Dietary flavonoids as cancer-preventive and therapeutic biofactors

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1. ABSTRACT

Flavonoids are present in many plants, and hence, in foods and ingredients derived from them. These polyphenolic compounds have attracted renewed attention as potential anticarcinogens, and the molecular mechanisms of their anticarcinogenic effects and their bioavailability have been extensively explored. In this review, we focus on the major dietary flavonoids; flavones, flavonols, and flavan-3-ols (catechins), and evaluate their roles in cancer prevention. After absorption with or without metabolic conjugation, flavonoids are transported to target organs where they exert their anticarcinogenic activity. The molecular mechanisms of the anticarcinogenic effects of flavonoids include their antagonistic effect on the aryl hydrocarbon receptor (AhR), and regulation of phase I and II drug metabolizing enzymes and phase III transporters. Experimental evidence suggests that flavonoids modulate signal transduction pathways at each stage of carcinogenesis. The interactions between flavonoids and biomolecules *in vivo* must be investigated in detail to identify specific targets. In addition, the potential side effects should be considered when flavonoid supplements are used for cancer prevention. Therefore, the use of flavonoids as chemopreventive agents should be further investigated to establish safe levels of flavonoid intake.

2. INTRODUCTION

Flavonoids are polyphenolic compounds that are produced as secondary metabolites throughout the plant kingdom. They are ubiquitously present in foods, ingredients, and medicines derived from plants, and have attracted much attention because of their possible roles in the prevention of a wide range of degenerative diseases. A common group of flavonoids is the diphenylpropanes, which have one or more phenolic groups (Figure 1). Numerous compounds from plant foods have been identified as flavonoids, and these compounds can be categorized into various subclasses, including flavones, flavonols, flavan-3-ols (catechins), isoflavones, flavanones, anthocyanidins, and some other minor components including chalcones, coumarins, and aurones (1). Vegetables such as onion, lettuce, cabbage, and broccoli are the major sources of dietary flavones and flavonols. Catechins include cacao polyphenols and tea catechins. Tea catechins mainly comprise (-)-epicatechin (EC), (-)-epicatechin gallate (ECg), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCg). In general, the gallate-type EGCg is the predominant tea catechin. Isoflavones are found only in legumes, and are present at particularly high concentrations in soybeans. Anthocyanidins and flavanones are the major flavonoid subclasses in fruits and citrus fruits.
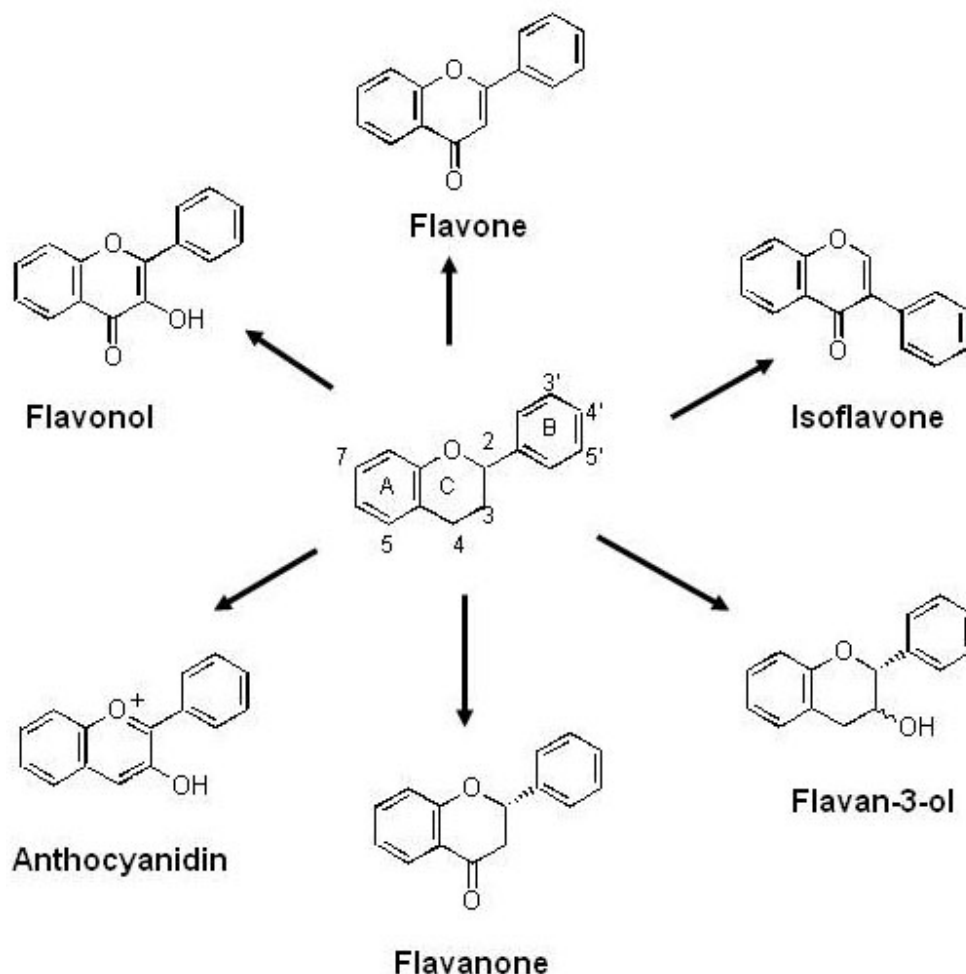
Figure 1. Basic structure of flavonoids.

Except for catechins, flavonoids generally exist as their glycoside form, in which a monosaccharide, disaccharide, or oligosaccharide binds to the hydroxyl group via a β-glycoside linkage (2). Thus, there are many flavonoid derivatives that differ according to their sugar group and the site of its attachment to the hydroxyl group. Quercetin (3,3',4',5,7-pentahydroxyflavone) is a typical flavonol present in vegetables. Previously (3), we described the anticarcinogenic effect of dietary quercetin, which involves its anti-/pro-oxidant chemistry and various molecular targets. Here, we evaluate the role of dietary flavonoids in cancer prevention focusing mainly on flavones, flavonols, and catechins.

Luteolin and apigenin are two representative flavones that are generally found as their 7-*O*-glycosides (Figure 2). They are distributed in celery, parsley, and some herbs but are not as widely distributed as flavonols. Interestingly, polymethoxylated flavones such as nobiletin and tangeretin are found in citrus species. Myricetin, quercetin, kaempferol, and isorhamnetin are common flavonols (Figure 2) that generally exist as *O*-glycosides in which the sugar group is frequently bound

to the hydroxyl group at the 3-positon of the C-ring. In onion, quercetin-4'-*O*-β-glucoside (Q4'G) and quercetin 3,4'-*O*-β-diglucoside (Q3,4'diG) are major flavonols in which the glucoside group is attached to the 4'-position of the B-ring. As mentioned above, tea catechins comprise EC, ECg, EGC, and EGCg (Figure 3). Gallate-type catechins, i.e., ECg and EGCg, are characteristic tea catechins. Cacao polyphenols consist of monomers of EC/(+)-catechin and their oligomeric proanthocyanidins.

It has long been believed that the antioxidant activity of dietary flavonoids contributes to their anticarcinogenic effects. The catechol structure at the B-ring (*o*-dihydroxy structure) should confer powerful radical scavenging activity (4). However, the antioxidant activity of dietary flavonoids does not necessarily contribute to their anticarcinogenic effects or other physiological functions. In this review, we first focus on the results of epidemiological studies on the relationship between dietary flavonoids and cancer prevention. Then, we focus on the bioavailability of flavonoids, and discuss their metabolic inactivation by phase II enzymes and reactivation through
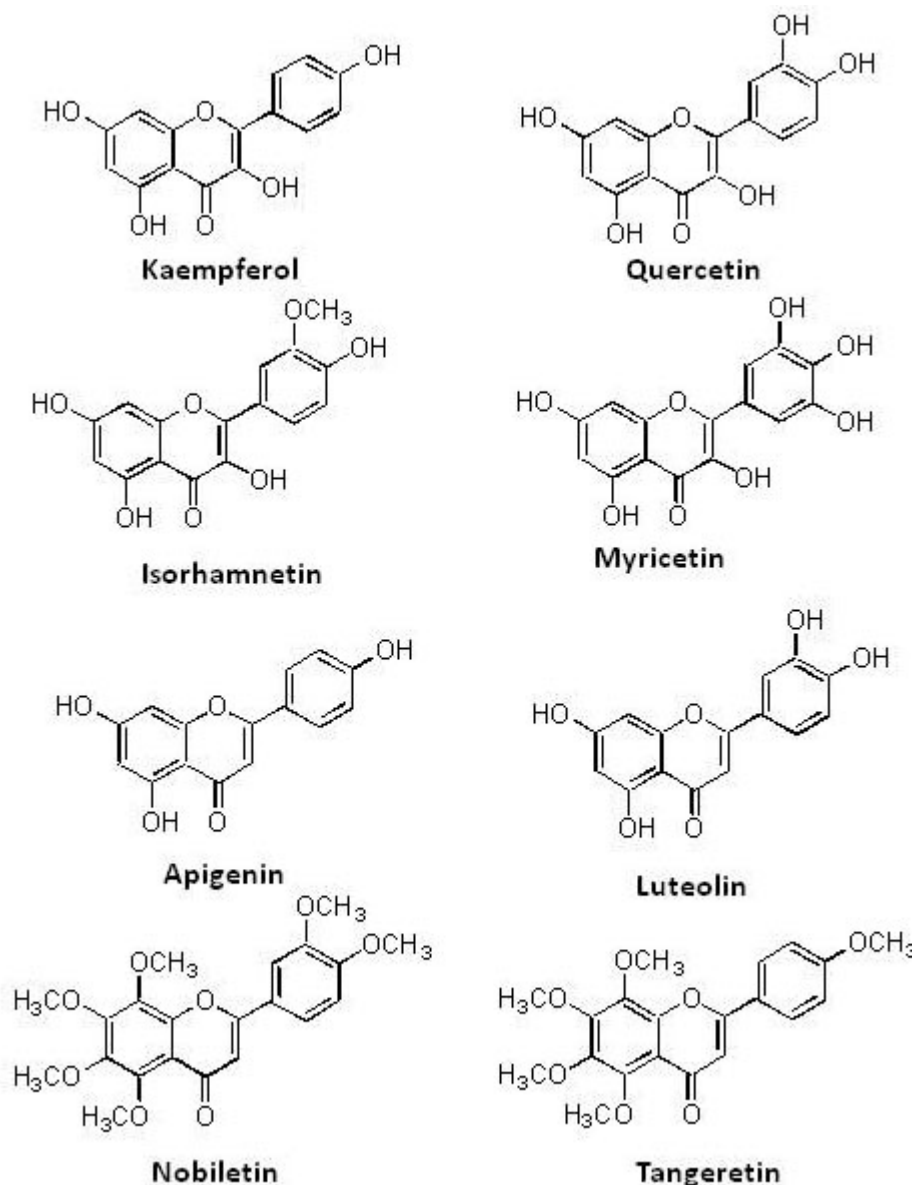
Figure 2. Major flavonols, flavones and polymethoxylated flavones.

deconjugation. In addition, we discuss the mechanisms of cancer prevention by flavonoids as determined by experiments utilizing animal models and cultured cell studies *in vitro*. Finally, we discuss the potential side effects of flavonoids, and consider the proper utilization of flavonoids as dietary supplements or other pharmacological applications.

