



# HHS Public Access

## Author manuscript

*Curr Pharmacol Rep.* Author manuscript; available in PMC 2018 December 01.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Published in final edited form as:

*Curr Pharmacol Rep.* 2017 December ; 3(6): 423–446. doi:10.1007/s40495-017-0113-2.

## Plant flavone apigenin: An emerging anticancer agent

Eswar Shankar<sup>a,b</sup>, Aditi Goel<sup>c</sup>, Karishma Gupta<sup>a,b</sup>, and Sanjay Gupta<sup>\*,a,b,d,e,f</sup>

<sup>a</sup>Department of Urology, The James and Eileen Dicke Laboratory, Case Western Reserve University, Cleveland, OH 44106, USA

<sup>b</sup>Department of Urology, The Urology Institute, University Hospitals Cleveland Medical Center, Cleveland, OH 44106, USA

<sup>c</sup>Department of Biology, School of Undergraduate Studies, Case Western Reserve University, Cleveland, OH 44106, USA

<sup>d</sup>Department of Nutrition, Case Western Reserve University, Cleveland, OH 44106, USA

<sup>e</sup>Division of General Medical Sciences, Case Comprehensive Cancer Center, Cleveland, OH 44106, USA

<sup>f</sup>Department of Urology, Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, OH 44106, USA

## Abstract

Research in cancer chemoprevention provides convincing evidence that increased intake of vegetables and fruits may reduce the risk of several human malignancies. Phytochemicals present therein provide beneficial anti-inflammatory and antioxidant properties that serve to improve the cellular microenvironment. Compounds known as flavonoids categorized anthocyanidins, flavonols, flavanones, flavonols, flavones, and isoflavones have shown considerable promise as chemopreventive agents. Apigenin (4', 5, 7-trihydroxyflavone), a major plant flavone, possessing antioxidant, anti-inflammatory, and anticancer properties affecting several molecular and cellular targets used to treat various human diseases. Epidemiologic and case-control studies have suggested apigenin reduces the risk of certain cancers. Studies demonstrate that apigenin retain potent therapeutic properties alone and/or increases the efficacy of several chemotherapeutic drugs in combination on a variety of human cancers. Apigenin's anticancer effects could also be due to its differential effects in causing minimal toxicity to normal cells with delayed plasma clearance and slow decomposition in liver increasing the systemic bioavailability in pharmacokinetic studies. Here we discuss the anticancer role of apigenin highlighting its potential activity as a chemopreventive and therapeutic agent. We also highlight the current caveats that preclude apigenin for its use in the human trials.

\*Correspondence to: Sanjay Gupta, Ph.D. sanjay.gupta@case.edu, Department of Urology, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106, Phone: (216) 368-6162, Fax: (216) 368-0213.

### Conflict of Interest

The authors have no competing interest.

## Keywords

cancer chemoprevention; dietary agents; plant flavonoids; health effects; nanoparticle; polyphenols

### 1.0 Introduction

Cancer is the nemesis in today's global vicissitudes despite progress, increase in life expectancy and rapidly increasing population. There will be an estimated 1,688,780 new cancer cases diagnosed and 600,920 cancer-related deaths occur in the United States in the year 2017 [1]. Global projections of new cancer cases are expected to increase from 16.8 million in 2017 to 21.7 million by 2030 due to increased prevalence and distribution of risk factors [2]. Despite recent advancements under development in the area of chemotherapeutic agents and other modalities to treat cancer, the side effects and chemoresistance obstruct recovery and survival. These shortcomings have led to changes in strategy that aim to reduce the incidence and burden of cancer through the development of agents to prevent, reverse, or delay the carcinogenic process [3]. The goal of cancer chemoprevention is to identify and design new compounds and determine their molecular targets [4]. Epidemiological research helps recognize natural dietary substances associated with reduced cancer mortality and incidence and clarifies variations in dietary patterns and lifestyle practices across a wide geographical range.

Diet is a major lifestyle factor that affects health; poor diets lead to chronic diseases and cancer. The World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR), recommend increased consumption of vegetables, fruits, and grains to help decrease the possibility of cancer development and progression [5]. Foods are not only a source of macronutrients (proteins, fats, and carbohydrates) and micronutrients (vitamins and minerals); they also contain various amounts of non-nutritional molecules called phytochemicals, which have beneficial health effects [6]. The most extensively studied groups of phytochemicals include the polyphenols that are present in plants, grains, fruits, and vegetables. Flavonoids are natural polyphenols produced by plants that can be subcategorized into anthocyanidins, flavanols, flavanones, flavonols, flavones, and isoflavones [7]. The beneficial properties attributed to plant flavonoids include antioxidant, anti-inflammatory, and anti-carcinogenic effects.

Several epidemiological studies have highlighted the benefits of a healthy diet and the number of phytochemicals consumed around the globe including a population-based analysis of the average intake of five flavonoids viz. quercetin, kaempferol, myricetin, luteolin, and apigenin among 7 countries [8–13]. These contents varies from 6 mg/day in Finland to 64 mg/day in Japan with intermediary intake in the United States (13 mg/day), Italy (27 mg/day), and the Netherlands (33 mg/day) [8]. Aherne and O'Brien (2002) assessed the number of flavonoids consumed within Europe and found lower rates of consumption among the Hungarian population as opposed to the Dutch (23 mg/day), Danish (28 mg/day), and Finnish (55 mg/day) populations [9]. A comparative study conducted on the flavonoid consumption between continents has revealed the lowest intake (1–9 mg/day) in the South African diet while the highest intake was from the Scandinavian diet (75–81

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

mg/day) [10]. The dietary sources for flavones and flavonols vary between various countries; for example, sources include teas in Japan (95%) and the Netherlands (65%) and beer and red wine in Italy (46%). Finland consumes 100% of flavonols and flavones from vegetables, whereas 80% of the dietary source in the United States is from fruits and vegetables. In the Australian sub-continent, tea provides a primary source of dietary flavonoids, especially flavan-3-ols (75%), with rates of consumption as high as 351mg/person/day [11]. A comparison between phenol-rich and phenol-depleted diets showed that a diet rich in flavonoids decreases oxidative stress markers in blood including antioxidant vitamins in plasma, erythrocyte superoxide dismutase (SOD), and diminution in lymphocyte DNA damage, endorsing the benefits of flavonoids and their effects on human health [12]. Apigenin is a major component of the Mediterranean diet associated with lower rates of numerous human diseases, including cancer [13]. In addition to other plant flavones, apigenin is an important compound found in fruit- and vegetable-rich diets. In this review, we discuss the anticancer properties of apigenin and the required efforts to strengthen its role as a chemopreventive and chemotherapeutic agent.

## 2.0 Sources of apigenin

The name apigenin is derived from the *Apium* genus in the Apiaceae (the celery, carrot, or parsley family, also known as Umbelliferae). Apigenin naturally occurs as a 4', 5, 7-trihydroxyflavone. Structurally, the compound possesses hydroxyl groups at positions C-5 and C-7 of the A-ring and C-4' of the B-ring. Apigenin is a yellow crystalline powder that is insoluble in water and soluble in dimethyl sulfoxide and hot ethanol. It has the molecular formula  $C_{15}H_{10}O_5$ , and its molecular weight is 270.24 Da. Biapigenin, an apigenin dimer, isolated from the buds and flowers of *Hypericum perforatum* exhibits neuroprotective effects. Naturally, apigenin exists as apigenin-7-O-glucoside and various acylated derivatives. Apigenin is found as a single ingredient in chamomile tea, obtained from the dried flowers of *Matricaria chamomilla*, an annual herb native to Western Asia and Europe; naturalized in Australia, Britain, and the United States. Drinks prepared from chamomile contains 0.8% to 1.2% apigenin and essential oils possessing aromatic, flavoring, and coloring properties. Apigenin is also a component of red wine and beer. Apigenin is abundant in a variety of natural sources, including fruits and vegetables. The best sources of apigenin are parsley, chamomile, celery, vine-spinach, artichokes, and oregano, and the richest sources are in the dried forms [14, 15]. Dried parsley has been reported to have the maximum quantity of apigenin, at 45,035 µg/g. Additional sources of apigenin are dried flower of chamomile, containing 3,000 to 5,000 µg/g; celery seeds, containing 786.5 µg/g; and vine spinach and Chinese celery, containing 622 µg/g and 240.2 µg/g [16].

## 3.0 Apigenin in human health and disease

Extensive studies performed in patients and healthy individuals have shown apigenin to possess anticancer properties. In the past, apigenin was documented as a chemopreventive agent with significant efforts in studying its efficacy in preventing cancer progression. A study conducted by Nielsen et al. [17], apigenin was systematically absorbed in subjects consuming parley-rich diets; subsequently, higher levels of erythrocyte glutathione reductase and superoxide dismutase was observed. However, erythrocyte catalase and glutathione

Author Manuscript  
Author Manuscript  
Author Manuscript  
Author Manuscript

peroxidase levels remained unchanged. Flavonoids have been shown to induce reductions in the plasma levels of low-density lipoproteins, inhibit platelet aggregation, and reduce cell proliferation [18–21]. A cross-sectional study in Japanese women demonstrate an inverse relationship between total flavonoid intake and total cholesterol and low-density lipoprotein concentration in the plasma [22]. Janssen et al. [23] evaluated the impact of daily dietary consumption of apigenin ( $84 \pm 6$  mg from parsley) and quercetin ( $377 \pm 10$  mmol from onions) on platelet aggregation and other hematologic measures in a seven-day study of eighteen healthy men and women. No significant alterations in collagen- or ADP-induced platelet numbers, factor VII, plasminogen and plasminogen activator inhibitor-1 activity, or fibrinogen concentrations were observed. These studies highlight the inherent properties of apigenin and help categorize it as a beneficial compound with health-promoting and disease-preventing properties.

Apigenin containing dietary source have found profound application in treating several ailments. The presence of apigenin in passion flower makes it useful to treat asthma, neuralgia, shingles, intransigent insomnia and Parkinson's disease [15]. Similarly high apigenin content in chamomile makes it a good antiphlogistic, antispasmodic, and antibacterial agent. For centuries chamomile tea has been used as a folk medicine remedy. Consumption of 3–4 cups of chamomile tea per day has been used to relieve indigestion or calming gastritis and as skincare product reducing cutaneous inflammation and dermatological problems [24]. Studies providing objective evidence of the beneficial properties of apigenin on human health are quite limited; however, some epidemiologic and clinical studies substantiate that apigenin is useful in counteracting coronary artery disease, gastrointestinal irritation, dermatological disorders, in alleviating labor pain, and in providing antidepressant, calming and relaxing effects. Also, several studies reveal that the anticancer properties of apigenin are mediated through responses to oxidative stress DNA damage, inhibition of angiogenesis and inflammation, suppression of cell growth, and induction of apoptosis and autophagy [16]. At the molecular level, apigenin remains a well-known inhibitor of several protein-tyrosine and serine- kinases including MAPK, PI3K-Akt, Src kinase, casein kinase 2, cell cycle regulated kinases, JAK kinases affecting various signaling pathways including IGF-growth axis, NF- $\kappa$ B, Stat-3, p53, etc. Other putative targets of apigenin include heat shock proteins [15, 25], telomerase [26], fatty acid synthase [27], matrix metalloproteinases [28], and aryl hydrocarbon receptor HER2/neu [29], all of which have relevance to the initiation of several human malignancies. A list of potential targets of apigenin in different human cancers is shown in Table 1.

#### 4.0 Epidemiological studies on apigenin in cancer

Epidemiologic and case-control studies have shown an inverse relationship between gastric, colorectal, breast, ovarian, and endometrial cancers and non-Hodgkin's lymphoma and intake of total flavonoids, flavonoid subgroups, or individual flavonoids [15]. The Zutphen study followed a cohort of 878 for 25 years following the intake of 5 flavonoids: apigenin, kaempferol, luteolin, myricetin, and quercetin and found reduced incidence of mortality from various cancers. These findings were encouraging in demonstrating that high flavonoids source from vegetables and fruits intake reduced the risk of cancer [30]. A large cohort of 9,959 Finnish men demonstrate stronger support for the protective effect of

flavonoids against lung cancer and other malignancies followed from 1967 to 1991 [31]. Another Zutphen study examined the relationship between five common dietary flavonoid intake on ovarian cancer. The study established that dietary supplementation of these flavonoids decreased ovarian cancer risk [32]. A multi-centric case-control study conducted between 1992 and 1999 in Italy further confirmed the inverse relationship between the consumption of common flavonoids and ovarian cancer risk by utilizing data from 1,031 cases with histologically confirmed epithelial ovarian cancer and 2,411 control cases [33]. Another case-control study from Italy conducted between 1991 and 1994 exhibited an inverse association between flavone intake and risk of breast cancer on 2,569 women with histologically confirmed breast cancer and 2,588 controls cases [34]. A study analyzing a group of five flavonoids (quercetin, kaempferol, myricetin, apigenin, and luteolin) in combined food samples, performed on 738 elderly patients, a median age of 65–84 years, and without a history of cancer. The subjects followed for five years and the study concluded that a high intake of flavonoids from fruits and vegetables was associated with a reduced risk of cancer [35]. To establish a dietary baseline and dietary history, the study, including 10,054 men, showed that males with higher myricetin intakes have a lower prostate cancer risk [36]. Conclusions drawn from other studies also support similar findings where flavonoid consumption was associated with decreased risk of sporadic colorectal cancer including reduction in disease recurrence [37]. Gates et al. [38] studied the association between apigenin, kaempferol, luteolin, myricetin and quercetin, and ovarian cancer risk in 1,141 patients with ovarian cancer and 1,183 frequency-matched control subjects. The study showed no clear relationship between the rates of ovarian cancer and the total ingestion of the five flavonoid compounds. An association between flavonoid intake and ovarian cancer risk among women with and without a history of tubal ligation revealed a stronger association with apigenin consumption and ovarian cancer without a history of tubal ligation [39]. Also, for women with no history of tubal ligation, apigenin consumption (quintiles four and five) showed a significant decrease in ovarian cancer risk [39]. Thus, analyses of individual flavonoids revealed that only apigenin intake was associated with reduced cancer risk (with significance), suggesting its strong role as an anticancer agent.

## 5.0 Effect of apigenin in various human cancers

### 5.1 Breast cancer

Apigenin has been shown to possess anti-proliferative effects on breast cancer cell lines exhibiting varying levels of HER2/neu [40, 41]. HER2/neu belongs to the family of human epidermal growth factor receptor (HER/EGFR/ERBB). Amplification or overexpression of HER2/neu plays an important role in the development and progression of breast cancer with aggressive behavior [40]. The anti-proliferative effects of apigenin was significantly higher in breast cancer cells over-expressing HER2/neu but was much less efficacious in restricting the growth of cell lines expressing HER2/neu at basal levels [40]. Apigenin induces apoptosis in a dose- and time-dependent fashion in breast cancer cells over-expressing HER2/neu [41]. Phosphatidylinositol 3-kinase (PI3K) and Akt/PKB play a significant role in inhibition of apoptosis in cells over-expressing HER2/neu. Apigenin interferes in the cell survival pathway by inhibiting Akt function by directly blocking PI3K activity [42]. Moreover, apigenin administration led to the depletion of HER2/neu protein in vivo,

Author Manuscript  
Author Manuscript  
Author Manuscript  
Author Manuscript

consequently inhibiting its auto phosphorylation and transphosphorylation. Additional studies demonstrate apigenin-mediated apoptosis in HER2/neu-expressing breast cancer cells resulted in decrease expression of HER2/neu protein and, subsequently, suppressing the signaling cascade of the HER2/HER3-PI3K/Akt cell survival pathway. Apigenin exposure to breast cancer cells resulted in apoptosis through the release of cytochrome c with the rapid increase of DNA fragmentation factor (GADD)-45. Apigenin treatment in breast cancer cells also results in decreased expression of cyclin D1, D3, and cdk4 and increased quantities of p27 protein [41]. Furthermore, groups have also uncovered the role of apigenin as an agent that induces autophagy during apoptosis [16]. Apigenin-induced autophagy in aggressive breast cancer cells simultaneously caused apoptosis suggesting the involvement of processes inhibiting proliferation and inducing cell death [43]. Low-dose apigenin has the potential to slow or prevent breast cancer progression [44]. Apigenin exerts its toxic effects on breast cancer cells while causing minimal damage to healthy cells, thereby indicating its selectivity in inhibiting tumor possession [45]. In triple-negative breast cancer cells, apigenin induces apoptosis by inhibiting the PI3K/Akt pathway thereby increasing FOXO3a expression that may attenuate cell migration and invasion-inducing apoptosis [46].

Protein kinase C (PKC)-activating phorbol ester (PMA) and peptide hormones prevent apoptosis through the stimulation of PI3K and MAPK pathways [42]. MCF-7 breast cancer cells treated with PMA resulted in the suppression of TNF $\alpha$ -induced apoptosis [42]. Other effects of apigenin on cell survival pathways relates to its suppression of PMA-induced AP-1 activity, thereby supporting its role as an anti-tumor agent. Apigenin treatment inhibited tumor cell invasion in estrogen-insensitive MDA-MB231 breast cancer cell line in a dose-dependent manner [47]. In breast carcinoma cells, apigenin prevents growth and induces G2/M arrest through its downstream effects on ERK MAP kinase and cyclin-CDK regulators [48]. Apigenin exerts inhibitory effects on breast cancer MCF-7 cells, expressing tumor suppressors' wild-type p53 and retinoblastoma (Rb) and expressing mutant p53 and no Rb in MDA-MB-468 cells. Apigenin caused G2/M phase cell cycle arrest which correlated with a marked decrease in CDK1 and cyclin B1 expression and reduction in CDK1 kinase activity. Apigenin exposure resulted in reduced levels of cyclin D1 and A, CDK4, and the inhibition of Rb-phosphorylation; however, cyclin E, CDK2, and CDK6 levels were unaffected. Moreover, apigenin treatment resulted in activation of ERK MAP kinase phosphorylation in MDA-MB-468 cells [48]. Wang & Kurzer [49, 50] analyzed the relationship between apigenin and DNA synthesis on estrogen-dependent MCF-7 cells exposed to estradiol (E2), epidermal growth factor, insulin, or tamoxifen and observed that apigenin inhibits E2-induced DNA synthesis in a variable and concentration-dependent manner. Collins-Burow et al. [51] assessed estrogenic and anti-estrogenic effects on flavonoids in the MCF-7 cells and demonstrated that apigenin exhibits anti-estrogenic activity facilitated through ER binding-dependent and independent- mechanisms [51]. The anti-estrogenic effect demonstrated by apigenin is important in the regulation of breast cancer cell proliferation.

