Targeting Inflammatory Pathways by Flavonoids for Prevention and Treatment of Cancer

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Abstract
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Observational studies have suggested that lifestyle risk factors such as tobacco, alcohol, high-fat diet, radiation, and infections can cause cancer and that a diet consisting of fruits and vegetables can prevent cancer. Evidence from our laboratory and others suggests that agents either causing or preventing cancer are linked through the regulation of inflammatory pathways. Genes regulated by the transcription factor NF-κB have been shown to mediate inflammation, cellular transformation, tumor cell survival, proliferation, invasion, angiogenesis, and metastasis. Whereas various lifestyle risk factors have been found to activate NF-κB and NF-κB-regulated gene products, flavonoids derived from fruits and vegetables have been found to suppress this pathway. The present review describes various flavones, flavanones, flavonols, isoflavones, anthocyanins, and chalcones derived from fruits, vegetables, legumes, spices, and nuts that can suppress the proinflammatory cell signaling pathways and thus can prevent and even treat the cancer.

Introduction
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Despite spending billions of dollars in research, a great deal of understanding of the causes and cell signaling pathways that lead to the disease, cancer continues to be a major killer worldwide. According to a recent statistical analysis, a total of 1 479 350 new cancer cases and 562 340 deaths from cancer occurred in the United States in 2009 [1]. Unlike heart disease, death rates for cancer remained approximately the same in the United States from 1975 through 2002. Indeed, it is predicted that by 2020 approximately 15 million new cancer cases will be diagnosed worldwide and 12 million cancer patients will die [2]. One of the most important findings to have emerged during the past three decades is that cancer is a preventable disease. Thus, people need to be educated about the risk factors for cancer and those that prevent the disease. As many as 90 % of all cancers have been shown to be due to environmental/acquired factors such as tobacco, diet, radiation, and infectious organisms, etc., and only the remaining 5–10 % of cases are caused by internal factors such as inherited mutations, hormones, and immune conditions [3].

A strong link between diet and cancer is indicated by the large variation in incidence rates of specific cancers and the observed changes in the incidence of cancer in migrating populations. For example, Ho [4] showed that although the Chinese in Shanghai will have a cancer incidence of 2 cases per 100 000 population, among those who migrate to the United States, the incidence increases to 23 per 100 000 within 5 years. In contrast, among US-born persons of Chinese descent the cancer incidence is 37 cases per 100 000 populations, compared with 58 per 100 000 among whites. This indicates that lifestyle plays a major role in the development of cancer. The important lifestyle factors that affect the incidence and mortality of cancer include tobacco, alcohol, diet, obesity, infectious agents, environmental pollutants, and radiation. All of these risk factors are linked to each other through inflammation.

Inflammation is a localized reaction of tissue to infection, irritation, or other injury. As defined by Rudolf Virchow in 1863, the key features of inflammation are redness, warmth, swelling, pain, and sometimes loss of movement or function. Inflammation is a necessary response to clear viral infections, repair tissue insults, and suppress tumor initiation/progression. However, when inflammation persists or control mechanisms are dysregulated, disease may develop, including cancer. Interestingly, inflammation functions at

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all three stages of tumor development: initiation, progression, and metastasis. During the initiation phase, inflammation induces the release of a variety of cytokines and chemokines that promote the activation of inflammatory cells and associated factors. This causes further oxidative damage, DNA mutations, and other changes in the tissue microenvironment, making it more conducive to cell transformation, increased survival, and proliferation. At the molecular level, inflammation, transformation, survival, and proliferation are regulated by nuclear factor κB (NF-κB), a family of ubiquitously expressed transcription factors. NF-κB is activated by various inducers such as tobacco, alcohol, infections, ionizing radiation, environmental pollutants, chemotherapeutic agents, and tumor necrosis factor-α (TNF-α). TNF-α is also one of the prime signals that induces apoptosis in many different types of cells. Whereas acute activation of NF-κB may be therapeutic, chronic activation may lead to the development of chronic inflammation, cancer, and other chronic diseases. NF-κB has been associated with increased survival in many tumor cells; thus, its inhibition could be a novel approach to breaking the vicious cycle of tumor cell proliferation.

What is NF-κB?

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NF-κB was first identified in 1986 by Sen and Baltimore [5]. In its resting stage, this factor resides in the cytoplasm as a heterotrimer consisting of p50, p65, and IκBα. The inhibitor IκBα masks the NF-κB nuclear localization domain and inhibits its DNA-binding activity. In response to a large variety of stimuli, the IκB inhibitor is rapidly phosphorylated and degraded. This allows NF-κB nuclear translocation, DNA binding to specific recognition sequences in promoters, and transcription of the target genes [6, 7]. The kinase that causes the phosphorylation of IκBα is called IκBα kinase (IKK). IKKβ mediates the classic/canonical NF-κB activation pathway, and IKKα mediates the non-canonical pathway. On activation, NF-κB induces the expression of more than 400 genes that have been shown to suppress apoptosis and induce cellular transformation, proliferation, invasion, metastasis, chemoresistance, radioresistance, and inflammation [8,9]. The activated form of NF-κB has been found to mediate different diseases, including cancer [8,10], atherosclerosis [11], myocardial infarction [12], diabetes [13] allergy [14,15], asthma [16], arthritis [17], Crohn's disease [18], multiple sclerosis [19], Alzheimer's disease [20,21], osteoporosis, psoriasis, septic shock, AIDS, and other inflammatory diseases [22,23]. It is not surprising that most of these diseases are caused by dysregulated inflammation [24]. Most of the traditional chemotherapeutic agents induce NF-kB, and subsequently make the tumors resistant to the drugs. Also, constitutive activation of NF-kB has been linked with chemoresistance and poor prognosis. Therefore, the flavonoids and other phytochemicals, which inhibit deregulated activation of NF-κB, offer a good promise for cancer prevention and therapy.

Therefore, agents are needed that can suppress inflammation through inhibition of NF-κB activation, which could potentially prevent or delay the onset of or treat NF-κB-linked diseases. Much evidence indicates that certain constituents of fruits, vegetables, nuts, legumes, and spices can downregulate inflammation and most chronic diseases (see **Fig. 1**). Flavonoids are one category of nutraceuticals present in these dietary products that can suppress inflammation and thus play an important role in inflammation-related diseases, including cancer. Flavonoids are believed to suppress inflammation, transformation, proliferation,

survival, invasion, and angiogenesis. Those that can suppress carcinogenesis are discussed in detail below.

Association of Inflammation with Cancer

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There is a strong association between chronic inflammatory conditions and cancer specific to the organ. Epidemiological evidence points to a connection between inflammation and a predisposition for the development of cancer, i.e., long-term inflammation leads to the development of dysplasia. Various factors are known to induce chronic inflammatory responses that further cause cancer. These include bacterial, viral, and parasitic infections (e.g., *Helicobacter pylori*, Epstein-Barr virus, human immunodeficiency virus, flukes, schistosomes), chemical irritants (i.e., tumor promoters, such as phorbol ester 12-*O*-tetradecanoyl-13-phorbol acetate, also known as phorbol myristate acetate), and non-digestible particles (e.g., asbestos, silica) [25,26]. Epidemiological studies estimate that nearly 15% of the worldwide cancer incidence is associated with microbial infection [27].

It is mechanistically proven that inflammation produces reactive oxygen species (ROS) and reactive nitrogen species (RNS). In particular, ROS and RNS lead to oxidative damage and nitration of DNA bases, which increases the risk of DNA mutations and further leads to cancer [28]. The most thoroughly studied examples of inflammation and cancer are chronic inflammatory bowel disease and the increased risk of colorectal cancer, chronic gastritis resulting from *H. pylori* infection and gastric adenocarcinoma, and chronic hepatitis and liver cancer. Chronic hepatitis B infection leads to about a 10-fold increase in the risk of liver cancer [29].

Inflammation results in the recruitment of leukocytes secreting a variety of proliferative cytokines and angiogenic factors to the site of tissue insult. These cytokines are known to be required for proper wound healing, and stimulate epithelial cell proliferation; however, if these are uncontrolled they could lead to dysplasias and ultimately cancer. Tumor cells themselves also produce various cytokines and chemokines that attract leukocytes, which in turn produce cytokines and chemokines that stimulate further tumor cell proliferation. Paradoxically, cytokine deficiency (e.g., GM-CSF, IL-2 and IFNγ) can also lead to tumor development. Immune homeostasis consists of a succession of pro- and anti-inflammatory signals. Loss of the anti-inflammatory signals leads to chronic inflammation and proliferative signaling.

The cytokines and chemokines secreted by tumor-associated macrophages and leukocytes cause metastasis and angiogenesis of cancer cells. These cytokines and chemokines promote cell motility and induce the growth of tumor-associated vessels. The leukocytes also promote angiogenesis by inducing vessel dilation and extravasations of tumor cells. Inflammation also promotes the establishment of metastases.

Biosynthesis and Metabolism of Flavonoids

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The typical flavone backbone in the flavonoids resembles other cognate heterocyclic molecules, which possess a flavin-like ring; and the word "flavin" is derived from the Latin word flavus, which means "yellow". It is the most common group of polyphenolic compounds, and their metabolites account for much of the red, blue, purple, and other color pigmentation in plants [30] (see **Fig. 1**). This class was the first to be termed "bioflavonoids." The
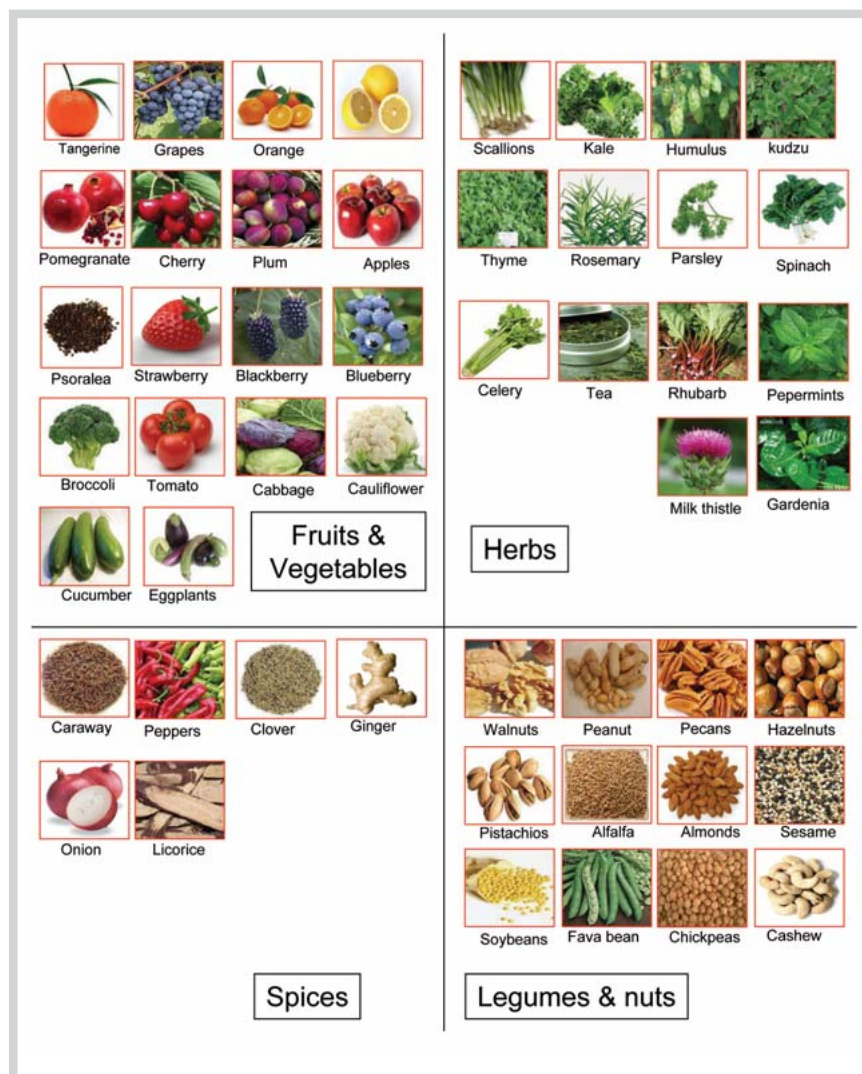
Fig. 1 Major sources of flavonoids. Flavonoids are found in a wide variety of edible fruits, vegetables, herbs, spices, legumes, nuts, etc. The list is only a representative and not the exhaustive.

health benefits of wine, chocolates, fruits, vegetables, nuts, spices, and legumes are attributable to their flavonoids (○ **Table 1**). Until 50 years ago, the mechanisms of flavonoids were hardly known. However, now it is widely known that flavonoids possess a broad spectrum of biological activity [31]. For example, flavonoids modify the body's reaction to allergens, viruses, and carcinogens. They also have antiallergic, anti-inflammatory, antimicrobial, and anticancer activity. Although it is unclear how flavonoids protect against cancer, many of their biological actions have been attributed to their anti-inflammatory and antioxidant activities.