3. EPIDEMIOLOGY

To date, more than 40 epidemiological studies have evaluated the effects of flavonoids (flavonols, flavones, and/or catechins) on cancer risk (summarized in Table 1). For flavonols and flavones, their protective effects against cancer incidence were revealed by prospective cohort studies and case-control studies. Two

prospective cohort studies (5, 6) and a case-control study (7) showed significant inverse relationships between flavonols and/or flavones intake and lung cancer risk. A meta-analysis also showed that flavonols, but not flavones, had a preventative effect against lung cancer (8). None of the prospective cohort studies for breast cancer risk showed an inverse association with high consumption of flavonoids (flavonols and/or flavones) (5, 9, 10), while two case-control studies found that flavones had a protective effect against breast cancer in all women (11, 12). For ovary cancer risk, only one out of two prospective cohort studies (9, 13) and one out of two case-control studies (14, 15) reported a significant inverse relationship with flavonols and/or flavones intake. Although five prospective cohort studies (5, 6, 9, 16, 17) and three case-control studies (18-20) evaluated the effects of intake of flavonols and flavones
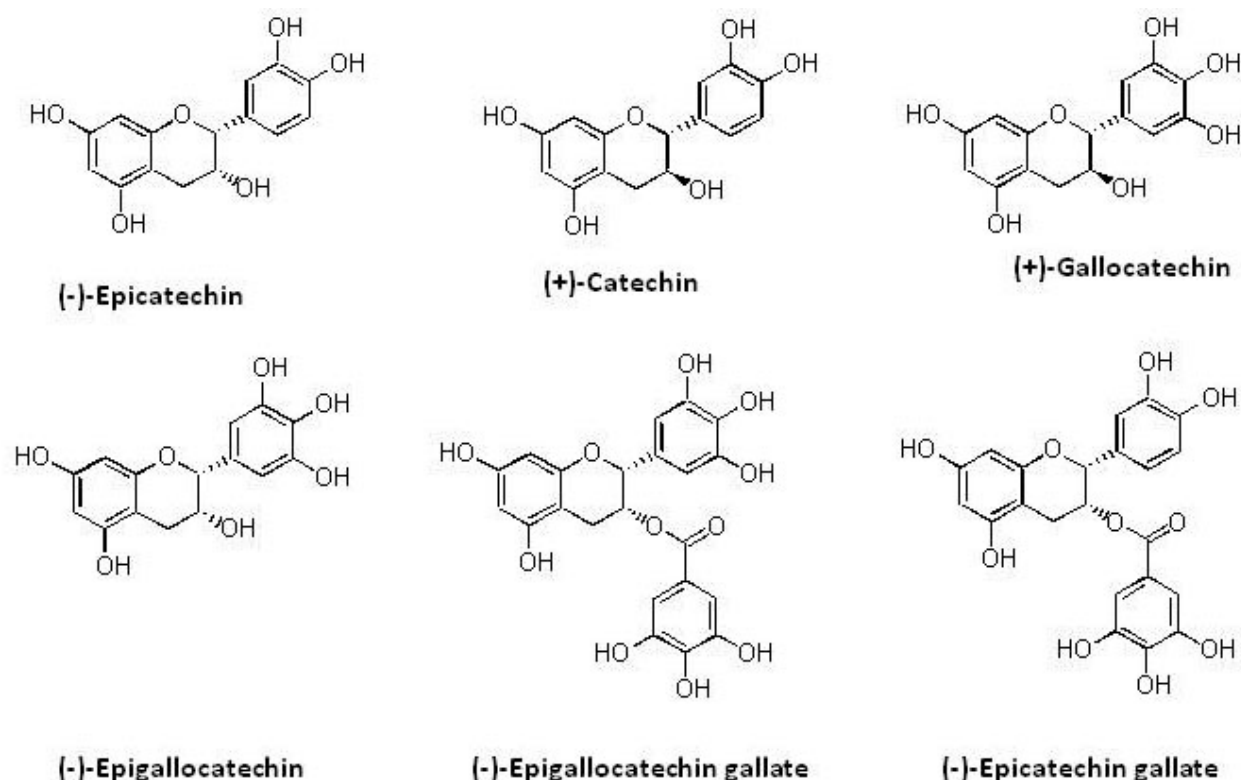
Figure 3. Structures of flavan-3-ol (catechins).

on colorectal cancer, four of the prospective cohort studies (6, 9, 16, 17) did not find an inverse association. On the other hand, the Polyp Prevention Trial showed that high consumption of flavonols decreased the reoccurrence of advanced colorectal adenoma (21). Significant inverse associations were also reported for cancers of the pancreas (22), larynx (23), mouth and pharynx (24), stomach (25), liver (26) and kidney (27), but not esophagus (28). In addition, flavonols and/or flavones showed some promise in reducing the incidence of cancer of the nervous system, although there was not a significant difference in relative risk, nor was the *p* value provided for the trend test (5).

Three prospective cohort studies [rectum (29), lung (16), and pancreas (22)] and two case-control studies [lung (7) and kidney (27)] showed protective effects of catechins against the incidence of cancer. However, most of the epidemiological studies demonstrated that catechins did not reduce the risk of several types of cancer. Moreover, meta-analyses of epidemiological studies also demonstrated that there is no correlation between tea consumption and cancer incidence (8, 30-37), though high levels of tea consumption had a potency to protect lung and endometrial cancers.

There are some limitations to the above epidemiological studies. The most common limitation is the accuracy of dietary data-based classification of the subjects. Although the Food Frequency Questionnaire was used in several studies, this was not designed to estimate the

flavonoid intake (13, 21, 23, 29, 38). In addition, if the food composition database was unsuitable, the flavonoid content would be over or underestimated (e.g US Department of Agliculture database to the Italian diet) (5, 14, 16-18, 22-28). Cultivation condition also causes the variation in the flavonoid content of vegetables and fruits (23, 28). The efficacy of flavonoids would be affected by their bioavailability, e.g., Kyle *et al.* (20) showed that there was a significantly reduced risk of developing colorectal and colon cancer associated with non-tea flavonoid intake, but not total flavonol intake.

Statistical data are usually adjusted for confounding factors including age, gender, smoking, education, energy intake and so on. In addition, supplements and genetic factor have been found to be potential and important confounding factors. In the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, β-carotene or α-tocopherol showed side effects because they increased relative risks of lung and pancreatic cancer, respectively (39, 40). A prospective cohort study following male smokers in the ATBC study reported that the supplement group-adjusted lung cancer risk was inversely associated with a high intake of flavonoids (flavonols and flavones) (6). In contrast, intake of flavonoid significantly reduced the risk of exocrine pancreatic cancer of a placebo group, whereas it had no beneficial effect on the group taking supplements (α-tocopherol and/or β-carotene) (22). These results suggest that the protective effects of flavonoids may be abolished in some tissues by

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Table 1. Flavonoid intake and cancer incidence

Flavonoid [1]	Cancer	Adjusted ratio [2] (95% CI)	P-trend	Country	Year (Follow-up)	Populations	Ref.
Prospective cohort studies							
Flavonoids [3]	Breast	0.72 (0.36-1.48)	–	Finland	1967 (24)	10,054MF	(5) [13]
	Lung	0.53 (0.29-0.97)	–				
	Stomach	1.15 (0.48-2.78)	–				
	Colorectum	0.74 (0.32-1.68)	–				
	Pancreas	1.46 (0.37-5.71)	–				
	Prostate	1.39 (0.56-3.46)	–				
	Urinary organs	0.84 (0.29-2.45)	–				
	Nervous system	0.32 (0.08-1.37)	–				
	Leukemia/lymphoma	0.90 (0.36-2.25)	–				
	Skin	0.91 (0.51-1.64)	–				
	All	0.87 (0.70-1.09)	–				
Flavonoids [3]	Lung	0.56 (0.45-0.69)	0.0001	Finland	1985 (8)	27,111M [11]	(6) [14]
	Stomach	1.2 (0.71-1.9)	0.51				
	Kidney	0.63 (0.36-1.1)	0.10				
	Urothelium	1.2 (0.73-1.8)	0.77				
	Prostate	1.3 (0.87-1.8)	0.24				
	Colorectum	1.7 (1.0-2.7)	0.10				
Flavan-3-ols [4]	Upper digestive tract	0.71 (0.46-1.11)	0.31	USA	1986 (8)	34,651F	(29) [15]
	Colon	1.10 (0.85-1.44)	0.63				
	Rectum	0.55 (0.32-0.95)	0.02				
	Pancreas	0.74 (0.46-1.20)	0.77				
	Bronchus and lung	0.94 (0.72-1.23)	0.94				
	Hematopoietic	0.65 (0.43-0.98)	0.14				
	Breast	1.04 (0.84-1.28)	1.00				
	Uterus	1.00 (0.73-1.36)	0.54				
	Ovary	0.73 (0.44-1.24)	0.21				
	Kidney	0.73 (0.40-1.32)	0.12				
	Bladder	1.12 (0.65-1.93)	0.93				
	Non-Hodgkin lymphoma	1.26 (0.87-1.85)	0.13				
Flavonols	Lung	0.29 (0.11-0.78)	0.055	Finland	1989 (16.2)	2,590M	(16) [16]
Flavones		1.36 (0.62-3.01)	0.876				
Flavan-3-ols		0.24 (0.09-0.64)	0.003				
Flavonols	Prostate	0.99 (0.61-1.62)	0.661				
Flavones		0.73 (0.44-1.22)	0.183				
Flavan-3-ols		1.13 (0.70-1.82)	0.941				
Flavonols	Colorecum	1.53 (0.72-3.23)	0.585				
Flavones		0.71 (0.30-1.65)	0.561				
Flavan-3-ols		1.37 (0.65-2.89)	0.820				
Flavonols	Breast	1.05 (0.83-1.34)	0.96	USA	1991 (8)	90,630F	(10) [17]
Quercetin		1.05 (0.83-1.33)	0.81				
Kaempferol		1.01 (0.80-1.27)	0.91				
Myricetin		0.99 (0.78-1.26)	0.35				
Flavonols	Pancreas	0.54 (0.27-1.07) [6]	0.04	Finland	1985 (19.4)	27,111M [11]	(22) [14]
		1.24 (0.87-1.78) [6,7]	0.11				
Flavones		0.89 (0.46-1.69) [6]	0.80				
		1.09 (0.75-1.56) [6,7]	0.50				
Flavan-3-ols [5]		0.42 (0.19-0.92) [6]	0.01				
		1.00 (0.69-1.44) [6,7]	0.54				
Total		0.36 (0.17-0.78) [6]	0.009				
		0.98 (0.69-1.39) [6,7]	0.39				
Flavonoids [3]	Ovary	0.75 (0.51-1.09)	0.02	USA	1984 (18)	66,940F	(13) [18]
Quercetin		0.80 (0.55-1.16)	0.04				
Kaempferol		0.60 (0.42-0.87)	0.002				
Myricetin		0.72 (0.50-1.04)	0.01				
Luteolin		0.66 (0.49-0.91)	0.01				
Apigenin		1.33 (0.96-1.83)	0.03				
Flavonoids [3]	Colorectum (M)	0.97 (0.76-1.23) [6]	0.83	Netherland	1986 (13.3)	120,852MF	(17) [19]
	Colon (M)	0.97 (0.74-1.27) [6]	0.72				
	Rectum (M)	1.04 (0.72-1.49) [6]	0.67				
Flavan-3-ols [4]	Colorectum (M)	0.99 (0.77-1.25) [6]	0.65				
	Colon (M)	1.13 (0.86-1.48) [6]	0.65				
	Rectum (M)	0.80 (0.56-1.14) [6]	0.24				
Flavonoids [3]	Colorectum (F)	0.90 (0.70-1.16) [6]	0.40				
	Colon (F)	0.85 (0.65-1.12) [6]	0.35				
	Rectum (F)	1.08 (0.67-1.75) [6]	0.94				
Flavan-3-ols [4]	Colorectum (F)	0.79 (0.61-1.02) [6]	0.20				
	Colon (F)	0.82 (0.62-1.09) [6]	0.25				
	Rectum (F)	0.80 (0.48-1.33) [6]	0.69				

