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Journal of Nutritional Biochemistry 18 (2007) 427–442
Journal of Nutritional Biochemistry

REVIEWS: CURRENT TOPICS

Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention

Sonia Ramos*

Department of Metabolism and Nutrition, Instituto del Frío, Consejo Superior de Investigaciones Científicas (CSIC), 28040 Madrid, Spain

Received 7 September 2006; received in revised form 6 November 2006; accepted 13 November 2006

Abstract

Epidemiological studies have described the beneficial effects of dietary polyphenols (flavonoids) on the reduction of the risk of chronic diseases, including cancer. Moreover, it has been shown that flavonoids, such as quercetin in apples, epigallocatechin-3-gallate in green tea and genistein in soya, induce apoptosis. This programmed cell death plays a critical role in physiological functions, but there is underlying dysregulation of apoptosis in numerous pathological situations such as Parkinson's disease, Alzheimer's disease and cancer. At the molecular level, flavonoids have been reported to modulate a number of key elements in cellular signal transduction pathways linked to the apoptotic process (caspases and *bcl-2* genes), but that regulation and induction of apoptosis are unclear.

The aim of this review is to provide insights into the molecular basis of the potential chemopreventive activities of representative flavonoids, with emphasis on their ability to control intracellular signaling cascades responsible for regulating apoptosis, a relevant target in cancer-preventive approach.

Keywords: Food-derived flavonoids; Apoptosis; Caspases; Bcl-2 family proteins; Cultured cells

1. Introduction

Polyphenolic compounds constitute one of the most numerous groups in the plant kingdom and can be divided into various classes on the basis of their molecular structure, with flavonoids being one of the main groups. Many studies dealing with flavonoids have focused on their antioxidant properties, but a number of reports in different cell lines, animal models and human epidemiological trials have pointed out an association between consumption of fruits, vegetables and certain beverages (such as tea and wine, which are rich in polyphenols) and reduced risk of chronic diseases, including cancer [1,2].

Carcinogenesis is a multistage process with an accumulation of genetic alterations, and that sequence of events has many phases for intervention, with the aim of preventing, slowing down or reversing the process; in this regard, induction of apoptosis is considered to be one of the important targets in a preventive approach. A regulated form of cell death is a complex process that involves the active participation of affected cells in a self-destruction cascade and is defined by a set of characteristic morphological hallmarks, including membrane blebbing, shrinkage of cell and nuclear volume, chromatin condensation and nuclear DNA fragmentation in cells due to endonuclease activation [3]. In addition, the apoptotic process is also defined by a group of characteristic biochemical features; at a molecular level, two effector mechanisms of apoptosis have been extensively characterized: the intrinsic or mitochondrial-mediated mechanism and the extrinsic or death-receptor-mediated [e.g., tumor necrosis factor (TNF) receptor, TNF-related apoptosis-inducing ligand and Fas] mechanism (Fig. 1); both cascades converge in a common executor mechanism involving activated proteases (caspases) and

Abbreviations: AKT, protein kinase B; CDK, cyclin-dependent kinase; *DAPK1*, death-associated protein kinase 1; EGCG, epigallocatechin-3-gallate; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular regulated kinase; IAP, inhibitor of apoptosis protein; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MIF, migration-inhibitory factor; MMP, metalloprotease; NF-κB, nuclear factor κB; *NIK*, NF-κB-inducing kinase; PA, plasminogen; PARP, poly ADP-ribose polymerase; *PI-3-kinase*, phosphatidylinositol-3-kinase; *RBQ1*, retinoblastoma-binding protein; *SKY*, tyrosine protein kinase; VEGF, vascular endothelial growth factor.

* Tel.: +34 91 544 56 07; fax: +34 91 549 36 27.
E-mail address: s.ramos@if.csic.es.