Flavonoids constitute a relatively diverse family of aromatic molecules that are derived from Phe and malonyl-coenzyme A (CoA). This malonyl-CoA yields the true backbone of flavonoids. According to IUPAC nomenclature based on structure, flavonoids are classified into three groups, i.e., flavones, isoflavones and neoflavones. These three flavonoid classes are all ketone-containing compounds. The flavonoids and bioflavonoids also contain non-ketone polyhydroxy polyphenol compounds which are more specifically termed flavanoids, flavan-3-ols, or catechins (although catechins are actually a subgroup of flavanoids). In general, flavonoids include six major subgroups that are found in most of the higher plants. These are chalcones, flavones (generally in herbaceous families, e.g., Labiatae, Umbelliferae, Compositae), flavonols (generally in woody angiosperms), flavanones, anthocyanins, and isoflavonoids. A seventh group, the aurones, is widespread but not ubiquitous. The flavonoid aglycone consists of a benzene ring (A) condensed with a six-membered ring (C), which in the 2-position carries a phenyl ring (B) as a substituent (○ **Fig. 2**). The six-membered ring condensed with the benzene ring is either an α-pyrone (flavonols and flavonones) or its dihydro derivative (flavanols and flavanones). The position of the benzenoid substituent divides the flavonoid class into flavonoids (2-position) and isoflavonoids (3-position). Flavonols differ from flavonones by a hydroxy group at the 3-position and C = C double bond [32]. Flavonoids, collectively known as vitamin P and citrin, are a class of plant secondary metabolites. They usually consist of two benzene rings joined together with a short three-carbon chain (○ **Fig. 2**). One of the carbons of the short chain is connected to the carbon of one of the benzene rings, either directly or through an oxygen bridge, thus forming a third middle ring, which can be five- or six-membered. ○ **Fig. 3** shows the different flavonoids in fruits, vegetables, legumes, nuts, spices and other herbs. The biosynthesis of flavonoids occurs in the cytoplasm through a series of enzymes found in membranes of the endoplasmic reticulum [33].

The pathway involved in biosynthesis is called the phenylpropanoid metabolic pathway. In this pathway phenylalanine, an amino acid, produces 4-coumaroyl-CoA [34] which is combined with

Table 1 Dietary flavonoids and their sources.

Flavonoid category	Dietary flavonoids	Sources
Chalcones	isoliquiritigenin, cardamomin, butein, 2′-hydroxychalcone, resveratrol, tris(methoxymethoxy)chalcone, xanthohumol	licorice, cashews, humulus, grapes, red wine
Flavanones	hesperetin, naringenin, naringin, eriodictyol, homoeriodictyol, poncirin, silibinin, bavachinin taxifolin	oranges, grapes, lemons, psoralea, milk thistle
Flavonols	quercetin, kaempferol, myricetin, isorhamnetin, morin, fisetin	onions, scallions, kale, broccoli, apples, berries, tea, tomatoes, cherries, spinach, almonds, cucumbers
Flavones	apigenin, luteolin, diosmetin, baicalein, wogonin, amentoflavone, biapigenin, baicalin, flavopiridol, isovitexin, ochnaflavone, acacetin, chrysin, tangeretin, pentamethoxyflavone, gossypin, nobiletin	parsley, thyme, celery, peppers, sesame, alfalfa, rosemary, tomatoes, thyme, tangerines, gardenia, oranges
Isoflavonoids	daidzein, genistein, glycitein, vitexin, irigenin, glabridin, equol, biochanin A, formononetin, coumestrol, nobiletin	soybeans, chickpeas, alfalfa, barley, broccoli, cauliflower, caraway, red clover, oranges, peanuts, fava beans, kudzu
Anthocyanidins	cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin	berries, grapes, cherries, plums, pomegranates, eggplant, cabbage, rhubarb, almonds, cashews, hazelnuts, pecans, pistachios, peanuts, walnuts, fava beans, kudzu, psoralea, blueberries, bilberries, mulberries, black raspberries
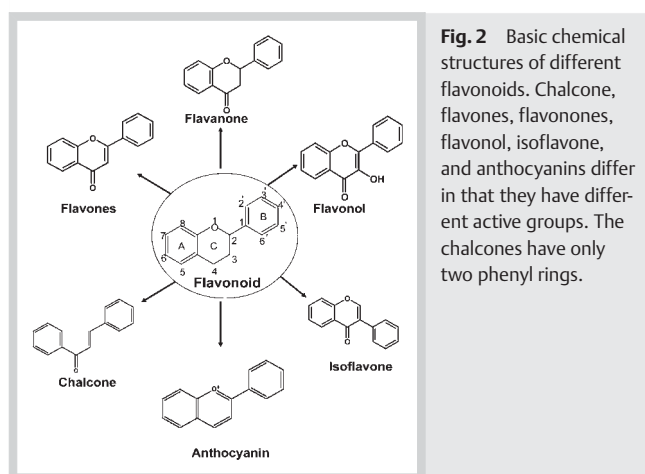
Fig. 2 Basic chemical structures of different flavonoids. Chalcone, flavones, flavonones, flavonol, isoflavone, and anthocyanins differ in that they have different active groups. The chalcones have only two phenyl rings.

malonyl-CoA. Chalcones contain two phenyl rings. They possess the monohydroxylated ring typical of all flavonoids. Flavanones, which are the important intermediates and branch point compounds in flavonoid biosynthesis, often occur in plants as glycosides. Flavones are known to arise by oxidative processes from flavanones. The oxidation of flavanones to flavones is catalyzed by an enzyme, flavone synthase, introducing a double bond between carbon atoms 2 and 3 of the third ring.

Interestingly, the biosynthesis of flavone is carried out by two completely different flavone synthases: flavone synthase I and flavone synthase II. Anthocyanin is synthesized by anthocyanidin synthase (ANS), a 2-oxoglutarate iron-dependent oxygenase, which catalyzes the penultimate step in its biosynthesis [35]. The synthesis of anthocyanidins is mediated through *in vitro* conversion of leucoanthocyanidins to dihydroflavonol and flavonol intermediates (*cis*- and *trans*-isoforms) catalyzed by ANS [36]. It has been shown that colored anthocyanidins such as pelargonidin and cyanidin are synthesized from colorless leucoanthocyanidins (leucopelargonidin and leucocyanidin) by the ANS-catalyzed reaction in the presence of ferrous ion, 2-oxoglutarate, and ascorbate, followed by acidification with HCl [37]. The other enzyme of the pathway, flavonoid 3-*O*-glucosyltransferase, is considered a final enzyme necessary for producing the stable anthocyanin molecules in plant cells [38].

When administered to rodents or humans, flavonoids have a relatively low bioavailability because of their limited absorption and rapid elimination. However, bioavailability differs among the various classes. It has been observed that isoflavones have the highest bioavailability, whereas flavonols (proanthocyanidins and tea catechins) and anthocyanins are very poorly absorbed [39]. Furthermore, it has been reported that even after cooking, most flavonoid glycosides reach the small intestine intact. Flavonoids are absorbed in the small intestine, where they are rapidly metabolized to form methylated, glucuronidated, or sulfated metabolites [39].

Hollman et al. [40] conducted a study to examine the absorption of quercetin (highly present in onions, particularly red onions) among nine patients who had undergone ileostomy. Patients received a quercetin-free diet for 12 days and were then randomized to receive one of the following supplemented diets over a 12-day period: 225 ± 43 (S.D.) µmol of fried onions (68 ± 13 mg quercetin glucosides), pure quercetin rutinoside (the major quercetin glycoside found in tea), or pure quercetin aglycone. The absorption of quercetin aglycone was found to be approximately 24%, whereas that of quercetin glycosides from onions was 52%. This study suggested that the glycoside moiety is present in quercetin enhanced absorption.

In another study on 12 men with benign prostatic hyperplasia, soy extract (3 Evestrel capsules), providing a total of 112.5 mg equiv./day of the isoflavone aglycone, was given for 3 days before prostate surgery. The metabolites were identified in blood and prostate tissues using electrospray ionization-liquid chromatography-tandem mass spectrometry. The concentration of total isoflavones in the prostate tissues was 1.05 nmol/g. However, concentrations in the prostate were lower than those in plasma in all volunteers: 0.47 µm vs. 0.66 µM for daidzein and 0.58 µm vs. 0.78 µM for genistein [41].

A study on a group of patients fed with 4 slices of bread containing soy grits 50 g/slice (n = 8) showed a statistically significant decrease in serum PSA concentrations compared with the control group that ingested a similar wheat bread without the soy supplementation (n = 8) [42]. A double-blind, placebo-controlled study was conducted in patients with high-grade prostatic intraepithelial neoplasia (HGPIN). The patients were administered 600 mg/day of mixed green tea catechins. The first-year follow-up of the 5-year study reported that the conversion rate from HGPIN to prostate cancer was 3% in the experimental group and
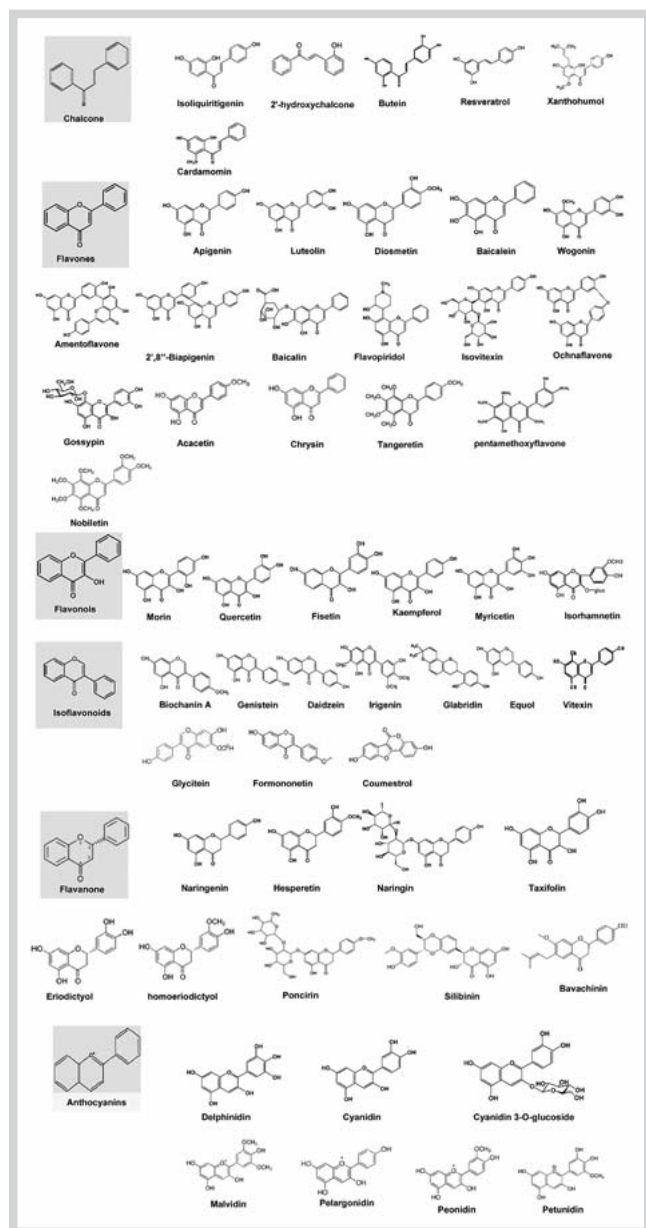
Fig. 3 Chemical structures of naturally occurring different types of flavonoids present in different fruits, vegetables herbs, spices, legumes, nuts, etc.