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Case-control studies							
Flavonols Flavones Flavan-3-ols	Breast	0.75 (0.59-0.95) 0.73 (0.57-0.93) 0.85 (0.67-1.09)	0.05 0.004 0.17	USA	1996-1997	1,434/1,440F [12]	(12)
Epicatechin Catechin Quercetin Kaempferol Myricetin	Lung	0.61 (0.40-0.93) [8] 0.44 (0.29-0.66) [8] 0.63 (0.39-1.0) [8] 0.66 (0.42-1.0) [8] 0.68 (0.45-1.0) [8]	0.0066 <0.0001 0.025 0.0079 0.22	USA	1999-2004	558/837MF [12]	(7)
Flavonols Flavones Flavan-3-ols [5]	Esophagus	0.68 (0.38-1.24) 0.97 (0.57-1.67) 1.06 (0.58-1.94)	0.060 0.877 0.773	Italy	1992-1197	304/743MF [12]	(28)
Flavonols Flavones Flavan-3-ols [5]	Larynx	0.32 (0.20-0.52) 0.76 (0.50-1.15) 0.64 (0.41-0.99)	<0.001 0.112 0.071	Italy	1992-2000	460/1,088MF [12]	(23)
Flavonols Flavones Flavan-3-ols [4]	Mouth and pharynx	0.62 (0.43-0.89) 0.75 (0.55-1.04) 0.84 (0.60-1.18)	0.002 0.073 0.15	Italy	1992-2005	805/2,081MF [12]	(24)
Flavonols Flavones Flavan-3-ols	Stomach	0.40 (0.25-0.64) 0.60 (0.40-0.89) 1.10 (0.76-1.60)	<0.001 0.012 0.623	Greece	1981-1984	110/100MF [12]	(25)
Flavonols Flavones Flavan-3-ols	Liver [9]	1.35 (0.75-2.44) 0.50 (0.27-0.94) 1.17 (0.65-2.12)	0.67 0.049 0.69	Greece	1995-1998	339/360MF [12]	(26)
Flavonols Flavones Flavan-3-ols [5]	Kidney	0.69 (0.50-0.95) 0.68 (0.50-0.93) 0.77 (0.56-1.06)	0.044 0.007 0.045	Italy	1994-2002	767/1,534MF [12]	(27)
Flavonols Flavones Flavan-3-ols [5]	Ovary	0.63 (0.47-0.84) 0.79 (0.60-1.04) 0.89 (0.67-1.17)	0.009 0.11 0.63	Italy	1992-1999	1,031/2,411F [12]	(14)
Flavonols Flavones Flavan-3-ols [5]	Colorectum	0.64 (0.54-0.77) 0.78 (0.65-0.93) 0.98 (0.82-1.18)	<0.001 0.004 0.736	Italy	1992-1996	1,953/4,154MF [12]	(18)
Flavonols (Total) Flavonols (Non-tea) Flavan-3-ols (Total) [4] Flavan-3-ols (Non-tea) [4] Flavonols (Total) Flavonols (Non-tea) Flavan-3-ols (Total) [4] Flavan-3-ols (Non-tea) [4] Flavonols (Total) Flavonols (Non-tea) Flavan-3-ols (Total) [4] Flavan-3-ols (Non-tea) [4]	Colorectum Colon Rectum	0.8 (0.5-1.3) 0.6 (0.4-1.0) 0.6 (0.4-1.0) 1.0 (0.6-1.8) 0.7 (0.4-2.1) 0.5 (0.3-0.8) 0.5 (0.3-1.0) 1.0 (0.6-1.9) 1.1 (0.5-2.3) 1.1 (0.5-2.4) 0.7 (0.4-1.4) 1.1 (0.5-2.4)	0.54 0.03 0.19 0.17 0.54 0.01 0.16 0.57 0.96 0.52 0.48 0.64	UK	1998-2000	261/404 MF [12]	(20)
Intervention study							
Flavonols Flavones Flavan-3-ols	Colorectal adenoma	0.24 (0.11-0.53) [10] 1.08 (0.55-2.10) [10] 0.65 (0.37-1.15) [10]	0.0006 0.82 0.12	USA	1994-1998	1,905MF	(21)

Abbreviations: CI, confidence interval; F, female, M, male. 1 Flavonols (quercetin, kaempferol and myricetin), flavones (luteolin and apigenin) and flavan-3-ols (catechin, gallocatehin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, theaflavin, theaflavin-3-gallate, theaflavin-3'gallate, theaflavin-3,3'-digallate and thearubigins) were estimated from the US Department of Agriculture databases, the Dutch food composition table or the UK McCance and Widdowson's food composiotion tabels. 2 Risk for the highest versus the lowest category of intake. Relative risk for prospective cohort study; odds ratio for case-control study. 3 Sum of flavonols and flavones. 4 Sum of catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate. 5 Sum of catechin and epicatechin. 6 Hazard ratios. 7 Supplement group (α-tocophenol, β-carotene or both) in the α-Tocophenol, β-Carotene Cancer Prevention Study. 8 Values shown were odds ratio of smokers. There was no association between lung cancer of nonsmokers and flavonoids. 9 Hepatitis B/C virus positive case and negative case give similar values. Results shown are virus positive case. 10 Odds ratio of advanced adenoma recurrence (≥ 1 cm in size). 11 Smokers. 12 Number of cases/controls. 13 Risk was assessed in the Finnish Mobile Clinic Health Examination Survey. 14 Risk was assessed in the α-Tocophenol, β-Carotene Cancer Prevention Study. 15 Risk was assessed in the Iowa Women's Health Study. 16 Risk was assessed in the Kuopio Ischaemic Heart Disease Risk Factor Study. 17 Risk was assessed in the Nurses' Health Study II. 18 Risk was assessed in the Nurses' Health Study. 19 Risk was assessed in the Netherland Cohort Study.

excessive intake of antioxidant supplements. The genotype of the metabolizing enzyme catechol-*O*-methyltransferase (COMT) may attenuate the beneficial effect of black and green tea on breast cancer incidence (high activity *COMT* allele, OR 1.02: 95% CI 0.66-1.60: *P* for trend = 0.85; low activity *COMT* allele, OR 0.48; 95% CI 0.29-0.77; *P* for trend = 0.002; non tea drinker *vs*. tea drinker) (41). Therefore, polymorphism of *COMT* may obscure the protective effect of catechins (also flavonols and flavones) on the occurrence of breast cancer and other types of cancer.

4. ABSORPTION AND METABOLISM

In general, the human body recognizes flavonoids as xenobiotics and protects itself against their invasion. Nevertheless, a variety of flavonoids can enter the blood stream in greater or lesser quantities. Therefore, the bioavailability of each flavonoid depends on its intestinal absorption and metabolic conversion, both of which should be determined to estimate its anticarcinogenic potential. In the small intestine, flavone/flavonol glucosides are hydrolyzed to their aglycone by the activity of lactose phlorizin hydrolase (LPH), which is located on the surface of epithelial cells. Thereafter, aglycones enter cells via a simple diffusion mechanism, and are then conjugated by phase II enzymes including uridin 5'-diphosphate glucuronosyl transferase (UGT) and phenol sulfotransferase (PST) (42). The kinetics and specificity of each conjugating enzyme depend on the structure of the respective flavonoid (43). In addition, the product specificity of UGT differs among animal species. For example, product profiles of UGT1A6 in human, monkey, and dog (beagle) are similar to each other, but substantially differ from that in rats (44). Intracellular conjugated metabolites are conveyed into the digestive tract and/or the circulation by multidrug resistance-associated proteins (MRP), which belong to the ATP-binding cassette (ABC) family of transporters. This transport system may vary depending on the structure of each metabolite, resulting in a surprising diversity of bioavailability (45, 46). Catechol *O*-methyl transferase also catalyzes *O*-methylation of hydroxyl groups in the catechol structure in catechol-type flavonoids, such as quercetin and luteolin. *O*-Methylation occurs mainly in the small intestine and the liver; however, it was also reported that quercetin undergoes *O*-methylation within platelet cells to produce tamarixetin (4'-*O*-methyl quercetin), indicating that metabolic conversion can also occur in the circulation process (47). Glucuronide conjugates were detected in the lymph of rats after oral administration of quercetin (48). Furthermore, rutin (quercetin rutinoside) and quercetin were found as conjugated quercetin metabolites in both portal blood and lymph, suggesting that dietary flavonoids are transported into the body through the lymph system as well as via the portal vein (49).

Conjugated metabolites present in human plasma after intake of quercetin-rich foods were identified as quercetin 3'-*O*-β-D-glucuronide (Q3'GA), quercetin 4'-*O*-β-D-glucuronide (Q4'GA), quercetin 3-*O*-β-D-glucuronide, 3'-*O*-methyl quercetin 4'-*O*-β-D-glucuronide, 3'-*O*-methyl

quercetin 3-*O*-β-D-glucuronide, and quercetin 3'-*O*-sulfate. The total amount of these metabolites was estimated at 4.7% of the ingested quercetin (50). The concentration of kaempferol in plasma after oral administration was 1,000 times lower than that in plasma after intravenous administration (51). This large difference between oral and intravenous administration indicates that the phase II enzyme-derived conjugation reaction largely blocks the anticarcinogenic functions of flavonoids. On the other hand, even if flavonoids are not absorbed in the small intestine, they could still reach the large intestine, where they are metabolized by enterobacteria and decomposed into their aglycones and/or ring-scission products. Intragastric administration of apigenin-7-glucoside resulted in formation of apigenin and its chain-scission products in free and conjugated forms, that is, 3-(3,4-dihydroxyphenyl)propionic acid, 3-(3-hydroxyphenyl) propionic acid, 3-(4-hydroxyphenyl)propionic acid and 4-hydroxycinnnamic acid, in urine and feces (52). In germ-free rats, only apigenin conjugates were predominant, suggesting that enterobacteria are solely responsible for the conversion of flavonoids into these phenolic acids. In an experiment of healthy volunteers consuming green tea, approximately 40% of tea catechins degraded into these phenolic acids in the large intestine, and were then excreted into the urine after passing into the circulation system (53). The amount of phenols and phenolic acids in the urine can serve as biomarkers of the absorbability of dietary flavonoids. 4-Ethylphenol, benzoic acid, and 4-ethylbenzoic acid, which are present in the urine after intestinal degradation of dietary quercetin, and 1,3,5-trimethylbenzene, 4-*O*-methylgallic acid, 3-*O*-methyl gallic acid, and gallic acid, which are products in urine that originate from EGCg, are positive indicators of absorption of quercetin and EGCg, respectively (54).

