression 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 *in vitro* by endothelial cells. Additionally, apigenin inhibited the activity of MAPK and PI3K in human ovarian carcinoma HO-8910PM cells (100).

Apigenin and prostate cancer. Next to skin cancer, prostate cancer has always been the most common cancer in males in Western countries (2,3). Prostate cancer is an attractive target for chemoprevention because of the prevalence of a long latency period between pre-malignant lesions and clinically evident cancer, and its defined molecular pathogenesis (11,12). Furthermore, curative treatments for established prostate cancers are associated with significant morbidities. Epidemiological studies suggest that plant-based diets decrease the risk of prostate cancer. Asian men have much lower incidences of prostate cancer and possibly of benign prostatic hyperplasia (BPH) than their Western counterparts. Vegetarian men also have a lower incidence of prostate cancer than omnivorous males. Both Asian and vegetarian men consume low-fat, high fiber diets which provide a rich supply of weak dietary estrogens. It has been postulated that these phyto-estrogens act as chemopreventive agents, particularly for Asian men and to a lesser extent, for vegetarian men also (20,101). Knowles *et al* (102) 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 (103).

Apigenin has the capability to significantly reduce cell number and induce apoptosis in PWR-1E, LNCaP, PC-3, and

DU145 cells (104). 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 overexpression of Bcl-2 in LNCaP B10 cells reduces the apoptotic effects of apigenin. Hessenauer *et al* (48) demonstrated a correlation between the activity of casein kinase (CK) 2 and certain growth properties of prostate cancer cells. Apigenin exposure led to the 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* (33) 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 a significant decrease in cell viability was observed in CA-HPV-10 cells. Gupta *et al* (52) 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 up-regulated and is p53-dependent. In addition, apigenin inhibited hyperphosphorylation of the pRb protein in these cells. Shukla and Gupta (53) 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 the 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 (105) examined whether apigenin was effective in inhibiting the expression of NF-κB, a gene that regulates several cell survival and antiapoptotic 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 metallo-proteinase 9, nitric oxide synthase-2, and VEGF. More recently, Shukla *et al* (64) 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.

Apigenin and skin cancer. The incidences of basal cell carcinoma, squamous cell carcinoma, and melanoma, the deadliest form of skin cancer, continue to increase in the United States and elsewhere (2,3). Solar ultraviolet (UV) B radiation has been implicated as the main cause of skin cancer. This adverse effect of UVB has become a major human health concern. Studies have shown that apigenin is effective in the prevention of UVA/B-induced skin carcinogenesis in SKH-1 mice (36). 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 (51). 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 (106). In human diploid fibroblasts apigenin produced G1 cell-cycle arrest by inhibiting cdk2 kinase activity and inducing p21/WAF1. Li *et al* (107) 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 local skin lesions. It was observed that topical application of apigenin was capable of targeting local tissue. Another study by Li and Birt (108) demonstrated the *in vivo* and *in vitro* percutaneous absorption of apigenin using different vehicles. Through these studies it was apparent that delivery of apigenin into viable epidermis appears to be a necessary property for an apigenin formulation to be effective in skin cancer prevention.

Caltagirone *et al* (109) 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 a B16-BL6 murine melanoma metastasis model, an effect that

was postulated to be due to the impairment of endothelial interactions in malignant cells.

Apigenin and thyroid cancer. Thyroid cancer is an uncommon tumor, accounting for ~1% of all new malignancies (3). Yin *et al* (47) 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* (56) demonstrated that the inhibitory effect of apigenin on ARO cell proliferation was associated with inhibition of both EGFR tyrosine auto-phosphorylation and phosphorylation of its downstream effector MAPK. Subsequently, Schroder-van der Elst *et al* (110) 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.

Apigenin and endometrial cancer. Endometrial cancer is most common in peri-menopausal and post-menopausal women. Endometrial carcinoma may be estrogen-related or non-estrogen associated (3). Some recent epidemiological findings in endometrial cancer suggest new avenues for possible chemoprevention of these cancers. O'Toole *et al* (111) identified genomic aberrations in endometrial cancer cells which were treated with 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 ß-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 post-menopausal women.

Apigenin and gastric cancer. Gastric cancer remains one of the most prevalent types of cancer worldwide (2,3). There is convincing evidence linking *Helicobacter pylori* infection to gastric cancer, suggesting that eradication of *H. pylori* infection is another promising potential preventive measure. It is postulated that micronutrients with an anti-oxidant role in the diet may exert influences on different phases of the carcinogenic process, interrupting the progression of precancerous lesions towards invasive cancer. Wu *et al* (112) 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.