30% in the control group; however, no significant change was observed in total prostate-specific antigen (PSA) between the 2 arms [43].

Silibinin, also known as silybin, is the major active constituent of silymarin, the mixture of flavonolignans extracted from blessed milk thistle *(Silybum marianum)*. In a clinical trial designed to assess the toxicity, silybin was administered orally to 13 prostate cancer patients, giving 2.5–20 g daily, in three divided doses. It was concluded that 13 g of oral silybin daily, in 3 divided doses, appeared to be well tolerated in patients with advanced prostate cancer, and was recommended as the phase II dose [44]. Furthermore, in 6 prostate cancer patients who received 13 g of silybin daily for 14–31 days (mean was 20 days) and six additional participants serving as control subjects showed increased absorption of silibilin. In contrast to the high peak levels of silibinin ob-

served in blood, the highest silibinin level observed in the harvested prostate tissue was 496.6 pmol/g. There were no significant differences noted in baseline and posttreatment blood levels of IGF-I and IGFBP-3. One of the treated patients developed a grade 4 postoperative thromboembolic event. The other observed toxicities in the treatment group were mild: four subjects had diarrhea and one had asymptomatic grade 2 hyperbilirubinemia which was transient [45].

The absorption of naringenin and its glycosides was studied by Felgines et al. [46] in rats given either a single dose or doses for 14 days. They observed that absorption of naringenin and naringenin 7-glucoside was similar in intestine and cecum, whereas naringenin 7-rhamnoglucoside exhibited delayed absorption, resulting in decreased bioavailability. Adaptation to flavanone diets did not induce the accumulation of naringenin in plasma. Moreover, flavanone cecal content markedly decreased after adaptation, and almost no naringenin 7-rhamnoglucoside was recovered after naringenin 7-rhamnoglucoside feeding, which suggests an adaptation of cecal microflora. Overall, these studies indicate that flavanones are efficiently absorbed after feeding to rats and human beings and that their bioavailability is related to their glycosidic moiety.

Interestingly, different flavonoinds effectively inhibit NF-κB and other inflammatory responses, in spite of having different bioavailability. This raises an interesting question that possibly, the *in vitro* and the *in vivo* effective concentrations are not the same. It is quite likely that an effective *in vitro* concentration may never be observed *in vivo* for a given compound, but still the compound may be effective in inhibiting inflammatory processes. Alternatively, it is also possible that the mechanisms through which these molecules function *in vitro* and *in vivo* are quite different. Flavonoids are mostly derived from fruits, vegetables, and edible plants and are considrd safe, but they do show toxicity when given in high doses. Son et al. [47] also revealed that small doses of phytochemicals ingested by humans are not toxic and instead induce mild cellular stress responses. Another flavonoid, quercetin, did not show toxicity when administered orally to male rats to a dose up to 2000 mg/kg body weight, probably because *in vivo* these compounds are metabolized and made available at lower doses [48].
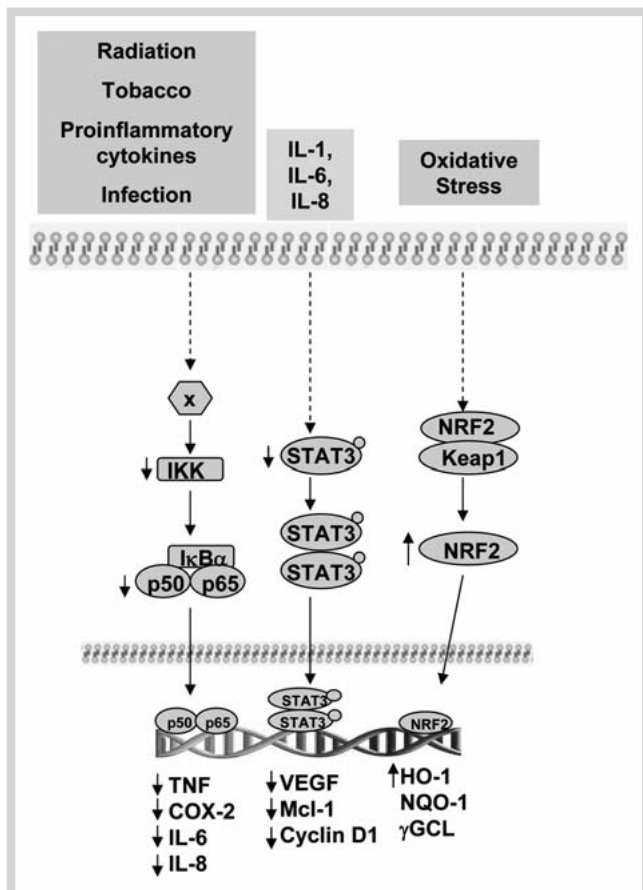
Molecular Targets of Flavonoids
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The human genome is made up of approximately 25 000 genes, approximately 3000 of which are linked with a total of 153 signaling pathways. More than 350 genes have been linked with any given cancer, indicating that cancer is a complex disease involving interactions between multiple genes that up- or down-regulate undesired protein products. Therefore, there is a need for novel and effective chemopreventive and chemotherapeutic drugs that normalize modulated genes. Modulation of the most common cellular responses, such as inflammation, proliferation, apoptosis, metastasis and angiogenesis, by flavonoids is discussed below.

Suppression of inflammatory pathways by flavonoids
Different flavonoids have a wide range of targets that lead to suppression of inflammation (○ Fig. 4), which has been closely linked to cancer (○ Table 2).
Regulation of inflammatory pathways by chalcones: Chalcones (1,3-diphenyl-2-propenones) are anti-inflammatory and cancer

Fig. 4 Major molecular targets of flavonoids for regulation of inflammatory pathways. Down and up arrow indicates the inhibition and induction, respectively, of the targets.

Table 2 Molecular targets of flavonoids in inhibition of inflammation.

Flavonoids	Targets
Chalcone	
▸ isoliquiritigenin	↓ NF-κB
▸ 2′-hydroxychalcone	↑ HO-1
▸ Cardamomin	↓ NF-κB, ↓ IKK
▸ tris(methoxymethoxy) chalcone	↓ NO, ↓ iNOS
▸ butein	↓ NF-κB, ↓ IKK
▸ xanthohumol	↓ NF-κB, ↓ IKK
Flavones	
▸ fisetin	↓ NF-κB, ↓ IKK
▸ gossypin	↓ NF-κB, ↓ IKK
▸ luteolin	↓ NF-κB
▸ acacetin	↓ NF-κB
▸ apigenin	↓ NF-κB, ↑ IκBα
Flavonols	
▸ quercetin	↓ NF-κB, ↓ AP-1
▸ myricetin	↓ COX-2, ↓ NF-κB
▸ kaempferol	↓ PGE2, ↓ COX-2, ↓ NF-κB
▸ isorhamnetin	↓ NF-κB, ↓ STAT-1
Flavanones	
▸ naringenin	↓ iNOS, ↓ NO, ↓ NF-κB, ↓ STAT-1
▸ poncirin	↓ PGE₂, ↓ IL-6, ↓ iNOS, ↓ COX-2, ↓ NF-κB-DNA binding
▸ hesperetin	↓ NF-κB, NIK/IKK, ERK, p38 and JNK
▸ silibinin	↓ NF-κB, ↓ NF-κB-DNA binding, ↓ COX-2
▸ taxifolin	↓ ICAM-1
Anthocyanins	
▸ cyanidin 3-rutinoside	↓ NF-κB, ↓ c-Jun
▸ cyanidin 3-glucoside	↓ NF-κB, ↓ c-Jun
▸ cyanidin	↓ c-Jun, ↓ NF-κB, ↓ PGE₂, ↓ COX-2
▸ delphinidin	↓ NF-κB, ↓ IKK
▸ malvidin	↓ IL-6 secretion
Isoflavonoids	
▸ genistein	↓ NF-κB, ↓ NF-κB-DNA binding, ↑ IκBα, ↓ IL-8
▸ daidzein	↓ NF-κB, ↓ STAT-1, ↓ iNOS
▸ glabridin	↓ NF-κB, ↓ NO
▸ irigenin	↓ iNOS, ↓ COX-2, ↓ NF-κB
▸ tangeretin	↓ COX-2, ↓ NF-κB
▸ nobiletin	↓ iNOS, ↓ COX-2

NF-κB, nuclear factor-kappaB; IκBα, inhibitor of kappaBalpha; HO-1, hemeoxygenase-1; NO, nitric oxide; AP-1, activator protein-1; COX-2, cyclooxygenase-2; IL-8, interleukin-8; iNOS, inductible nitric oxide synthase

preventive food components found in a human diet rich in fruits and vegetables. Chalcone induces the mitochondrial pathway of apoptosis and inhibits the NF-κB inflammatory system in T24 and HT-1376 cells [49]. In IL-6- and LPS-treated endothelial cells, chalcone has been found to abrogate inflammation by inhibiting the activation of STAT3 and NF-κB. Chalcone also upregulates levels of the transcription factor Nrf2 in nuclear extracts and increases antioxidant response element (ARE)-luciferase and thioredoxin reductase promoter activity in endothelial cells [50]. Isoliquiritigenin with a chalcone structure, an active component present in plants such as *Glycyrrhiza* and *Dalbergia* spp., shows various biological activities, including anti-inflammatory activity. Isoliquiritigenin was found to inhibit NF-κB activation in endothelial cells by blocking the phosphorylation and subsequent degradation of IκBα [9]. The chalcone derivative 2′-hydroxychalcone showed anti-inflammatory action in LPS-induced inflammation in RAW 264.7 macrophages via activation of the HO-1 pathway [51]. In these cells, cardamomin (2′,4′-dihydroxy-6′-methoxychalcone) from *Alpinia conchigera* Griff (Zingiberaceae) also inhibited LPS-induced NF-κB activation.

Another chalcone, cardamomin, inhibited not only LPS-induced degradation and phosphorylation of IκBα but also activation of IKK and nuclear translocation of NF-κB [52]. Other chalcone derivatives, such as 2′-hydroxy-4′-methoxychalcone, 2′,4-dihydroxy-4′-methoxychalcone, and 2′,4-dihydroxy-6′-methoxychalcone, also inhibited LPS-induced NF-κB. The inhibition of NF-κB was

found to be mediated through inhibition of IκBα degradation and the phosphorylation of c-jun N-terminal kinase (JNK) and c-jun [53]. The synthetic chalcone derivatives 2′-methoxy-3,4-dichlorochalcone and 2′-hydroxy-3-bromo-6′-methoxychalcone were also reported to suppress the NF-κB activation in LPS-treated RAW 264.7 cells [54]. The synthetic chalcone 2′,4′,6′-tris(methoxymethoxy)chalcone showed anti-inflammatory activities by decreasing the degradation of IκBα, which further inhibited NF-κB translocation into the nucleus; it also reduces nitric oxide (NO) production by inhibition of inducible NO synthase (iNOS) expression in LPS-activated RAW 264.7 macrophages [52]. **Regulation of inflammatory pathways by flavones:** Flavones are a class of flavonoids with a backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). Flavones are mainly found in cereals and herbs and cause yellow pigments in plants. Natural flavones include apigenin, luteolin, tangeritin, chrysin, baicalein, scutellarein, wogonin, and fisetin. Two synthetic flavones are di-

osmin and flavoxate. Flavones have several putative beneficial effects against a number of diseases, such as atherosclerosis, osteoporosis, diabetes mellitus, inflammation, and cancers.