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doi:10.1016/j.jnutbio.2006.11.004

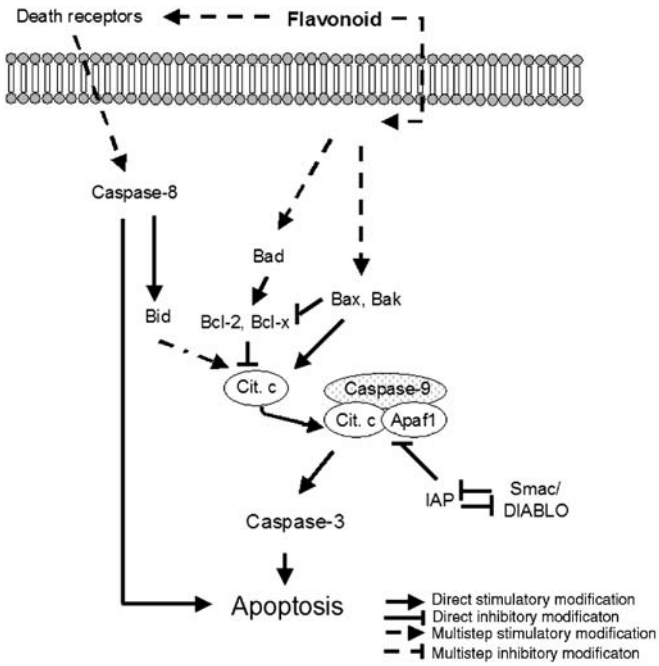


Fig. 1. Initiation and regulation of the two principal known apoptotic pathways. The intrinsic or mitochondrial pathway of apoptosis is initiated by the release of cytochrome *c* to the mitochondria, which binds to Apaf1, dATP and procaspase-9 to form the apoptosome. Then procaspase-9 is cleaved and effector caspases are activated. The extrinsic or cytoplasmatic apoptotic pathway is activated at the cell surface by the binding of a specific ligand to its corresponding cell surface death receptor. Death receptors are clustered and promote the recruitment of adapter proteins (e.g., FADD), which can interact with procaspase-8 to generate its active form; subsequently, downstream effector caspases are activated. Caspase-8 can also interact with the intrinsic apoptotic pathway by cleaving Bid, which results in cytochrome *c* release. Antiapoptotic members of the Bcl-2 family (Bcl-2 and Bcl-x_L) can block apoptosis, but its proapoptotic members (Bax and Bid) can also regulate programmed cell death. Both initiator and effector caspases can be inhibited by other mitochondrial proteins [e.g., Smac/DIABLO or by inhibitor of apoptosis proteins (IAPs)].

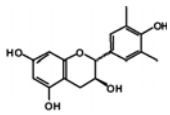
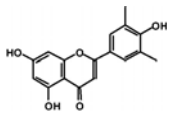
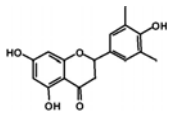
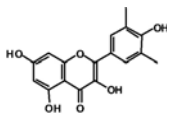
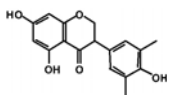
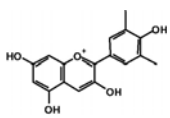
DNA endonucleases, which cleave regulatory and structural molecules and lead to cellular death [4,5].

Apoptosis plays a critical role in physiological functions such as cell deletion during embryonic development, balancing of cell number in continuously renewing tissues and immune system development [1,6]. Additionally, enhanced rates of apoptosis can contribute to degenerative diseases such as myocardial infarction, atherosclerosis, diabetes, reperfusion injury, Parkinson's disease or Alzheimer's disease [1,4], whereas inhibition of apoptosis can lead to proliferative diseases such as autoimmune disorders or cancer [1,4,7]. Over the past few years, it has been shown that flavonoids can trigger apoptosis through the modulation of a number of key elements in cellular signal transduction pathways linked to apoptosis (caspases and *bcl-2* genes) [1,6,7]. However, how flavonoids regulate and induce the apoptotic process remains to be elucidated. In this overview, recent studies on representative dietary components (flavonoids) and apoptosis in relation to cancer are reviewed.