Dietary flavonoids are thought to be involved in the dietary prevention of gastrointestinal cancer. The compounds themselves may exert an anticarcinogenic effect on the stomach and the small intestine, whereas microflora-dependent degradation products may be responsible for their effects in the large intestine (55). In contrast, their anticarcinogenic effects on other target organs depend largely on their distribution in the body (Figure 4). De Boer *et al* (56) detected conjugated quercetin (isorhamnetin and tamarixetin) in conjugated and free forms in the liver, kidney, muscle, heart, lung, brain, testes, spleen, thymus, bone, brown fat, and white fat of rats after dietary intake of quercetin (0.1% and 1%) for 11 weeks. They also found these conjugated metabolites and aglycones in the liver, lung, white fat, muscle, brain, kidney, heart, and spleen of pigs consuming a high quercetin diet (500 mg/kg body weight/day) for 3 days (56). These studies imply that dietary flavonoids are distributed into many organs, where they exert their anticarcinogenic effects. The concentration of quercetin and its metabolites in the kidney and jejunum increased with longer feeding periods, whereas the duration of feeding did not affect their accumulation in the liver, lung, and muscle (57). This phenomenon suggests that factors other than the duration of feeding are important for bioavailability of flavonoids in such organs. Recently,
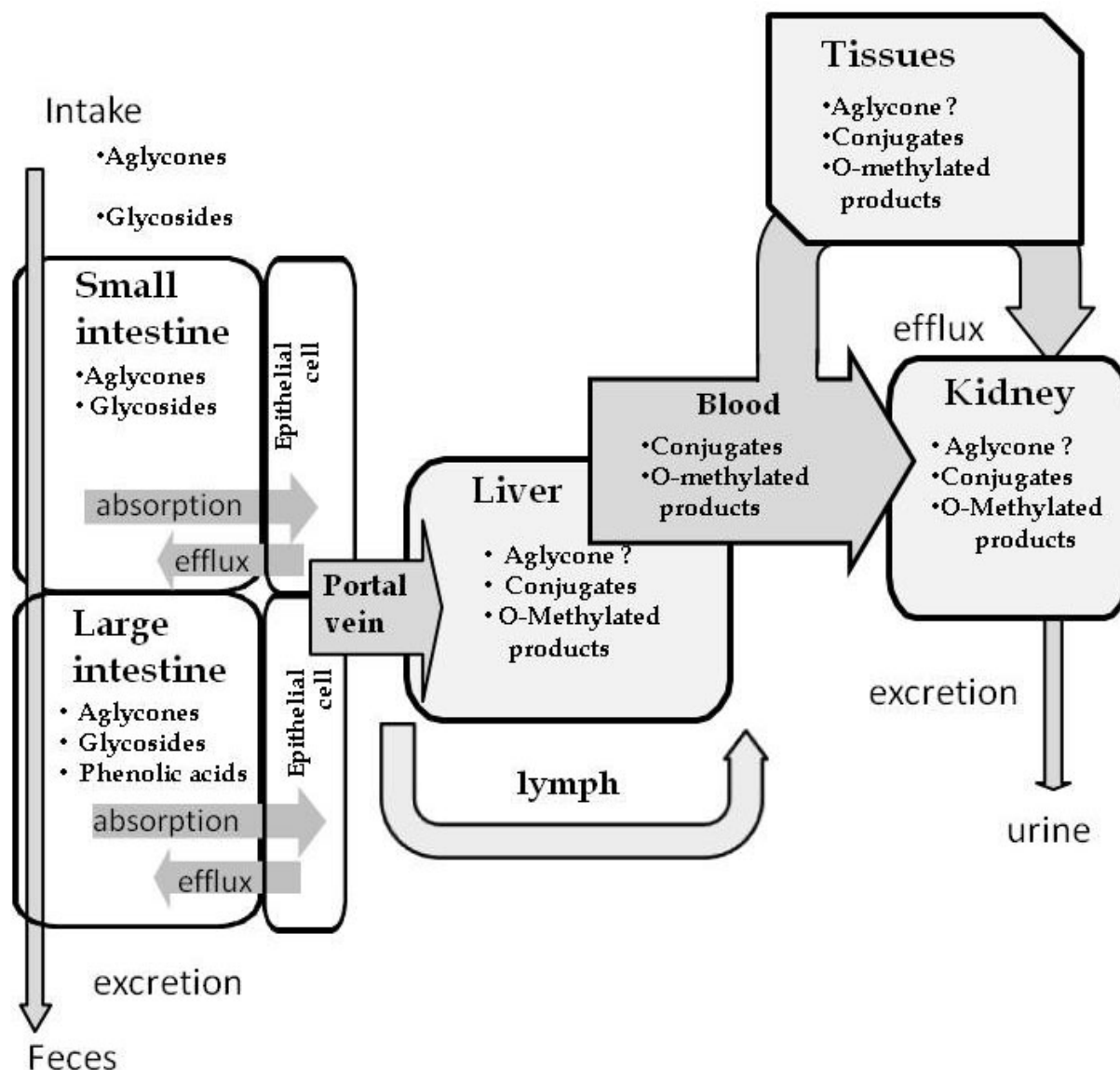
Figure 4. Transport pathway of flavonoid aglycones and glycosides after oral intake.

Mullen *et al.* (42) reported that very little radioactivity was detected in body tissues other than the gastrointestinal tract in rats fed by gavage with radiolabeled quercetin (4 mg/kg body weight). Their results suggested that at normal intake levels, ingested flavonoids are mostly converted to phenolic acids without accumulating in organs. Thus, enhancing the accumulation of dietary flavonoids in the target organ is an attractive strategy for dietary prevention of carcinogenesis.

Inflammation often activates β-glucuronidase (58, 59), indicating that this process and its related oxidative stress may alter the bioavailability and distribution of flavonoids to the target tissues. When the bioavailability of naringin (naringenin 7-*O*-neohesperidioside) was compared between healthy rats and tumor-bearing rats, the concentration of conjugated metabolites in the plasma of tumor-bearing rats was significantly lower than that in plasma of healthy rats

(60). Thus, the disease condition itself may influence flavonoid bioavailability. Shimoi *et al* (59, 61) demonstrated that the plasma concentration of luteolin aglycone was higher in lipopolysaccharide (LPS)-injected rats than in control rats. This result clearly indicates that LPS-induced inflammation enhances β-glucuronidase activity. Taken together, these results show that changes of expression and activity of metabolic enzymes affected by dietary flavonoids are dependent on internal and external factors. The complex interactions among such factors and their targets will greatly affect the anticarcinogenic activity of flavonoids.

On the other hand, it is of great interest to indicate that Siddiqui *et al.* have developed a unique delivery system by using polylactic acid-polyethylene glycol nanoparticles to enhance the bioavailability and limit

any unwanted toxicity of EGCg, and observed that encapsulated EGCg retains its biological effectiveness with over 10-fold dose advantage for exerting its proapoptotic and angiogenesis inhibitory effects (62). This approach may take the breakthrough to solve the problems underlying poor bioavailability of polyphenolic compounds with pronounced cancer preventive potentials.

5. CHEMOPREVENTION (RODENT AND HUMAN STUDIES)

Numerous reports on the chemopreventive effects of flavonoids have been published, and there is a substantial body of evidence that flavonoids inhibit carcinogenesis not only *in vitro* but also *in vivo*. They may inhibit carcinogenesis by affecting molecular events at the initiation, promotion, and/or progression stages. Above all, there is abundant evidence that the colon is a target site for chemoprevention by flavonoids (Table 2a).

Gee *et al.* investigated the effects of dietary quercetin on aberrant crypt foci (ACF) formation induced by the carcinogen, 1,2-dimethylhydrazine (DMH), in a short-term colon carcinogenesis model in rats (63). ACF is a putative precursor of colon cancer. The diet containing 0.1% quercetin dramatically suppressed ACF formation and proliferation of colonic epithelial cells induced by DMH. In a long-term model, a diet containing 0.1–2% quercetin suppressed focal areas of dysplasia and tumor multiplicity induced by azoxymethane (AOM), a metabolite of DMH (64). In that report, quercetin also significantly suppressed AOM-induced hyperproliferation of colonic epithelial cells. Meanwhile, a diet containing 0.01–1% quercetin, but not its glycoside rutin, decreased tumor incidence, multiplicity, and size in a dose-dependent manner (65). This observation was supported by Volate *et al.*, who demonstrated that the number of ACF was markedly suppressed by quercetin but not by rutin in AOM-treated rats (66). Thus, quercetin glycosides are less effective than its aglycon (quercetin) for the prevention of experimental carcinogenesis in the rodent colon (3).

Other flavonoids can also inhibit colon carcinogenesis. Dietary administration of the polymethoxyflavonoid nobiletin (0.01 or 0.05%) significantly reduced the number of tumors (67) in AOM-treated rats, and suppressed cell proliferation activity. Furthermore, in the colitis-related carcinogenesis model, nobiletin also dramatically decreased the incidence and number of colon tumors (68). It is also reported that the administration of apigenin and luteolin significantly inhibited colon tumorigenesis in rodent model, respectively (69, 70).

As shown in the review by Yang *et al* (71), green tea (and its constituents) is one of the most frequently studied compounds for cancer chemoprevention *in vivo*, including colon carcinogenesis model. In 1991, Yamane *et al.* administrated AOM to rats and then treated them with 0.01 or 0.1 % GTP as the sole source of drinking water, and observed that GTP significantly decreased tumor incidence and multiplicity (72). Hao *et al.* studied the cancer

preventive activities of Polyphenon E (PPE), a standardized green tea polyphenol preparation. In that study, the effects of PPE and other individual catechins were compared between consumption via food and consumption via drinking fluid (73). APC$^{Min/+}$ mice, spontaneously form benign adenomas in the small intestine, were supplied with 0.08% EGCg, 0.08% ECg, or 0.12% PPE in drinking fluid or in food. Interestingly, the number of tumors was significantly decreased in only two groups of mice; those given 0.12% PPE in food and those given 0.08% EGCg in drinking fluid. Thus, the cancer chemopreventive activity of green tea was mainly attributed to EGCg, and diet appeared to be a better route for PPE administration than drinking fluid.

Administration of a combination of flavonoids with other agents is a new strategy in chemoprevention. This is expected to reduce the dose of each compound and to decrease unexpected side-effects. Ohishi *et al.* examined synergistic effects of EGCg and sulindac, a non-steroidal anti-inflammatory drug, on AOM-induced colon carcinogenesis in rats (74). Interestingly, co-administration of EGCg and sulindac significantly reduced ACF formation and enhanced apoptosis in colonic epithelial cells as compared with administration of individual compounds. Recently, the chemopreventive effects of combined or individual administrations of PPE and curcumin, a hydrophobic polyphenol derived from turmeric, were studied in a DMH-induced colon carcinogenesis model in rats (75). The combined treatment showed the most potent inhibitory effect on ACF formation. The results of this and other studies suggest that a combination of flavonoids and other chemopreventive compounds can result in a synergistic colon cancer-preventive effect that is more efficient than that of each individual compound.

Obesity is an important risk factor for several types of cancer, including colon cancer. Administration of quercetin, chrysin, nobiletin, (76) and EGCg (77) suppressed the development of AOM-induced precursor lesions related to colorectal cancer, ACF and β-catenin accumulated crypt (BCAC), in genetically obese (db/db) mice. It is also reported that 0.12 and 0.24% PPE significantly decreased the total number of ACF in rats consuming a 20% high-fat diet (78). Therefore, these flavonoids may also be useful in the chemoprevention or treatment of obesity-related colorectal cancer.

In addition to the colon, several other organs are target sites for chemopreventive efficacy of flavonoids. This has been demonstrated in numerous animal studies (Table 2b). These results suggest that flavonoids can exert their chemopreventive effect not only in the gastrointestinal tract but also in various tissues, even after the absorption. These encouraging results of anticancer effects have led to clinical trials of some flavonoids in humans (Table 2c).

Cruz-Correa *et al.* evaluated the combined efficacy of quercetin and curcumin to regress adenomas in patients with familial adenomatous polyposis (79). Five patients who had undergone colectomies received oral administrations of quercetin (20 mg) and curcumin (480

Dietary flavonoids as cancer-preventive and therapeutic biofactors

Table 2. Studies demonstrating chemopreventive effects in (a) colon and (b) other organs in rodents and (c) results from clinical trials