We have shown that fisetin, present in vegetables and fruits such as strawberry, apple, persimmon, grape, onion, and cucumber and wine, is a potent inflammatory agent through its suppression of NF-κB activation induced by various inflammatory agents and carcinogens. The inhibition of NF-κB was found to be mediated through blocking of phosphorylation and degradation of IκBα by inhibiting IKK activation and suppression of the phosphorylation and nuclear translocation of p65 [55]. Gossypin, a flavone originally isolated from *Hibiscus vitifolius,* has been shown to suppress inflammation as well as angiogenesis and carcinogenesis. In one study we have shown that gossypin inhibited NF-κB activation as induced by inflammatory stimuli and carcinogens. It also inhibited constitutive NF-κB activation in tumor cells. The NF-κB inhibition by gossypin was also found to be mediated through inhibition of IKK, which led to the suppression of IκBα phosphorylation and degradation, p65 nuclear translocation, and NF-κB-regulated gene expression [56]. The flavone luteolin effectively suppressed NF-κB induced by TNF in lung cancer cells [57]. Acacetin (5,7-dihydroxy-4′-methoxyflavone) was also found to have anti-inflammatory and antiperoxidative effects in RAW 264.7 cells activated with LPS. One study showed that treatment with acacetin reduced the translocation of NF-κB. The activation of NF-κB was inhibited by prevention of the degradation of IκB [58]. Apigenin, another flavone found in parsley, thyme, and peppermint, showed down-modulation of the constitutive expression of NF-κB with an decrease in IκBα expression in prostate cancer cells [59]. Chrysin, an active component of *Oroxylum indicum*, inhibited NF-κB activity through the inhibition of IκBα phosphorylation and diminished IL-8 secretion in human intestinal Caco-2 cells [60].

Baicalein, a component of *Scutellaria baicalensis*, was found to inhibit inflammation by suppressing LPS-induced degradation of IκBα and formation of NF-κB-DNA complex. Baicalein also decreased levels of iNOS and the overproduction of NO and superoxide anions caused by LPS [61]. Along with baicalein, wogonin and baicalin which are also the components of *Scutellaria baicalensis* showed anti-inflammatory activities. Wogonin suppressed IL-1b-induced IL-6 and IL-8 production in ARPE-19 cells. Wogonin also suppressed the NF-κB binding activities in ARPE-19 cells indicating anti-inflammatory effects of baicalein and wogonin [62]. Baicalin inhibited COX-1 and COX-2 peroxidase and 5-LOX enzyme activities to reduce production of proinflammatory eicosanoids and attenuated edema in an *in vivo* model of inflammation [63]. The anti-inflammatory activity of baicalin was also found by inhibiting the binding of a number of chemokines such as CXC, IL-8, CC [macrophage inflammatory protein (MIP)-1β, monocyte chemotactic protein (MCP)-2], and C [lymphotactin (Ltn)] to human leukocytes which was further associated with a reduced capacity of the chemokines to induce cell migration [64]. *Cayaponia tayuya* root extract which contains the flavone isovitexin as a bioactive component showed an anti-inflammatory response in models of topical mouse ear edema. The anti-inflammatory activity of *C. tayuya* root extract is most likely due to inhibition of the induction of the enzymes COX-2 and iNOS [65]. Another study showed that isovitexin extracted from *Oryza sativa* inhibited the release of TNF-α, a proinflammatory cytokine and PGE2 production, upon LPS activation [66].

Flavopiridol, a synthetic flavone closely related to a compound originally isolated from the stem bark of the native Indian plant *Dysoxylum binectariferum*, has been found to suppress inflammation, inhibit cyclin-dependent kinases, induce apoptosis, and modulate the immune response. The inhibition of TNF-induced inflammation by flavopiridol was associated with inhibition of NF-κB activation. This effect was mediated through inhibition of IKK, phosphorylation, ubiquitination, and degradation of IκBα, and suppression of phosphorylation, acylation, and nuclear translocation of the p65 subunit of NF-κB [67]. However, biochemical analysis of plasma in patients undergoing infusional flavopiridol found a significant induction of IL-6 leading to the appearance of the proinflammatory syndrome [68]. Ochnaflavone exhibits anti-inflammatory activity through the regulation of proinflammatory gene expression *in vitro* and *in vivo*. This molecule also exhibits phospholipase A2, COX-2 and 5-LOX inhibitory activity [69]. Suh et al. [70] also showed that LPS-induced iNOS expression is abolished by ochnaflavone in RAW 264.7 cells by blocking the inhibition of transcription factor NF-κB binding activities. These activities of ochnaflavone were found to be associated with the downregulation of IKK activity, thus inhibiting LPS-induced phosphorylation as well as the degradation of IκBα.

Regulation of inflammatory pathways by flavonols: Flavonols are a class of flavonoids that have a 3-hydroxyflavone backbone and are present in a wide variety of fruits and vegetables, including onion, kale, broccoli, lettuce, tomato, apple, grape, berries, tea, and red wine. The greener a leaf is, the more flavonols it contains. The major dietary flavonols are quercetin, kaempferol, myricetin, and isorhamnetin. Various studies have shown that flavonols have properties that reduce several diseases, including inflammation and cancer.

In one example, it was reported that the red wine flavonoid quercetin inhibits 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced activation of activator protein-1 and NF-κB. Quercetin also inhibited the transformation of mouse skin epidermal (JB6 P+) cells [71]. Another major flavonol present in red wine, myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone), inhibited TPA-induced COX-2 expression in JB6 P+ cells by suppressing the activation of NF-κB [72]. Muñoz-Espada and Watkins [73] showed that the flavonols cyanidin and kaempferol reduced the level of PGE$_2$ and COX-2 protein in LNCaP cell cultures. They further showed that the reduction of COX-2 by kaempferol and cyanidin may be mediated through the actions of NF-κB and PPARγ as nuclear factors that bind to the COX-2 gene promoter. Isorhamnetin isolated from *Smilax bockii warb* showed moderate activity in inhibiting TNF-α-induced NF-κB activation with an IC$_{50}$ value of 166.6 µg/mL [74]. Another study found that isorhamnetin inhibited both NF-κB and STAT-1 in activated macrophages [75].

Regulation of inflammatory pathways by flavanones: Flavanones are a subgroup of flavonoids with the basic structure 2,3-dihydro-2-phenylchromen-4-one. The major flavanones are hesperetin, naringenin, eriodictyol, and homoeriodictyol, and they play an important role in the inhibition of inflammation and cancer. For instance, naringenin present in orange and grape juice was found to inhibit iNOS protein and NO production through down-modulation of NF-κB in activated macrophages [75]. Another compound, poncirin, a flavanone glycoside isolated from the dried immature fruits of *Poncirus trifoliata*, showed anti-inflammatory effects by inhibiting PGE$_2$ and IL-6 production. Poncirin also reduced LPS-induced protein levels of iNOS and COX-2 and the DNA binding activity of NF-κB [54]. Hesperetin, another flavanone abundant in citrus fruits, has shown variety of pharmacological properties like antioxidant, cholesterol-lowering, and

anti-inflammatory properties. In one animal study, hesperetin fed to 6- and 24-month-old rats modulated NF-κB in their kidneys. Interestingly, hesperetin-induced suppression of NF-κB was found to occur through four signal transduction pathways: NIK/IKK, extracellular signal-regulated kinase (ERK), p38, and JNK [76]. In an A549 xenograft athymic BALB/c nu/nu model, silibinin (an active flavanone of silymarin, milk thistle) treatment decreased the doxorubicin-induced NF-κB DNA binding. Further study to determine the mechanism of action showed that silibinin inhibited the increased translocation of p65 and p50 from cytosol to nucleus caused by doxorubicin. Silibinin also inhibited COX-2, an NF-κB target, in combination with doxorubicin [77]. Another component of milk thistle, taxifolin, also potently inhibited IFN gamma-induced ICAM-1 expression in a reconstructed human skin suggesting therapeutic potential of taxifolin in pathological skin conditions related to increased cell adhesion and inflammation [78]. *Psoralea corylifolia*, an Ayurvedic medicine in India (Babchi) and BuGuZhi in China, possesses anti-inflammatory, antipyretic, and analgesic activity due to its bakuchiol content. In a study it has been observed that topical administration of this compound effectively inhibited TPA-induced ear edema and myeloperoxidase activity and also reduced the PGE$_2$ content in the arachidonic acid-induced response [79].

Regulation of inflammatory pathways by anthocyanins: Anthocyanins are a subclass of flavonoids synthesized via the phenylpropanoid pathway. Anthocyanins are odorless and nearly flavorless, and they contribute to taste as a moderately stringent sensation. Anthocyanins are found in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits, and they cause a red-blue color. For example, cherries, plums, cabbage, rhubarb, eggplants, almonds, cashews, hazelnuts, pecans, pistachios, peanuts, walnuts, lupine, fava beans, kudzu, psoralea blueberries, bilberries, mulberries and black raspberries are rich sources of anthocyanins. Anthocyanins are derivatives of anthocyanidins, which include pendant sugars. Nearly 600 anthocyanins have been reported. Anthocyanins present in white currant berries showed chemopreventive potential in an experimental model of intestinal tumorigenesis. Treatment in a diet containing 10% freeze-dried white currant for 10 weeks fed to multiple intestinal neoplasia (Min) mice showed a reduced number of tumors in the small intestine of study mice, compared with controls. Further study showed that currant reduced nuclear β-catenin and NF-κB protein levels in the intestinal adenomas [80]. Juices of other berries also induced apoptosis and inhibited inflammation in various cancer cells, including those of the stomach, prostate, intestine, and breast. The apoptosis and anti-inflammatory response are mediated by anthocyanins through inhibition of COX-2 expression and suppression of NF-κB activation in cancer cell lines [81]. Anthocyanins from *Oryza sativa* have been shown to inhibit the growth and invasion of SKHep-1 cells. These effects were found to be associated with a reduced expression of MMP-9 and urokinase plasminogen activator (u-PA) [82]. The anthocyanins cyanidin 3-rutinoside and cyanidin 3-glucoside (extracted from *Morus alba* L.) have been reported to exert an inhibitory effect on the migration and invasion of highly metastatic A549 human lung carcinoma cells through reduced expression of MMP-9 and u-PA. It has also been shown that treatment with cyanidin 3-rutinoside and cyanidin 3-glucoside resulted in an inhibition of the activation of c-Jun and NF-κB [83]. Anthocyanins (such as delphinidin, cyanidin, and pelargonidin) found in pomegranates *(Punica granatum)* possesses strong antioxidant and anti-inflammatory properties. Pomegranate fruit extract (PFE) inhibited UV-B-mediated

activation of the NF-κB pathway. Treatment with PFE in normal human epidermal keratinocytes inhibited UV-B-mediated degradation and phosphorylation of IκBα and activation of IKKα which further inhibited nuclear translocation and phosphorylation of NF-κB/p65 at Ser(536) [84].

Regulation of inflammatory pathways by isoflavonoids: Isoflavonoids (or isoflavones) are produced via a branch of the general phenylpropanoid pathway that produces flavonoid compounds in higher plants. Most members of the Fabaceae/Leguminosae (bean) family, including various legumes such as kudzu *(Pueraria lobata)*, lupine *(Lupinus* spp.*)*, broccoli (*Brassica oleracea*), cauliflower (*Brassica oleracea*), barley (*Hordeum vulgare*), fava beans *(Vicia faba)*, and soy *(Glycine max)*, are the major source of isoflavones. Among these, the soybean is the most common source of isoflavones in human food. The major isoflavones in soybean are genistein and daidzein. Other dietary sources of isoflavones are chick peas (biochanin A), alfalfa (formononetin and coumestrol), and peanuts (genistein). Studies have shown that isoflavones have diverse effects on human and animal health, including inflammation and cancer. Genistein, a major isoflavone, was found to inhibit NF-κB activation during acute lung injury induced by LPS in rats. Intratracheal treatment of rats with LPS (6 mg/kg) resulted in increased DNA-binding activity of NF-κB in alveolar macrophages and lung tissue. However, 2-h pretreatment with genistein (50 mg/kg, intraperitoneally) inhibited the LPS-induced induction of NF-κB activation and changes in lung injury [85]. Equol, an isoflavonoid, inhibited the LPS-induced activation of NF-κB as well as binding to DNA. A study in RAW 264.7 cells showed that inhibition of NF-κB by equol was mediated through inhibition in degradation of IκBα and IκBβ and nuclear translocation of the p65 subunit of NF-κB [86]. We found that the isoflavonoid morin, isolated from mulberry figs, almond hulls and old fustic (*Chlorophora tinctoria*), inhibited NF-κB activation induced by different inducers such as TNF, phorbol 12-myristate 13-acetate, LPS, ceramide, IL-1, and H$_2$O$_2$ in different cancer cells. The suppression of NF-κB by morin was mediated through inhibition of IKK, leading to suppression of phosphorylation and degradation of IκBα and consequent nuclear translocation of p65 [87].