2. Polyphenols

Phenolic compounds constitute one of the most numerous groups in the plant kingdom. They can be divided into 10 general classes based on their chemical structure, and more than 8000 different compounds have been described [8,9]. The most abundantly occurring polyphenols in plants are phenolic acids, flavonoids, stilbenes and lignans, of which flavonoids and phenolic acids account for 60% and 30%, respectively, of dietary polyphenols. More than 4000 varieties of flavonoids have been identified, and they share a common carbon skeleton of dyphenylpropanes (C_6–C_3–C_6; i.e., two benzene rings joined by a linear three-carbon chain that forms an oxygenated heterocycle) (Table 1). Flavonoids

Table 1
Basic chemical structure and major dietary sources of commonly occurring flavonoids

Flavonoid subgroup	Representative flavonoids	Food source
Flavanols 	EGCG Epigallocatechin Catechin Epicatechin	Chocolate, beans, tea, red wine, apple, cherry, apricot
Flavones 	Luteolin Apigenin Chrysin	Capsicum pepper, thyme Celery Parsley
Flavanones 	Naringenin Hesperidin Eriodictyol	Orange Grapefruit
Flavonols 	Quercetin Myricetin Kaempherol Rutin	Onion, apple, cherry, broccoli, tomato, berries, tea, red wine, leek
Isoflavonoids 	Genistein Daidzein	Soya beans, legumes
Anthocyanindins 	Pelargonidin Cyanidin Malvidin	Rhubarb, cherry, strawberry, red wine

can be divided into various classes according to their molecular structure; the main groups are flavanols, flavones, flavanones, flavonols, isoflavones and anthocyanidins [8,9]. Representative groups of flavonoids are listed in Table 1 together with their molecular structure, the best-known members of each group and food sources in which they are present.

Flavonoid ingestion depends on habits and food preferences, but the average daily intake of flavonoids in UK and in the United States has been estimated to range between 20 mg and 1 g [10]. Therefore, since flavonoids and polyphenols, in general, are absorbed and extensively metabolized [8–10] and due to the nutritional significance and biological activities of these polyphenolic compounds, it is important to know their bioavailability after ingestion.

Although the metabolism of flavonoids has not been well characterized, studies have shown that there is great variability in preferential pathways among individuals [9,11], which may be due to differences in gut microflora populations. In brief, aglycones can be absorbed in the small intestine. However, in food, most of the phenolic compounds are present as esters, glycosides or polymers, which can either be absorbed in these forms or be hydrolyzed by intestinal enzymes or the colonic microflora; consequently, aglycone or hydrolyzed products can be absorbed. During absorption, flavonoids undergo extensive metabolism in the small intestine and later in the liver and other organs: phenolic compounds are conjugated by methylation, sulfation, glucuronidation or a combination in order to decrease their hydrophobicity and to facilitate their urinary and biliary excretion. Manach et al. [11] have reported the existence of intermolecular bonds between albumin and quercetin conjugates, which supports its slow elimination from the body. Similarly, (−)-epigallocatechin-3-gallate (EGCG) possesses a high affinity for blood proteins [12] and, consequently, contributes to extend its half-life. Moreover, flavonoids may be secreted in bile to the duodenum and then reabsorbed, which results in enterohepatic cycling and evokes a longer half-life for conjugates.