(a)	Animal (gender)	Organs	Carcinogens	Dose and administration	Treatment (Stage/weeks)	Biomarker and result	Ref.
	Wistar rats (M)	Colon	DMH	Quercetin, 0.1% (diet)	Post-initiation/6	AC and large ACF ↓	(63)
	CF1 mice (F)	Colon	AOM	Quercetin, 0.1-2.0% (diet) Rutin, 1.0 and 4.0% (diet)	Initiation+Post-initiation/50	Tumor incidence/multiplicity ↓ Tumor incidence →, multiplicity ↓	(64)
	F344 rats (M)	Colon	AOM	Quercetin, 0.01-1% (diet) Rutin, 4% (diet)	Initiation+Post-initiation/40	Tumor incidence/multiplicity/size ↓ Tumor incidence ↓, multiplicity/size →	(65)
	F344 rats (M)	Colon	AOM	Quercetin, 1.5 and 3% (diet) Rutin, 1.5 and 3% (diet)	Initiation+Post-initiation/5	ACF multiplicity ↓ ACF multiplicity →	(66)
	F344 rats (M)	Colon	AOM	Nobiletin, 0.01 and 0.05% (diet)	Post-initiation/34	ADC incidence ↓	(67)
	ICR mice (M)	Colon	AOM/DSS	Nobiletin, 0.01% (diet)	Post-initiation/17	ADC incidence ↓	(68)
	CF1 mice (F)	Colon	AOM	Apigenin, 0.025 and 0.1% (diet)	Initiation+Post-initiation/25	Tumor incidence/multiplicity ↓	(69)
	Wistar rats (M)	Colon	DMH	Luteolin, 0.2 mg/kg BW (orally)	Post-initiation /15	Tumor incidence/size ↓	(70)
	Fischer rats (M)	Colon	AOM	GTP, 0.01 and 0.1% (water)	Post-initiation/15	Tumor incidence/multiplicity ↓	(72)
	APC$^{Min/+}$ mice (M,F)	SI	Spontaneous	EGCg, 0.08% (water) ECg, 0.08% (water) PPE, 0.12% (water) PPE, 0.12% (diet)	Post-initiation/9	Tumor multiplicity ↓ (only in Female) Tumor multiplicity → Tumor multiplicity → Tumor multiplicity ↓ (only in Female)	(73)
	F344 rats (M)	Colon	AOM	EGCg, 0.01% (water) Sulindac, 10 mg/kg BW (orally) EGCg, 0.01% (water) +Sulindac, 10 mg/kg BW (orally)	Initiation+Post-initiation/4	ACF multiplicity ↓ ACF multiplicity ↓ ACF multiplicity ↓↓	(74)
	Wistar rats (M)	Colon	DMH	GTC, 0.2% (diet) Curcumin, 0.2% (diet) GTC, 0.1% (diet)+Curcumin, 0.1% (diet)	Initiation+Post-initiation/32	ACF multiplicity ↓, Tumor multiplicity → ACF multiplicity ↓, Tumor multiplicity → ACF and Tumor multiplicity ↓	(75)
	db/db mice (M)	Colon	AOM	Quercetin, 0.01% (diet) Chrysin, 0.01% (diet) Nobiletin, 0.01% (diet)	Initiation+Post-initiation/10	ACF, BCAC multiplicity ↓ ACF, BCAC multiplicity ↓ ACF, BCAC multiplicity ↓	(76)
	db/db mice (M)	Colon	AOM	EGCg, 0.01, 0.1% (water)	Post-initiation/7	ACF, BCAC multiplicity ↓	(77)
	F344 rats (M)	Colon	AOM	PPE, 0.12, 0.24% (High-fat diet)	Post-initiation/8	ACF multiplicity ↓	(78)
(b)	Animal (gender)	Organs	Carcinogens	Dose and administration	Treatment (Stage/weeks)	Biomarker and result	Ref.
	ICR mice (F)	Skin	DMBA/TPA	Luteolin, 1 mg (topically)	Post-initiation/20	Tumor incidence/multiplicity ↓	(85)
	Balb/c mice (M)	Skin	UVB	EGCg, 10 and 50 mg (topically) EGCg, 100 and 500 mg (water)	Initiation+Post-initiation/28	Tumor incidence ↓ Tumor incidence →	(86)
	SKH-1 mice (F)	Skin	UVB	GTE, 4-6 mg/ml (water)	Post-initiation/18-23	SCC multiplicity ↓	(87)
	SD rats (F)	Mammary gland	DMBA NMU	Quercetin, 5% (diet) Quercetin, 5% (diet)	Initiation+Post-initiation/20 Initiation+Post-initiation/20	Tumor incidence/multiplicity ↓ Tumor incidence/multiplicity ↓	(88)
	SD rats (F)	Mammary gland	DMBA	GTC, 0.01 and 1% (diet) GTC, 0.1% (diet)	Post-initiation/35	Tumor incidence →/multiplicity ↓ Tumor incidence →/multiplicity →	(89)
	Swiss mice	Lung	NDEA	Quercetin, 9 μg/ml (water)	Post-initiation/28	Tumor incidence ↓/size →	(90)
	A/J mice (F)	Lung	NNK	PPE, 0.5% (water)	After tumor formation/32	Tumor incidence/multiplicity ↓	(91)
	F344 rats (M)	Tongue	4-NQO	Quercetin, 0.05% (water) Quercetin, 0.05% (water)	Initiation /10 Post-initiation/22	SCC incidence ↓ SCC incidence ↓	(92)
	Wistar rats (M)	Tongue	4-NQO	GTP, 200 mg/kg (orally) GTP, 200 mg/kg (orally)	Initiation /10 Post-initiation/12	SCC multiplicity/volume ↓ SCC multiplicity/volume ↓	(93)
(c)	Disease		Patients	Dose and administration	Duration	Biomarker and result	Ref.

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	Familial adenomatous polyposis	5	Quercetin 20mg and Curcumin 480 mg, 3 times/day (orally)	6 months	Decrease in the number and size of ileal and rectal adenomas	(79)
	Colorectal adenoma	125	GTE 1.5g (orally)	12 months	Reduction of incidence and size of metachronous adenomas	(80)
	Oral mucosal leukoplakia	59	GTP 1.2g (orally) and tea in glycerine (topically)	6 months	Reduction of the lesion size and cell proliferation	(81)
	Cervical cancer	88	200 mg PPE or EGCg (orally) and PPE ointment (topically)	12 weeks	Improvement in histology/cytology	(82)
	Prostate intraepithelial neoplasia	60	GTC 200 mg, 3 times/day (orally)	12 months	Suppress the progression to prostate cancer	(83)

Abbreviations: DMH: 1,2-dimethylhydrazine; AC: aberrant crypt; ACF: aberrant crypt foci; AOM: azoxymethane; ADC: adenocarcinoma; BW: body weight; GTP: green tea polyphenol fraction; SI: small intestine; EGCg: (-)-epigallocatechin gallate; ECg: (-)-epicatechin gallate; PPE: polyphenon E; GTC: green tea catechins; BCAC: β-catenin-accumulated crypt; DMBA: 7,12-dimethylbenz[*a*]anthracene; TPA: 12-*O*-tetradecanoylphorbol-13-acetate; UVB: ultraviolet B; GTE: green tea extract; SCC: squamous cell carcinoma; NMU: *N*-nitrosomethylurea; NDEA: *N*-nitrosodiethylamine; NNK: (4-methylnitrosamino)-1-(3-pyridyl)-1-butanone; 4-NQO: 4-nitroquinoline-*N*-oxide.

mg) three times a day. After a mean of 6 months of treatment with these natural compounds, the number and size of ileal and rectal adenomas were significantly decreased compared with the baseline in all five patients, and no toxic effects were observed.

The beneficial effects of green tea constituents have been also demonstrated in several human studies. Shimizu *et al.* conducted a randomized trial to evaluate the preventive effect of GTE on metachronous colorectal adenomas (80). Patients whose colorectal adenomas had been removed by endoscopic polypectomy were randomized into two groups; one receiving 1.5 g GTE supplementation per day for 12 months, and the other receiving none. At the end-point colonoscopy, the incidence of metachronous colorectal adenomas was significantly reduced in the GTE group, and no serious adverse effects were observed. The size of relapsed adenomas was also smaller in the GTE group than in the control group. In the recent studies, green tea constituents show the preventive effects in human oral precancerous mucosa lesions (81) and cervical lesions (82). Moreover, administration of 600 mg of GTC daily for 12 months dramatically reduced the progression of high-grade prostate intraepitherial neoplasia to prostate cancer (83). These findings support a potential role for green tea components, especially EGCg, in the treatment or prevention of cancer. However, a relatively large phase II trial conducted in China detected no appreciable effects of decaffeinated green tea on intermediate biomarkers of esophageal squamous carcinogenesis (84).

There are ongoing clinical trials to clarify whether and to what extent flavonoids, including quercetin and green tea catechins, prevent or ameliorate cancer. At present, however, there are limited data on the anticancer effects of flavonoids. Further research is required to clarify the clinical potential of flavonoids for chemoprevention and/or chemotherapy applications.

6. MECHANISMS

6.1. AhR/transformation
6.1.1. Interactions of flavonoids with AhR
The aryl hydrocarbon receptor (AhR), also known as the dioxin receptor, is a ligand-activated transcription factor belonging to the basic helix-loop-helix (bHLH)/Per-Arnt-Sim (PAS) protein family (94, 95). Toxic chemicals such as halogenated aromatic hydrocarbons (HAHs) and non-halogenated polycyclic aromatic hydrocarbons (PAHs) exert multiple toxic effects including carcinogenesis through binding to AhR, which results in excessive and consecutive AhR activation (96-98). Certain natural products, especially dietary compounds, can also bind to AhR and/or regulate the AhR-dependent signaling pathways (96). Some flavonoids, as well as resveratrol (99, 100) and curcumin (101, 102) , also interact with AhR. Natural flavonoids function as agonists or antagonists of AhR. Diosmin, diosmetin, quercetin, chrysin, galangin, genistein, baicalein, daidzein, and apigenin (103-105) show AhR-agonist activity. Regarding the antagonistic activity of flavonoids, galangin, kaempferol, quercetin, luteolin, apigenin, and naringenin inhibited the activation of AhR induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) (105-107). In a cell-free system using rat hepatic cytosol, it was also found that their antagonistic effects were dependent on their subclasses; the order of the antagonistic activity was flavones = flavonols > flavanones > catechins >> isoflavones (108). On the contrary, anthocyanins did not affect the transformation of AhR (109). In the case of catechins, EGCg suppressed the transformation of AhR more effectively than other catechins (110). In those experimental conditions, flavone, tamarixetin, chrysin, flavonol, quercetin, flavanone and daidzein showed weak agonistic effects when supplied at higher concentrations than their respective physiological levels, but catechins had no agonistic effect (108). These results indicated that dietary flavonoids act as antagonists, rather than agonists, of AhR.

The interactions between flavonoids and AhR depend on the subclass of the flavonoid. Compounds belonging to flavone, flavonol, flavanone, and catechin subclasses directly bound to the intact AhR complex, which consists of two molecules of hsp90, XAP2, and p23, in the cytoplasm (111). Flavone, flavonol and flavanone subclasses competitively inhibited binding to AhR (112), strongly suggesting that they can bind to the ligand-binding site of AhR. In addition, fewer hydroxyl and methoxy groups in the flavonoid structure (except in catechins) resulted in stronger suppressive effects on AhR activation. Therefore,hydrophobicity of flavonoids may be an

important factor for their interactions with the AhR complex. On the contrary, EGCg exhibited an interesting behavior. It did not competitively inhibit binding of agonists to AhR (112), but it did bind to hsp90, a component of the AhR complex (113). EGCg also inhibited the 2,3,7,8-TCDD-induced nuclear translocation of AhR (113). Interestingly, EGCg was able to translocate AhR into the nucleus, although it did not enhance the binding of AhR to DNA (113). In a recent study, it was also suggested that flavonoids regulate AhR-related signal transduction via inhibition of extracellular signal-regulated kinase (ERK). The degree of this inhibition varied among the different flavonoids, and appeared to depend on their structure (114). Taken together, these results indicate that flavonoids can regulate the AhR-related signaling pathway through multiple mechanisms, which vary according to the flavonoid subclass.

6.1.2. Chemoprevention of carcinogenesis through regulation of AhR and/or drug-metabolizing enzymes by flavonoids

In the body, exogenous chemicals, which are typically hydrophobic compounds, are converted to polar molecules through metabolic conversions such as oxidation, reduction, hydrolysis, cyclization, and decyclization. Such conversions are catalyzed by phase I drug-metabolizing enzymes, such as cytochrome P450 (CYP) monooxygenase, alcohol dehydrogenase, aldehyde dehydrogenase (ALDH), NAD(P)H-CYP reductase, esterase, and epoxide hydrolase. The polar molecules are then conjugated with glucuronic acid, sulfonates, glutathione, or amino acids by phase II drug-metabolizing enzymes, including methyltransferase, glutathione *S*-transferase (GST), sulfotransferase (SULT), amino acid *N*-acyl transferase, NAD(P)H:quinone oxidoreductase (QR), and UGT. Finally, the conjugated polar molecules are excreted into urine and/or bile by phase III transporters, also known as phase III drug-metabolizing enzymes, such as multidrug resistance (MDR), MRP, breast cancer resistance protein (BCRP), and organic anion-transporting polypeptide (OATP). Procarcinogens and carcinogens are also converted to more hydrophilic compounds; i.e., ultimate carcinogens, by certain drug-metabolizing enzymes. Ultimate carcinogens covalently bind to DNA, resulting in carcinogenesis. Ultimate carcinogens can be metabolized into inactive molecules by certain drug-metabolizing enzymes, resulting in excretion into urine and/or bile. Therefore, regulation of drug-metabolizing enzymes can enhance detoxification of carcinogens. An *in vivo* study showed that inducers of CYP (one of the drug-metabolizing enzymes) can decrease the carcinogenicity of chemical carcinogens (115), and also suggested that the amount of carcinogens was more dependent on detoxification pathways than on carcinogen synthesis pathways (115).