Zhang et al. [52] studied the effects of several plant flavonoids used alone or in combinations of breast cancer resistance protein (BCRP) and found that the combination at equimolar concentrations was highly effective in inhibiting BCRP. Additionally, Stroheker et al. [53] examined the endocrine disruption capabilities of bisphenol derivatives on apigenin

Author Manuscript  
Author Manuscript  
Author Manuscript  
Author Manuscript

and other plant flavonoids, and the impact on ER(+) MCF-7 and AR(+) and GR(+) MDA-MB453 human breast carcinoma cells. The results suggest that these compounds exert a biphasic effect, acting as partial androgen receptor (AR) agonists at low levels and as GR agonists at high concentrations [53]. Apigenin and genistein have demonstrated anti-proliferative activity on MCF-7 and T47D cells (ER $\alpha$ -positive), but not the ER $\alpha$ -negative MDA-MB-435 cell line [54]. Therefore, the estrogenicity of the phytochemicals contributes to the stimulation of cellular proliferation or reduction in aromatase activity, suggesting that these flavonoids must be used with caution [55]. In HER2-overexpressing breast cancer cells, apigenin-exerted anti-proliferative activity by inhibiting STAT3 signaling, suggesting its potential as a preventive agent [56]. In addition, apigenin also down-regulated STAT3 target genes MMP-2, MMP-9, VEGF and Twist1, which are involved in cell migration and invasion of breast cancer cells [56–58]. Combination treatment of apigenin with chrysin in triple negative breast cancer cells caused marked decrease in cell motility as a consequence of the inhibition of matrix metalloproteinases (MMP)-2 and MMP-9, leading to apoptosis [59].

Brusselmans et al. [60] demonstrated that plant flavonoids induce apoptosis in prostate and breast carcinoma cells due to their ability to inhibit fatty acid synthase, an enzyme that catalyzes long chain fatty acid synthesis and is over-expressed in several cancer cells. This study provided indication that six plant flavonoids, including apigenin, inhibit cancer cell growth and survival due to their effects on fatty acid synthesis [60]. Identification of new target protein interactions through phage display, coupled with second generation sequencing reported in breast cancer cells, discovered three main pathways affected by apigenin, including GTPase activation, membrane transport, and mRNA metabolism/alternative splicing. Apigenin binds to the glycine-rich domain of hnRNPA2, thereby inhibiting its dimerization, a mechanism that is essential for RNA binding. hnRNPA2 is involved in mRNA metabolism and splicing associated with various malignancies. The interaction between apigenin and hnRNPA2 explains that apigenin exerts anti-carcinogenic activity by increasing apoptosis together with the use of chemotherapeutic drugs [61]. Also, studies suggest that combining apigenin with other flavones such as genistein inhibited tumor growth by complexing with Cu (II), thereby significantly enhancing the antitumor properties [62]. In triple-negative breast cancer cells, apigenin results in epigenetic changes by inducing histone H3 acetylation, thereby decreasing cyclin A and cyclin B and increasing p21/Waf1/Cip1 levels [63]. A sub-cytotoxic concentration of apigenin inhibits IFN- $\gamma$ -induced PD-L1 expression in MDA-MB-468 and 4T1 breast cancer cells, associated with reduced phosphorylation of STAT1, transiently at Tyr701 and persistently at Ser727. However, apigenin exposure did not affect constitutive PD-L1 expression in triple-negative MDA-MB-231 BC cells but was more susceptible to T-cell-mediated antitumor immune responses [64].

## 5.2 Colon cancer

Wang et al. [65] were the first to report the effect of apigenin on cell growth in various human colon cancer cell lines [65]. Apigenin exposure to colon cancer cells demonstrated anti-proliferative effects, followed by reversible G2/M phase arrest in the cell cycle, and led to decreased levels of cyclin B1 and p34 (cdc2) protein levels. Wang et al. [66] further

analyzed the effects of seven analogs of apigenin on cell viability and cell cycle in human colon cancer cell lines. The results of this study suggest that five of the seven apigenin analogs caused induction of cell-cycle arrest and their combination at small doses synergistically protect against colorectal cancer by collectively inhibiting cell-cycle progression. Apigenin has been shown to stabilize the tumor suppressor p53 in normal cells. In fact, cancer preventive effects of apigenin may be due to its modifying effects on tumor suppressor p53 [67]. Apigenin exposure to two p53-mutant HT-29 and MG63 cancer cell lines leads to growth inhibition and G2/M cell cycle arrest, accompanied by increase in p21/Waf1 expression in a dose- and time-dependent manner [68]. The result indicate the possibility of p53-independent pathway responsible for apigenin-mediated growth inhibition through induction in p21/Waf1 expression in p53-mutant cells.

Farah et al. [69] demonstrated apigenin- and 5,6-dichloro-ribifuranosylbenzimidazole-(DRB)-induced sensitization of HCT-116 and HT-29 colon cancer cells to TNF $\alpha$ -mediated apoptosis. Apigenin and DRB inhibited casein kinase (CK)-2 expression in these cells resulting in a synergistic diminution in cell survival after exposure to TNF $\alpha$ . [69]. VanDross et al. [70] highlighted that modulation of the MAPK cascade, may in part, be responsible for the chemopreventive activity of apigenin. Induction of ERK and p38 kinase phosphorylation was noted after apigenin exposure, which was dose-dependent; with little effect on the phosphorylation of c-Jun amino-terminal kinase (JNK). In the azoxymethane (AOM)-induced CF-1 mice, apigenin treatment resulted in the inhibition of formation of aberrant crypt foci and ornithine decarboxylase (ODC), a rate-limiting enzyme of the polyamine synthesis pathway, along with mutant Adenomatous Polyposis Coli (APC) gene [71].

Svehlikova et al. [72] demonstrated the relationship between apigenin and sulforaphane in the induction of UDP-glucuronosyltransferase 1-1 (UGT1A1) and glutathione S-transferase A1 (GSTA1) and the phase II detoxifying enzymes in human colorectal adenocarcinoma CaCo-2 cells. Apigenin induces UGT1A1 transcription but not GSTA1, whereas sulforaphane-induced both UGT1A1 and GSTA1 transcription in a time- and dose-dependent fashion. Therefore, apigenin and sulforaphane synergistically induce UGT1A1 mRNA expression but not GSTA1. The results suggest that diverse signal transduction pathways might regulate the expression of detoxification enzymes [73]. Al-Fayez et al. [74] determined that apigenin exhibits more potency than quercetin or tricin in downregulating inducible COX-2 levels in HCEC cells. Apigenin also contributes to TRAIL-mediated cell death by activating the DR5 death receptor in colon cancer cells [75]. TRAIL, a member of the TNF family, triggers apoptosis in cancer cells via interacting with death receptor 4 (DR4) and death receptor 5 (DR5) leading to the formation of the death-inducing signaling complex (DISC) with a subsequent binding of caspase-8. Recruitment of caspase-8 to the DISC activates its proteolytic properties initiating terminal caspase-3 activation, promoting the cleavage of death substrates and thereby inducing apoptosis. Further investigation shown that apigenin inhibits ODC activity and the formation of aberrant crypt foci (ACF) but fails to inhibit adenoma formation in the Min mouse, showing promise as a chemopreventive agent [74]. Studies demonstrate that apigenin mediates apoptosis and G2M cell cycle arrest in colon cancer cells involving APC tumor suppressor [76]. Feeding rats with azoxymethane, an inducer of colon cancer, with a diet of 97% pure apigenin isolated from Citrus aurantium L. protected the animals from colon cancer, reducing the incidence of ACF

Author Manuscript  
Author Manuscript  
Author Manuscript  
Author Manuscript

significantly [77]. One of the mechanisms of action of apigenin in colon cancer cells to induce apoptosis suggests the involvement of PKC $\delta$  that activates p21 and growth/differentiation factor 15 (NAG-1), followed by apoptosis and inhibition cell proliferation in a p53-independent manner [78]. The same study also exhibited that apigenin could activate p53 by phosphorylating it at Ser-37 and Ser-15 via the ataxia telangiectasia mutated (ATM) pathway to potentiate apoptosis. Turketekin et al. [79] reported that apigenin-induced cell cycle arrest and apoptosis in p53 mutant colon cancer cells. Similar studies also show that apigenin possesses autophagy-inducing effects in HCT116 colon cancer cells, and combined treatment with inhibitor 3-methyladenine (3-MA), an autophagy inhibitor, potentiates apoptosis [80]. CD26, a multifunctional cell-surface protein, in humans, is encoded by the DPPIV gene. The substrates of CD26/DPPIV are proline (or alanine)-containing peptides that binds with enzyme adenosine deaminase and appears to work as tumor suppressor inhibiting pathways involved in tumor metastasis. CD26/DPPIV is down-regulated in various cancers including colorectal carcinoma. Apigenin substantially upregulates CD26/DPPIV on human colorectal cancer HT-29 and HRT-18 cells to inhibit metastasis [81]. Synergistic interaction between apigenin and ABT-263, a BH3 mimetic inhibitor of the Bcl-2 family, was more potent than apigenin or ABT-263 alone in inhibiting tumor growth in xenograft tumors of colon cancer cells [82].

Wang et al. [83] demonstrated that apigenin at 20 mg/kg caused the antitumor activity of human colorectal carcinoma (CRC) SW480 xenografts implanted in nude mice. In an orthotopic CRC model, apigenin at a dose of 10 mg/kg inhibited tumor growth and metastasis to the liver and lungs. Apigenin treatment up-regulated transgelin, suppressed MMP-9 expression by attenuating phosphorylation of Akt at Ser473 and in particular Thr308 to prevent cell proliferation and migration [84].

### 5.3 Gastric cancer

Wu et al. [85] examined the effect of apigenin on gastric cancer by utilizing human gastric carcinoma SGC-7901 cells. Apigenin treatment resulted in dose-dependent cell growth inhibition, clone formation via induction of apoptosis [85]. In *Helicobacter pylori*-infected gastric adenocarcinoma cells, apigenin treatment effectively inhibited NF- $\kappa$ B activation, scavenged free radicals, and stimulated MUC-2 secretion. Its effect on the NF- $\kappa$ B pathway impacted relevant inflammatory factors such as cyclooxygenase (COX)-2, intercellular adhesion molecule (ICAM)-1, reactive oxygen species (ROS), interleukin (IL)-6, and IL-8. Apigenin exhibits higher potential for the prevention of *H. pylori*-induced gastric epithelial inflammation [86]. Apigenin remarkably inhibited *H. pylori*-induced atrophic gastritis and gastric cancer progression in eight-week-old Mongolian gerbils. The study also reports apigenin to possess potent anti-gastric cancer activity [86]. Apigenin treatment of HGC-27 and SGC-7901 gastric cancer cells resulted in the inhibition of proliferation followed by mitochondrial depolarization resulting in apoptosis [87].

### 5.4 Liver Cancer

The 7-hydroxyl group present in plant flavonoids is a putative inhibitor of the human P-form phenol sulfotransferase, which plays an important role in drugs metabolism. A major function of the P-form phenol sulfotransferase is to inactivate and rapid eliminate sulfuric

acid ester conjugates or facilitate the formation of conjugates possessing higher pharmacological activity [88]. Studies demonstrate that addition of a prenyl group to apigenin increases the hydrophobicity, thereby enhancing its pharmacological and biochemical properties. C8-prenylation of apigenin augments the cytotoxic effects and induces apoptosis in H4IE hepatoma cells without modifying anti-oxidative properties [89]. Yee et al. [90] demonstrated that treatment of human hepatocellular carcinoma HepG2 cells with apigenin and luteolin resulted in inhibition of cell growth, cell cycle arrest, and downregulation of CDK4 expression, along with induction of p53 and p21 [90]. In Wistar albino rats, Jeyabal et al. [91] found apigenin to exert protective effects against N-nitroso-diethylamine-induced and phenobarbital promoted hepatocarcinogenesis. Two weeks of 25 mg/kg apigenin supplementation led to protection against the oxidative stress and DNA damage caused by exposure to the carcinogen [91]. Anti-proliferative effects of apigenin in HepG cells revealed the activation of p53 mediated the induction of p21, causing G2M cell cycle arrest [90]. Further studies revealed apigenin-induced apoptosis in hepatoma tumor cells by utilizing ROS generated through the activation of the NADPH oxidase [92]. The inhibitory role of apigenin in hepatoma cell growth is reported to be mediated by alterations in gene expression profiles confirmed through cDNA microarrays [93]. Apigenin significantly sensitized doxorubicin-resistant BEL-7402 (BEL-7402/ADM) cells to doxorubicin (ADM) and increased the intracellular concentration of ADM by reducing Nrf2-mediated genes through the downregulation of the PI3K/Akt pathway. Apigenin and ADM co-treatment resulted in reduced proliferation, tumor growth inhibition, and apoptosis induction, compared to ADM treatment alone. These results underline the role of apigenin as an adjuvant to overcome chemoresistance [95]. In hepatocellular carcinoma tumors, apigenin enhanced the cytotoxicity of fluorouracil (5-FU), overcoming resistance by inhibiting ROS-mediated drug resistance, leading to mitochondrial depolarization and apoptosis [96]. Cell-based assays demonstrated apigenin as a potent inhibitor of human 26S proteasome. Apigenin treatment led to increase in the ubiquitination of endogenous proteins and inhibition of chymotrypsin-like, trypsin-like, and caspase-like activities of the human 26S proteasome. Apigenin also enhanced the phosphorylation of signal transducer and activator of transcription proteins (STAT1 and STAT2) and promoted the endogenous IFN $\alpha$ -regulated gene expression preventing the ubiquitination of type I interferon receptor 1 (IFNAR1) [97].

### 5.5 Lung cancer

Liu et al. [98] determined whether apigenin had a dose-dependent inverse relationship between proliferation of A549 lung cancer cells and transcriptional activation of the vascular endothelial growth factor (VEGF). VEGF is the most important growth factor that provides the tissue surrounding the tumor with nutrients for vascular permeability. Apigenin acts on the HIF-1 binding site, which decreases HIF-1 $\alpha$ , but not the HIF-1 $\beta$  subunit, thereby inhibiting VEGF. Apigenin further acts to inhibit AKT and p70S6K1 activation, factors that play a role in mediating VEGF transcriptional activation. Additionally, apigenin at 15  $\mu$ M treatment in mice carrying A549 lung cancer xenografts reduced the tumor volume by partially inhibiting the HIF-1 $\alpha$ -vascular endothelial growth factor pathway and subsequent suppression of angiogenesis and cell proliferation [98]. Lung cancer SQ-5 cells exposed to apigenin exhibited greater radiosensitivity and apoptosis compared to cells without apigenin

Author Manuscript  
Author Manuscript  
Author Manuscript  
Author Manuscript

exposure [99]. Moreover, apigenin was found to inhibit angiogenesis, as suggested by decreased HIF-1 $\alpha$  and VEGF expression in cancer cells after exposure in nude mice with implanted lung tumors. Another study by Engelmann et al. [100] demonstrated the effects of apigenin treatment in experimental Lewis lung carcinomas (LLC), C-6 gliomas and DHDK 12 colonic cancers *in vivo*. Mice with tumors received an apigenin dose of 50 mg/kg/day in three different galenical formulations over 12 days in 8-h intervals. Although LLC, C-6, and DHDK 12 and endothelial cells demonstrated high sensitivity to apigenin in cell culture with marked growth suppression at concentrations beyond 30  $\mu$ g/ml; however, no *in vivo* response was evident [100]. Mice on B57BL/6N background implanted with B16-BL6 tumors exhibited a decreased quantity of tumor cells adhered to lung vessels after treatment with apigenin and quercetin in a single dose [101]. Apigenin seems to be a promising radiosensitizer for use in human lung carcinomas. Apigenin sensitized SQ-5 spheroids (cell aggregates growing in a three-dimensional structure that simulate growth and microenvironment conditions of *in vivo* tumors) to radiation [99]. In lung cancer cells, apigenin treatment caused dysfunction of mitochondria leading to Bax activation, cytochrome c release, AIF, and Endo G, resulting in caspase-mediated apoptosis [102, 103]. Similar studies by Das et al. [104] reveal that apigenin treatment in lung cancer cells caused DNA interaction, damage, and mitochondrial dysfunction either by direct or indirect action on mitochondrial oxidative phosphorylation system.

Bruno et al. [105] demonstrate that apigenin upregulates leptin receptors to cause apoptosis in lung cancer cells while co-treatment with leptin inhibited cell proliferation. Synergistic administration of curcumin and apigenin may be beneficial for further development as cost-effective anticancer drug combination. Combined treatment with these agents being applied to lung cancer cells induced apoptosis and blocked cell cycle progression at the G2/M phase. Co-administration of apigenin and curcumin, exhibited strong depolymerizing effects on interphase microtubules and inhibited reassembly of cold depolymerized microtubules. This outcome suggests that these agents bind to tubulin at diverse locations [106]. Apigenin exposure NSCLC lung cancer cell resulted in inhibition of proliferation and downregulation of Axl expression, with subsequent alterations in p21 and XIAP expression [107]. Apigenin induces apoptosis and slows cell growth through metabolic and oxidative stress as a consequence of the down-regulation of glucose transporter 1 (GLUT1). Such action leads to a decreased glucose utilization in lung cancer cells. On the contrary, the activation of pentose phosphate pathway-mediated NADPH reversed the effects of apigenin by ectopic GLUT1 overexpression and galactose supplementation. The combined treatment of apigenin with a glutaminase inhibitor, compound 968, sensitized lung cancer cells and caused severe metabolic stress [108]. A small concentration of apigenin synergistically induced cell apoptosis through multiple targets that included caspases and NF- $\kappa$ B pathways in NSCLC cell lines in combination with tumor necrosis factor related apoptosis-inducing ligands (TRAIL). These studies suggest that apigenin possesses substantial therapeutic value for use in conjunction with TRAIL against lung cancer cells [109].

## 5.6 Pancreatic cancer

Pancreatic cancer remains one of the most deadly forms of human cancer with poor prognoses in spite of attempts to resection and adjuvant therapy. Studies with apigenin in

Author Manuscript  
Author Manuscript  
Author Manuscript  
Author Manuscript

combination with cell cycle inhibitor flavopiridol have shown to inhibit pancreatic tumor growth through suppression of cyclin B-associated cdc2 activity and G2/M arrest [110]. Apigenin administered in combination with gemcitabine enhanced anti-tumor efficacy through suppression of Akt and NF- $\kappa$ B activity and apoptosis induction in human pancreatic cancer MiaPaca-2 and AsPC-1 cells and pancreatic tumors from nude mice [111]. In a study conducted by Strouch et al. [112], co-treatment with apigenin and gemcitabine, led to cell cycle arrest, down-regulation of p-Akt, and induction of apoptosis in pancreatic cancer cells. Individually, apigenin regressed pancreatic tumors by inhibiting the key members of the NF- $\kappa$ B pathway [113]. In both hypoxic and normoxic conditions, apigenin inhibited GLUT-1, HIF-1 $\alpha$ , and VEGF at mRNA and protein levels in pancreatic cancer cells. The study suggests that apigenin has a potential to be developed as a future chemopreventive agent [114]. King et al. [115] demonstrated that treatment of pancreatic cells with apigenin enhanced the acetylation of p53 at Lysine382 causing increased nuclear translocation increasing its DNA binding. In this study, six weeks treatment of orthotopically implanted nude mouse model of human pancreatic cancer with apigenin in diet at 0.2% significantly caused antitumor activity. Furthermore, apigenin increased the functions of mutant p53 in pancreatic cell lines [115]. Apigenin inhibited the tobacco-derived carcinogen-mediated cell proliferation and migration involving the  $\beta$ -AR and its downstream signals FAK and ERK activation [116]. Another study suggests apigenin causing inhibition of GSK-3 $\beta$  and NF- $\kappa$ B pathway in pancreatic cancer cells to mediate apoptosis [117]. Restriction of the NF- $\kappa$ B pathway by apigenin has been shown to involve the inhibition of its upstream kinase IKK $\beta$  and overcome TNF $\alpha$ -induced NF- $\kappa$ B activity [113].