In a study of *in situ* human DeltaF508 cystic fibrosis bronchial tissues, isoflavones showed an anti-inflammatory response. It has been observed that treatment of cystic fibrosis gland cells with the isoflavone genistein resulted in decreased IL-8 production and that genistein reversed the effects of LPS *Pseudomonas aeruginosa*-induced nuclear translocation of NF-κB by increasing IκBα protein levels in cystic fibrosis gland cells [88]. The isoflavones daidzein and genistein have also been shown to suppress NF-κB and STAT-1, which are significant transcription factors for iNOS involved in inflammation [75]. Glabridin, a isoflavonoid present in licorice extract, is also known to have anti-inflammatory, antimicrobial, and cardiovascular protective activities. One study showed that glabridin attenuated LPS-induced NO production and NF-κB activation in isolated mouse peritoneal macrophages and RAW 264.7 cells. The inhibition of NF-κB by glabridin in RAW 264.7 cells was mediated through the inhibition of IκBα degradation and p65 nuclear translocation [89]. Irigenin, another isoflavonoid from the rhizomes of *Belamcanda chinensis* (Iridaceae), was found to have anti-inflammatory effects in RAW 264.7 cells. It has been shown that irigenin inhibited the expression of iNOS and COX-2 proteins. Treatment of the transfectant RAW 264.7 cells with irigenin also reduced the level of NF-κB activity associated with decreased p65 protein levels in the nucleus [90]. Vitexin which is found in caraway (*Eleusine indica*) inhibits

inflammation by preventing lung neutrophil recruitment in mice exposed to aerosols of LPS from gram-negative bacteria [91]. Recent studies have shown that tangeretin (5,6,7,8,4′-pentamethoxyflavone), a polymethoxylated flavonoid concentrated in the peel of citrus fruits, exhibits antiproliferative, anti-invasive, antimetastatic, and antioxidant activities. It down-modulated IL-1 induced COX-2 expression. Pretreatment of cells with tangeretin inhibited IL-1β-induced p38 MAPK, JNK, and AKT phosphorylation and the downstream activation of NF-κB [92]. Since NF-κB is also required for the replication of HIV, it is possible that anti-HIV activity associated with 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone (PMF) is due to suppression of NF-κB [93].

It is very evident from the foregoing discussion, and as also summarized in **Table 2**, that the anti-inflammatory activity of flavonoids, in general, is attributed to their ability to inhibit NF-κB activation by a wide variety of inflammatory stimuli. This is brought by inhibiting the phosphorylation of IκBα, and its subsequent degradation. Phosphorylation of IκBα is inhibited by inhibition of IKK activity. However, the pathway through which IKK activity is inhibited has not been delineated unequivocally, in most of the cases. However, the involvement of ROS has been postulated in the case of some of the flavonoids. Once the NF-κB is inhibited, the expression of all the downstream targets genes, such as COX2, MMP-9, ICAM1, iNOS, etc. are brought down. In some instances, in addition to inhibiting the NF-κB pathway, the NRF-2 pathway and its downstream target HO-1 were shown to be activated. The above pathways invariably have been worked out *in vitro*, but their *in vivo* validation is lacking.

Role of Flavonoids in Apoptosis, Proliferation, and Angiogenesis

Apoptosis especially plays a crucial role in the regulation of tissue homeostasis, and an imbalance between cell death and proliferation may result in tumor formation [94]. Tumor cells can break away, leak, or spill from a primary tumor, enter lymphatic and blood vessels, circulate through the bloodstream, and be deposited within normal tissue elsewhere in the body. After metastasis, a new tumor is formed, called a secondary or metastatic tumor, and its cells are like those in the original tumor. In the tumor, new blood vessel formation starts from preexisting vessels in a process called angiogenesis. Metastatic cancer cells exhibit a resistance to apoptosis. NF-κB activation leads to the expression of genes that are involved in the proliferation, survival, angiogenesis, invasion, and metastasis of cancer [95]. Thus, there is growing interest in the potential of flavonoids to modulate NF-κB-regulated gene products and fight cancer; however, it is not yet clear how flavonoid consumption affects the cancer risk in humans. Various *in vitro, in vivo*, and clinical studies have shown that flavonoids modulate different genes involved in the regulation of apoptosis, proliferation, metastasis and angiogenesis (**Fig. 5**).

Role of chalcones in apoptosis, proliferation, and angiogenesis: A study on chalcone showed that it inhibits the proliferation of T24 and HT-1376 cells by blocking the cell cycle progression in the G2/M phase through the regulation of p21, p27, cyclin B1, cyclin A, and Cdc2 proteins and inducing apoptosis through the induction of Bax and Bak, decreasing the levels of Bcl-2 and Bcl-xL, and inducing the release of cytochrome c and activation of caspase-9 and caspase-3 [49]. The major prenylated chalcone, xanthohumol, found in hops (*Humulus*), is well-known to exert anticancer effects. In fact, xanthohumol at a concentration of 25 µM repressed proliferation and migration and induced apoptosis in two HCC cell lines (HepG2 and Huh7). Xanthohumol also inhibits
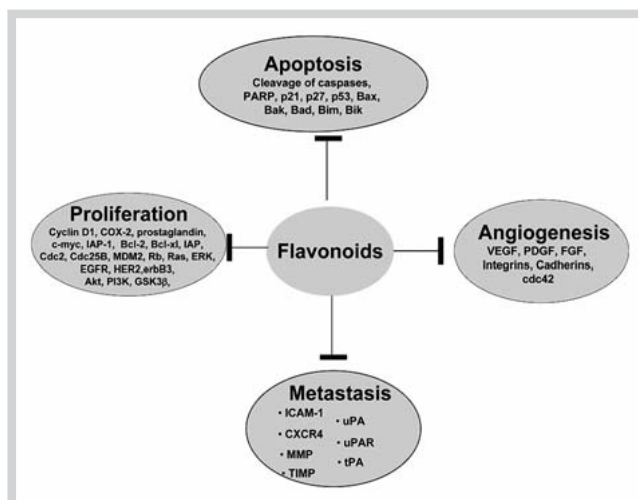
Fig. 5 Molecular targets of flavonoids for inhibition of proliferation, metastasis, and angiogenesis, and induction of apoptosis. VEGF, vascular endothelial growth factor; PDGF, platlets derived growth factor; PARP, poly (ADP-ribose) polymerase; MDM2, murine double minute 2; EGFR, epidermal growth factor receptor; PI3K, phosphoinositide 3-kinases; GSK-3β, glycogen synthase kinase-3β; ICAM-1, intercellular adhesion molecule-1; CXCR4, CXC chemokine receptor; MMP-9, matrix metallopeptidase-9; TIMP, tissue inhibitor of metalloproteinases.

TNF-induced NF-κB activity and IL-8 expression in both cell lines at even lower concentrations [96, 97].

Butein (3,4,2′,4′-tetrahydroxychalcone), another chalcone (isolated from stem bark of cashew), exhibits antiproliferative and apoptotic effects against tumor cells through suppression of the STAT3 activation pathway. The suppression of STAT3 was mediated through the inhibition of activation of the upstream kinases c-Src, Janus-like kinase (JAK) 1, and JAK2 [98]. It also inhibited the proliferation of breast cancer cells expressing aromatase. Thus, butein was found to be the strongest aromatase inhibitor among the compounds tested in that study − 2-hydroxychalcone, 2′-hydroxychalcone, 4-hydroxychalcone, 4,2′,4′-trihydroxychalcone (isoquiritigenin), and 3,4,2′,4′-tetrahydroxychalcone (butein) – and its IC$_{50}$ value was 3.75 µM [99].

Isoliquiritigenin, isolated from licorice and shallots, inhibits the proliferation of A549 human non-small cell lung cancer cells. Isoliquiritigenin also induced apoptosis and blocked cell cycle progression in the G1 phase through increased expression of Fas/FasL, p53, and p21/WAF1 protein [100]. Isoliquiritigenin was also found to inhibit cell cycle progression at the G2/M phase through the enhanced expression of p21(CIP1/WAF1) in lung cancer cells and GADD153 in prostate cancer cells [101]. In MGC-803 cells, isoliquiritigenin treatment showed apoptosis as indicated by the typical morphological and biochemical features of apoptosis including cell shrinkage, chromatin condensation, DNA ladder formation, and the appearance of apoptotic peaks (subG1) [102]. In addition, isoliquiritigenin reduced pulmonary metastasis of mouse renal cell carcinoma without any weight loss or leukocytopenia [103].

The chalcone derivatives xanthoangelol and 4-hydroxyderricin, isolated as main components from the *Angelica keiskei* Koizumi (Umbelliferae) root, have antitumor and antimetastatic properties to the lung or liver in subcutaneous or intrasplenic LLC-implanted C57BL/6J female mice. 4-Hydroxyderricin at a dose of 50 mg/kg × 2/day orally inhibited tumor growth in subcutaneous

LLC-implanted mice, inhibited lung metastasis, and prolonged survival time in mice [104]. Xanthoangelol also inhibited tumor growth in LLC-bearing mice as well as lung and liver metastasis and prolonged survival time in carcinectomized mice at a daily dose of 50 mg/kg [105]. The antitumor and/or antimetastatic activities of xanthoangelol and 4-hydroxyderricin may be due to inhibition of DNA synthesis and of tumor-induced neovascularization through inhibition of the formation of capillary-like tubes by vascular endothelial cells and inhibition of the binding of VEGF to vascular endothelial cells [104, 105]. In addition, 2′-hydroxy-4′-methoxychalcone showed antiangiogenic and antitumor activities in *in vitro* and *in vivo* studies. This compound decreased the angiogenesis in chick embryos and mice implanted with murine Lewis lung carcinoma. It has been observed that the antiangiogenic activities of 2′-hydroxy-4′-methoxychalcone might be due to antiproliferative activity under inhibition of COX-2 enzyme [106].

Role of flavones in apoptosis, proliferation, and angiogenesis: Apigenin, a common dietary flavone, has been shown to possess antitumor properties. In one study of human ovarian cancer A2780 cells, apigenin was found to inhibit proliferation and tumorigenesis through Id1 (inhibitor of differentiation or DNA binding protein 1). Apigenin suppressed the expression of Id1 through activating transcription factor 3 (ATF3) [107]. Apigenin also inhibits motility and invasion, disrupts actin cytoskeleton organization, and modulates focal adhesion kinase (FAK)/Src signaling of PC3 cells [108]. In a study of human ovarian cancer A2780 cells, apigenin was found to inhibit the expression of FAK, which plays an important role in the migration and invasion of cancer cells. An *in vivo* study also showed that apigenin inhibited the spontaneous metastasis of A2780 cells implanted onto the ovary of nude mice [109]. HGF-induced cell motility, scattering, cell migration, and invasion were repressed by apigenin through blocking of Akt phosphorylation but not Met, ERK, and JNK phosphorylation in hepatoma SK-Hep1 cells and lung carcinoma A549 cells [110].