Some phase I and II drug-metabolizing enzymes and phase III transporters are induced through the AhR-related signaling pathway. For example, CYP1A1 (116), 1A2 (116), and 1B1 (117) are phase I drug-metabolizing enzymes that are induced by AhR activation. CYP1A1 and 1B1 metabolize PAHs and HAHs into carcinogenic

metabolites (118). A previous study showed that upregulation of CYP1A1 in lung tissue was related to a high risk of lung cancer (119). High CYP1A1 activity was also associated with colorectal cancer (120). The level of nuclear-translocated AhR and the expression level of CYP1A1 were increased in gastric tissues of gastric cancer patients (121). CYP1A2 activates aminofluorenes and nitrosamines (118, 122). It also converts some procarcinogens such as PAHs, nitrosamines, and arylacetamides into carcinogens (122), and plays a role in human tobacco-related cancers (123). Moreover, CYP1A1 and 1A2 convert chemical carcinogens such as benzo[*a*]pyrene and heterocyclic amines into their ultimate carcinogens, leading to the formation of DNA adducts (124, 125). Elevated levels of CYP1B1, which is an extrahepatic estradiol 4-hydroxylase, were associated with estrogen carcinogenesis (126). CYP1B1 expressed in healthy and tumor tissues of humans produced carcinogenic 4-hydroxy estrogen, and inhibition of CYP1B1 regulated the production of mutagenic estrogen 3,4-catechols (127). Regarding phase II drug-metabolizing enzymes, UGT1A1 (128), UGT1A4 (129), UGT1A6 (130, 131), GSTA1 (132), and GSTA2 (132) are induced through AhR activation. The UGT superfamily catalyzes the glucuronidation of many compounds, including xenobiotics such as drugs and environmental carcinogens, as well as endogenous compounds including bilirubin and steroid hormones. UGT1A1, 1A4, and 1A6 are mainly expressed in the liver (133). UGT1A1 can glucuronidate metabolites of tobacco carcinogens including benzo[*a*]pyrene (134). Human GSTA1 and A2 are most highly expressed in the liver and testis (135), and catalyze the detoxification of N-acetoxy-2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (N-acetoxy-PhIP), which is the carcinogen metabolite that allows reaction with DNA (136, 137). Regarding phase III transporters, AhR activation was reported to induce MDR1 (138), MRP2, 3, 5, 6, and 7 (139), BCRP (140), and OATP3 and 8 (141, 142). MDR1, a so-called *p*-glycoprotein, is mainly expressed in the liver and kidney, and acts as an efflux pump to eliminate xenobiotics (143). MDR1 also exists in epithelial tissues to protect the organs from entry of xenobiotics (143). MRP2 is mainly expressed in liver and epithelial tissues, and is involved in efflux of carcinogens (144). In fact, the plasma level of the food-derived carcinogen 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) was significantly higher in Mrp2-null mice than in wild-types (145). BCRP can also transfer dietary carcinogens, such as aflatoxin B1 and PhIP. Aflatoxin B1 accumulated in the plasma, lung, kidney, and brain of Bcrp-null mice (146).

The effects of flavonoids on the CYP system were first reported by Wattenberg *et al.* more than 40 years ago (147). Since then, many studies have focused on the actions of flavonoids on drug-metabolizing enzymes and transporters, especially CYPs (Table 3). Quercetin and kaempferol, which belong to the flavonol subclass and are dietary ligands of AhR, exerted different effects on expression of CYP1A1 (104). Treatment of human breast adenocarcinoma MCF-7 cells with quercetin led to dose- and time-dependent increases in the level of CYP1A1 mRNA and enzyme activity of CYP1A1 (104). On the

Table 3. Regulation of drug-metabolizing enzymes and transporters by flavones, flavonols, and catechins

Flavonoid		Drug-metabolizing enzyme or transpoter	Regulation	Ref. No.
Flavones	Apigenin	SULT1A1	Activity↓	(167)
		CYP19	Activity↓	(168)
	Baicalein	CYP1B1	mRNA expression↓	(169)
		CYP19	Activity↓	(168)
		SULT1A3	Activity↓	(170)
	Chrysin	CYP1A1	Activity↑	(149)
		SULT1A1	Activity↓	(167)
	5,7-Dimethoxyflavone	CYP1A1	B[a]P-induced mRNA expression↓	(149)
	Diosmetin	CYP1A1	mRNA expression↑	(103)
		CYP1A1	DMBA-induced mRNA expression↓	(103)
		CYP1A1	Activity↓	(171)
		CYP1A2	Activity↓	(171)
	Diosmin	CYP1A1	mRNA expression↑	(103)
	Flavone	CYP1A1	Activity↓	(148)
		CYP1A2	Activity↓	(148)
		CYP19	Activity↓	(149)
		UGT1A1	Activity↓	(173)
		UGT	Activity↑	(160, 174)
		GST	Activity↑	(175)
		GSTA2	mRNA expression↑	(176)
		QR	Activity↑	(177)
		SULT1A1	Activity↓	(178)
	Luteolin	CYP1A1	mRNA expression↑	(179)
	Tangeretin	CYP1A2	Activity↓	(180)
	6,2',4'-Trimethoxyflavone	CYP1A1	B[a]P-induced mRNA expression↓	(150)
Flavonols	Fisetin	SULT1A1	Activity↓	(167)
	Galangin	CYP1A1	Activity↓	(106)
		CYP1A1	mRNA expression↑	(106)
		CYP1A1	DBMA-induced mRNA expression↓	(106)
		CYP1A2	Activity↓	(148)
		CYP19	Activity↓	(168)
		UGT1A1	mRNA expression↑	(164)
		UGT	Activity↑	(161)
		QR	Activity↑	(181)
		SULT1A1	Activity↓	(167)
	Isorhamnetin	UGT1A1	mRNA expression↑	(164)
	Kaempferol	CYP1A1	TCDD-induced mRNA expression↓	(104)
		CYP1A1	B[a]P-induced mRNA expression↓	(176)
		UGT	Activity↑	(161)
		SULT1A1	Activity↓	(167)
	Morin	GST	Activity↑	(183, 184)
		QR	Activity↑	(183, 184)
	Myricetin	CYP3A4	Activity↓	(185)
		SULT1A1	Activity↓	(167)

	Quercetin	CYP1A1	mRNA expression↑	(104)
		CYP1A1	Activity↑	(104)
		CYP1A1	TCDD-induced mRNA expression↓	(104)
		CYP1A2	Activity↓	(186)
		CYP3A4	Activity↓	(169)
		UGT1A1	Activity↓	(173)
		UGT	Activity↑	(161, 174)
		GSTP1-1	Activity↓	(187)
		QR	Activity↑	(181)
		SULT1A1	Activity↓	(167, 170, 178)
		SULT1E1	Activity↓	(188)
		BCRP	mRNA expression↑	(166)
		MRP1	Activity↓	(189)
		MRP2	Activity↓	(189)
Catechins	(+)-Catechin	SULT1A1	Activity↓	(178)
	EGCg	CYP1A1	B[a]P-induced activity↓	(154)
		CYP1A2	PhIP-induced activity↓	(154)
		CYP3A4	AFB$_1$-induced activity↓	(154)
		CYP2A6	Activity↓	(154)
		CYP2C9	Activity↓	(154)
		CYP2E1	Activity↓	(154)
	ECg	CYP1A1	B[a]P-induced activity↓	(154)
		CYP1A2	PhIP-induced activity↓	(154)
		CYP3A4	AFB$_1$-induced activity↓	(154)
		CYP2A6	Activity↓	(154)
		CYP2C9	Activity↓	(154)
		CYP2E1	Activity↓	(154)
	EGCg+EGC	GST	4-NQO-downregulated Activity↑	(93)

Abbreviations: EGCg, (−)-epigallocatechin-3-gallate; ECg, (−)-epicatechin-3-gallate; EGC, (−)-epigallocatechin; SULT, sulfotransferase; CYP, cytochrome; UGT, uridine diphosphate glucuronosyltransferase; GST, glutathione *S*-transferase; QR, quinone reductase; BCRP, breast cancer resistance protein; MRP, multidrug resistance protein; B[a]P, benzo[a]pyrene; DMBA, 7,12-dimethylbenz[a]anthracene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, AFB$_1$, aflatoxin B1; 4-NQO, 4-nitroquinoline 1-oxide.

other hand, kaempferol did not affect CYP1A1 expression, although it bound to AhR and exerted an antagonistic effect against 2,3,7,8-TCDD-induced upregulation of CYP1A1 (104). Despite the structural similarities between quercetin and kaempferol, they exerted different effects, suggesting that different functional groups of flavonoids affect their ability to regulate CYP1A1.

The flavonol galangin is a potent inhibitor of CYP1A1. Galangin increased the level of CYP1A1 mRNA, but dose-dependently inhibited expression of CYP1A1 mRNA induced by 7,12-dimethylbenz[a]anthracene (DMBA) and 2,3,5,7-tetrachlorodibenzo-p-dioxin (2,3,5,7-TCDD) (106). In addition, analysis of the inhibition kinetics by a double-reciprocal plot revealed that galangin noncompetitively inhibited the enzymatic activity of CYP1A1 (106). Thus, galangin is a potent inhibitor of DMBA metabolism, as well as an agonist and antagonist of AhR. Galangin is also a potent mixed-type inhibitor of CYP1A2 (148). Therefore, galangin can compete for substrate binding at the active site, and may also bind to a region that does not participate directly in substrate binding.

5,7-Dimethoxyflavone, which is a major constituent of the leaves of a *Malaysian Piper* species, inhibited the benzo[*a*]pyrene-upregulated enzymatic activity of CYP1A1 as well as expressions of CYP1A1 protein and mRNA (149). In addition, 6,2',4'-trimethoxyflavone antagonized expression of CYP1A1 induced by benzo[a]pyrene, although 6,2',4'-trimethoxyflavone itself did not induce expression of CYP1A1 mRNA (150). On the contrary, 5,7-dihydroxyflavone, also known as chrysin, was a potent inducer of CYP1A1 (149). These results suggest that methylation of flavonoids may play an important role in inhibition of CYP1A1 activity.

CYP1A2 is regulated by activator protein-1 (AP-1) as well as AhR. In human, the CYP1A2 gene contains AP-1 binding sites (151). Green tea extracts inhibited the phorbol 12-otetradecanoate 13-acetate-induced AP-1 transcriptional activation of a human CYP1A2 enhancer element, although green tea extracts could not enhance its activation (151). In these experimental conditions, quercetin activated the transcriptional activity of AP-1 (151). Tea catechins inhibit the microsomal CYP enzyme system (152, 153). Green tea catechins inhibited the metabolic activation of procarcinogens by human CYP1A1, CYP1A2, and CYP3A4, and EGCg and ECg also inhibited other CYPs such as CYP2A6, CYP2C9, and CYP2E1, suggesting that EGCg has non-specific inhibitory effects on human CYPs. (154). On the contrary, long-term consumption of green tea (833 mg/kg/day for 6 months) increased enzymatic activities of hepatic CYP1A1 and 1A2 in Wistar rats (155). Thus, flavonoids can modulate the expression and/or enzymatic activity of phase I drug-metabolizing enzymes.