Pancreatic cancer cells evade immune destruction through the development of regulatory T cells (Tregs) that inhibit effector T cells through regulation of transcription factor Ikaros. The decrease in Ikaros expression causes a reduction in the CD4+ and CD8+ T cell expression, and an increase in CD4+ CD25+ Tregs in tumor-bearing mice. In pancreatic cancer cells, CK2 regulates Ikaros expression, and apigenin has shown to stabilize Ikaros by downregulating CK2 to central T cell homeostasis [118].

### 5.7 Prostate cancer

Knowles et al. [119] determined the effects of plant flavonoids including apigenin on the androgen-refractory human prostate cancer PC-3 cell line, and observed that apigenin exposure led to complete growth restriction of these cells. Studies have also investigated the effects of flavonoids on the activity and phosphotyrosine content of proline-directed protein kinase FA (PDPK FA), an oncogene utilizing human prostate cancer cells. Low doses treatment of human prostate cancer cells with quercetin, apigenin, and kaempferol for prolonged times resulted in tyrosine dephosphorylation and inactivation of oncogenic PDPK FA [120]. Furthermore, apigenin treatment to various human prostate cancer LNCaP, PC-3, and DU145 cells and in transformed human prostate epithelial PWR-1E cells resulted in decrease proliferation and induction of apoptosis [121]. In the study, the LNCaP and PWR-1E cells were more sensitive to apigenin-induced apoptosis than PC-3 and DU145 cells. Apigenin also causes caspase-dependent apoptosis in prostate cancer cells. Apigenin exposure resulted in increased ROS generation, loss of mitochondrial Bcl-2 expression, increases mitochondrial permeability, causing cytochrome C release, and cleavage of

caspase 3, 7, 8, and 9 with the concomitant cleavage of the inhibitor of apoptosis protein, cIAP-2 [121]. Over-expression of Bcl-2 in LNCaP B10 cells decreases the apoptotic effects of apigenin. Hessenauer et al. [122] determined that apigenin exposure inhibited CK2 activity in both hormone responsive LNCaP cells and hormone refractory PC-3 cells; however, only LNCaP cells exhibited apoptosis. [122].

Gupta et al. [123] evaluated the growth-inhibitory effects of apigenin on normal (NHPE), virally transformed (PZ-HPV-7) and prostate cancer (CA-HPV-10) cells. Apigenin exposure caused similar levels of mild growth inhibition in NHPE and PZ-HPV-7 cells. In contrast, a marked reduction in cell viability was noted in CA-HPV-10 cells. Gupta et al. [124] elucidated the molecular basis for the apigenin-induced growth restrictions of androgen-responsive LNCaP cells. Apigenin treatment resulted in decreased intracellular and secreted forms of PSA as well as AR protein expression. Apigenin exposure further resulted in G1 cell cycle arrest after, together with marked reduction in cyclin D1, D2, and E levels as well as CDK2, 4, and 6. Furthermore, there was a concomitant induction of Waf1/p21 (in p53 dependent manner) and Kip1/p27 after the apigenin treatment. Moreover, apigenin restricted the hyperphosphorylation of the pRb protein in LNCaP cells [124]. Shukla and Gupta [125] studied apigenin-mediated effects on androgen-insensitive DU145 cells harboring mutations in p53 and pRb. Apigenin treatment of DU145 cells, in both dose- and time-dependent fashion, inhibited growth and colony formation and resulted in G1 cell cycle arrest in these cells. Apigenin exposure further altered the Bax/Bcl2 ratio in favor of apoptosis through induction of apoptotic protease-activating factor-1 (Apaf-1). This cascade led to an upsurge in cleaved products of caspase-9, -3, and poly (ADP-ribose) polymerase (PARP). Treatment with apigenin caused downregulation in the nuclear expression of NF- $\kappa$ B/p65 and NF- $\kappa$ B/p50, associated with upregulation of cytosolic I $\kappa$ B $\alpha$ . [125]. Additional investigations by Shukla & Gupta [126] analyzed the effectiveness of apigenin in moderating NF- $\kappa$ B expression, a ubiquitous transcription factor that regulates cell survival, apoptosis and immune functions. Apigenin treatment of PC-3 cells reduced DNA binding and nuclear levels of the NF- $\kappa$ B/p65 and NF- $\kappa$ B/p50 subunits with a simultaneous decrease in I $\kappa$ B $\alpha$  degradation, I $\kappa$ B $\alpha$  phosphorylation, and IKK $\alpha$  kinase activity. Moreover, apigenin was determined to reduce TNF $\alpha$ -induced NF- $\kappa$ B activation via the I $\kappa$ B $\alpha$  pathway, sensitizing cells to TNF $\alpha$ -induced apoptosis. Inhibition of NF- $\kappa$ B corresponded with reduced levels of NF- $\kappa$ B-dependent reporter gene, as well as NF- $\kappa$ B-regulated genes including Bcl2, cyclin D1, cyclooxygenase-2, matrix metalloproteinase 9, nitric oxide synthase-2, and VEGF. Additional evidence supported that apigenin-mediated reduction in cell proliferation, invasiveness, and decrease in tumor growth results from the downregulation of IKK $\alpha$  and downstream targets affecting NF- $\kappa$ B signaling pathways [126]. Shukla et al. [127] elucidated that apigenin directly binds to IKK $\alpha$ , inhibiting its kinase activity. Apigenin treatment restored I $\kappa$ B $\alpha$  expression, preventing its phosphorylation by upstream kinase IKK which undergoes proteasomal degradation [127]. Further assessment by Shukla et al. [128] examined the apigenin-induced 22Rv1 tumor growth inhibition subcutaneously implanted in athymic male nude mice. The results of this study suggested that apigenin restricted tumor growth via increased accumulation of human IGFBP-3 and apoptosis induction. Apigenin supplementation also led to low levels of serum IGF-I in tumor xenografts, signifying that the downstream effects of apigenin involve regulation of IGF-signaling in prostate cancer

[128]. In further studies, oral supplementation of apigenin resulted in increased expression of Waf1/p21, Kip1/p27, INK4a/p16, and INK4c/p18 along with decrease in expression of cyclins D1, D2, and E, in dose-dependent manner. A decrease in cyclin-dependent kinases (cdk) cdk2, cdk4, and cdk6; p-Rb (Ser780); increase in the binding of cyclin D1 toward Waf1/p21 and Kip1/p27; and decrease in the binding of cyclin E toward cdk2 were noted in tumor specimens [129]. Furthermore apigenin treatment resulted in G0-G1 cell cycle arrest, decreased total retinoblastoma (Rb) levels and p-Rb at Ser780 and Ser807/811 in a dose- and time-dependent manner in LNCaP and PC-3 cells. Apigenin was found to increase ERK1/2 and JNK1/2 phosphorylation, resulting in reduction of ELK-1 phosphorylation and c-FOS expression, thereby preventing cell survival. Also, apigenin exposure led to decreased levels of cell cycle regulatory proteins including cyclin D1, D2 and E and their regulatory partners CDK2, 4, and 6, with the loss of RNA polymerase II phosphorylation. The downstream effects of apigenin exposure reinforce its effectiveness in inhibiting transcription of integral proteins [130]. Shukla et al. [131] evaluated the effect of apigenin on TRansgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice. Apigenin supplementation at doses of 20 and 50 mg/mouse/day, 6 days per week for 20 weeks, significantly decreased prostate cancer tumor volumes and complete elimination of metastases. Apigenin treatment resulted in higher levels of E-cadherin and reduced levels of nuclear  $\beta$ -catenin, c-Myc, and cyclin D1 in the prostates of TRAMP mice. These studies suggest that apigenin has ability to block  $\beta$ -catenin signaling, suppressing prostate carcinogenesis *in vivo* [131].

Our investigations of apigenin in cell culture and *in vivo* models suggest that apigenin exerts its anticancer effects by perturbing various pathways leading to inhibition of prostate cancer. Pandey et al. [132] demonstrated the role of apigenin as a histone deacetylase inhibitor. As such, apigenin acts on HDAC1 and HDAC3 and increases the global histone acetylation and the localized hyperacetylation of histone H3 on the p21/Waf1 promoter [132]. A study by Kanwal et al. [133] demonstrated that apigenin could also function as a dual epigenetic inhibitor having ability to alter the DNA methyltransferase and histone deacetylase activity. It does so by reversing both DNA methylation and the trimethylation of lysine 27 at the H3 histone in cultured cells and in an artificial *in vitro* system [133]. This study further supported an investigation on apigenin and its role as a chemopreventive agent.

Abnormal alterations to the insulin-like growth factor (IGF) pathway has been reported to stimulate prostate cancer progression, adaptation, and survival in a castrated environment and metastasis. A study by Shukla et al. [134] demonstrated that apigenin hindered cancer progression in TRAMP mice by altering IGF-I/IGFBP-3 signaling pathway with an effect on the inhibition of angiogenesis and metastasis. A reduction in the IGF-1 and increase in IGFBP-3 levels in the serum and the dorsolateral prostate was observed in apigenin-treated mice. Furthermore, apigenin intake resulted in marked inhibition of p-Akt, p-ERK1/2, VEGF, uPA, MMP-2 and MMP-9, corresponding with tumor growth and metastasis inhibition in TRAMP mice [134]. Further studies on apigenin in TRAMP mice suggested that reduction in PI3K/Akt signaling could also activate FoxO3a and its DNA binding ability, increasing BIM and p27/Kip1 protein expression, and ultimately resulting in cell cycle arrest and reduced proliferation and metastasis in prostate tumors [135]. Studies evaluating apigenin treatment alone or in combination with additional modalities such as radiation and chemotherapy suggest apigenin may be beneficial in managing advance-stage

Author Manuscript  
Author Manuscript  
Author Manuscript  
Author Manuscript

prostate cancer. Combination of apigenin with cisplatin showed synergistic cytotoxic and anti-migration activity of CD44 positive prostate cancer stem cells. This treatment induced apoptosis through downregulation of Bcl-2, upregulation of Apaf-1, p21/Waf1 and p53 expression, and inhibition of PI3K/Akt and NF-κB signaling pathways [136]. Shukla et al. [137] demonstrated the ability of apigenin in inhibiting the IAP family of proteins, thereby making prostate cancer cells more susceptible to Bax-mediated apoptosis. Apigenin treatment to prostate cancer cells caused decrease in HDAC1 thereby altering acetylation status, resulting in increased acetylation of the lysine residues of Ku70, thereby releasing Bax from the complex facilitating apoptosis [137]. In further studies of apigenin-mediated apoptosis in prostate cancer cells, Sharma et al. [138] demonstrate preferential uptake and accumulation of apigenin in the nuclear matrix, binding it with the DNA to reduce oxidative DNA damage and apoptosis in prostate epithelial cells.

### 5.8 Skin cancer

Apigenin displayed beneficial effects in SKH-1 mice in preventing UVA/B-induced skin carcinogenesis [139]. Lepley et al. [140] demonstrated that apigenin, when applied topically, inhibits UV-mediated stimulation of ornithine decarboxylase activity, reduces tumor incidence, and increases tumor-free survival in mice. Apigenin also prevents UV-induced skin tumorigenesis by inhibiting cyclins and cyclin-dependent kinases driving cell cycle progression. [140]. In mouse keratinocytes, apigenin causes G2/M cell cycle arrest, accumulation of p53, and induction of p21/Waf1. Further, apigenin-mediated cell cycle arrest led to reduction in p34 (cdk2) kinase activity, independent of p21/Waf1 [141]. Apigenin halts the cell cycle at G0/G1 phase by inhibiting cdk2 kinase and inducing p21/Waf1 in human diploid fibroblasts. Li et al. [142] developed a short-term *in vivo* model to evaluate the efficacy of topical apigenin when applied to local skin lesions. This study noted that topical application of apigenin was capable of targeting local tissue [140]. Li et al. [143] further demonstrated the response of percutaneous absorption of apigenin using different vehicles both *in vivo* and *in vitro* models.

Recent observations suggest that apigenin suppresses UVB-induced increase in COX-2 protein and mRNA in mouse and human keratinocytes [144,145]. COX-2 is an enzyme that converts arachidonic acid to prostaglandins, and its overexpression leads to carcinogenesis. Caltagirone et al. [146] determined that the combined treatment of quercetin and apigenin *in vivo* inhibited B16-BL6 melanoma lung tumor metastasis. This action was attributed to the reduction of endothelial interaction in tumor cells [146]. Topical application of apigenin to mouse skin effectively reduces the incidence and size of skin tumors caused by UVB exposure inducing apoptosis via the intrinsic and extrinsic apoptotic pathways [147]. Exposure of apigenin and luteolin to human keratinocytes inhibited UVA-induced collagenolytic MMP-1 production through interference with Ca(2+)-dependent MAPKs and AP-1 signaling [148]. Apigenin modifies membrane fluidity by altering the motional freedom of polar head groups, thereby decreasing penetration of Pr3+ ions to the membrane. The structural and dynamic changes to the membrane caused by apigenin are crucial for tumor suppression, signal transduction pathways and cell cycle regulation [149]. The *in vivo* skin model developed by Byun et al. [150] supports that apigenin prevents UVB-induced ear edema development, COX-2 expression and Src kinase activity in SKH-1 hairless mice

[150]. These results indicate that apigenin prevents skin cancer by epigenetic modifications. Apigenin inhibits UVB-induced cutaneous angiogenesis through maintenance of the normal high levels of endogenous TSP1 attenuating neo-angiogenesis, proliferation and epidermal thickening in mice exposed to UVB irradiation [152]. The protective role of apigenin was further deciphered using non-melanoma skin cancer model where apigenin inhibited COX-2 that promotes proliferation and tumorigenesis. Thus, inhibition of COX-2 averts skin tumor development [153]. Aberrant activation of Akt/mTOR characterizes skin cancer development as a result of UVB radiation. Apigenin inhibited UVB-mediated mTOR activation in mouse skin and in mouse epidermal keratinocytes independent of Akt, and this led to autophagy [154]. Using 7,12-dimethyl benz[a]anthracene (DMBA)-induced experimental oral carcinogenesis golden hamsters buccal pouch model by painting 0.5% DMBA three times a week for 14 weeks, Silvan et al. [155] demonstrated that oral administration of 2.5 mg/kg apigenin reduced tumor volume causing inhibition of cell proliferation, apoptosis inflammation, and angiogenesis markers, and modulation of phase I and II detoxification cascades. In another study, topical application of apigenin in murine skin tumorigenesis initiated by DMBA and promoted by 12-O-tetradecanoylphorbol-13-acetate in SENCAR mice caused marked reduction of incidence, number of papillomas and carcinomas [156]. Mafuvadze et al. [157] demonstrated that treatment of 50 mg/kg apigenin to mice bearing BT-474 xenograft tumors exposed to medroxyprogesterone acetate resulted in the progression and development of xenograft tumors by inducing apoptosis, inhibiting cell proliferation, and reducing Her2/neu expression.

### 5.9 Cervical cancer

Zheng et al. [158] first reported that apigenin hinders the progression of human cervical carcinoma HeLa cells through apoptosis. Apigenin prevents cell growth, causes cell cycle arrest in the G1 phase, and prompts p53-dependent apoptosis associated with p21/Waf1 induction and upregulation of Fas/APO-1 and caspase-3. Apigenin exposure also resulted in downregulation of Bcl-2 protein [158]. Czyz et al. [159] established that apigenin interferes with cell proliferation and survival through gap junctional coupling. Apigenin treatment of HeLa cells (wild-type variant) and their connexin43 (Cx43) transfected counterparts caused marked inhibition of cell translocation. Apigenin, at low concentrations, did not demonstrate significant effects on cell proliferation whereas was effective on cell motility and invasiveness in HeLa Cx43 cells [159]. An extract containing parthenolide, camphor, luteolin, and apigenin prepared from the medicinal herb feverfew (*Tanacetum parthenium*) exhibited anti-proliferative activity against human cervical cancer SiHa cells [160]. A study by Liu et al. [161] demonstrated that in HeLa cells, casein kinase 2 (CK2) is a positive regulator in the self-renewal of cervical cancer stem-like cells. They also revealed that apigenin inhibits self-renewal capability through the downregulation of CK2α protein expression. These findings provide evidence for the potential benefits of apigenin as a CK2 inhibitor in the treatment of human cervical cancer by targeting cancer stem cells [161]. The consequence of apigenin on cell proliferation was less pronounced, especially at low concentrations, whereas the effect of apigenin on cell motility corresponded with a blunting of the invasive potential of HeLa Cx43 cells [159]. Another recent study with the medicinal herb feverfew (*Tanacetum parthenium*) extract containing parthenolide, camphor, luteolin, and apigenin, showed anti-proliferative activity against human cervical cancer SiHa cells.

[160]. A study by Liu et al. [161] demonstrated that in HeLa cells, casein kinase 2 (CK2) is a positive regulator in the self-renewal of cervical cancer stem-like cells. They also revealed that apigenin inhibits self-renewal capability through the downregulation of CK2α protein expression. These findings provide evidence for the potential benefits of apigenin as a CK2 inhibitor in the treatment of human cervical cancer by targeting cancer stem cells [161]. Apigenin has been shown to exert a selective dose-dependent cytotoxic effect in cervical cancer cells inducing apoptosis as a result of changes in mitochondrial redox potential impairment and inhibition reductions in of cancer cell migration and invasion. These results show that apigenin had a strong and selective anti-tumor effect on cervical cancer cells immortalized by infected with human papilloma virus, especially HPV16 and HPV18. These results indicate that apigenin has potential to be developed as therapeutic agent for (HPV) 16, HPV 18, and HPV 16 and 18 indicating its potential to be a powerful candidate in developing therapeutic agent for all cervical cancer types [162]. Apigenin enhances the inhibitory effect of IFN- $\alpha$  on cell viability in HeLa cancer cells but did not exhibit an effect on cell proliferation and apoptosis in HeLa cancer cells [163]. These results support that additional preclinical and clinical studies are required for further validation of antitumor effects of apigenin applicable to cervical cancer.