The natural flavone diosmetin which is present in plants belonging to the genus *Teucrium* (Lamiaceae) and in Portuguese olive leaves along with other fruits and vegetables, was also found to inhibit the proliferation of breast adenocarcinoma MDA-MB 468, which caused an arrest of the cell cycle at the G1 phase at 10 μM [111]. The flavone luteolin present in tea, tomatoes, apples and other fruits and vegetables, has multiple anti-inflammation, antiallergy, and anticancer effects. The anticancer properties of luteolin have been associated with the induction of apoptosis and inhibition of cell proliferation, metastasis, and angiogenesis through suppression of cell survival pathways such as phosphatidylinositol 3′-kinase (PI3K)/Akt, NF-κB, and XIAP and stimulation of apoptosis pathways, including those that induce the tumor suppressor p53 [112]. Another study showed that the antiproliferative effect of luteolin is associated with the arrest of cells at the G2/M phase regulated through the proteins Cdc2, cyclin B1, Cdc25C, and p21/cip1 in gastric cancer AGS cells. In these cells, luteolin also induced apoptosis through increasing the levels of proapoptotic proteins, including caspases-3, -6, and -9, Bax, and p53 and decreasing levels of the antiapoptotic protein Bcl-2 [113]. An *in vivo* study of murine xenografts showed that luteolin inhibited tumor growth and angiogenesis induced by VEGF. Luteolin inhibited VEGF-induced PI3K and abolished activation of Akt in human umbilical vein endothelial cells [114]. Another study showed that luteolin inhibits the invasion of prostate cancer PC3 cells through E-cadherin. The luteolin-induced expression of E-cadherin was mediated through the Akt/mdm2 pathway. *In vivo* experiments also showed that luteolin inhibited spontaneous lung metastasis of PC3 cells implanted into nude mice [115].

Protoapigenone, a novel flavonoid isolated from the whole plant *Thelypteris torresiana,* was found to have cytotoxic effects on ovarian cancer MDAH-2774 and SKOV3 cells [116]. Protoapigenone arrested MDAH-2774 and SKOV3 cells at the S and G2/M phases via decreasing the expression of p-Cdk2, Cdk2, p-cyclin B1, and cyclin B1, as well as increasing the expression of inactive p-Cdc25C and inducing apoptosis through decreasing protein levels of Bcl-xL and Bcl-2 and increasing levels of cleaved poly(ADP-ribose) polymerase (PARP) by activating caspase-3. In one *in vivo* study in nude mice, protoapigenone treatment significantly suppressed tumor growth without major side effects [116].

Nobiletin, an effective component of citrus fruit, showed antiproliferative activity on lung cancer cells both *in vitro* and *in vivo.* Nobiletin-induced cell p53-mediated cycle arrest at the G2/M phase and further apoptosis via modulated the Bax: Bcl-2 protein ratio in A549 cells. Nobiletin had an overt inhibitory effect on tumor growth in the nude mice model as observed *in vivo* [117]. Nobiletin inhibits TPA-stimulated tumor metastasis by inhibiting the phosphorylation of MEK and thereby suppressing MMP expression in human fibrosarcoma HT-1080 cells [118]. The decrease in MEK activity caused by nobiletin was found to inhibit the phosphorylation of ERK, a downstream signaling factor for MEK [119]. Another citrus component, tangeretin, also inhibits proliferation of human breast cancer cell lines MDA-MB-435 and MCF-7 and human colon cancer line HT-29 up to 80 % over the course of 4 days, by cell cycle arrest without inducing apoptosis [120]. An *in vitro* study showed that tangeretin inhibits the invasion of MO4 cells into embryonic chick heart fragments. That study showed a decrease in ATP content in MO4 cells after tangeretin treatment; thus, it was deduced that this flavonoid inhibits a number of intracellular processes, which leads to an inhibition of cell motility and, hence, of invasion [121]. Zapotin (5,6,2′,6′-tetramethoxyflavone), found in the tropical fruit zapote blanco *(Casimiroa edulis),* showed a marked suppression of cell proliferation in the HT-29 cells through accumulation of cells in the G2/M phase, with a concomitant decrease of cells in the G0/G1 phase. It also inhibits the generation of aberrant crypt foci in mice [122].

Fisetin and gossypin regulate the proteins involved in antiapoptosis (cIAP-1/2, Bcl-2, Bcl-xL, XIAP, survivin, and TRAF1), proliferation (cyclin D1, c-Myc, and COX-2), invasion (ICAM-1 and MMP-9), and angiogenesis (VEGF) and exhibits anticancer effects [55]. In a study it has been shown that acacetin (5,7-dihydrocy-4′-methoxyflavone) possesses the ability to inhibit cell proliferation and induce apoptosis in human gastric carcinoma AGS cells. The induction of apoptosis by acacetin was mediated through the mitochondrial cell death pathway. Acacetin-induced cell death was characterized by a loss of mitochondrial transmembrane potential, stimulation of ROS, release of mitochondrial cytochrome c into the cytosol, induction of procaspases-9 and -3, and subsequently DNA fragmentation [123]. Acacetin also showed strong cell growth inhibition by cell cycle arrest at the G2/M phase accompanied by cell death in human prostate cancer LNCaP and DU145 cells. G2/M arrest was associated with a decrease in Cdc25C, Cdc2/p34, and cyclin B1, which were more prominent in LNCaP than in DU145 cells [124].

Other flavones, 7-hydroxyflavanone, 5,6,7-trihydroxyflavanone, and 4′,5,7-trihydroxyflavanone, were found to exert an inhibi-

tory effect on the invasion and migration of SCC-4 cells. Treatment of flavones decreased the expression of MMP-2 and u-PA together with altered expression levels of their endogenous inhibitors, such as the tissue inhibitor of metalloproteinase-2 (TIMP-2) and plasminogen activator inhibitor-1 (PAI-1). An *in vivo* chorioallantoic membrane (CAM) intravasation also revealed the antimetastatic effect of flavones [125]. In combination, tangeritin enhanced the ability of cisplatin to inhibit the proliferation and apoptosis of A2780/CP70 and 2008/C13 cisplatin-resistant human ovarian cancer cells. Tangeritin activates apoptosis via the caspase cascade and by arresting the cell cycle at the G2/M phase. Upon tangeretin-cisplatin treatment, phospho-Akt, NF-κB, phospho-GSK-3β, and phospho-BAD were downregulated [126]. Baicalein inhibits the proliferation of cells by blocking cell cycle progression in the S/G2/M phase and inhibits cyclin D1, p42/44 MAPK, and Akt phosphorylation without changes in other cell cycle regulatory proteins [127]. In contrast, baicalein, chrysin, and scutellarein inhibited the proliferation of MDA-MB-468 cells to a lesser extent than sinensetin and genkwanin [128]. Baicalin inhibited cell proliferation of human lung carcinoma A549 and mouse Lewis lung cancer. An *in vivo* study showed that baicalin suppressed tumor growth and prolonged survival in C57BL/6 mice bearing LLC tumor and nude mice bearing A549 carcinoma without systemic toxicity [129].

Flavopiridol which is in clinical phase I/II trial inhibits the growth of MDA-MB-435 breast cancer cells, induces apoptosis, regulates the expression of genes, and inhibits invasion and metastasis of breast cancer cells. Induction of apoptosis by flavopiridol was measured by DNA laddering, PARP, and CPP32 cleavages. It also showed modest upregulation of Bax and downregulation of Bcl-2, as well as significant downregulation of c-erbB-2 in flavopiridol-treated cells. Flavopiridol inhibits cell invasion by inhibiting the secretion of erbB-2, MMP-2 and 9 in the breast cancer cells [130]. Ochnaflavone, a biflavonoid present in the human diet, is known to inhibit angiotensin II-induced hypertrophy and serum-induced smooth muscle cell proliferation. Further study showed that its inhibitory effects are associated with reduced ERK1/2 activity and G1 cell cycle arrest. The cell cycle block was found to be mediated through induced downregulation of cyclins and CDKs and upregulation of the CDK inhibitor p21(waf1) expression. It also showed antimetastatic properties by the inhibition of MMP-9 gene expression in human aortic smooth muscle cells [70].

Role of flavonols in apoptosis, proliferation, and angiogenesis: Kaempferol, a natural flavonoid present in many fruits and vegetables like spinach, tea, onions, curly kale, leeks, broccoli, and blueberries, has been found to only slightly induce cytotoxicity in OVCAR-3 and A2780/CP70 human ovarian cancer cells but to inhibit angiogenesis, VEGF expression, and Akt phosphorylation. HIF-1α, a regulator of VEGF, is also downregulated by kaempferol in ovarian cancer cell lines [131]. In the human HCT116 colon cancer cell line, kaempferol induced p53-dependent growth inhibition and apoptosis. It induced ATM and H2AX phosphorylation and cytochrome c release from mitochondria and activated caspase-3 cleavage [132]. Quercetin, a ubiquitous bioactive flavonol present in scallions, tea, tomatoes, alfalfa seeds, and apples, inhibits cell proliferation and induces cell cycle arrest and apoptosis in different cancer cell types. Quercetin induced apoptosis promoted by the activation of caspases-3, -8, and -9 in MDA-MB-231 cells [133]. Another study showed that, instead of cleavage of caspases, quercetin also decreased the levels of survivin and Bcl-2 protein expression in HepG2 cells and concurrently increased

the levels of p53 [134]. Quercetin induced cell cycle arrest at G2/M of SW480 cells in a process mediated through cyclin D1 and survivin gene regulation [135]. Beside apoptotic and antiproliferative effects, quercetin was found to inhibit adhesion and migration and invasion of HeLa cells [136]. Quercetin sensitized TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis in U87-MG, U251, A172, and LN229 glioma cells. The TRAIL-induced enhanced apoptosis by quercetin was mediated through reduction of the survivin protein [137]. In a study on glioma cells, quercetin caused a rapid reduction in phosphorylation of ERK and Akt. Quercetin-induced depolarization of the mitochondrial membrane is thought to lead to a decrease in expression of anti-apoptotic proteins and caspase activation and, finally, to apoptosis [138]. Quercetin also inhibits cell proliferation in ER-positive MCF-7 and T47D cells. It has been found that quercetin stimulates ERβ responses more than ERα, the receptor possibly involved in adverse cell proliferative effects [139].

In addition to quercetin, isoquercetin and rutin also affect the proliferation of Gbm cells. Isoquercetin did not show apoptosis, but inhibition of cell proliferation was found to be associated with a reduction in cyclin D1 levels and an increase in p27 levels [140]. Rutin was shown to induce apoptosis in leukemia WEHI-3 cells implanted in BALB/c mice, which showed a decreased percentage of the Mac-3 marker after rutin treatment, indicating that the differentiation of the precursor of macrophage and T cells was inhibited [141]. Pachypodol (5,4′-dihydroxy-3,7,3′-trimethoxyflavone), a flavonol isolated from the leaves of *Calycopteris floribunda,* has been found to cytotoxic as evaluated by the Promega's cell titer 96 nonradioactive cell proliferation assay using CaCo-2 colon cancer cells [142].

Resveratrol is also known to inhibit proliferation and induce apoptosis in tumor cells. In one study, resveratrol was tested in mouse xenograft models of human neuroblastoma and *in vitro* using human cell lines. Results of this study showed that resveratrol inhibited the outgrowth of tumors and decreased tumor cell viability *in vitro* by inhibiting cell proliferation and inducing apoptosis. Resveratrol caused depolarization of mitochondrial membrane potential, release of cytochrome c and Smac/Diablo from the mitochondria, and subsequently activation of caspases-9 and -3 [143]. *In vitro* results also showed that resveratrol inhibits survival and induces apoptosis in MOLT-4 acute lymphoblastic leukemia cells through modulating signaling pathway Notch and its downstream effector. This flavonol induces levels of the proapoptotic protein p53 and its effectors p21waf and Bax, inhibits the PI3K/Akt pathway, and activates Gsk-3β [144]. The antiproliferative activity of resveratrol is due to induced cell cycle arrest and finally apoptosis [145, 146]. The cell cycle arrest by resveratrol of SPC-A-1/CDDP cells was found between the G0/G1 and S phase or at the G2/M phase. It showed antitumour effects in nude mice that had been implanted with SPC-A-1/CDDP cells [147]. Resveratrol triggered c-Jun-NH$_2$-terminal-kinase activation, followed by the activation of intrinsic and extrinsic caspase pathways in Waldenström's macroglobulinemia cells [146].

It has been found that grape polyphenols at physiologically relevant concentrations are more effective than individual compounds at inhibition of ERa(−), ERb(+) MDA-MB-231 breast cancer cell proliferation, cell cycle progression, and primary mammary tumor growth [148]. It has also been found that combined grape polyphenols induced apoptosis, inhibition of cell proliferation, cell cycle progression, and cell migration more effectively than individual resveratrol, quercetin, or catechin in metastatic ER(−) MDA-MB-435 cells [149]. Myricetin exhibited a reduction

of cell vitality and induction of apoptosis via the caspase-dependent pathway in DU-145 cells [150]. A clinical study showed that there is a strong association between the intake of flavonoids and colorectal cancer incidence. In 71 976 women from the Nurses' Health Study and 35 425 men from the Health Professionals Study, results showed that the intake of flavonols, including quercetin, myricetin, and kaempferol, was related to a lower risk of colorectal cancer [151].