So far, various studies using NF-E2 related factor 2 (Nrf2)-deficient mice have revealed that induction of phase II drug-metabolizing enzymes is beneficial in cancer prevention (156). In the absence of stimuli, Nrf2 is localized in the cytoplasm where it interacts with kelch-like ECH-associated protein 1 (Keap1). Inducers disrupt the Nrf2-Keap 1 complex, resulting in the translocation of Nrf2 into the nucleus where it is heterodimerized with small Maf proteins (157). The Nrf2-Maf complex binds to an antioxidant response element (ARE), subsequently inducing phase II drug-metabolizing enzymes (157). Nrf2-deficient mice are more susceptible to benzo[*a*]pyrene-evoked carcinogenesis than are wild-type mice, and cancer prevention by phase II inducers was not observed in Nrf2-deficient mice (156). In previous studies, it was reported that inducers of Phase II drug-metabolizing enzymes were Michael reaction acceptors (158), and the potency of their inducing activity paralleled their efficiency in Michael reactions (159). Various flavonoids have Michael acceptor centers, and flavone (160), kaempferol (161), green tea catechins (93), and genistein (162) have been shown to induce phase II drug-metabolizing enzymes in *in vitro* or *in vivo* experiments. Taken together, these results suggest that induction of phase II drug-metabolizing enzymes by flavonoids leads to detoxification of carcinogens. Recently, a relationship between AhR and Nrf2 was found. DNA sequence analysis revealed that the mouse Nrf2 promoter contained three functional xenobiotics response elements (XREs), which are binding sites for AhR and two AREs (163). In that study, AhR activation increased the expression level of Nrf2 protein (163). Multi-copy XREs were also identified in the rat and human Nrf2 promoters, indicating that Nrf2 is a downstream factor of AhR. Chrysin enhanced the enzymatic activity of UGT1A1 and UGT1A6, respectively, more strongly than 2,3,7,8-TCDD, which is a classical ligand of AhR (164, 165). These results suggest that certain flavonoids can enhance some phase II drug-metabolizing enzymes through signaling pathways that are dependent on AhR as well as Nrf2.

The relationship between cancer and phase III transporters has not been elucidated yet. However, the functions of phase III transporters may be associated with cancer, because they import and export carcinogenic metabolites as well as a variety of molecules such as amino acids, ions, sugars, lipids, and so on. ATP binding cassette (ABC) transporters, so-called phase III transporters, mainly comprise MRPs, MDRs, and OATPs, and are expressed in various tissues. The expression of BCRP, which is a cellular efflux pump for many compounds including carcinogens, was enhanced in human colon adenocarcinoma Caco-2 cells by AhR agonists, and an AhR antagonist inhibited its upregulation (140). In addition, quercetin, which acts as an AhR agonist, increased the level of BCRP in human breast cancer MCF-7 cells, while quercetin could not upregulate BCRP in AhR-deficient MCF-7 cells (166). These results suggested that quercetin induces the expression of BCRP via an AhR-related signaling pathway. Therefore, flavonoids may promote efflux of carcinogens through the AhR-dependent induction of phase III transporters, resulting in cancer prevention. Further studies are required to clarify the interrelationships among flavonoids, phase III transporters, and cancer prevention.

Through their interactions with AhR, flavonoids can control the functions of phase I and II drug-metabolizing enzymes, and probably those of phase III transporters as well. Inducers of phase I drug-metabolizing enzymes can reduce the carcinogenicity of chemical carcinogens (115). A deficiency of phase II drug-metabolizing enzyme-related factors resulted in increased sensitivity to carcinogens and increased risks of carcinogenesis (156). The plasma level of carcinogens was increased in phase III transporter-deficient mice (145, 146). Therefore, regulation of drug-metabolizing enzymes and/or transporters is associated with cancer prevention, and the drug-metabolizing systems are key targets for cancer prevention by flavonoids.

6.2. Signal transduction

The process of carcinogenesis consists of three stages; initiation, promotion, and progression. These stages are associated with dysregulation of multiple signal transduction pathways involved in detoxification, proinflammation, cell cycle, apoptosis, migration, and other cellular functions. There have been numerous reports on the effects of flavonoids on these signal transduction pathways. In this review, we highlight the effects of catechin (EGCg), flavonols (quercetin and myricetin), and flavone (nobiletin) on signal transduction pathways at each stage of carcinogenesis.

At the initiation stage of carcinogenesis, tissues are exposed to carcinogenic agents. This results in various metabolic activations, leading to covalent interactions between reactive metabolites and DNA. The detoxification of carcinogens is often catalyzed by a series of phase II detoxifying and antioxidant enzymes such as glutathione peroxidase (GPx), glutamate cysteine ligase (GCL), GST, superoxide dismutase (SOD), hemeoxygenase-1 (HO-1), and QR. The expressions of these genes are chiefly regulated by the transcription factor Nrf2 (190). Activation of Nrf2 is regulated through two distinct mechanisms, the

first of which is the direct oxidation or covalent modification of the thiol group in Keap1. This leads to translocation of Nrf2 to the nucleus for transactivations of its target genes (191). The second mechanism involves the phosphorylation of Nrf2 at its serine and threonine residues by several kinases which promotes its transcriptional activity.

EGCg can induce a set of detoxifying enzymes not only in *in vitro* but also *in vivo* systems. For example, the intraperitoneal administration of EGCg to Swiss albino mice elevated the hepatic activity of GST, GPx, and SOD (192). EGCg also induced the expression of GCL and HO-1 in the wild-type, but not Nrf2-deficient mice (193), indicating that expressions of those genes were Nrf2-dependent. Wu *et al.* reported that EGCg-induced HO-1 expression was mediated by activation of Akt and ERK in endothelial cells (194). The molecular mechanism underlying Nrf2 activation by EGCg remains uncertain. *o*-Quinone at the B- or D-(gallate) ring produced by auto-oxidation of EGCg is susceptible to conjugation with GSH (195). This lowers the intracellular GSH level, which may disrupt the cellular redox status and activate Nrf2 by Keap1 oxidation and/or phosphorylations of several protein kinases responsible for Nrf2 activation. Abnormally elevated, constitutive expression of HO-1 in human cancers can confer a growth advantage and resistance to chemotherapy (196). In human A549 cells, a relatively high concentration of EGCg (>150 μM) down-regulated HO-1, whereas it induced HO-1 at a concentration lower than 50 μM (197). Thus, the regulation of Nrf2 activity by EGCg appears to be concentration-dependent. Quercetin was also reported to induce HO-1 expression in human hepatocytes through the activation of Nrf2 (198). Inhibitors of MAPKs such as ERK and p38 suppressed induction of HO-1, indicating that these kinases participate in Nrf2 activation.

Previous and current studies have demonstrated the close relationship between chronic inflammation and carcinogenesis. The expressions of proinflammatory mediators are mainly regulated by several key transcription factors such as nuclear factor-κB (NF-κB) and AP-1, both of which are activated through transduction of signaling molecules and MAPKs (199). EGCg reduced LPS-induced expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in the murine cell line RAW264.7 through suppression of NF-κB (200, 201). It also inhibited the DNA binding activity of AP-1 in human chondrocytes (202). The effects of EGCg on MAPK pathways are controversial, and vary among different experimental systems. For instance, when human epidermal keratinocytes were pretreated with EGCg, H_2O_2-induced phosphorylation of ERK1/2, JNK, and p38 was significantly inhibited (203). In contrast, EGCg activated all three MAPKs in dose- and time-dependent manners in human HepG2-C8 cells (204).

Quercetin also reduced LPS-induced production of proinflammatory cytokines and expression of iNOS and COX-2 via suppression of ERK- and p38-, but not JNK-, mediated phosphorylation, together with disruption of IκB degradation (205). Myricetin down-regulated phorbol ester-induced COX-2 expression by blocking activation of NF-κB in mouse epidermal cells (206). Although the effects of myricetin on activities of MAPKs are unknown, Kumamoto *et al.* reported that it suppressed other protein kinases such as PI3K, Akt, MEK1, and MKK4 (207-209). Nobiletin suppressed the phosphorylation of ERK induced by phorbol ester in human HT-1080 cells (210).

As compared with normal cells, malignant or transformed cells show dysregulated cell cycles and growth. The epidermal growth factor receptor (EGFR) is activated in a constitutive manner and overexpressed in several cancer cell lines, leading to activation of the Ras-Raf-MEK-ERK mitogenic signaling pathway. This cascade regulates progression of the cell cycle, possibly by activating cyclin-dependent kinases (CDKs), which hyperphosphorylate the retinoblastoma (RB) protein together with their catalytic subunit cyclins. Subsequent activation of the E2F transcription factor is a rate-limiting step for cell growth. EGCg blocked the cell cycle at the G1 phase in MCF-7 via suppressing the activities of CDK2 and 4 and inducing expression of the CDK inhibitors p21[WAF1] and p27 [Kip1] (211). Shimizu *et al.* reported that EGCg inhibited the phosphorylation of EGFR and subsequently ERK in human HT29 cells (212). Quercetin inhibited the phosphorylation of EGFR in human MiaPaCa-2 cells (213), and in another study, caused G(1)/S cell cycle arrest through induction of p21 with a concomitant decrease of phosphorylation of RB. In addition, it also inhibited G(2)/M cell cycle progression by down-regulating cyclin B1 and CDK1 (214). Myricetin inhibited EGF-induced phosphorylation of EGFR in mouse JB6 P(+) cells (208). Zhang *et al.* reported that myricetin caused G(2)/M cell cycle arrest in KYSE-510 cells through up-regulation of p21 and down-regulation of cyclin B1 at the mRNA and protein levels (214). Nobiletin blocked cell cycle progression at the G1 phase in MDA-MB-435, MCF-7, and HT-29 cells (215). The mechanism underlying cell cycle arrest by nobiletin has yet to be reported, except for the down-regulation of ERK (210).

A variety of cancer cell types show disrupted regulation of apoptosis. Apoptosis is induced by activation of caspase-3 and 9, which requires release of cytochrome c from mitochondria. This is regulated by antiapoptosis genes such as Bcl-2 and Bcl-X_L as well as proapoptosis genes such as Bax and p21[WAF1]. The expressions of these apoptotic proteins are regulated by three major transcription factors; NF-κB and Stat3, which upregulate the expression of Bcl-2 and Bcl-X_L, and p53, which activates Bax and p21[WAF1]. EGCg was able to induce apoptosis in KM3 cells by activating caspase-3 and 9 (216), and abrogated the expression of Bcl-2 and Bcl-X_L proteins while enhancing the levels of Bax proteins and activating caspase-3 (217). Inhibition of NF-κB and Stat3 activities and stabilization of p53 may account for regulation of apoptotic proteins by EGCg (218). Moreover, the antioxidant *N*-acetyl-L-cysteine suppressed the production of intracellular reactive oxygen species (ROS) and apoptosis through preventing EGCg-induced activation of MAPK (219). Therefore, EGCg-induced ROS may initiate the apoptosis signaling cascade.
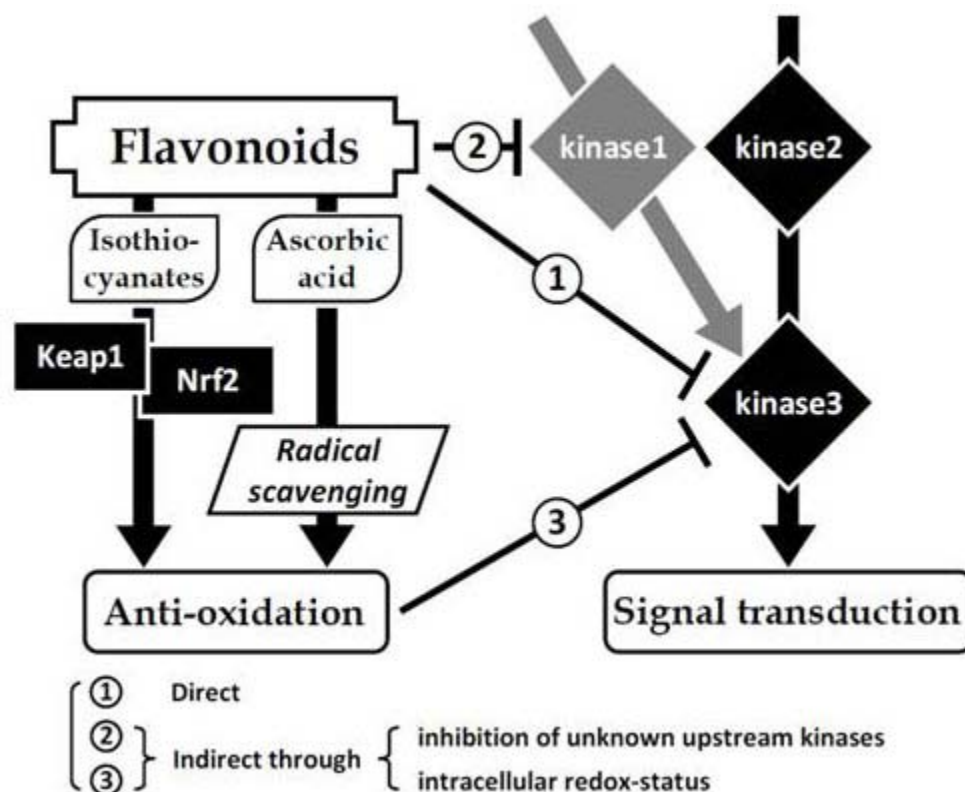
Figure 5. Flavonoids' modes of action for modulating protein kinase activity. Flavonoids regulate protein kinase activities via at least three pathways: (1) direct binding to kinase3, thereby suppressing its activity; (2) direct binding to kinase1, thereby down-regulating kinase3; and (3) regulating cellular redox-status via the Keap1-Nrf2 system and/or their own auto-oxidation properties, thereby suppressing kinase3.