### 5.10 Endometrial cancer

O'Toole et al. [164] performed array-based comparative genomic hybridization on treated endometrial cancer cells treated with phyto-estrogenic compounds agents including apigenin using array-based comparative genomic hybridization. The results of this study found that  $\beta$ -estradiol modified over 20% of the array genes involving involved in insulin metabolism compared to those after treatment with apigenin at similar treated with the same concentration of apigenin. Therefore, this evidence suggests that apigenin may could be beneficial to in the treatment of endometrial cancer.

### 5.11 Ovarian cancer

Studies in patients with ovarian cancer have shown that intake of apigenin is significantly associated with a lower risk [38, 39]. Fang et al. [165, 166] demonstrated that exposure of human ovarian cancer cells with apigenin decreases invasiveness through suppression of VEGF expression at the transcriptional level in these cells along with expression of HIF-1 $\alpha$  via the PI3K/AKT/p70S6K1 and HDM2/p53 pathways [165–167]. Apigenin also contributes to the prevention of tube formation by endothelial cells *in vitro*. Additionally, in human ovarian carcinoma HO-8910PM cells, apigenin restricts MAPK and PI3K the activity of MAPK and PI3K [168]. Due to adenoviral toxicity, most of the patients become immunocompromised. Treatment of ovarian cancer cell lines with apigenin after adenoviral infection employing Ad5/3-Delta24 that targets the Rb pathway revealed the reduction of adenovirus replication and associated toxicity in *in vitro* cell culture and *in vivo* models. This suggested that apigenin was able to overcome toxicity due to adenovirus infection [169]. Further studies also show apigenin regulating the focal adhesion kinase (FAK) in A2780 ovarian cancer cells to attenuate migration and invasion, suggesting that targeting FAK may be a useful strategy for chemoprevention and/or chemotherapeutics of ovarian cancers [170]. Apigenin's effectiveness is further supported by studies that show its inhibitory effect on Id1 (inhibitor of differentiation or DNA binding protein 1) through

activating transcription factor 3 (ATF3) to retract proliferation and tumorigenesis of human ovarian cancer A2780 cells [171]. In another study, apigenin has shown effectiveness in overcoming chemo-resistance in doxorubicin (DOX) and etoposide (VP16) resistance in 2008/MRP1 ovarian carcinoma cells to DOX by altering the multidrug resistance protein 1 (MRP-1) [172]. The Hedgehog (Hh) signaling pathway plays a critical role in the stimulation of cancer stem cell growth and casein kinase 2 (CK2), a protein kinase frequently activated in cancers. SKOV3 derived SFCs express high levels of CK2α and glioma-associated oncogene 1 (Gli1) proteins. Apigenin inhibited the self-renewal capacity of SKOV3 sphere-forming cells (SFC) by downregulating Gli1 regulated by CK2α [173]. In orthotropic tumors induced using ovarian epithelial cancer cells, OVCAR-3 cells oral treatment of apigenin caused downregulation of MMP-9 mediated by the AKT/p70S6K1 pathway to inhibit tumor progression [174]. In taxol-resistant ovarian cancer cells, apigenin caused down regulation of TAM family of tyrosine kinase receptors and also caused inhibition of IL-6/STAT3 axis, thereby attenuating proliferation. This study suggests that apigenin has ability to overcome taxol resistance in ovarian cancer cells [175].

### 5.12 Hematologic cancer

Human leukemia cells were evaluated to determine the effects of apigenin treatment. Compared to other flavonoids, apigenin was more effective in leukemia cells in terms of inducing apoptosis [176]. Additional studies have demonstrated that a combination treatment of apigenin and quercetin inhibits topoisomerase-catalyzed DNA irregularities, which are often seen in leukemia cell DNA metabolism, especially in replication and transcription. Vargo et al. [177] determined that apigenin treatment provoked differential anti-proliferative and apoptotic response in monocytic and lymphocytic leukemia cell lines, attributed to protein kinase C delta induction [177]. Utilizing human leukemia cells, Chen et al. [178] examined several flavonoids including apigenin for their proteasome-inhibitory and apoptosis-inducing abilities. The results of this study suggest that apigenin and quercetin exhibit stronger potency in inhibiting chymotrypsin-like activity of purified 20S and 26S proteasome, as well as altering the ubiquitinated forms of two proteasome target proteins, Bax and IκBα, caspase-3 and poly ADP-ribose polymerase (PARP) in Jurkat T cells. Furthermore, the level of proteasome inhibition of these flavonoids corresponds with their potency in inducing apoptosis [178].

Wang et al. [176] demonstrated that human promyelocytic leukemia HL-60 cells underwent apoptosis after exposure to structurally related flavonoids, including apigenin, quercetin, myricetin, and kaempferol due to induction of caspase-3 and PARP cleavage. Consequently, exposure to flavonoids led to the mitochondrial transmembrane potential loss, with a spike in reactive oxygen species, and cytochrome c release into the cytosol with induction of pro-caspase-9. Apigenin ranked higher than other flavonoids in inducing apoptosis. In another study, apigenin-7-O-glucoside extracted from seven principal Tunisian olive varieties reduced the differential marker nitro blue tetrazolium in HL-60 cells [179]. In another study, Monasterio et al. [180] examined the apoptotic potential of twenty two flavonoids and related compounds in leukemic U937 cells. The results of this study revealed that apigenin and additional flavones induced apoptosis in U937 cells; however, isoflavones and flavonones were not as effective in provoking apoptosis.

Horvathova et al. [181] studied the protective effects of various plant flavonoids including apigenin on H<sub>2</sub>O<sub>2</sub>-induced DNA damage in murine leukemia L1210 cells. Apigenin at low doses was slightly effective in reducing the scope of DNA damage. Comparatively, at higher concentrations, apigenin exposure led to DNA single strand breaks, suggesting its potential role as a pro-oxidant [181]. In another study, Strick et al. [182] examined the ability of dietary bioflavonoids to cause MLL gene cleavage, which may lead to infant leukemia. Using primary progenitor hematopoietic cells from healthy newborns and adults, apigenin was shown to induce DNA cleavage by targeting topoisomerase II, an enzyme that alters DNA topology. However, it is uncertain whether or not this in-vitro study can be extrapolated to humans due to dose and bioavailability concerns [182]. Apigenin caused the apoptosis in human lymphoma B cells in vitro and prevented the reverted mutations with a high hindrance percentage. This suggests that it has anti-mutagenic properties [183]. In elucidating the mechanism of apoptosis as a result of apigenin treatment in leukemia cells, Gonzalez-Mejia et al. [184] revealed the phosphorylation of Hsp27 as an important event in causing cell death. Additionally, it was shown that apigenin treatment in a late phase involves the activation of p38 and PKC $\delta$  to modulate Hsp27, thus leading to apoptosis [184]. A study by Jayasoorya et al. [185] demonstrated that apigenin inhibits cell growth and diminishes telomerase activity in human-derived leukemia cells, ultimately leading to apoptosis. In addition to inducing apoptosis in leukemia cells, apigenin-7 glycoside has been shown to induce granulocyte differentiation that was confirmed by the presence of significant amounts of CD11b positive cells [186]. Evidence also suggests that PI3K/Akt and JNK kinases and their related pathways are potential targets for apigenin-induced apoptosis in leukemia cells [187]. Apigenin-induced expressions of  $\alpha$ ,  $\beta$ , and  $\gamma$  globin genes increased the expression of glycosporin as a marker for differentiation. The hydroxyl groups are likely to render apigenin effective for inducing cell differentiation [188].

### 5.13 Adrenal cortical cancer

Previous studies of adrenocortical cancers have established the existence of aberrations in numerous signaling pathways and enzymes, including aromatase, an enzyme involved in the conversion of androgens to estrogen. Sanderson et al. [189], utilizing human adenocortical cancer H295R cells, studied the effect of plant flavonoids on the catalytic and promoter specific expression of aromatase. Results revealed that plant flavonoids are potent aromatase inhibitors, an action related to increased intracellular concentrations of cAMP [189]. Ohno et al. [190] also evaluated the relationship between exposure of plant flavonoids and cortisol production in H295R cells, and determined that apigenin-exposed cells exhibited reduced cortisol production, and decrease in 3 $\beta$ -HSD II and P450c21 activity.

### 5.14 Thyroid cancer

Yin et al. [191] studied the effect of apigenin on various human thyroid carcinoma cell lines including UCLA NPA-87-1 (NPA) (papillary carcinoma), UCLA RO-82W-1 (WRO) (follicular carcinoma), and UCLA RO-81A-1 (ARO) (anaplastic carcinoma). Apigenin exposure to these cells resulted in inhibition of cell proliferation amongst other plant flavonoids [191]. Yin et al. [192] further illustrated the apigenin-induced inhibition of ARO cell proliferation was associated with the disruption of EGFR tyrosine auto-phosphorylation and its downstream effector MAPK phosphorylation [192]. Schroder-van der Elst et al. [193]

examined the effect of various flavonoid on iodide transport and growth utilizing human follicular thyroid FTC133 cancer cells, stably transfected with the human Na (+)/I (−) symporter (hNIS). Apigenin exposure to these cells prevented NIS mRNA expression, which may have therapeutic implications for the radioiodide treatment of thyroid carcinoma [193]. Apigenin induces apoptosis in ATC cells, mediated through c-Myc, with concomitant changes in p53 and p38 in FRO ATC cells. Apigenin, together with BRAFV600E inhibitor PLX4032, induced cytotoxicity, suppressing Akt in ATC cells which harbor BRAFV600E [194]. The apigenin-enhanced iodide influx rate is increased by Akt inhibition in thyroid cells under acute TSH stimulation and requires p38 MAPK activity. Treatment with apigenin increases radioiodide accumulation in thyroid cells expressing BRAFV600E and in primary cultured thyroid tumor cells from TR $\beta$  (PV/PV) mice. Thus, along with Akt inhibitors, apigenin can further enhance the efficacy of radioiodine therapy for thyroid cancer patients [195]. Apigenin-induced cell death has been shown to involve autophagy in papillary thyroid cancer, perhaps because of ROS stimulation, induction of DNA damage and G2/M phase cell cycle arrest [196]. Targeted radioiodine therapy, used in thyroid cancer, depends on thyrotropin-mediated selective stimulation of Na+/I<sup>−</sup> Symporter (NIS)-mediated radioactive iodide uptake (RAIU) by thyroid cells. Unfortunately, patients with advanced thyroid cancer do not benefit from radioiodine therapy due to reduced or absent NIS expression. Though PI3K inhibitors could induce RAIU by diminished iodide efflux rate where TGF- $\beta$ , a secreted cytokine facilitates the growth of thyroid cancers, has been observed to reverse the effect. A combination treatment of apigenin with PI3K inhibitor GDC-0941 attenuated the effect of TGF- $\beta$  increasing RAIU in both BRAFV600E and RET/PTC3 expressing cells [197].

### 5.15 Neuroblastoma

Torkin et al. [198] studied the downstream effect of apigenin on various neuroblastoma cell lines of human origin. Apigenin exposure to cells resulted in reduced colony-forming ability and survival, leading to induction of apoptosis which was accompanied by an increase in tumor suppressor p53 and its downstream targets including p21/Waf1 and Bax. Furthermore, apigenin demonstrated differential response by causing cell death and apoptosis of neuroblastoma cells expressing wild-type p53, but not mutant p53. Apigenin also augmented caspase-3 activity and PARP cleavage [198]. Neuroblastoma SH-SY5Y cells treated with apigenin led to induction of apoptosis, accompanied by higher levels of intracellular free [Ca(2+)] and shift in Bax:Bcl-2 ratio in favor of apoptosis, cytochrome c release, followed by activation caspase-9, calpain, caspase-3 and caspase-12 [199]. In neuroblastoma SK-N-DZ cells, a combination of the small molecule Bcl-2 inhibitor HA14-1 (HA) and apigenin worked synergistically to decrease cell viability and suppress the expression of angiogenic factors thereby activating extrinsic and intrinsic apoptotic pathways [200]. A combination treatment of apigenin with synthetic retinoid N-(4-hydroxyphenyl) retinamide (4-HPR) in serum-starved human malignant neuroblastoma cells suppressed autophagy and promoted apoptosis [201]. In malignant neuroblastoma cells, ectopic expression of Krüpple-like factor 4 (KLF4) in combination with apigenin treatment resulted in induction of apoptosis downregulating Bcl-2 expression and impairment of transcription and translation of MMP-2 and MMP-9 leading to prevention of tumor cell migration [202]. Apigenin treatment of malignant neuroblastoma cells led to sequential telomerase reverse transcriptase (hTERT)

Author Manuscript  
Author Manuscript  
Author Manuscript  
Author Manuscript

knockdown, inhibited cell invasion and proliferation and induced apoptosis [203]. Further studies revealed that apigenin involved miR-138 more effectively than hTERT to induce apoptosis in malignant neuroblastoma cancer cell lines [204]. Similar studies using the oncogene N-Myc silencing in combination with apigenin prevented cell migration and decreased N-Myc driven survival, angiogenesis, and invasive factors. This activity suggests that using the said process is a promising strategy for controlling the growth of N-Myc amplified human malignant neuroblastoma cells [205]. Apigenin diminished insulin fibril-induced reactive oxygen species (ROS) production and lipid peroxidation in neuroblastoma cells. These effects led to increased catalase activity and alterations in intracellular glutathione levels, subsequently reducing nitric oxide production and NF- $\kappa$ B activity. Inhibition of NF- $\kappa$ B pathway caused a concomitant reduction in TNF $\alpha$  and IL-6 levels [206]. The suppressive nature of apigenin to inhibit ROS in human neuroblastoma SH-SY5Y cells has been shown to involve reduction in the oxidation of cellular glutathione and subsequent formation of malondialdehyde and carbonyls [207].

### 5.16 Bladder cancer

Using MEKK1 overexpression in bladder smooth muscle (SM) cell, Liu et al. [208] demonstrated the effect of apigenin in the phosphorylation of MAPKs, ERK, JNK and p38, which are the downstream molecules of MEKK1. Apigenin exposure at 50  $\mu$ M to these cells significantly inhibited activation/phosphorylation of MAPKs and migration of SM cells induced by MEKK1 overexpression. Furthermore, apigenin also inhibited actin polymerization, which underlines muscle contraction and cell migration [208]. In another study, apigenin suppressed proliferation and inhibited the migration and invasion potential of T24 bladder cancer cells in a dose- and time-dependent manner, which was associated with induced G2/M phase cell cycle arrest and apoptosis through the involvement of PI3K/Akt pathway and Bcl-2 family proteins. In addition, apigenin increased caspase-3 activity and PARP cleavage, indicating that apigenin induced apoptosis in a caspase-dependent way [209]. Zhu et al. [210] demonstrated that apigenin treatment caused decrease proliferation and induction of apoptosis in human bladder T24 cells, associated with an increase in the phospho-p53, p53, p21, and p27 levels, and with a decrease in the cyclin A, cyclin B1, cyclin E, CDK2, Cdc2, and Cdc25C expression, thereby blocking cell cycle progression. In addition, apigenin increased the Bax, Bad, and Bak levels, but reduced the Bcl-xL, Bcl-2, and Mcl-1 levels, and subsequently triggered the mitochondrial apoptotic pathway through release of cytochrome c and activation of caspase-9, caspase-3, caspase-7, and PARP cleavage [210].

### 5.17 Mesothelioma

Malignant mesothelioma (MM) is a tumor arising from mesothelium. Masuelli et al. [211] demonstrated that apigenin in MM cells caused apoptosis and not autophagy by altering Bax/Bcl2 ratio. Apigenin also caused the activation of p53 and caspase-9. The authors further evidenced that apigenin inhibited Akt and NF- $\kappa$ B/p65 pathways because of MAPK attenuation in these cells. Additionally, tumors with MM cells implanted in C57BL/6 mice treated 20 mg/kg apigenin prolonged the survival time. These evidences suggests the anti-metastatic role of apigenin in a wide variety of *in vitro* and preclinical MM models.

### 5.18 Osteosarcoma

The effect of apigenin on human osteosarcoma U2OS cells has been demonstrated. Apigenin significantly decrease cell viability, induced apoptosis through the activations of caspase-3, -8, -9, and BAX and promoted the release of AIF in U2OS cells. Furthermore, nude mice bearing U2OS xenograft tumors, 2 mg/kg apigenin every 3 day for 30 days inhibited tumor growth [212]. Bumke-Vogt et al. [213] demonstrated the impact of apigenin and luteolin on U2OS cells in rapid intracellular translocation of the forkhead box transcription factor O1 (FOXO1), an important mediator of insulin signal transduction. Treatment of human hepatoma HepG2 cells with apigenin and luteolin on the expression of the gluconeogenic enzymes viz. phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pc), the lipogenic enzymes fatty-acid synthase (FASN) and acetyl-CoA-carboxylase (ACC) which were downregulated by both flavones with smaller effective dosages of apigenin than for luteolin. Furthermore, apigenin and luteolin reduced the expression of PKB/AKT-, PRAS40-, p70S6K-, and S6-phosphorylation was reduced by and luteolin but not that of the insulin-like growth factor receptor IGF-1R. Liu et al. [214] demonstrated that apigenin inhibited proliferation and reduced invasion in human U2OS and MG63 osteosarcoma cell lines through downregulation of the expression of  $\beta$ -catenin in these cells.