Role of flavanones in apoptosis, proliferation, and angiogenesis: Flavanones have been found to inhibit TPA-induced proliferation of NIH3T3 cells. The structurally related 2′-hydroxyflavanone, 4′-hydroxyflavanone, and 6-hydroxyflavanone showed inhibition of TPA-stimulated MAPK phosphorylation, COX-2, ODC, c-Jun protein expressions and PGE$_2$ production. Thus, the blocking TPA-induced intracellular signaling responses might be involved in the antipromotive mechanism of flavanones [152]. Despite an influence on cell viability, flavanone and 2′-hydroxyflavanone markedly inhibited the invasion, motility, and cell-matrix adhesion of A549 cells. This was associated with a reduced expression of MMP-2 and u-PA in A549 cells. The flavanone and 2′-hydroxyflavanone also potently attenuated the phosphorylation of ERK 1/2 and p38MAPK, as well as the activation of NF-κB and AP-1 [153]. The flavanone naringenin was shown to exhibit antimitogenic effects by modulating ERα-mediated rapid signaling. Naringenin induced ERα activities in the human cervix epitheloid carcinoma cell line (HeLa) devoid of any estrogen receptors and acted as an E2 mimetic on ERα transcriptional activity, whereas it impaired the activation of rapid signaling pathways committed to E2-induced proliferation [154]. Naringenin decreased the binding of E2 to ERα without impairing the estrogen response element (ERE). Naringenin also prevented E2-induced ERK1/2 and Akt activation and induced the activation of p38, the proapoptotic member of the MAPK family. As a consequence, naringenin stimulation impeded the E2-induced transcription of cyclin D1 promoter and reverted E2-induced cell proliferation, driving cancer cells to apoptosis [155]. Naringenin-induced apoptosis has been demonstrated by the activation of p38/MAPK leading to the proapoptotic caspase-3 activation and to the PARP cleavage in cancer cell lines [156].

Silymarin and silibinin (50–100 μg/mL) inhibited cell proliferation, apoptosis, and cell cycle arrest. G1 arrest was found to be associated with regulation of cyclin D1, cyclin D3, cyclin E, cyclin-dependent kinase (CDK)4, CDK6, and CDK2 protein CDK inhibitors (CDKIs) Kip1/p27 and Cip1/p21. Silibinin and silymarin also induced cell cycle arrest at the G2/M phase, and this was associated with decreased levels of cyclin B1, cyclin A, pCdc2 (Tyr15), Cdc2, Cdc25B, and Cdc25C [157]. Taxifolin induced apoptosis by affecting mitochondrial membrane potential. A study suggested that the 2,3 double bond/3-hydroxy group of this flavanone in conjugation with the 4-oxo function on the ring seem to favor the interaction of these compounds with the mitochondrial membrane, decreasing its fluidity by either inhibiting the respiratory chain of mitochondria or causing uncoupling [158]. The synthetic flavanone derivative 4′-chloroflavanone was found to inhibit cell proliferation through G1/S phase disruption and to induce apoptosis in both MCF-7 and MDA-MB-453 cells. The cell cycle arrest by 4′-chloroflavanone was associated with decreased CDK4 and cyclin D and with increased p21/Cip1 expression. The activation of p53 also played a role in 4′-chloroflavanone-induced cell cycle arrest of human breast cancer cells. The apoptosis induced by 4′-chloroflavanone was due to increased cytochrome c without any change in the expression of Bcl-2 and Bax [159].

Another flavanone, the 2′,5,7-trihydroxy-4′,5′-(2,2-dimethylchromeno)-8-(3-hydroxy-3-methylbutyl) derivative, isolated from the root bark of *Cudrania tricuspidata,* was found to cause apoptotic cell death of human leukemia U937 cells. Treatment with this compound in human U937 cancer cells inhibited the activity of topoisomerase I and caused growth inhibition of cells. It induced apoptosis as characterized by cleavage of PARP and procaspase-3 [160]. Another synthesized flavanone derivative, (±)-(3a*RS*,4*SR*)-2-(2-chloro-4-methyl- sulfonylphenyl)-4′-chloro-3a,4-diethoxyflavane[4,3-*d*]-Δ-1,2,3-thiadiazoline, was shown to have antileukemic activity. It inhibited the proliferation of leukemic cells and induced apoptosis by inducing procaspase-9 and procaspase-3 cleavage, XIAP and Bcl-xL downregulation, and PARP degradation and by reducing the phosphorylation of ERK1/2 and increasing the phosphorylation of Jun N-terminal kinase [161].

Role of anthocyanins in apoptosis, proliferation, and angiogenesis: The widespread consumption of diets rich in flavonoids including anthocyanins contributes to the prevention of cancer. Animal studies have shown that grape juice, which contains anthocyanin, inhibited mammary adenocarcinoma multiplicity compared with that in controls through the inhibition of DNA synthesis of rat mammary adenocarcinoma [162]. Recently, it was reported that anthocyanin-rich red grape extract containing oenocyanin interferes with intestinal adenoma development in the Apc(Min) mouse. The development of adenoma was found to be reduced by oenocyanin-induced modulation of Akt in small intestinal adenomas [163]. Cranberry extract, which contains both anthocyanins and the flavonol glycosides proanthocyanidins, have synergistic or additive antiproliferative interactions. Treatment with cranberry extracts in human oral (KB, CAL27), colon (HT-29, HCT116, SW480, SW620), and prostate (RWPE-1, RWPE-2, 22Rv1) cancer cell lines showed inhibition of proliferation [164]. Anthocyanin-rich extracts from berries and grapes and several pure anthocyanins and anthocyanidins have exhibited proapoptotic effects in multiple cell types *in vitro* [165–168]. Prodelphinidin B2 3′-*O*-gallate, a proanthocyanidin gallate isolated from green tea leaves, also has antiproliferative activity in human non-small cell lung cancer A549 cells. Prodelphinidin B2 3′-*O*-gallate blocked cell cycle progression in the G0/G1 phase and effectively induced apoptosis of A549 cells [169]. Anthocyanidins, the aglycones of anthocyanins, have also been shown to have inhibitory effects on human cancer cell proliferation. The anthocyanidins – cyanidin, delphinidin, pelargonidin, petunidin, and malvidin – inhibited proliferation of human cancer cell lines AGS (stomach), HCT-116 (colon), MCF-7 (breast), NCI H460 (lung), and SF-268 (central nervous system) [170]. Malvidin exhibited a potent antiproliferative effect on AGS cells. The malvidin-induced inhibition of proliferation was accompanied by the arrest of AGS cells at the G0/G1 phase. The occurrence of apoptosis induced by malvidin was confirmed by morphological and biochemical features, including apoptotic body formation, loss of mitochondrial membrane potential, elevation of the Bax: Bcl-2 ratio, caspase 3 activation, and PARP proteolysis [171]. The anthocyanins present in black raspberries contribute to the chemopreventive activity *in vivo.* Anthocyanin treatment in F344 rats was effective in reducing NMBA-induced tumorigenesis in the esophagus [172].

Delphinidin, another anthocyanin, showed G2/M phase cell cycle arrest, apoptosis, and inhibition of NF-κB signaling in 22Rnu1 cells. Delphinidin treatment of human prostate cancer LNCaP, C4-2, 22Rnu1, and PC3 cells resulted in a dose-dependent inhibi-

tion of cell growth without showing any substantial effect on normal human prostate epithelial cells. The induction of apoptosis by delphinidin was mediated via activation of caspases [173]. Delphinidin also inhibited VEGF-stimulated human umbilical endothelial cell migration and proliferation and neovascularisation *in vivo* in a chorioallantoic membrane model [174]. Anthocyanins inhibit metastasis through regulation of MMP-2 and MMP-9, and it also modulates the expression levels of Ras, PI3K, phospho-Akt, and NF-κB as studied in B16-F1 cells [175]. Peonidin 3-glucoside and cyanidin 3-glucoside, major anthocyanins extracted from black rice, also inhibited invasion and motility of SKHep-1 cells through the mechanism of MMP-9u-PA inhibition [82].

Role of isoflavonoids in apoptosis, proliferation, and angiogenesis: Isoflavonoids, flavonoids, and lignans are natural estrogenic compounds derived from soy, tea, fruits, and vegetables, and they have been proposed as chemopreventive agents. It has been known that consumption of a plant-based diet can prevent the development and progression of chronic diseases and also extensive neovascularization of tumor cells. In an epidemiological study it has been observed that the urine of healthy human subjects consuming a plant-based diet had a high content of the isoflavonoid genistein in the micromolar range, which is 30-fold higher than that of subjects consuming a traditional Western diet. Furthermore, it has been observed that genistein inhibited endothelial cell proliferation and *in vitro* angiogenesis [176]. Because many Western diseases are hormone-dependent cancers, a vegetarian or semivegetarian diet containing many fruits and vegetables may alter hormone production, metabolism, or action at the cellular level. Soybean products (mainly isoflavonoids but also lignans), as well as whole grain cereals, seeds, and probably berries and nuts (mainly lignans) inhibit growth factor action, malignant cell proliferation, differentiation, and angiogenesis, making them strong candidates for a role as natural cancer protective compounds.

Studies have shown that soy protein preparations containing isoflavonoid conjugates have chemopreventive activity. Rats treated neonatally or prepuberally with genistein have a longer latency and a reduced number of 7,12-dimethylbenz*[a]*anthracene-induced mammary tumors. Genistein was found to inhibit the rapid proliferation and differentiation of the mammary gland by the inhibition of estrogenic activity [177]. Besides genistein, other isoflavonoids, such as daidzein, biochanin A, formononetin, and equol, also inhibted the proliferation of estrogen receptor ER-positive MCF-7 cells induced by synthetic chemicals 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2,2-trichloroethane (*o*,*p*'-DDT), 4-nonylphenol (4-NP), and 5-octylphenol (5-OP). Among these isoflavonoids, genistein was the most potent inhibitor of MCF-7 cell proliferation [178]. Jung et al. [179] showed that tectorigenin and tectoridin isolated from the rhizomes of *Belamcanda chinensis* (Iridaceae) inhibited angiogenesis in both *in vitro* and *in vivo* models. They observed in a chorioallantoic membrane assay that tectorigenin and tectoridin decreased both angiogenesis of chick embryos and vessel formation in the mouse Matrigel plug assay. Along with these properties, tectorigenin also exhibited strong antiproliferative activity in mice implanted with murine Lewis lung carcinoma. Pterocarpans, natural isoflavonoids, have received considerable interest because of their medicinal properties. Erybraedin C and bitucarpin A are two recently discovered structurally related pterocarpans that show antiproliferative and apoptosis-inducing mechanisms on human adenocarcinoma cell lines LoVo and HT29. Erybraedin C similarly affected the survival of HT29 (MMR +/+, p53 −/−, and Bcl-2 +/+) and LoVo (MMR −/−,

p53 +/+, and Bcl-2 −/−) cells, and bitucarpin A affected cell cycle progression on both cell lines, inducing a transient block in G0/G1 and finally inducing apoptosis [180]. Vitexin, an isoflavonoid, showed antitumor effects and cytotoxic activity exerted through a proapoptotic process, which is mediated by a decreased Bcl-2/Bax ratio and activation of caspases [115]. It also diminished the hypoxia-inducible genes such as VEGF, smad3, aldolase A, enolase 1, and collagen type III in the PC12 cells which indicates its antimetastatic potential [181]. Vitexicarpin, a flavonoid from the fruits of *Vitex rotundifolia*, inhibited T-lymphocyte proliferation as well as B-lymphocyte proliferation at > 0.1 microM. Vitexicarpin also inhibited the growth of certain cancer cell lines, EL-4 and P815.9 [182]. Morin inhibited the proliferation of cancer cells by inhibiting the gene products IAP-1, IAP-2, XIAP, Bcl-xL, survivin, cyclin D1, and COX-2 and invasion by inhibiting MMP-9 [87].