Quercetin induced apoptotic cell death in HepG2 cells via activation of caspase-3 and 9 caused by reduction of Bcl-X_L and activation of Bax (220). It also induced inactivation of NF-κB, stabilization of p53, and activation of JNK in HepG2 cells (221). Lee *et al.* showed that quercetin increased apoptotic cell death in MCF-7 cells through ROS generation (222), whereas myricetin did so in HL-60 cells through a ROS-independent pathway (223). Nobiletin triggered p53-mediated apoptosis in A549 cells via modulating the Bax:Bcl-2 protein ratio (224). Collectively, it appears that ROS generation by flavonoids (except nobiletin) is closely related to their effects on apoptosis. Interestingly, Ferraresi *et al.* found that short-term treatment with quercetin resulted in antioxidative and antiapoptotic effects, whilst long-term treatment resulted in pro-oxidative and proapoptotic effects (225). This finding suggests that the effects of flavonoids on apoptosis depend on their concentrations and other experimental conditions.

Metastasis is a crucial step in cancer cell progression. The metastasis cascade consists of the multiple and sequential steps including invasion, intravasation, circulation in blood or lymphatic vessels, extravasation at distant sites, and then growth of secondary cancer. The effects of flavonoids on matrix metalloproteinases (MMPs), which degrade the extracellular matrix (ECM), have been extensively studied. The intraperitoneal administration of EGCg to Balb/c mouse inoculated with B16 cells reduced lung metastases through inhibition of activity of MMP-9 (226). EGCg inhibited invasion via repression of phosphorylation of EGFR and ERK followed by downregulation of MMP-2 and 9 in MCF-7 cells (227). Lin *et al.* reported that quercetin inhibited phorbol ester-induced MMP-9 via suppressing PKC-δ and ERK (228). Myricetin suppressed invasion through ERK inactivation in A549 cells (229). Miyata *et al.* demonstrated that nobiletin also inhibited MMP-9 production via suppression of phosphorylation of MEK and ERK in HT-1080 cells (210). The signal transduction pathway involved in EGFR-induced metastasis chiefly includes the MEK-ERK pathway, which overlapped with that of cell growth signaling.

As mentioned above, flavonoids modulate the signal transduction pathways involved in carcinogenesis by targeting not one but many molecules in non-specific manners. For example, the four flavonoids discussed in this section all down-regulate ERK1/2, JNK1/2, and p38 MAPK. These broad effects may depend on multiple molecular mechanisms underlying suppression of protein kinases. Flavonoids regulate activities of protein kinases both directly and indirectly (Figure 5). It is presumed that

suppression of the phosphorylations of protein kinases might be due to inactivation of known and/or unidentified upstream kinases. The phosphorylation of a certain protein kinase results from activation of and crosstalk among multiple upstream kinases. In addition, the cellular redox status is a major factor in the phosphorylation of protein kinases. Flavonoids that have the potential to form o-quinones may alter the cellular redox status by auto-oxidation and/or by Nrf2 activation through electrophilic addition, which may also modulate protein kinase activities. Mechanisms of flavonoids to exhibit dual anti-oxidative activities are characteristic when compared with those of other representative anti-oxidants, *e.g.*, ascorbic acid which is a free radical scavenger and isothiocyanates which have potentials to activate Nrf2 but not scavenge any free radicals. These mechanisms partially explain the complex modes of action by which flavonoids modulate the signal transduction pathways involved in carcinogenesis.

6.3. Target molecules

As noted above, flavonoids exhibit various beneficial effects, such as cancer prevention. Such effects are associated with their many properties, including redox modulation and protein kinase inhibition. The conventional view has been that the bioactivities of flavonoids are largely due to their antioxidant properties; however, there is now ample evidence that this cannot fully account for their physiological functions. For example, flavonoids and their metabolites accumulate in the plasma or organs at much lower concentrations than ascorbic acid (230). Nobiletin, a polymethoxyflavonoid lacking antioxidant activity, was able to inhibit phorbol ester-induced skin tumor promotion in ICR mice (231). These reports indicate the significance of the direct interactions between flavonoids and biomolecules, which are independent of their redox regulatory effects.

In 2004, Tachibana *et al.* identified the 67-kDa laminin receptor (67LR) as a receptor of EGCg that mediates its anticancer activity (232). The signaling pathway induced by the direct binding of EGCg to 67LR involves eukaryotic translation elongation factor 1A (eEF1A) and myosin phosphatase targeting subunit 1 (MYPT1), whose activation leads to rearrangement of the actin cytoskeleton (213). Importantly, 67LR recognizes EGCg and ECg, but not EC and EGC (232), suggesting that there is a specific interaction between 67LR and their gallate moieties. Following this pioneering work, there have been further studies on the direct interactions between flavonoids and biomolecules, resulting in the identification of various target molecules. For example, EGCg binds to vimentin, which is an intermediate filament protein involved in maintenance of cell structure and function in JB6 Cl41 cells (233). Li *et al.* found that EGCg bound to the insulin-like growth factor-I receptor (IGF-IR), which activated the Ras-Raf-MEK-ERK mitogenic signaling pathway in mouse fibroblasts (234). EGCg also bound to a ζ chain-associated 70-kDa protein (ZAP-70) in lysate from Jurkat leukemia cells (binding affinity, $Kd = 0.62$ μM), leading to suppression of ERK activity and induction of interleukin-2 (235). Fyn, a tyrosine kinase involved in cell transformation, is a target molecule of EGCg in JB6 Cl41

cells, and shows a remarkably high binding affinity for EGCg ($Kd = 0.37$ μM) (236).

Quercetin has been reported to bind to Raf and MEK1 in lysate from JB6 P(+) cells (237). Lee *et al.* reported that quercetin bound to the p85 subunit of PI3K, and subsequently inactivated PI3K, in lysate from rat liver epithelial cells (238). Quercetin also binds to tubulin, a component of microtubules, which are essential for cell growth and division (239). In addition, myricetin interacts with various protein kinases. Kumamoto *et al.* showed that myricetin bound directly to JAK1, Akt, MEK1, and Stat3, but not EGFR, in lysate from JB6 cells (207, 208). JAK1, a tyrosine kinase, plays a critical role in cellular survival, proliferation, and apoptosis through the phosphorylation of Stat3. Myricetin can bind to Fyn and MKK4, the latter of which is known to phosphorylate JNK in lysate from JB6 P(+) cells(209, 240).

Competitive pull-down studies with ATP suggested that flavonoids bind to the ATP-binding site of some protein kinases. For example, the interaction between EGCg and IGF-IR is attenuated by competition with ATP (234). Similarly, excess ATP inhibited the interactions of EGCg-ZAP-70 and myricetin-Akt, Fyn, and MKK4, but not myricetin-MEK1 (235). However, one cannot precisely identify the flavonoid binding sites from competitive studies, because as well as disrupting ATP binding sites, addition of ATP could also alter the tertiary structure of proteins.

Studies on the direct interactions between flavonoids and biomolecules have revealed that flavonoids can act as chemical inhibitors. This previously unknown property of flavonoids is independent of their antioxidant activity. Unfortunately, however, there is little information about the binding selectivity of flavonoids, except for the case of EGCg and 67LR. The broad range of bioactivities of flavonoids that have been demonstrated in numerous studies could be due to non-specific and moderate binding properties to various functional proteins. In addition, many of the effects observed *in vitro* have not been demonstrated *in vivo*. Some of the results of flavonoid-protein binding from *in vitro* investigations may represent experimental artifacts, because of alterations in the tertiary structure of proteins in cell lysate. Thus, the interactions between flavonoids and biomolecules must be verified *in vivo*.

7. POTENTIAL SIDE-EFFECTS

As mentioned above, numerous reports have suggested that dietary flavonoids are promising compounds for cancer prevention and/or therapy. These anticancer effects may be partly due to the antioxidant properties of the flavonoids, although recent studies suggest that interactions with essential signal transduction pathways may be more important.

The antioxidant activity of some flavonoids is attributed to two mechanisms; the ability of the flavonoid to scavenge free radicals and ROS, and/or the ability to form complexes with metal ions, thus preventing oxidation

of the metals yielding ROS (241). However, because of these biological properties, it has been suggested that some antioxidant compounds, including quercetin and EGCg, can act as both antioxidants and pro-oxidants in certain conditions (241). These paradoxical activities can result in unexpected effects; for instance, ascorbic acid (vitamin C) and the cruciferous vegetable constituent benzyl isothiocyanate (242-244), both of which are effective antioxidants and chemopreventive agents, have also been reported to induce carcinogenesis in experimental models (245, 246).

In fact, there are several studies on the effects of flavonoids *in vitro* and *in vivo* with controversial results. Quercetin was one of the strongest mutagenic flavonoids *in vitro* studies (247). Pereira *et al.* also demonstrated that dietary administration of 1.68 or 3.36% quercetin significantly increased the number of AOM-induced tumors in rat colon in a dose-dependent manner (248).

In addition, EGCg induced expression of pro-MMP-7, which plays pivotal role in the early stage of colon cancer, via production of O_2^- in HT-29 human colon cancer cells (249). In the acute colitis model in mice, the colon shortening induced by dextran sulfate sodium (DSS) was unchanged by 0.1 and 0.25% dietary GTP, but increased by 0.5 and 1% dietary GTP (250). In that study, dietary GTP at 0.5 and 1% did not prevent the development of multiple colon tumors induced by DMH/DSS, rather, they tended to increase it. These results indicate that excess intake of GTP or GTP-containing supplements does not have chemopreventive effects in certain high-risk patients, i.e., those who are at particular risk of developing epithelial malignancies in the inflamed large bowel. Moreover, in a 4-week clinical trial assessing the safety of PPE (800 mg/day of EGCg), a few of the otherwise healthy individuals reported mild nausea, stomach upset, dizziness, or muscle pain (251).

These reports suggest that flavonoids have potential side effects, a fact that may have been overlooked in human applications. Therefore, further evaluations of flavonoids as chemopreventive agents should proceed very cautiously until their mechanisms of action and side effects are well understood.

8. CONCLUSIONS AND PERSPECTIVES

Flavonoids are promising compounds for diet-based cancer prevention. These polyphenolic compounds are ubiquitously present in fruits and vegetables; therefore, their anticarcinogenic effects may be at least partly responsible for the epidemiological observation that increased fruit and vegetable intake reduces the risk of several types of cancers. In recent years, the development of molecular biology methods has advanced our understanding of the molecular mechanisms that underlie the anticarcinogenic effects of flavonoids. Nevertheless, the exact roles of dietary flavonoids in cancer prevention remain unclear. At present, we cannot state unequivocally that flavonoid-rich fruits and vegetables are beneficial for our health by preventing carcinogenesis. The molecular mechanisms for their anticarcinogenic effects should be further clarified by identifying the target molecule for each

flavonoid *in vivo*. The effects of combining flavonoids with other food ingredients should be further examined to evaluate the efficacy of the anticarcinogenic effect and the effects on their bioavailability. When the mechanisms by which dietary flavonoids prevent against cancer are fully understood, we can adapt the diet to achieve optimum intake of these compounds. We believe that this goal is achievable in the near future.

9. ACKNOWLEDGMENTS

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