## 6.0 Combinational studies with apigenin

Apigenin has also shown to have a significant effect when combined with various chemotherapeutic agents where various cancer cells exhibit differential effects [215–222]. Studies with pancreatic cancer cells reveal that 24 h pretreatment with low concentrations of apigenin for 24 h followed by cisplatin (10  $\mu$ M), 5-Flurouracil (50  $\mu$ M), oxaliplatin (0.1  $\mu$ M), gemcitabine hydrochloride (10  $\mu$ M) for 36 h resulted in increased growth inhibition of pancreatic cancer cells, compared to co-treatment or individual treatments, where the effects were less than additive [215]. In human multiple myleoma U266 and RPMI 8226 cell lines, apigenin significantly decreased Hsp90 clients when combined with the Hsp90 inhibitor geldanamycin and the histone deacetylase inhibitor vorinostat to induce apoptosis [216]. Similarly combination of synthetic retinoid N-(4-hydroxyphenyl) retinamide (4-HPR) (0.5  $\mu$ M) and apigenin (50  $\mu$ M) in the serum-starved human malignant neuroblastoma cells inhibited autophagy inducing apoptosis [217]. The same group had earlier published that combination of apigenin with Bcl-2 inhibitor, small molecule HA14-1 (HA) induces apoptosis in human malignant neuroblastoma cells inhibiting angiogenic factors [218]. In human cervical epithelial carcinoma HeLa cells combination of apigenin and paclitaxel significantly increased inhibition of cell proliferation, suppressing the activity of SOD, inducing ROS accumulation leading to apoptosis by activation of caspase-2 [219]. In MiaPaCa-2 subcutaneous xenograft model of pancreatic cancer, combination treatment of gemcitabine (125 mg/kg) with apigenin (50 mg/kg) exhibited significant tumor growth inhibition, decrease in tumor volume and weight. This combination caused a significant decrease in the activation of Akt and inhibited the DNA binding of NF- $\kappa$ B/p65 in the nucleus [220]. Zhu et al. [221] demonstrated that apigenin inhibits expression of ABCB1, the ATP-binding cassette (ABC) transporter family, and resensitizes docetaxel-resistant prostate cancer cells to docetaxel treatment. In another study by Hu et al. [222] sub-toxic concentrations of apigenin (4  $\mu$ mol/L) significantly enhanced the cytotoxicity of 5-FU (100

µg/mL) in hepatocellular carcinoma (HCC) cells through mitochondrial membrane potential ( $\Psi_m$ )-mediated apoptosis. Furthermore, *in vivo*, combined treatment with 20 mg/kg apigenin five times per week in 3 week protocol and 20 mg/kg 5-FU for 5 consecutive days significantly inhibited the growth of HCC xenograft tumors [222]. Gao et al. [95] demonstrated that apigenin significantly sensitizes doxorubicin-resistant BEL-7402 (BEL-7402/ADM) cells to doxorubicin (ADM) and increases intracellular concentration of ADM through downregulation of PI3K/Akt pathway, leading to a reduction of Nrf2-downstream genes. In BEL-7402 xenografts, apigenin and ADM cotreatment inhibited tumor growth, reduced cell proliferation and induced apoptosis more substantially when compared with ADM treatment alone.

## 7.0 Clinical trials with apigenin

Early clinical trial with apigenin was based on the epidemiologic studies demonstrating that dietary intake of flavonols and flavones was inversely associated with risk for cardiovascular disease. Janssen et al. [23] performed a study feeding 18 healthy volunteers with 220 g onions and 5 g dried parsley per day providing 114 mg quercetin and 84 mg apigenin respectively, or a placebo for 7 day each in a randomized crossover experiment to determine their effect on platelet aggregation. Although onion consumption raised mean plasma quercetin concentrations to 1.5 µmol/L; plasma apigenin could not be measured. No significant effects of onions or parsley were found on platelet aggregation, thromboxane B2 production, factor VII, or other hemostatic variables. In another study, Hoensch et al. [223] investigated the preventive effects of dietary flavonoid mixture composed of 20 mg apigenin and 20 mg epigallocatechin-3-gallate on recurrence risk on 31 patients with resected colorectal cancer or adenoma polypectomy and compared with matched control group consisting of 56 patients observed for 3–4 years by surveillance colonoscopy. No cancer recurrence was noted and only one adenoma developed in resected colon cancer group. In untreated control, 20% patients developed recurrence and 27% evolved adenomas, suggesting that continuous long-term supplementation of flavonoid mixture could reduce the recurrence of colon neoplasia. In another double-blind, placebo-controlled study, Amsterdam et al. [224] examined the antianxiety and antidepressant action of 220 mg oral chamomile (*Matricaria recutita*) extract standardized to 1.2% apigenin in participants with symptoms of comorbid anxiety and depression evaluated by the scores from the Hamilton Depression Rating (HAM-D) questionnaire among treatment groups. A significant reduction over time in total HAM-D scores for chamomile versus placebo in all participants was noted. Furthermore, a clinically meaningful but nonsignificant trend was noted for a greater reduction in total HAM-D scores for chamomile *versus* placebo in participants with current comorbid depression provided evidence that chamomile may provide clinically meaningful antidepressant activity. Choi et al. [225] demonstrated the clinical efficacy of apigenin on aged skin, using an apigenin-containing cream on forty women, aged over 30 years, through a randomized and double-blinded clinical trial with four weeks of treatment. Application of apigenin-containing cream increased dermal density and elasticity, and reduced fine wrinkle length along with improved skin evenness, moisture content and transepidermal water loss, compared to the placebo group. These results suggest that apigenin possess anti-aging

properties. Based on the published literature, no studies in humans have been conducted solely with apigenin with respect to solid cancer.

## 8.0 Major limitations of apigenin

Apigenin is unstable and is not very soluble in water or organic solvents. These properties restrict the use of apigenin in its pure forms. Naturally, apigenin is available in foods as glycoside and acylated derivatives, having higher solubility in water compared to the parent compound [226, 227]. The moiety conjugating apigenin helps determine its absorption and bioavailability, facilitating enzymatic cleavage by mammalian or microbial glucosidases [227]. Consequently, it seems likely that apigenin in the natural form bound to  $\beta$ -glycosides may provide better bioavailability. In the intestines, apigenin is extensively metabolized in a method involving both enteric and enterohepatic recycling [228, 229]. Apigenin rapidly metabolizes via UDP-glucuronosyltransferase UGT1A1 as glucuronide and sulfate conjugates that are more readily transported in the blood and excreted in bile or urine [230]. Oral intake of radio-labeled apigenin in a single dose by rats demonstrated 51% recovery of radioactivity in urine, 12% in feces, 1.2% in blood, 9.4% in the intestine, 1.2% in liver, 0.4% in the kidneys, and 24.8% in the rest of the body within 10 days. The radioactivity appeared in blood 24 h after oral apigenin intake. A relatively high elimination of apigenin with a half-time of 91.8 h was noted in the blood in kinetics study, compared to other dietary flavonoids [231]. These results endorse the fact that although the bioavailability of apigenin is limited, however the slow pharmacokinetics may be the reason for possible accumulation of this flavone in the peripheral tissues for its effective chemopreventive effects. In an attempt to achieve better functionality, nano-formulations of apigenin are under investigation in *in vitro* and *in vivo* models. Gold nanoparticles (AuNPs) as biosensors, in photothermal therapy and in imaging have gained popularity due to their possible applications in cancer treatment and in drug delivery [232]. The ability of apigenin to reduce Au<sup>3+</sup> ions to form AuNPs is attributed to -OH and C=O groups present in apigenin that reduce Au<sup>3+</sup> facilitating the stabilization of the AuNPs [233]. Studies on the synthesis of apigenin-AuNPs have revealed that Au<sup>3+</sup> can be reduced by apigenin at a pH of 10 and at room temperature forming highly stable and spherical apigenin-AuNPs. These apigenin-AuNPs are biocompatible towards normal human epidermoid HaCat cells while inducing apoptosis of A431 and SiHa cells. The apigenin-AuNPs also exhibit decent antiangiogenic property. Hence, apigenin-AuNPs is a promising candidate for use in skin cancer treatment [234]. Further studies on nano-apigenin using poly (lactic-co-glycolide) (PLGA) was effective in oral and topical application for skin cancer, achieving a higher efficacy and potency with reduced toxicity [235]. Because of its reduced solubility in water and other lipid compounds, the oral bioavailability of apigenin remains relatively low. Formulations using carbon nanopowder solid dispersions for apigenin have proven to enhance its bioavailability. These apigenin nanoparticles showed low levels of toxicity in animals [236]. Apigenin-loaded lipid nanocapsules prepared with phase inversion method showed high efficacy in restricting breast and liver cancer growth [237]. Cochran et al. [238] demonstrate that a long-term releasing apigenin-based polymer and subsequent nanoparticle delivery system has ability to inhibit tumor cell adhesion through the suppression of endothelial cell adhesion molecule

expression. These studies show promise that nano-formulations of apigenin could become an effective drug-delivery system.

## 9.0 Conclusion and future directions

In this review, we aim to justify the role of apigenin as an anticancer agent. The fact that apigenin impacts numerous essential pathways and targets associated with cancer is well established. In fact, current research also underlines apigenin as an epigenetic modulator that could act as a dual DNA methyltransferase and histone methyltransferase inhibitor. Nano formulations of apigenin have shown to increase the bioavailability leading to its possible accumulation in tissues. Being the most available bioactive compound from various plants, vegetables, and fruits, dietary apigenin supplementation is highly recommended.

Bioavailability of apigenin following oral administration in rats and mice have been reported. So far, this information in humans, including pharmacokinetic and pharmacodynamics profiles, is not available. Further research is necessary concerning the bioavailability and safety profile in humans. A generation of scientific evidence is necessary to confirm the beneficial effects of apigenin in cancer patients to establish its role in chemoprevention and therapy better. However, the currently available reports suggest that apigenin possess potential to be developed as a chemopreventive or chemotherapeutic agent in the future.

## Acknowledgments

We sincerely apologize to those investigators whose work could not be cited due to space constraints. The original work from author's laboratory outlined in this review was supported by VA Merit Review 1I01BX002494; United States Public Health Service Grants RO1CA108512, R21CA193080 and R03CA186179 and Department of Defense grant W81XWH-15-1-0558 to SG.

## Abbreviations

<b>4-HPR</b>	N-(4-hydroxyphenyl) retinamide
<b>5-FU</b>	Fluorouracil
<b>ACF</b>	Aberrant crypt foci
<b>ADM</b>	Doxorubicin
<b>ADP</b>	Adenosine di-phosphate
<b>AICR</b>	American Institute for Cancer Research
<b>AOM</b>	Azoxymethane
<b>APC</b>	Adenomatous polyposis coli
<b>ATF3</b>	Activating transcription factor 3
<b>BCRP</b>	Breast cancer resistance protein
<b>CK2</b>	Casein kinase 2

<b>COX-2</b>	Cyclooxygenase-2
<b>DISC</b>	Death-inducing signaling complex
<b>DOX</b>	Doxorubicin
<b>DR4</b>	Death receptor 4
<b>EGFR</b>	Epidermal growth factor receptor
<b>ERK</b>	Extracellular regulated kinase
<b>FAK</b>	Focal adhesion kinase
<b>GADD45</b>	DNA fragmentation factor-45
<b>Gli1</b>	Glioma-associated oncogene 1
<b>GLUT1</b>	Glucose transporter 1
<b>GSTA1</b>	Glutathione S-transferase A1
<b>HDAC</b>	Histone deacetylase
<b>HIF-1<math>\alpha</math></b>	Hypoxia-inducible factor 1-alpha
<b>hTERT</b>	Telomerase reverse transcriptase
<b>ICAM-1</b>	Intercellular adhesion molecule-1
<b>IFNAR1</b>	Type I interferon receptor 1
<b>IFN-<math>\gamma</math></b>	Interferon gamma
<b>IGF</b>	Insulin-like growth factor
<b>IL6</b>	Interleukin-6
<b>JAK</b>	Janus kinase
<b>JNK</b>	c-Jun amino-terminal kinase
<b>KLF4</b>	Krüpple-like factor 4
<b>MAPK</b>	Mitogen-activated protein kinases
<b>MMP</b>	Matrix metalloproteinases
<b>NADPH</b>	Nicotinamide adenine dinucleotide phosphate
<b>NF-<math>\kappa</math>B</b>	Nuclear factor- <i>kappa</i> B
<b>NIS</b>	Na+/I- symporter
<b>NSCLC</b>	Non-small cell lung cancer
<b>ODC</b>	Ornithine decarboxylase

<b>PARP</b>	Poly (ADP-ribose) polymerase
<b>PD-L1</b>	Programmed death-ligand 1
<b>PDPK FA</b>	Proline-directed protein kinase FA
<b>PI3K</b>	Phosphatidylinositol-4,5-bisphosphate 3-kinase
<b>PKC</b>	Protein kinase C
<b>PLGA</b>	Poly-lactic-co-glycolide
<b>PMA</b>	Protein kinase C-activating phorbol ester
<b>ROS</b>	Reactive oxygen species
<b>SOD</b>	Superoxide dismutase
<b>Stat</b>	Signal transducer and activator of transcription
<b>TGF-β</b>	Transforming growth factor-beta
<b>TRAIL</b>	Tumor necrosis factor related apoptosis-inducing ligands
<b>TRAMP</b>	Transgenic adenocarcinoma of the mouse prostate
<b>UGT1A1</b>	UDP-glucuronosyltransferase 1-1
<b>VEGF</b>	Vascular endothelial growth factor
<b>WCRF</b>	World Cancer Research Fund

## References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016; 66:7–30. [PubMed: 26742998]
2. Fidler MM, Soerjomataram I, Bray F. A global view on cancer incidence and national levels of the human development index. Int J Cancer. 2016; 139:2436–2446. [PubMed: 27522007]
3. Lippman SM, Hong WK. Cancer prevention science and practice. Cancer Res. 2002; 62:5119–5125. [PubMed: 12234971]
4. Gupta S. Prostate cancer chemoprevention: current status and future prospects. Toxicol Appl Pharmacol. 2007; 224:369–376. [PubMed: 17189645]
5. Key TJ, Schatzkin A, Willett WC, Allen NE, Spencer EA, Travis RC. Diet, nutrition and the prevention of cancer. Public Health Nutr. 2004; 7:187–200. [PubMed: 14972060]
6. Newell-McGloughlin M. Nutritionally improved agricultural crops. Plant Physiol. 2008; 147:939–953. [PubMed: 18612071]
7. Manach C, Scalbert A, Morand C, Rémesy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004; 79:727–747. [PubMed: 15113710]
8. Hertog MG. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Int Medicine. 1995; 155:381–386.
9. Aherne SA, O'Brien NM. Dietary flavonols: chemistry, food content, and metabolism. Nutrition. 2002; 18:75–81. [PubMed: 11827770]
10. de Vries JH, Janssen PL, Hollman PC, van Staveren WA, Katan MB. Consumption of quercetin and kaempferol in free-living subjects eating a variety of diets. Cancer Lett. 1997; 114:114–141.

11. Johannot L, Somerset SM. Age-related variations in flavonoid intake and sources in the Australian population. *Public Health Nutrition*. 2006; 9:1045–1054. [PubMed: 17125569]
12. Kim H-Y, 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. *Journal of the American College of Nutrition*. 2003; 22:217–223. [PubMed: 12805248]
13. Arango D, Morohashi K, Yilmaz A, Kuramochi K, Parihar A, Brahimaj B, Grotewold E, Doseff AI. Molecular basis for the action of a dietary flavonoid revealed by the comprehensive identification of apigenin human targets. *Proc Natl Acad Sci USA*. 2013; 110:E2153–62. [PubMed: 23697369]
14. McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita* L.). *Phytother Res*. 2006; 20:519–530. [PubMed: 16628544]
15. Shukla S, Gupta S. Apigenin: a promising molecule for cancer prevention. *Pharm Res*. 2010; 27:962–978. [PubMed: 20306120]
16. Sung B, Chung HY, Kim ND. Role of Apigenin in Cancer Prevention via the Induction of Apoptosis and Autophagy. *J Cancer Prev*. 2016; 21:216–226. [PubMed: 28053955]
17. Nielsen SE, Young JF, Daneshvar B, Lauridsen ST, Knuthsen P, Sandström B, Dragsted LO. Effect of parsley (*Petroselinum crispum*) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. *British Journal of Nutrition*. 1999; 81:447–455. [PubMed: 10615220]
18. Surh YJ. Cancer chemoprevention with dietary phytochemicals. *Nature Rev Cancer*. 2003; 3:768–780. [PubMed: 14570043]
19. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annual Review of Nutrition*. 2001; 21:381–406.
20. 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; 66:2075–2088. [PubMed: 14609732]
21. Thiery-Vuillemain A, Nguyen T, Pivot X, Spano JP, Dufresne A, Soria JC. Molecularly targeted agents: Their promise as cancer chemopreventive interventions. *European J Cancer*. 2005; 41:2003–2015. [PubMed: 16098739]
22. 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; 130:2243–2250. [PubMed: 10958819]
23. Janssen K, Mensink R, Cox F, Harryvan J, Hovenier R, Hollman P, Katan M. Effects of the flavonoids quercetin and apigenin on hemostasis in healthy volunteers: Results from an in vitro and a dietary supplement study. *Amer J Clin Nutr*. 1998; 67:255–262. [PubMed: 9459373]
24. Graf J. Herbal anti-inflammatory agents for skin disease. *Skin Therapy Lett*. 2000; 5:3–5.
25. 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. [PubMed: 15388333]
26. Menichincheri M, Ballinari D, Bargiotti A, Bonomini L, Ceccarelli W, D’Alessio R, Fretta A, Moll J, Polucci P, Soncini C, Tibolla M, Trosset JY, Vanotti E. Catecholic flavonoids acting as telomerase inhibitors. *J Med Chem*. 2004; 47:6466–6475. [PubMed: 15588081]
27. 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; 280:5636–5645. [PubMed: 15533929]
28. Kim MH. Flavonoids inhibit VEGF/bFGF-induced angiogenesis in vitro by inhibiting the matrix-degrading proteases. *J Cell Biochem*. 2003; 89:529–538. [PubMed: 12761886]
29. Way TD, Kao MC, Lin JK. Apigenin induces apoptosis through proteasomal degradation of HER2/neu in HER2/neu-overexpressing breast cancer cells via the phosphatidylinositol 3-kinase/Akt-dependent pathway. *J Biol Chem*. 2004; 279:4479–4489. [PubMed: 14602723]
30. 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; 19:945–952. [PubMed: 2084026]