Apigenin and hepatocellular cancer. Hepatocellular carcinoma is one of the most frequent cancers worldwide and is one of the leading causes of cancer-related deaths (3). Hepatitis B virus infection and exposure to aflatoxins in the diet act synergistically to amplify hepatocellular cancer risk. Plant flavonoids may be beneficial in preventing this disease. Initial studies on plant flavonoids have shown that structural analogs designated the flavonoid 7-hydroxyl group are potent inhibitors of the human P-form phenolsulfotransferase, which is of major importance in the metabolism of many drugs, resulting in either inactivation and rapid renal elimination of the highly ionized sulfuric acid ester conjugates formed or, in some instances, formation of conjugates with increased pharmacological activity (113). Yee *et al* (114) investigated the inhibitory effects of luteolin and apigenin on human hepatocellular carcinoma HepG2 cells. The 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. In addition, Jeyabal *et al* (115) have shown the *in vivo* protective effects of apigenin on N-nitroso-diethylamine-induced and phenobarbitol promoted hepato-carcinogenesis in Wistar albino rats. Apigenin treatment at 25 mg/kg body weight for two weeks to these rats provided protection against the oxidative stress and DNA damage caused by the carcinogen.

Apigenin and neuroblastoma. Neuroblastoma is a pediatric tumor accounting for ~15% of childhood cancer deaths. It portends a poor prognosis in children >1 year of age (2,3). Torkin *et al* (116) 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 of 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.

Apigenin and adrenocortical cancer. Adrenocortical carcinoma is a rare tumor with a dismal prognosis (2,3). Laboratory studies of adrenocortical cancers have revealed aberrations in a wide variety of signaling pathways and enzymes including aromatose, a key enzyme in the synthesis of estrogen from androgens, are altered in these neoplasms. Sanderson *et al* (117) investigated the effect 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* (118) 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 3ß-HSD II and P450c21 activity.

6. Major limitations of apigenin

Apigenin in its pure form is unstable and is not very soluble in water or organic solvents. These properties restrict the use of apigenin in its pure forms. In its natural form, apigenin is present in foods mostly as glycoside conjugates and various

acylated derivatives, which are more water soluble than the parent compound (119, 120 and references therein). The moiety with which apigenin is conjugated is an important determinant of its absorption and bioavailability, since these attributes may require enzymatic cleavage by mammalian or microbial glucosidases (120). Studies have shown that human absorption of quercetin glycoside from onions is far better than that of pure quercetin (121,122). Consequently it seems likely that apigenin in natural form bound to ß-glycosides may be provide its best bioavailability.

Upon reaching the gut, apigenin is extensively metabolized via the dual recycling scheme involving both enteric and enterohepatic recycling (123,124). Apigenin has been shown to be rapidly metabolized via UDP glucuronosyltransferase UGT1A1 as glucoroside and sulfate conjugates which are more readily transported in the blood and excreted in bile or urine (125). Gradolatto *et al* (126) have shown that oral intake after a single dose of radio-labeled apigenin in rats resulted in 51% recovery of radioactivity in urine, 12% in feces, 1.2% in blood, 0.4% in the kidneys, 9.4% in the intestine, 1.2% in liver and 24.8% in the rest of the body within 10 days. The radioactivity appeared in blood 24 h after oral apigenin intake. The kinetics of apigenin in blood exhibited a relatively high elimination half-time of 91.8 h compared to other dietary flavonoids. These results suggest that although the bioavailability of apigenin is limited, the slow pharmacokinetics may lead to possible accumulation of this flavonoid in the tissues to effectively impart its chemopreventive effects.

7. Conclusions

There is considerable evidence that plant flavonoids may provide important health benefits. The development of diet-derived chemopreventive strategies requires a thoughtful and structured approach. The most rationale approach for agent development is to test new agents on specific molecular and cellular targets in an appropriate animal model to determine the efficacy and bioavailability of the agents before initiation of clinical trials. Much information can be garnered from epidemiological studies, which can provide valuable suggestions for the development of chemopreventive agents. However, it is critically important to confirm the resultant hypothesis with experimental data, in cell culture and appropriate animal models before initiation of clinical trials. Apigenin is a common non-mutagenic plant flavonoid abundantly present in fruits and vegetables that has shown remarkable promise as a potent chemopreventive agent. Many mechanisms of action have been identified for apigenin-mediated cancer prevention and therapy, including estrogenic/anti-estrogenic activity, anti-proliferative activity, induction of cell-cycle arrest and apoptosis, prevention of oxidation, induction of detoxification enzymes, regulation of the host immune system, and changes in cellular signaling. Recent progress has been made in testing the efficacy of apigenin in pre-clinical models of cancer. Continued efforts are needed, focusing on additional pre-clinical studies of various animal models of cancer that closely simulate human cancers, which may be subsequently validated in clinical trials. A large body of accumulated evidence suggests that apigenin possesses enormous potential for development as a promising cancer chemopreventive agent in the near future.

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