Considerable evidence has shown that increased consumption of soy and soy isoflavones is associated with a reduced risk for cancer. In a study it has been reported that glycitein, a component of soy, significantly inhibited cellular proliferation of prostate cancer RWPE-1 cells [183]. In the same cancer cells, Clubbs and Bomser [184] showed that glycitein activates ERK1/2, and decreased RWPE-1 cell proliferation. Glycitein-induced ERK1/2 activation was found to be dependent, in part, on tyrosine kinase activity associated with VEGFR. Targeretin has been shown to induce G1 cell cycle arrest in colorectal cancer cells through upregulation of p21, p27 and p53 [185]. Numerous reports have previously shown that the compound isolated from the *Gardenia* species, 5,3′-dihydroxy-3,6,7,8,4′ pentamethoxyflavone, is cytotoxic to various tumor cells [186, 187]. Thus, these isoflavonoids may act as anticancer agents and could enhance the apoptosis induced by different chemotherapeutic agents.

Flavonoids inhibit cell proliferation and induce apoptosis, in general, through up-modulation of p53 and its regulated gene products (CDK inhibitors), p21/cip1/WAF and p27/Kip1, and down-modulation of cyclin D1, cyclin A, cyclin B1, cdc25C, and cdc2. Consequently, the cell cycle is arrested at the G2/M boundary, and apoptosis is induced by the induction of Bax and Bak, down-moduation of Bcl-2 and Bcl-xL, the release of cytochrome C and activation of caspases-9 and -3. In addition to this, the apoptosis induced by 4′-chloroflavanone is due to increased cytochrome c release, without any change in the expression of Bcl-2 and Bax. Resveratrol (a flavonol) involves the activation of the mitochondrial pathway, release of cytochrome c and Smac/Diablo from the mitochondria, and subsequently activation of caspases-9 and -3. Resveratrol further inhibits survival, and induces apoptosis through modulating the signaling pathway of Notch and its downstream effectors. The apoptotic pathway is further accentuated by inhibition of the NF-kB-dependent survival pathway. A chalcone (butein) exhibits antiproliferative and apoptotic effects against tumor cells through suppression of the STAT3 activation pathway, which is mediated through the inhibition of the upstream kinases c-Src, Janus kinase (JAK)1 and 2. Some flavonoids inhibit proliferation of breast cancer cells by inhibiting E2-dependent proliferation and subsequently driving cancer cells to apoptosis. In some other cases (as in the case of quercetin), involvement of TRAIL-mediated apoptosis through DR 4/5, and reduction in the expression of survivin protein have been shown. The inhibition of angiogenesis by flavonoids proceeds primarily through down-modulation of VEGF expression.

Regulation of inflammatory cytokines and chemokines by flavonoids: Cytokines and chemokines have been postulated to play

an important role in the maintenance of cell homeostasis and the development of cancers. Therefore, excess production of cytokines and chemokines can be minimized by various flavonoids. Xanthohumol has been shown to repress interleukin-8 expression and proliferation and migration of hepatocellular carcinoma [188]. Amentoflavone, a biflavonoid from *Biophytum sensitivum*, inhibits proinflammatory cytokine (IL-1beta, IL-6, GM-CSF and TNF-alpha) production in B16F-10 cells and peritoneal macrophages [189]. It also induces apoptosis and inhibits NO production in B16F-10 cells. Ashwagandha *(Withania somnifera)* treatment in prostate cancer cells downregulated the gene and protein expression of proinflammatory cytokines IL-6, IL-1beta, chemokine IL-8, Hsp70 and STAT-2 and prevented prostate cancer progression [190]. The elevated levels of TNF-alpha in LPS- and Con-A stimulated BALB/c mice were found to be lowered by pretreatment with rutin. Rutin also inhibits nitrite production by activated macrophages *in vitro* to the normal level [191].

Kaempferol and quercetin activate an immune response in the prostate cancer cells by stimulating GM-CSF production. The stimulated GM-CSF production involves PLC, PKC, and MEK1/2 activation. This GM-CSF production in turn could result in the recruitment of DCs to the tumor site [192]. Flavonoids, kaempferol, quercetin, fisetin, and chrysin have also been shown to block the TNF-alpha induced IL-8 promoter activation and gene expression in HEK 293 cells [193]. Kaempferol suppressed the expression of proinflammatory cytokine interleukin-6 and chemokines interleukin-8, monocyte chemoattractant protein-1, and regulated, on activation, normal T-cell expression and secretion. Kaempferol inhibited glioma cell migration in a ROS-dependent manner [194]. Flavonoids not only suppress cytokines production, but also inhibit cytokines regulated gene products. A flavonoid, baicalein, inhibits the protein phosphorylation such as that of Jak, STAT3, MAPK, and Akt induced by IL-6 [195]. Silibinin also strongly decreased cytokine mixture-induced phosphorylation of ERK1/2 but only marginally affected JNK1/2 phosphorylation silibinin and downregulated iNOS expression in lung cancer cells [196]. Treatment of hemopoietic cells from human and mouse origin with kaempferol prevents the IL-4-induced phosphorylation of JAK1 and JAK3. It also blocked the *in vitro* phosphorylation activity of JAK3 without affecting JAK1, suggesting that it specifically targeted JAK3 activity [197].

### Flavonoids as a kinase inhibitor via direct binding

Protein kinases, which phosphorylate a specific substrate, play crucial roles in the regulation of multiple cell signaling pathways and cellular functions. However, deregulation of protein kinases under certain pathological conditions, leads to perturbation of protein kinase-mediated cell signaling pathways and results in various disorders including inflammation and cancer [198]. Because of the involvement of these processes in tumor cell proliferation and survival, regulation of protein kinases would be a promising strategy for the prevention and treatment of cancer [199–201]. It is evident that natural compounds have a great potential in the regulation of multiple cellular signaling pathways and cancer prevention and treatment.

Flavonoids are one of the biggest families of natural products and have been proposed to exert beneficial effects in a multitude of diseases, including cancer. It has been shown that they regulate various cells signaling pathways. Recent studies have suggested that the cellular effects of flavonoids may be mediated by their interactions with specific proteins central to intracellular signaling cascades [202–204]. Studies on the functional activity of fla-

vonoids showed that it may interact selectively at different components of a number of protein products including kinases. Interaction or direct binding of flavonoids with kinases such as phosphoinositide 3-kinase (PI3K) [205, 206], Akt/protein kinase B (Akt/PKB) [207], protein kinase C (PKC) [205, 208], and mitogen-activated protein kinase (MAPKs) [209] may alter their phosphorylation state to regulate multiple cell signaling pathways (◉ Fig. 6).

It is known that JAK kinase, one of the non-receptor tyrosine protein kinases, is required for the tyrosine phosphorylation STAT3. The flavonol myricetin is found to bind with JAK1 with rapid formation of the JAK1-myricetin complex. When it is compared with piceatannol, which is a specific inhibitor for JAK1, it slowly reacts with JAK1 [210]. Other than JAK kinase, it also binds to Akt, MEK1, Fyn, MKK4 and PI3K. The results of *ex vivo* and *in vitro* binding data showed that myricetin bound directly to the ATP-binding site of Akt with higher binding affinity ($K_d = 0.26\,\mu M$) [210]. The direct binding of MEK1 was revealed by an *ex vivo* binding assay and it was found that binding was distinct from the ATP-binding site [72]. However, it directly binds to the ATP-binding site of Fyn [211]. The binding of Fyn was found to be associated with the formation of hydrogen bonds with the backbone of the hinge region of Fyn. As we know, Fyn is a ubiquitously expressed member of the Src family of non-receptor tyrosine kinases that is involved in transmitting signals from various cell surface receptors to cytoplasmic signal transduction cascades. Myricetin also binds to the ATP-binding site of MKK4 as revealed by *ex vivo* and *in vitro* binding data [212]. The docking data revealed that myricetin fits easily onto the ATP-binding site of MKK4, located between the N- and C-lobes of the kinase domain. Like Fyn, myricetin forms hydrogen bonds with the backbone of the hinge region in MKK4. The direct binding of myrecetin with PI3Kγ revealed that it fits into the ATP-binding pocket with a K_d of $0.17\,\mu M$, which is slightly lower than that of quercetin as shown by X-ray crystallography.

Tea flavonoid epigallocatechin gallate (EGCG) also has direct binding properties to kinases. An *in vitro* protein binding assay revealed that it bound directly to Fyn ($K_d = 0.367\,\mu M$) of the GST-Fyn-SH2 domain but not the GST-Fyn-SH3 domain, which leads to inhibition of its kinase activity [213]. This binding of Fyn by EGCG could inhibit the phosphorylation of EGF-induced p38, activating transcription factor-2 (ATF-2) and STAT1 with attenuated cell transformation. Another flavonol, quercetin, is found to bind directly with Raf1 and MEK1 as observed in *ex vivo* and *in vitro* studies. Further study showed that quercetin has a stronger kinase inhibitory activity on MEK1 than Raf1 [71]. Interestingly, quercetin could be docked to the pocket separate from but adjacent to the ATP-binding site of MEK1. However, MEK1 inhibition by resveratrol and kaempferol was found to be weaker than that of quercetin. Although the binding model of resveratrol appears similar to that of quercetin. However, the lack of the hydroxy group at the 3′ position of resveratrol would result in the failure of the formation of the hydrogen bond between resveratrol and the backbone amide group of Ser212 [71]. The direct binding of kinase MEK1 was also observed with quercetin. The X-ray crystallographic structure showed that quercetin fits into the ATP-binding site of PI3Kγ with a K_d value of $0.28\,\mu M$, which is slightly higher than that of myricetin [214]. Isoflavonoid equol bound directly to MEK1 without competing with ATP as observed by pulldown assay. Modeling data further suggest that equol is docked to a pocket separate from but adjacent to the ATP-binding site [215].
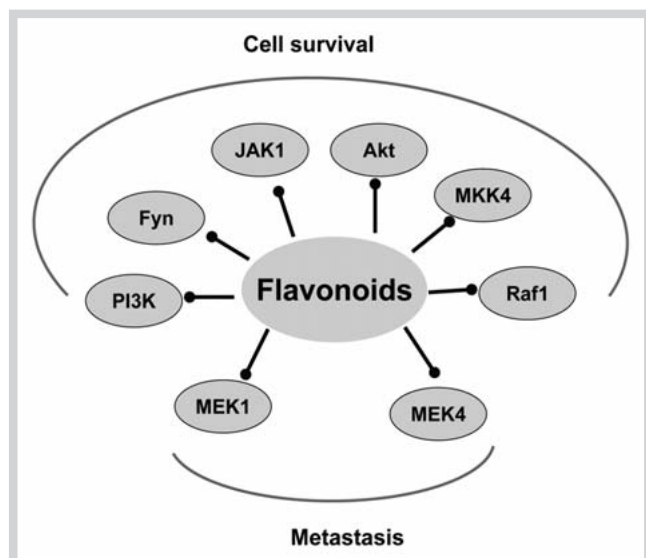
Fig. 6 Inhibition of different kinases by direct binding of flavonoids. The kinases invoved in cell survival, proliferation and metastasis of cells are grouped together.

Conclusion
▼

Overall, the studies described above provide evidence that fruits, vegetables, spices, nuts, and legumes comprise a vast array of biologically active flavonoids, many of which have been used in traditional medicine for thousands of years. The considerable evidence suggests that flavonoids in a diet rich in fruits and vegetables are associated with a decreased risk of inflammation-regulated chronic diseases, including cancer. Most of this evidence is preclinical, and more clinical trials are needed to further strengthen this evidence. Because most chronic diseases, including cancer, incubate for 20–30 years before they manifest, structuring such clinical trials will be difficult. It remains unknown what amount of flavonoids are needed and for how long and whether it is better to consume food with flavonoids or if supplements will suffice. The evidence, however, that these flavonoids are safe, multitargeted, efficacious, and affordable demands further investigation in cancer and other chronic diseases.

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