31. Knekt P, Järvinen R, Seppänen R, Hellövaara M, Teppo L, Pukkala E, Aromaa A. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *Am J Epidemiol.* 1997; 146:223–230. [PubMed: 9247006]
32. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary flavonoids and cancer risk in the Zutphen Elderly Study. *Nutr Cancer.* 1994; 22:175–184. [PubMed: 14502846]
33. Rossi M, Negri E, Lagiou P, Talamini R, Dal Maso L, Montella M, Franceschi S, La Vecchia C. Flavonoids and ovarian cancer risk: A case-control study in Italy. *Int J Cancer.* 2008; 123:895–898. [PubMed: 18491402]
34. Bosetti C, Sperti L, Parpinel M, Gnagnarella P, Lagiou P, Negri E, Franceschi S, Montella M, Peterson J, Dwyer J, Giacosa A, La Vecchia C. Flavonoids and breast cancer risk in Italy. *Cancer Epidemiol Biomarkers Prev.* 2005; 14:805–808. [PubMed: 15824147]
35. 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. [PubMed: 14502846]
36. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, Hakulinen T, Aromaa A. Flavonoid intake and risk of chronic diseases. *Am J Clin Nutr.* 2002; 76:560–568. [PubMed: 12198000]
37. Hoensch H, Grob B, Edler L, Kirch W. Prospective cohort comparison of flavonoid treatment in patients with resected colorectal cancer to prevent recurrence. *World J Gastroenterol.* 2008; 14:2187–2193. [PubMed: 18407592]
38. Gates MA, Tworoger SS, Hecht JL, De Vivo I, Rosner B, Hankinson SE. A prospective study of dietary flavonoid intake and incidence of epithelial ovarian cancer. *Int J Cancer.* 2007; 121:2225–2232. [PubMed: 17471564]
39. Gates MA, Vitonis AF, Tworoger SS, Rosner B, Titus-Ernstoff L, Hankinson SE, Cramer DW. Flavonoid intake and ovarian cancer risk in a population-based case-control study. *Int J Cancer.* 2009; 124:1918–1925. [PubMed: 19117058]
40. Way TD, Kao MC, Lin JK. Apigenin induces apoptosis through proteasomal degradation of HER2/neu in HER2/neu-overexpressing breast cancer cells via the phosphatidylinositol 3-kinase/Akt-dependent pathway. *J Biol Chem.* 2004; 279:4479–4489. [PubMed: 14602723]
41. 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; 579:145–152. [PubMed: 15620704]
42. Weldon CB, McKee A, Collins-Burow BM, Melnik LI, Scandurro AB, McLachlan JA, Burow ME, Beckman BS. PKC-mediated survival signaling in breast carcinoma cells: a role for MEK1-AP1 signaling. *Int J Oncol.* 2005; 26:763–768. [PubMed: 15703835]
43. Cao X, Liu B, Cao W, Zhang W, Zhang F, Zhao H, Niu R. Autophagy inhibition enhances apigenin-induced apoptosis in human breast cancer cells. *Chin J Cancer Res.* 2013; 25:212–222. [PubMed: 23592903]
44. Harrison ME, Power Coombs MR, Delaney LM, Hoskin DW. Exposure of breast cancer cells to a subcytotoxic dose of apigenin causes growth inhibition, oxidative stress, and hypophosphorylation of Akt. *Exp Mol Pathol.* 2014; 97:211–217. [PubMed: 25019465]
45. Bai H, Jin H, Yang F, Zhu H, Cai J. Apigenin induced MCF-7 cell apoptosis-associated reactive oxygen species. *Scanning.* 2014; 36:622–631. [PubMed: 25327419]
46. Lin CH, Chang CY, Lee KR, Lin HJ, Chen TH, Wan L. Flavones inhibit breast cancer proliferation through the Akt/FOXO3a signaling pathway. *BMC Cancer.* 2015; 15:958. [PubMed: 26675309]
47. 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; 39:139–147. [PubMed: 11588896]
48. Yin F, Giuliano AE, Law RE, Van Herle AJ. Apigenin inhibits growth and induces G2/M arrest by modulating cyclin-cCDK regulators and ERK MAP kinase activation in breast carcinoma cells. *Anticancer Res.* 2001; 21:413–420. [PubMed: 11299771]
49. Wang C, Kurzer MS. Phytoestrogen concentration determines effects on DNA synthesis in human breast cancer cells. *Nutr Cancer.* 1997; 28:236–247. [PubMed: 9343831]
50. 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. [PubMed: 9770719]

51. Collins-Burow B, Burow M, Duong B, McLachlan J. Estrogenic and antiestrogenic activities of flavonoid phytochemicals through estrogen receptor binding-dependent and -independent mechanisms. *Nutr Cancer*. 2001; 38:229–44.
52. Zhang S, Yang X, Morris ME. Combined effects of multiple Flavonoids on breast cancer resistance protein (ABCG2)-mediated transport. *Pharm Res*. 2004; 21:1263–1273. [PubMed: 15290869]
53. 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 and Chem Toxicol*. 2004; 42:887–897. [PubMed: 15110097]
54. Seo HS, DeNardo DG, Jacquot Y, Laios I, Vidal DS, Zambrana CR, Brown PH. Stimulatory effect of genistein and apigenin on the growth of breast cancer cells correlates with their ability to activate ER alpha. *Breast Cancer Res Treatment*. 2006; 99:121–134.
55. 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; 221:372–383. [PubMed: 17482226]
56. Seo HS, Ku JM, Choi HS, Woo JK, Jang BH, Shin YC, Ko SG. Induction of Caspase-dependent Apoptosis by Apigenin by inhibiting STAT3 signaling in HER2-overexpressing MDA-MB-453 breast cancer cells. *Anticancer Res*. 2014; 34:2869–2882. [PubMed: 24922650]
57. Seo HS, Jo JK, Ku JM, Choi HS, Choi YK, Woo JK, Kim HI, Kang SY, Lee KM, Nam KW, Park N, Jang BH, Shin YC, Ko SG. Induction of caspase-dependent extrinsic apoptosis by apigenin through inhibition of signal transducer and activator of transcription 3 (STAT3) signalling in HER2-overexpressing BT-474 breast cancer cells. *Biosci Rep*. 2015; 35(6)
58. Scherbakov AM, Andreeva OE. Apigenin Inhibits Growth of Breast Cancer Cells: The Role of ER $\alpha$  and HER2/neu. *Acta Naturae*. 2015; 7:133–139. [PubMed: 26483970]
59. Huang C, Wei YX, Shen MC, Tu YH, Wang CC, Huang HC. Chrysin, Abundant in Morinda citrifolia Fruit Water-EtOAc Extracts, Combined with Apigenin Synergistically Induced Apoptosis and Inhibited Migration in Human Breast and Liver Cancer Cells. *J Agric Food Chem*. 2016; 64:4235–4245. [PubMed: 27137679]
60. 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; 280:5636–5645. [PubMed: 15533929]
61. Arango D, Morohashi K, Yilmaz A, Kuramochi K, Parihar A, Brahimaj B, Grotewold E, Doseff AI. Molecular basis for the action of a dietary flavonoid revealed by the comprehensive identification of apigenin human targets. *Proc Natl Acad Sci U S A*. 2013; 110:E2153–2162. [PubMed: 23697369]
62. Spoerlein C, Mahal K, Schmidt H, Schobert R. Effects of chrysin, apigenin, genistein and their homoleptic copper(II) complexes on the growth and metastatic potential of cancer cells. *J Inorg Biochem*. 2013; 127:107–115. [PubMed: 23973682]
63. Tseng TH, Chien MH, Lin WL, Wen YC, Chow JM, Chen CK, Kuo TC, Lee WJ. Inhibition of MDA-MB-231 breast cancer cell proliferation and tumor growth by apigenin through induction of G2/M arrest and histone H3 acetylation-mediated p21(WAF1/CIP1) expression. *Environ Toxicol*. 2017; 32:434–444. [PubMed: 26872304]
64. Coombs MR, Harrison ME, Hoskin DW. Apigenin inhibits the inducible expression of programmed death ligand 1 by human and mouse mammary carcinoma cells. *Cancer Lett*. 2016; 380:424–433. [PubMed: 27378243]
65. 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. *Molecular Carcinogenesis*. 2000; 28:102–110. [PubMed: 10900467]
66. 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. *Nut Cancer*. 2004; 48:106–114.
67. Plaumann B, Fritzsche M, Rimpler H, Brandner G, Hess R. Flavonoids activate wild-type p53. *Oncogene*. 1996; 13:1605–1614. [PubMed: 8895505]

68. 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; 26:1629–1637. [PubMed: 15870879]
69. 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. *Amer J Physiol - Gastrointestinal and Liver Physiology.* 2003; 285:G919–G928.
70. 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–3804S. [PubMed: 14608117]
71. 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; 54:243–251. [PubMed: 16898869]
72. Svehliková V, Bennett RN, Mellon FA, Needs PW, Piacente S, Kroon PA, Bao Y. Isolation, identification and stability of acylated derivatives of apigenin 7-O-glucoside from chamomile (*Chamomilla recutita* [L.] Rauschert). *Phytochemistry.* 2004; 65:2323–2332. [PubMed: 15381003]
73. Svehlikova V, Wang S, Jakubikova J, Williamson G, Mithen R, Bao Y. Interactions between sulforaphane and apigenin in the induction of UGT1A1 and GSTA1 in CaCo-2 cells. *Carcinogenesis.* 2004; 25:1629–1637. [PubMed: 15090468]
74. 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; 58:816–825. [PubMed: 16552572]
75. Horinaka M, Yoshida T, Shiraishi T, Nakata S, Wakada M, Sakai T. The dietary flavonoid apigenin sensitizes malignant tumor cells to tumor necrosis factor-related apoptosis-inducing ligand. *Mol Cancer Ther.* 2006; 5:945–951. [PubMed: 16648565]
76. Chung CS, Jiang Y, Cheng D, Birt DF. Impact of adenomatous polyposis coli (APC) tumor suppressor gene in human colon cancer cell lines on cell cycle arrest by apigenin. *Molecular Carcinogenesis.* 2007; 46:773–782. [PubMed: 17620292]
77. Leonardi T, Vanamala J, Taddeo SS, Davidson LA, Murphy ME, Patil BS, Wang N, Carroll RJ, Chapkin RS, Lupton JR, Turner ND. Apigenin and naringenin suppress colon carcinogenesis through the aberrant crypt stage in azoxymethane-treated rats. *Exp Biol Med.* 2010; 235:710–717.
78. Zhong Y, Krisanapun C, Lee SH, Nuansanit T, Sams C, Peungvicha P, Baek SJ. Molecular targets of apigenin in colorectal cancer cells: involvement of p21, NAG-1 and p53. *Eur J Cancer.* 2010; 46:3365–3374. [PubMed: 20709524]
79. Turktekin M, Konac E, Onen HI, Alp E, Yilmaz A, Menevse S. Evaluation of the effects of the flavonoid apigenin on apoptotic pathway gene expression on the colon cancer cell line (HT29). *J Med Food.* 2011; 14:1107–1117. [PubMed: 21548803]
80. Lee Y, Sung B, Kang YJ, Kim DH, Jang JY, Hwang SY, Kim M, Lim HS, Yoon JH, Chung HY, Kim ND. Apigenin-induced apoptosis is enhanced by inhibition of autophagy formation in HCT116 human colon cancer cells. *Int J Oncol.* 2014; 44:1599–1606. [PubMed: 24626522]
81. Lefort EC, Blay J. The dietary flavonoid apigenin enhances the activities of the anti-metastatic protein CD26 on human colon carcinoma cells. *Clin Exp Metastasis.* 2011; 28:337–349. [PubMed: 21298326]
82. Shao H, Jing K, Mahmoud E, Huang H, Fang X, Yu C. Apigenin sensitizes colon cancer cells to antitumor activity of ABT-263. *Mol Cancer Ther.* 2013; 12:2640–2650. [PubMed: 24126433]
83. Wang QR, Yao XQ, Wen G, Fan Q, Li YJ, Fu XQ, Li CK, Sun XG. Apigenin suppresses the growth of colorectal cancer xenografts via phosphorylation and up-regulated FADD expression. *Oncol Lett.* 2011; 2:43–47. [PubMed: 22870126]
84. Chunhua L, Donglan L, Xiuqiong F, Lihua Z, Qin F, Yawei L, Liang Z, Ge W, Linlin J, Ping Z, Kun L, Xuegang S. Apigenin up-regulates transgelin and inhibits invasion and migration of colorectal cancer through decreased phosphorylation of AKT. *J Nutr Biochem.* 2013; 24:1766–75. [PubMed: 23773626]
85. Wu K, Yuan L, Xia W. Inhibitory effects of apigenin on the growth of gastric carcinoma SGC-7901 cells. *World J Gastroenterol.* 2005; 11:4461–4464. [PubMed: 16052672]

86. Wang YC. Medicinal plant activity on *Helicobacter pylori* related diseases. *World J Gastroenterol.* 2014; 20:10368–10382. [PubMed: 25132753]
87. Chen J, Chen J, Li Z, Liu C, Yin L. The apoptotic effect of apigenin on human gastric carcinoma cells through mitochondrial signal pathway. *Tumour Biol.* 2014; 35:7719–7726. [PubMed: 24805829]
88. Eaton EA, Walle UK, Lewis AJ, Hudson T, Wilson AA, Walle T. Flavonoids, potent inhibitors of the human p-form phenolsulfotransferase. Potential role in drug metabolism and chemoprevention. *Drug Metabol Disp.* 1996; 24:232–237.
89. Wätjen W, Weber N, Lou YJ, Wang ZQ, Chovolou Y, Kampkötter A, Kahl R, Proksch P. Prenylation enhances cytotoxicity of apigenin and liquiritigenin in rat H4IE hepatoma and C6 glioma cells. *Food Chem Toxicol.* 2007; 45:119–124. [PubMed: 17045382]
90. Yee SB, Lee JH, Chung HY, Im KS, Bae SJ, Choi JS, Kim ND. Inhibitory effects of luteolin isolated from *ixeris sonchifolia* hance on the proliferation of hepg2 human hepatocellular carcinoma cells. *Arch Pharm Res.* 2003; 26:151–156. [PubMed: 12643593]
91. Jeyabal PVS, 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. *Molecular Carcinogenesis.* 2005; 44:11–20. [PubMed: 15924350]
92. Chiang LC, Ng LT, Lin IC, Kuo PL, Lin CC. Anti-proliferative effect of apigenin and its apoptotic induction in human Hep G2 cells. *Cancer Lett.* 2006; 237:207–214. [PubMed: 16023288]
93. Choi SI, Jeong CS, Cho SY, Lee YS. Mechanism of apoptosis induced by apigenin in HepG2 human hepatoma cells: involvement of reactive oxygen species generated by NADPH oxidase. *Arch Pharm Res.* 2007; 30:1328–1335. [PubMed: 18038912]
94. Cai J, Zhao XL, Liu AW, Nian H, Zhang SH. Apigenin inhibits hepatoma cell growth through alteration of gene expression patterns. *Phytomedicine.* 2011; 18:366–373. [PubMed: 20850954]
95. Gao AM, Ke ZP, Wang JN, Yang JY, Chen SY, Chen H. Apigenin sensitizes doxorubicin-resistant hepatocellular carcinoma BEL-7402/ADM cells to doxorubicin via inhibiting PI3K/Akt/Nrf2 pathway. *Carcinogenesis.* 2013; 34:1806–1814. [PubMed: 23563091]
96. Hu XY, Liang JY, Guo XJ, Liu L, Guo YB. 5-Fluorouracil combined with apigenin enhances anticancer activity through mitochondrial membrane potential ( $\Psi_m$ )-mediated apoptosis in hepatocellular carcinoma. *Clin. Exp. Pharmacol. Physiol.* 2015; 42:146–153.
97. Li S, Yang LJ, Wang P, He YJ, Huang JM, Liu HW, Shen XF, Wang F. Dietary apigenin potentiates the inhibitory effect of interferon- $\alpha$  on cancer cell viability through inhibition of 26S proteasome-mediated interferon receptor degradation. *Food Nutr Res.* 2016; 60:31288. [PubMed: 27356910]
98. Liu LZ, Fang J, Zhou Q, Hu X, Shi X, Jiang BH. Apigenin inhibits expression of vascular endothelial growth factor and angiogenesis in human lung cancer cells: implication of chemoprevention of lung cancer. *Mol Pharmacol.* 2005; 68:635–43. [PubMed: 15947208]
99. 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.* 2007; 48:45–50. [PubMed: 17132915]
100. Engelmann C, Blot E, Panis Y, Bauer S, Trochon V, Nagy HJ, Lu H, Soria C. Apigenin—strong cytostatic and anti-angiogenic action in vitro contrasted by lack of efficacy in vivo. *Phytomedicine.* 2002; 9:489–495. [PubMed: 12403156]
101. Piantelli M, Rossi C, Iezzi M, La Sorda R, Iacobelli S, Alberti S, Natali PG. Flavonoids inhibit melanoma lung metastasis by impairing tumor cells endothelium interactions. *J Cell Physiol.* 2006; 207:23–29. [PubMed: 16222712]
102. Lu HF, Chie YJ, Yang MS, Lee CS, Fu JJ, Yang JS, Tan TW, Wu SH, Ma YS, Ip SW, Chung JG. Apigenin induces caspase-dependent apoptosis in human lung cancer A549 cells through Bax- and Bcl-2-triggered mitochondrial pathway. *Int J Oncol.* 2010; 36:1477–1484. [PubMed: 20428772]
103. Lu HF, Chie YJ, Yang MS, Lu KW, Fu JJ, Yang JS, Chen HY, Hsia TC, Ma CY, Ip SW, Chung JG. Apigenin induces apoptosis in human lung cancer H460 cells through caspase- and mitochondria-dependent pathways. *Hum Exp Toxicol.* 2011; 30:1053–1061. [PubMed: 20937639]

104. Das S, Das J, Samadder A, Boujedaini N, Khuda-Bukhsh AR. Apigenin-induced apoptosis in A375 and A549 cells through selective action and dysfunction of mitochondria. *Exp Biol Med*. 2012; 237:1433–1448.
105. Bruno A, Siena L, Gerbino S, Ferraro M, Chanez P, Giammanco M, Gjomarkaj M, Pace E. Apigenin affects leptin/leptin receptor pathway and induces cell apoptosis in lung adenocarcinoma cell line. *Eur J Cancer*. 2011; 47:2042–2051. [PubMed: 21550230]
106. Choudhury D, Ganguli A, Dastidar DG, Acharya BR, Das A, Chakrabarti G. Apigenin shows synergistic anticancer activity with curcumin by binding at different sites of tubulin. *Biochimie*. 2013; 95:1297–1309. [PubMed: 23485682]
107. Kim KC, Choi EH, Lee C. Axl receptor tyrosine kinase is a novel target of apigenin for the inhibition of cell proliferation. *Int J Mol Med*. 2014; 34:592–598. [PubMed: 24926787]
108. Lee YM, Lee G, Oh TI, Kim BM, Shim DW, Lee KH, Kim YJ, Lim BO, Lim JH. Inhibition of glutamine utilization sensitizes lung cancer cells to apigenin-induced apoptosis resulting from metabolic and oxidative stress. *Int J Oncol*. 2016; 48:399–408. [PubMed: 26573871]
109. Chen M, Wang X, Zha D, Cai F, Zhang W, He Y, Huang Q, Zhuang H, Hua ZC. Apigenin potentiates TRAIL therapy of non-small cell lung cancer via upregulating DR4/DR5 expression in a p53-dependent manner. *Sci Rep*. 2016; 6:35468. [PubMed: 27752089]
110. Ujiki MB, Ding XZ, Salabat MR, Bentrem DJ, Golkar L, Milam B, Talamonti MS, Bell RH Jr, Iwamura T, Adrian TE. Apigenin inhibits pancreatic cancer cell through G2/M cell cycle arrest. *Mol Cancer*. 2006; 5:76. [PubMed: 17196098]
111. Lee S, Ryu J, Lee K, Woo S, Park J, Yoo J, Yoon Y. Enhanced anti-tumor effect of combination therapy with gemcitabine and apigenin in pancreatic cancer. *Cancer Lett*. 2008; 259:39–49. [PubMed: 17967505]
112. Strouch MJ, Milam BM, Melstrom LG, McGill JJ, Salabat MR, Ujiki MB, Ding XZ, Bentrem DJ. The flavonoid apigenin potentiates the growth inhibitory effects of gemcitabine and abrogates gemcitabine resistance in human pancreatic cancer cells. *Pancreas*. 2009; 38:409–415. [PubMed: 19142175]
113. Wu DG, Yu P, Li JW, Jiang P, Sun J, Wang HZ, Zhang LD, Wen MB, Bie P. Apigenin potentiates the growth inhibitory effects by IKK- $\beta$ -mediated NF- $\kappa$ B activation in pancreatic cancer cells. *Toxicol Lett*. 2014; 224:157–164. [PubMed: 24148603]
114. Melstrom LG, Salabat MR, Ding XZ, Strouch MJ, Grippo PJ, Mirzoeva S, Pelling JC, Bentrem DJ. Apigenin down-regulates the hypoxia response genes: HIF-1 $\alpha$ , GLUT-1, and VEGF in human pancreatic cancer cells. *J Surg Res*. 2011; 167:173–181. [PubMed: 21227456]
115. King JC, Lu QY, Li G, Moro A, Takahashi H, Chen M, Go VL, Reber HA, Eibl G, Hines OJ. Evidence for activation of mutated p53 by apigenin in human pancreatic cancer. *Biochim Biophys Acta*. 2012; 1823:593–604. [PubMed: 22227579]
116. Pham H, Chen M, Takahashi H, King J, Reber HA, Hines OJ, Pandol S, Eibl G. Apigenin inhibits NNK-induced focal adhesion kinase activation in pancreatic cancer cells. *Pancreas*. 2012; 41:1306–1315. [PubMed: 22889981]
117. Johnson JL, Gonzalez de Mejia E. Interactions between dietary flavonoids apigenin or luteolin and chemotherapeutic drugs to potentiate anti-proliferative effect on human pancreatic cancer cells, in vitro. *Food Chem Toxicol*. 2013; 60:83–91. [PubMed: 23871783]
118. Nelson N, Szekeres K, Iclozan C, Rivera IO, McGill A, Johnson G, Nwogu O, Ghansah T. Apigenin: Selective CK2 inhibitor increases Ikaros expression and improves T cell homeostasis and function in murine pancreatic cancer. *PLoS One*. 2017; 12:e0170197. [PubMed: 28152014]
119. Knowles LM, Zigrossi DA, Tauber RA, Hightower C, Milner JA. Flavonoids suppress androgen-independent human prostate tumor proliferation. *Nutr Cancer*. 2000; 38:116–122. [PubMed: 11341036]
120. 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; 18:1117–1121. [PubMed: 9615775]
121. 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; 63:131–142. [PubMed: 15486995]

122. Hessenauer A, Montenarh M, Götz C. Inhibition of CK2 activity provokes different responses in hormone-sensitive and hormone-refractory prostate cancer cells. *Int J Oncol.* 2003; 22:1263–1270. [PubMed: 12738992]
123. 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; 287:914–920. [PubMed: 11573952]
124. 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; 21:3727–3738. [PubMed: 12032841]
125. 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; 39:114–126. [PubMed: 14750216]
126. 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; 10:3169–3178. [PubMed: 15131058]
127. Shukla S, Kanwal R, Shankar E, Datt M, Chance MR, Fu P, Gupta S. Apigenin blocks IKK $\alpha$  activation and suppresses prostate cancer progression. *Oncotarget.* 2015; 6:31216–31232. [PubMed: 26435478]
128. Shukla S, Mishra A, Fu P, MacLennan G, Resnick M, 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; 19:2042–2044. [PubMed: 16230333]
129. Shukla S, Gupta S. Molecular targets for apigenin-induced cell cycle arrest and apoptosis in prostate cancer cell xenograft. *Mol Cancer Ther.* 2006; 5:843–852. [PubMed: 16648554]
130. 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; 6:1102–1114. [PubMed: 17457054]
131. Shukla S, MacLennan GT, Flask CA, Fu P, Mishra A, Resnick MI, Gupta S. Blockade of beta-Catenin signaling by plant Flavonoid Apigenin suppresses prostate Carcinogenesis in TRAMP mice. *Cancer Res.* 2007; 67:6925–6935. [PubMed: 17638904]
132. Pandey M, Kaur P, Shukla S, Abbas A, Fu P, Gupta S. Plant flavone apigenin inhibits HDAC and remodels chromatin to induce growth arrest and apoptosis in human prostate cancer cells: in vitro and in vivo study. *Mol Carcinog.* 2012; 51:952–962. [PubMed: 22006862]
133. Kanwal R, Datt M, Liu X, Gupta S. Dietary Flavones as Dual Inhibitors of DNA Methyltransferases and Histone Methyltransferases. *PLoS One.* 2016; 11:e0162956. [PubMed: 27658199]
134. Shukla S, MacLennan GT, Fu P, Gupta S. Apigenin attenuates insulin-like growth factor-I signaling in an autochthonous mouse prostate cancer model. *Pharm Res.* 2012; 29:1506–1517. [PubMed: 22139534]
135. Shukla S, Bhaskaran N, Babcock MA, Fu P, MacLennan GT, Gupta S. Apigenin inhibits prostate cancer progression in TRAMP mice via targeting PI3K/Akt/FoxO pathway. *Carcinogenesis.* 2014; 35:452–460. [PubMed: 24067903]
136. Erdogan S, Turkekul K, Serttas R, Erdogan Z. The natural flavonoid apigenin sensitizes human CD44(+) prostate cancer stem cells to cisplatin therapy. *Biomed Pharmacother.* 2017; 88:210–217. [PubMed: 28107698]
137. Shukla S, Fu P, Gupta S. Apigenin induces apoptosis by targeting inhibitor of apoptosis proteins and Ku70-Bax interaction in prostate cancer. *Apoptosis.* 2014; 19:883–894. [PubMed: 24563225]
138. Sharma H, Kanwal R, Bhaskaran N, Gupta S. Plant Flavone Apigenin binds to nucleic acid bases and reduces oxidative DNA damage in prostate epithelial cells. *PLoS ONE.* 2014; 9:e91588. [PubMed: 24614817]
139. 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. [PubMed: 9066634]

140. Lepley DM, Li B, Birt DF, Pelling JC. The chemopreventive flavonoid apigenin induces G2/M arrest in keratinocytes. *Carcinogenesis*. 1996; 17:2367–2375. [PubMed: 8968050]
141. 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; 21:633–639. [PubMed: 10753197]
142. 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; 13:1530–1534. [PubMed: 8899846]
143. 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; 13:1710–1715. [PubMed: 8956339]
144. 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*. 2006; 27:283–296. [PubMed: 17074806]
145. 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; 46:303–314. [PubMed: 17186551]
146. Caltagirone S, Rossi C, Poggi A, Ranelletti FO, Natali PG, Brunetti M, Piantelli M. Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential. *Int J Cancer*. 2000; 87:595–600. [PubMed: 10918203]
147. Abu-Yousif AO, Smith KA, Getsios S, Green KJ, Van Dross RT, Pelling JC. Enhancement of UVB-induced apoptosis by apigenin in human keratinocytes and organotypic keratinocyte cultures. *Cancer Res*. 2008; 68:3057–3065. [PubMed: 18413777]
148. Hwang YP, Oh KN, Yun HJ, Jeong HG. The flavonoids apigenin and luteolin suppress ultraviolet A-induced matrix metalloproteinase-1 expression via MAPKs and AP-1-dependent signaling in HaCaT cells. *J Dermatol Sci*. 2011; 61:23–31. [PubMed: 21112745]
149. Pawlikowska-Pawl ga B, Misiak LE, Zarzyka B, Paduch R, Gawron A, Gruszecki WI. FTIR, <sup>1</sup>H NMR and EPR spectroscopy studies on the interaction of flavone apigenin with dipalmitoylphosphatidylcholine liposomes. *Biochim Biophys Acta*. 2013; 1828:518–527. [PubMed: 23088916]
150. Byun S, Park J, Lee E, Lim S, Yu JG, Lee SJ, Chen H, Dong Z, Lee KW, Lee HJ. Src kinase is a direct target of apigenin against UVB-induced skin inflammation. *Carcinogenesis*. 2013; 34:397–405. [PubMed: 23161610]
151. Paredes-Gonzalez X, Fuentes F, Su ZY, Kong AN. Apigenin reactivates Nrf2 anti-oxidative stress signaling in mouse skin epidermal JB6 P+ cells through epigenetics modifications. *AAPS J*. 2014; 16:727–735. [PubMed: 24830944]
152. Tong X, Mirzoeva S, Veliceasa D, Bridgeman BB, Fitchev P, Cornwell ML, Crawford SE, Pelling JC, Volpert OV. Chemopreventive apigenin controls UVB-induced cutaneous proliferation and angiogenesis through HuR and thrombospondin-1. *Oncotarget*. 2014; 5:11413–11427. [PubMed: 25526033]
153. Kiraly AJ, Soliman E, Jenkins A, Van Dross RT. Apigenin inhibits COX-2, PGE2, and EP1 and also initiates terminal differentiation in the epidermis of tumor bearing mice. *Prostaglandins Leukot Essent Fatty Acids*. 2016; 104:44–53. [PubMed: 26802941]
154. Bridgeman BB, Wang P, Ye B, Pelling JC, Volpert OV, Tong X. Inhibition of mTOR by apigenin in UVB-irradiated keratinocytes: A new implication of skin cancer prevention. *Cell Signal*. 2016; 28:460–468. [PubMed: 26876613]
155. Silvan S, Manoharan S, Baskaran N, Anusuya C, Karthikeyan S, Prabhakar MM. Chemopreventive potential of apigenin in 7,12-dimethylbenz(a)anthracene induced experimental oral carcinogenesis. *Eur J Pharmacol*. 2011; 670:571–577. [PubMed: 21970806]
156. 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. [PubMed: 2105157]

157. Mafuvadze B, Liang Y, Besch-Williford C, Zhang X, Hyder SM. Apigenin induces apoptosis and blocks growth of medroxyprogesterone acetate-dependent BT-474 xenograft tumors. *Horm Cancer*. 2012; 3:160–171. [PubMed: 22569706]
158. Zheng PW, Chiang LC, Lin CC. Apigenin induced apoptosis through p53-dependent pathway in human cervical carcinoma cells. *Life Sci*. 2005; 76:1367–1379. [PubMed: 15670616]
159. Czyz J, Madeja Z, Irmer U, Korohoda W, Hülser DF. Flavonoid apigenin inhibits motility and invasiveness of carcinoma cells in vitro. *Int J Cancer*. 2005; 114:12–18. [PubMed: 15523693]
160. Wu C, Chen F, Rushing JW, Wang X, Kim HJ, Huang G, He G. Antiproliferative activities of Parthenolide and Golden Feverfew extract against Three human cancer cell lines. *J Med Food*. 2006; 9:55–61. [PubMed: 16579729]
161. Liu J, Cao XC, Xiao Q, Quan MF. Apigenin inhibits HeLa sphere-forming cells through inactivation of casein kinase 2α. *Mol Med Rep*. 2015; 11:665–669. [PubMed: 25334018]
162. Souza RP, Bonfim-Mendonça PS, Gimenes F, Ratti BA, Kaplum V, Bruschi ML, Nakamura CV, Silva SO, Maria-Engler SS, Consolaro ME. Oxidative Stress Triggered by Apigenin Induces Apoptosis in a Comprehensive Panel of Human Cervical Cancer-Derived Cell Lines. *Oxid Med Cell Longev*. 2017;1512745. [PubMed: 28191273]
163. Li S, Yang LJ, Wang P, He YJ, Huang JM, Liu HW, Shen XF, Wang F. Dietary apigenin potentiates the inhibitory effect of interferon- $\alpha$  on cancer cell viability through inhibition of 26S proteasome-mediated interferon receptor degradation. *Food Nutr Res*. 2016; 60:31288. [PubMed: 27356910]
164. 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 Medica*. 2005; 71:435–439. [PubMed: 15931582]
165. Fang J. Apigenin inhibits VEGF and HIF-1 expression via PI3K/AKT/p70S6K1 and HDM2/p53 pathways. *FASEB Journal*. 2005; 19:342–353. [PubMed: 15746177]
166. 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; 19:342–353. [PubMed: 15746177]
167. Fang J, Zhou Q, Liu LZ, Xia C, Hu X, Shi X, Jiang BH. Apigenin inhibits tumor angiogenesis through decreasing HIF-1alpha and VEGF expression. *Carcinogenesis*. 2007; 28:858–856. [PubMed: 17071632]
168. 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; 22:358–362. [PubMed: 12703988]
169. Kanerva A, Raki M, Ranki T, Särkioja M, Koponen J, Desmond RA, Helin A, Stenman UH, Isoniemi H, Höckerstedt K, Ristimäki A, Hemminki A. Chlorpromazine and apigenin reduce adenovirus replication and decrease replication associated toxicity. *J Gene Med*. 2007; 9:3–9. [PubMed: 17149790]
170. Hu XW, Meng D, Fang J. Apigenin inhibited migration and invasion of human ovarian cancer A2780 cells through focal adhesion kinase. *Carcinogenesis*. 2008; 29:2369–2376. [PubMed: 18974065]
171. Li ZD, Hu XW, Wang YT, Fang J. Apigenin inhibits proliferation of ovarian cancer A2780 cells through Id1. *FEBS Lett*. 2009; 583:1999–2003. [PubMed: 19447105]
172. Wong IL, Chan KF, Tsang KH, Lam CY, Zhao Y, Chan TH, Chow LM. Modulation of multidrug resistance protein 1 (MRP1/ABCC1)-mediated multidrug resistance by bivalent apigenin homodimers and their derivatives. *J Med Chem*. 2009; 52:5311–5322. [PubMed: 19725578]
173. Tang AQ, Cao XC, Tian L, He L, Liu F. Apigenin inhibits the self-renewal capacity of human ovarian cancer SKOV3-derived sphere-forming cells. *Mol Med Rep*. 2015; 11:2221–2226. [PubMed: 25405327]
174. He J, Xu Q, Wang M, Li C, Qian X, Shi Z, Liu LZ, Jiang BH. Oral administration of apigenin inhibits metastasis through AKT/P70S6K1/MMP-9 pathway in orthotopic ovarian tumor model. *Int J Mol Sci*. 2012; 13:7271–7282. [PubMed: 22837693]
175. Suh YA, Jo SY, Lee HY, Lee C. Inhibition of IL-6/STAT3 axis and targeting Axl and Tyro3 receptor tyrosine kinases by apigenin circumvent taxol resistance in ovarian cancer cells. *Int J Oncol*. 2015; 46:1405–1411. [PubMed: 25544427]

176. 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; 35:1517–1525. [PubMed: 10673981]
177. 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. [PubMed: 16844095]
178. 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; 69:1421–1432. [PubMed: 15857606]
179. 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; 71:1306–1312. [PubMed: 17485840]
180. 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.
181. Horvathova K, Novotny L, Vachalkova A. The free radical scavenging activity of four flavonoids determined by the comet assay. *Neoplasma*. 2003; 50:291–295. [PubMed: 12937843]
182. 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; 97:4790–4795. [PubMed: 10758153]
183. Hashemi M, Nouri Long M, Entezari M, Nafisi S, Nowroozii H. Anti-mutagenic and pro-apoptotic effects of apigenin on human chronic lymphocytic leukemia cells. *Acta Med Iran*. 2010; 48:283–288. [PubMed: 21287458]
184. Gonzalez-Mejia ME, Voss OH, Murnan EJ, Doseff AI. Apigenin-induced apoptosis of leukemia cells is mediated by a bimodal and differentially regulated residue-specific phosphorylation of heat-shock protein-27. *Cell Death Dis*. 2010; 1:e64. [PubMed: 21364669]
185. Jayasooriya RG, Kang SH, Kang CH, Choi YH, Moon DO, Hyun JW, Chang WY, Kim GY. Apigenin decreases cell viability and telomerase activity in human leukemia cell lines. *Food Chem Toxicol*. 2012; 50:2605–2611. [PubMed: 22617349]
186. Nakazaki E, Tsolmon S, Han J, Isoda H. Proteomic study of granulocytic differentiation induced by apigenin 7-glucoside in human promyelocytic leukemia HL-60 cells. *Eur J Nutr*. 2013; 52:25–35. [PubMed: 22113421]
187. Budhraja A, Gao N, Zhang Z, Son YO, Cheng S, Wang X, Ding S, Hitron A, Chen G, Luo J, Shi X. Apigenin induces apoptosis in human leukemia cells and exhibits anti-leukemic activity in vivo. *Mol Cancer Ther*. 2012; 11:132–142. [PubMed: 22084167]
188. Isoda H, Motojima H, Onaga S, Samet I, Villareal MO, Han J. Analysis of the erythroid differentiation effect of flavonoid apigenin on K562 human chronic leukemia cells. *Chem Biol Interact*. 2014; 220:269–277. [PubMed: 25058688]
189. 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; 82:70–79. [PubMed: 15319488]
190. 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; 80:355–363.
191. 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; 19:4297–4303. [PubMed: 10628390]
192. 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; 21:413–420. [PubMed: 11299771]
193. Schröder-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<sup>+</sup>/I<sup>-</sup> symporter-transfected follicular thyroid carcinoma cell line. *Eur J Endocrinol*. 2004; 150:557–564. [PubMed: 15080787]

194. Kim SH, Kang JG, Kim CS, Ihm SH, Choi MG, Yoo HJ, Lee SJ. Apigenin induces c-Myc-mediated apoptosis in FRO anaplastic thyroid carcinoma cells. *Mol Cell Endocrinol.* 2013; 369:130–139. [PubMed: 23376608]
195. Lakshmanan A, Doseff AI, Ringel MD, Saji M, Rousset B, Zhang X, Jhiang SM. Apigenin in combination with Akt inhibition significantly enhances thyrotropin-stimulated radioiodide accumulation in thyroid cells. *Thyroid.* 2014; 24:878–887. [PubMed: 24400871]
196. Zhang L, Cheng X, Gao Y, Zheng J, Xu Q, Sun Y, Guan H, Yu H, Sun Z. Apigenin induces autophagic cell death in human papillary thyroid carcinoma BCPAP cells. *Food Funct.* 2015; 6:3464–3472. [PubMed: 26292725]
197. Lakshmanan A, Scarberry D, Green JA, Zhang X, Selmi-Ruby S, Jhiang SM. Modulation of thyroidal radioiodide uptake by oncological pipeline inhibitors and apigenin. *Oncotarget.* 2015; 6:31792–31804. [PubMed: 26397139]
198. 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. [PubMed: 15657348]
199. 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; 119:2575–2585. [PubMed: 16988947]
200. Karmakar S, Davis KA, Choudhury SR, Deeca A, Banik NL, Ray SK. Bcl-2 inhibitor and apigenin worked synergistically in human malignant neuroblastoma cell lines and increased apoptosis with activation of extrinsic and intrinsic pathways. *Biochem Biophys Res Commun.* 2009; 388:705–710. [PubMed: 19695221]
201. Mohan N, Banik NL, Ray SK. Combination of N-(4-hydroxyphenyl) retinamide and apigenin suppressed starvation-induced autophagy and promoted apoptosis in malignant neuroblastoma cells. *Neurosci Lett.* 2011; 502:24–29. [PubMed: 21801811]
202. Mohan N, Ai W, Chakrabarti M, Banik NL, Ray SK. KLF4 overexpression and apigenin treatment down regulated anti-apoptotic Bcl-2 proteins and matrix metalloproteinases to control growth of human malignant neuroblastoma SK-N-DZ and IMR-32 cells. *Mol Oncol.* 2013; 7:464–474. [PubMed: 23317647]
203. Chakrabarti M, Banik NL, Ray SK. Sequential hTERT knockdown and apigenin treatment inhibited invasion and proliferation and induced apoptosis in human malignant neuroblastoma SK-N-DZ and SK-N-BE2 cells. *J Mol Neurosci.* 2013; 51:187–198. [PubMed: 23417743]
204. Chakrabarti M, Banik NL, Ray SK. miR-138 overexpression is more powerful than hTERT knockdown to potentiate apigenin for apoptosis in neuroblastoma in vitro and in vivo. *Exp Cell Res.* 2013; 319:1575–1585. [PubMed: 23562653]
205. Hossain MM, Banik NL, Ray SK. N-Myc knockdown and apigenin treatment controlled growth of malignant neuroblastoma cells having N-Myc amplification. *Gene.* 2013; 529:27–36. [PubMed: 23941992]
206. Amini R, Yazdanparast R, Ghaffari SH. Apigenin modulates the expression levels of pro-inflammatory mediators to reduce the human insulin amyloid-induced oxidant damages in SK-N-MC cells. *Hum Exp Toxicol.* 2015; 34:642–653. [PubMed: 25304968]
207. Kim A, Nam YJ, Lee MS, Shin YK, Sohn DS, Lee CS. Apigenin Reduces Proteasome Inhibition-Induced Neuronal Apoptosis by Suppressing the Cell Death Process. *Neurochem Res.* 2016; 41:2969–2980. [PubMed: 27473386]
208. Liu Q, Chen X, Yang G, Min X, Deng M. Apigenin inhibits cell migration through MAPK pathways in human bladder smooth muscle cells. *Biocell.* 2011; 35:71–79. [PubMed: 22423483]
209. Zhu Y, Mao Y, Chen H, Lin Y, Hu Z, Wu J, Xu X, Xu X, Qin J, Xie L. Apigenin promotes apoptosis, inhibits invasion and induces cell cycle arrest of T24 human bladder cancer cells. *Cancer Cell Int.* 2013; 13:54. [PubMed: 23724790]
210. Shi MD, Shiao CK, Lee YC, Shih YW. Apigenin, a dietary flavonoid, inhibits proliferation of human bladder cancer T-24 cells via blocking cell cycle progression and inducing apoptosis. *Cancer Cell Int.* 2015; 15:33. [PubMed: 25859163]
211. Masuelli L, Benvenuto M, Mattera R, Di Stefano E, Zago E, Taffera G, Tresoldi I, Giganti MG, Frajese GV, Berardi G, Modesti A, Bei R. In Vitro and In Vivo Anti-tumoral Effects of the

- Flavonoid Apigenin in Malignant Mesothelioma. *Front Pharmacol.* 2017; 8:373. [PubMed: 28674496]
212. Lin CC, Chuang YJ, Yu CC, Yang JS, Lu CC, Chiang JH, Lin JP, Tang NY, Huang AC, Chung JG. Apigenin induces apoptosis through mitochondrial dysfunction in U-2 OS human osteosarcoma cells and inhibits osteosarcoma xenograft tumor growth in vivo. *J Agric Food Chem.* 2012; 60:11395–11402. [PubMed: 23066961]
213. Bumke-Vogt C, Osterhoff MA, Borchert A, Guzman-Perez V, Sarem Z, Birkenfeld AL, Bähr V, Pfeiffer AF. The flavones apigenin and luteolin induce FOXO1 translocation but inhibit gluconeogenic and lipogenic gene expression in human cells. *PLoS One.* 2014; 9:e104321. [PubMed: 25136826]
214. Liu X, Li L, Lv L, Chen D, Shen L, Xie Z. Apigenin inhibits the proliferation and invasion of osteosarcoma cells by suppressing the Wnt/β-catenin signaling pathway. *Oncol Rep.* 2015; 34:1035–1041. [PubMed: 26035210]
215. Johnson JL, Gonzalez de Mejia E. Interactions between dietary flavonoids apigenin or luteolin and chemotherapeutic drugs to potentiate anti-proliferative effect on human pancreatic cancer cells, *in vitro*. *Food Chem Toxicol.* 2013; 60:83–91. [PubMed: 23871783]
216. Zhao M, Ma J, Zhu HY, Zhang XH, Du ZY, Xu YJ, Yu XD. Apigenin inhibits proliferation and induces apoptosis in human multiple myeloma cells through targeting the trinity of CK2, Cdc37 and Hsp90. *Mol Cancer.* 2011; 10:104. [PubMed: 21871133]
217. Mohan N, Banik NL, Ray SK. Combination of N-(4-hydroxyphenyl) retinamide and apigenin suppressed starvation-induced autophagy and promoted apoptosis in malignant neuroblastoma cells. *Neurosci Lett.* 2011; 502:24–29. [PubMed: 21801811]
218. Karmakar S, Davis KA, Choudhury SR, Deeconda A, Banik NL, Ray SK. Bcl-2 inhibitor and apigenin worked synergistically in human malignant neuroblastoma cell lines and increased apoptosis with activation of extrinsic and intrinsic pathways. *Biochem Biophys Res Commun.* 2009; 388:705–710. [PubMed: 19695221]
219. Xu Y, Xin Y, Diao Y, Lu C, Fu J, Luo L, Yin Z. Synergistic effects of apigenin and paclitaxel on apoptosis of cancer cells. *PLoS One.* 2011; 6(12):e29169. [PubMed: 22216199]
220. Lee SH, Ryu JK, Lee KY, Woo SM, Park JK, Yoo JW, Kim YT, Yoon YB. Enhanced anti-tumor effect of combination therapy with gemcitabine and apigenin in pancreatic cancer. *Cancer Lett.* 2008; 259:39–49. [PubMed: 17967505]
221. Zhu Y, Liu C, Nadiminty N, Lou W, Tummala R, Evans CP, Gao AC. Inhibition of ABCB1 expression overcomes acquired docetaxel resistance in prostate cancer. *Mol Cancer Ther.* 2013; 12:1829–1836. [PubMed: 23861346]
222. Hu XY, Liang JY, Guo XJ, Liu L, Guo YB. 5-Fluorouracil combined with apigenin enhances anticancer activity through mitochondrial membrane potential ( $\Psi_m$ )-mediated apoptosis in hepatocellular carcinoma. *Clin Exp Pharmacol Physiol.* 2015; 42:146–153. [PubMed: 25363523]
223. 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; 14:2187–2193. [PubMed: 18407592]
224. Amsterdam JD, Shults J, Soeller I, Mao JJ, Rockwell K, Newberg AB. Chamomile (Matricaria recutita) may provide antidepressant activity in anxious, depressed humans: an exploratory study. *Altern Ther Health Med.* 2012; 18:44–49.
225. Choi S, Youn J, Kim K, Joo da H, Shin S, Lee J, Lee HK, An IS, Kwon S, Youn HJ, Ahn KJ, An S, Cha HJ. Apigenin inhibits UVA-induced cytotoxicity in vitro and prevents signs of skin aging in vivo. *Int J Mol Med.* 2016; 38:627–634. [PubMed: 27279007]
226. Hollman PC, Katan MB. Health effects and bioavailability of dietary flavonols. *Free Radical Res.* 1999; 31:S75–80. [PubMed: 10694044]
227. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu Rev Nutr.* 2002; 22:19–34. [PubMed: 12055336]
228. Chen J, Lin H, Hu M. Metabolism of flavonoids via enteric recycling: Role of intestinal disposition. *J Pharmacol Exp Ther.* 2003; 304:1228–1235. [PubMed: 12604700]
229. Chen J, Wang S, Jia X, Bajimaya S, Lin H, Tam V, Hu M. Disposition of flavonoids via recycling: Comparison of intestinal versus hepatic disposition. *Drug Metabol Disp.* 2005; 33:1777–1784.

230. Walle UK, Walle T. Induction of human UDP-Glucuronosyltransferase UGT1A1 by Flavonoids—Structural requirements. *Drug Metab Disp.* 2002; 30:564–569.
231. Gradolatto A, Basly JP, Berges R, Teyssier C, Chagnon MC, Siess MH, Canivenc-Lavier MC. Pharmacokinetics and metabolism of apigenin in female and male rats after a single oral administration. *Drug Metabol Dispos.* 2005; 33:49–54.
232. Llevot A, Astruc D. Applications of vectorized gold nanoparticles to the diagnosis and therapy of cancer. *Chem Soc Rev.* 2012; 41:242–257. [PubMed: 21785769]
233. Guo C, Zhu Y, Weng Y, Wang S, Guan Y, Wei G, Yin Y, Xi M, Wen A. Therapeutic time window and underlying therapeutic mechanism of breviscapine injection against cerebral ischemia/reperfusion injury in rats. *J Ethnopharmacol.* 2014; 151:660–666. [PubMed: 24291152]
234. Rajendran, I., Dhandapani, H., Anantharaman, R., Rajaram, R. Apigenin mediated gold nanoparticle synthesis and their anti-cancer effect on human epidermoid carcinoma (A431) cells. 2015. <https://www.researchgate.net/publication/277604036,10.1039/C5RA04303D>
235. Das S, Das J, Samadder A, Paul A, Khuda-Bukhsh AR. Strategic formulation of apigenin-loaded PLGA nanoparticles for intracellular trafficking, DNA targeting and improved therapeutic effects in skin melanoma in vitro. *Toxicol Lett.* 2013; 223:124–138. [PubMed: 24070738]
236. Ding S, Zhang Z, Song J, Cheng X, Jiang J, Jia X. Enhanced bioavailability of apigenin via preparation of a carbon nanopowder solid dispersion. *International journal of nanomedicine.* 2014; 9:2327–33. [PubMed: 24872695]
237. Ding B, Chen H, Wang C, Zhai Y. Preparation and in vitro evaluation of apigenin loaded lipid nanocapsules. *J Nanosci Nanotechnol.* 2013; 13:6546–6552. [PubMed: 24245113]
238. Cochran DB, Gray LN, Anderson KW, Dziubla TD. Degradable poly(apigenin) polymer inhibits tumor cell adhesion to vascular endothelial cells. *J Biomed Mater Res B Appl Biomater.* 2016; 104:1438–1447. [PubMed: 26251070]

**Table 1**

Effect of apigenin on various molecular targets in human cancers

Human Cancers	Molecular Targets	Apigenin concentrations	Ref.
Breast cancer	PD-L1, ErbB2, Bax, Bcl2, p450 CYP1, CYP19, Caspases, p21/Waf1, p53, Aromatase, VEGF, ERK, JNK, PI3K-Akt, Foxo3a, COX-2, ER $\beta$ , CK2, PKC, MAPK, Cyclin D1 and HER2/neu, JAK-STAT3, NF- $\kappa$ B, AP-1, c-Fos, cyclinB1	0–100 $\mu$ M	40–64
Colon Cancer	APC, ODC, TRAIL, COX-2, PGE-2, MMP, GST, UDP-glucuronosyltransferases (UGT)1A1, p34(cdc2), cdc25, caspases -3, -8, -9, Bax, Bcl2, p21/Waf1, mTOR, CD26, JNK, p38, Elk	0–200 $\mu$ M	65–84
Gastric Cancer	NF- $\kappa$ B, cyclinD1, COX-2, VEGF, Bcl2, I $\kappa$ B $\alpha$ , ICAM-1, IL6, IL8, Bax	9–80 $\mu$ mol/L	85–87
Liver cancer	NF- $\kappa$ B, Snail, PIG3, cytochrome c, Bax, Bcl2, caspases -3, -9, Nrf2, PI3K/Akt, cyclin-dependent kinases (cdk)	0–200 $\mu$ mol/L	88–97
Lung Cancer	p21/Waf1, XIAP, uPAR, GLUT1, NADPH, Bax, Bcl2, caspases -3, -9, cytochrome c, leptin, leptin receptor, Bid, AIF, GRP78, GADD153, HIF-1 $\alpha$ , VEGF, Akt and p70S6K1	In vitro: 0–160 $\mu$ M In vivo: 1 mg/kg/day	98–109
Pancreatic Cancer	NF- $\kappa$ B, I $\kappa$ B $\alpha$ , GSK3 $\beta$ , cyclinB1, IL17, IFNB1, p53, p21/Waf1, PUMA, HIF-1 $\alpha$ , VEGF, GLUT1, Cde6, cyclin A, cdc2, cdc25	0–100 $\mu$ M	110–118
Prostate Cancer	FAK, Src, PTEN, IGF1R, IGFBP-3, IGF-1, GSK-3 $\beta$ , Akt, p21/Waf1, p27, VEGF, IKK $\alpha$ , NF- $\kappa$ B, CK2, IAPs, TRAIL, p53, caspases -3, -8, -9, HIF-1, FAK, $\beta$ -Catenin, c-Myc, cyclinD1, cyclin-dependent kinases -2, -4, -6, MAPK, PI3K-Akt, ER $\beta$ , AP-1, Bax, Bcl2, Bcl-XL, 17 $\beta$ -hydroxysteroid oxidoreductase, E-cadherin, GLUT1, HDAC-1, -3, XIAP, Ku70, FoxO3a, ABCB1, FAK, Smad -2, -3, IGF-1, IGFBP3, ERK, H3 and H4 acetylation, BAD, 14–3–3 $\beta$ , p14ARF, MDM2, c-Myc, Rb, RNA polymerase	In vitro: 0–40 $\mu$ M In vivo: 20 & 50 $\mu$ g/mouse/day	119–138
Skin Cancer	PGE2, EP1, EP2, cytochrome c, Bax, Bcl2, caspase -3, -9, PARP, p21/Waf1, COX-2, PKC, STAT3, MMP-2, MMP-9, VEGF, Twist1	In vitro :0–100 $\mu$ M In vivo: 150 mg/day	139–157
Cervical Cancer	CK2 $\alpha$ , Bax, Bcl2, p53, p21, Fas/Apo-1, caspase3	0–75 $\mu$ M	158–163
Endometrial Cancer	Id1, VEGF, p70S6K1, HIF1 and FAK	0–40 $\mu$ mol/L	164
Ovarian Cancer	Axl, tyrosine receptor kinase, Akt, MMP-9, p70S6K1, Id1, ATF3, FAK, VEGF, HIF-1 $\alpha$ , HDM2/p53, platelet aggregation, proteasome degradation -20S, -26S, Bax and I $\kappa$ B $\alpha$	In vitro: 0–60 $\mu$ M In vivo: 5–150 mg/kg/day	165–175
Hematologic cancer/Leukemia	P34(cdc2), p21/Waf1, p38, PKC $\delta$ , ATM, caspases, telomere activity (hTERT), c-Myc,	0–100 $\mu$ M	176–188
Adrenal cortical cancer	aromatase, cytochrome p450	0–20 $\mu$ M	189, 190
Thyroid cancer	Beclin-1, LC3, Cdc25c, c-Myc, p53, p38, EGFR, MAPK, ERK, MAPK	0–80 $\mu$ M	191–197
Neuroblastoma	Bid, Bcl2, Bax, cytochrome c, caspase -3, -8, -9, PARP-1, p53, p21/Waf1	0–60 $\mu$ M	198–207
Bladder cancer	p53, p21/Waf1, p27/Kip1, CyclinA, cyclinB1, cyclinE, CDK2, cdc2, cdc25, Bax, Bad, Bak, Bcl2, Bcl-XL, Mcl-1, cytochrome c, caspase -3, -7, -9, PARP cleavage, GSH	0–80 $\mu$ M	208–210
Mesothelioma	Bax/Bcl-2 ratio, p53, caspase-9, Akt, NF- $\kappa$ B/p65, MAPK	In vitro: 6.25–100 $\mu$ M In vivo: 20 mg/kg	211
Osteosarcoma	$\beta$ -catenin, Bax, caspase -3, -8, -9, AIF	0–75 $\mu$ M	212–214

AIF Apoptosis inducing factor, AP-1 Activator protein-1, APC Adenomatous polyposis coli, Bax bcl-2-like protein 4, Bcl2 B-cell lymphoma 2, BID BH3 interacting-domain death agonist, CK2 casein kinase 2, COX-2 Cyclooxygenase-2, CYP cytochrome p, ErbB2 Erythroblastic leukemia viral oncogene homolog 2, ERK Extracellular Signal-Regulated Kinase, ER $\beta$  Estrogen receptor beta, FAK Focal Adhesion Kinase, Foxo3a

Forkhead box O3, GADD Growth Arrest DNA Damage, GLUT1 Glucose transporter 1, GRP78 glucose-regulated protein78 kDa, GSK3 $\beta$  Glycogen synthase kinase 3, GST Glutathione S-transferases, H3 Histone H3, HDAC Histone deacetylases, HER2/neu Receptor tyrosine-protein kinase erbB-2, HIF-1 $\alpha$  Hypoxia-inducible factor 1-alpha, ICAM-1 Intercellular Adhesion Molecule 1, Id1 Inhibitor of differentiation or DNA binding protein 1, IFNB1 Interferon beta 1, IGF-1 Insulin-like growth factor 1, IGF1R Insulin-like growth factor 1 receptor, IGFBP-3 Insulin-like growth factor binding protein 3, IKK $\alpha$  I $\kappa$ B kinase alpha, IL Interleukin, JAK-STAT3 Janus kinase/signal transducers and activators of transcription 3, JNK c-Jun N-terminal kinase, MAPK Mitogen-activated protein kinase, MDM2 Mouse double minute 2 homolog, MMP Matrix metalloproteinase, NADPH Nicotinamide adenine dinucleotide phosphate, NF- $\kappa$ B Nuclear factor-kappa B, Nrf2 Nuclear factor (erythroid-derived 2)-like 2, ODC Ornithine decarboxylase, PARP Poly ADP ribose polymerase, PD-L1 Programmed death-ligand 1, PGE2 Prostaglandin E2, PI3K Phosphatidylinositol-4,5-bisphosphate 3-kinase, PIG3 p53-inducible gene 3, PKC Protein Kinase C, PTEN Phosphatase and tensin homolog, PUMA p53 upregulated modulator of apoptosis, Rb Retinoblastoma, Src v-src sarcoma, TRAIL TNF-related apoptosis-inducing ligand; UDP-glucuronosyltransferases, uPAR urokinase-type plasminogen activator receptor, VEGF Vascular endothelial growth factor, XIAP X-linked inhibitor of apoptosis protein