3. Anticarcinogenic effects: epidemiological data

Many epidemiological and experimental studies have shown the effect of diet on health; thus, the relation between the consumption of certain foods and a reduced risk of some chronic diseases, including cancer, is becoming obvious. A wide range of dietary constituents show potential biological activities; in the past few years, a number of reports have focused on polyphenols and their health-related properties. In this regard, many studies in different cell lines, animal models and human epidemiological trials have shown the potential of dietary polyphenols as anticarcinogenic agents [1,2,6]. These phenolic compounds may inhibit various stages in the carcinogenesis process by affecting molecular events in the initiation, promotion and progression stages; they may increase the expression of proapoptotic components in initiated proliferating cells and thereby prevent or delay tumor development. Although induction of apoptosis seems to be rather specific for cancer cells, it should be mentioned that certain human studies have shown no beneficial effects (Table 2). In The Netherlands Cohort Study on Diet and Cancer, consumption of black tea was not found to affect the risk for colorectal, stomach, lung and breast cancers [13]. In a cohort study in Japan involving more than 25,000 stomach cancer patients, no association was observed between gastric cancer risk and consumption of green tea [14]. Similarly, other reports did not find a positive association between intake of flavonoids and reduced risk for different types of cancer [15–17]. On the contrary, a decreased risk for different types of cancer [18–26] or a diminished recurrence of lung [27] or breast [28] cancer has been reported after the consumption of

Table 2
Epidemiologic studies: association between flavonoids or foods rich in phenolic compounds and cancer

	Effect	Sample	References
Flavonoids	Decreased cancer risk in all sites combined	9959 Men	[23]
	Decreased cancer risk in oral cavity, pharynx, larynx and esophagus	540 People	[19]
	Nonreduced risk of bladder cancer	497 People	[15]
	Nonreduced risk of cancer incidence	728 Men	[16]
	Nonreduced risk of lung cancer	103 Women	[17]
Quercetin, onions, white grapes	Decreased recurrence of lung cancer	582 People	[27]
Quercetin	Decreased incidence of lung cancer	10,054 Men	[24]
Myricetin	Decreased risk of prostate cancer		
Quercetin, kaempferol	Decreased risk of gastric cancer	354 People	[20]
Catechins	Decreased incidence of rectal cancer	34651 Women	[18]
Tea	Decreased risk of colon cancer	12,170 People	[25]
Green tea	Reduced risk of cancer in different organs	8552 People	[21]
	Decreased risk of breast cancer recurrence and metastasis	472 People	[28]
Black tea	No association with gastric cancer	11,902 Men and 14,409 women	[14]
	No association with risk of colorectal, stomach, lung and breast cancers	58,279 Men and 62,573 women	[13]
Soya	Decreased risk of lung cancer	999 Men	[26]
	Decreased risk of breast cancer	34,759 Women	[22]

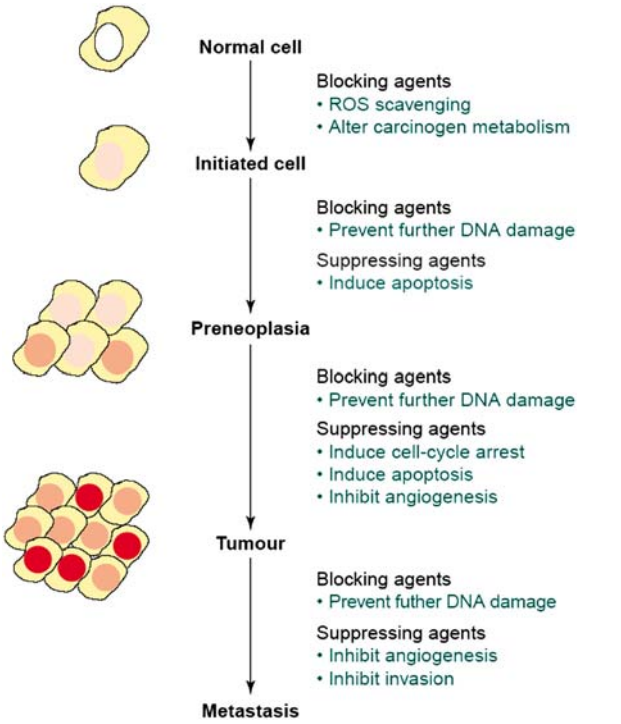


Fig. 2. Multistage model of carcinogenesis and potential effects of polyphenols on cancer progression. (Reprinted from Manson [7] with permission from Elsevier.)

flavonoids or certain foods or drinks (tea) rich in these phenolic compounds.

4. Flavonoids and cancer

Carcinogenesis is a multistage genetic change affecting proto-oncogenes or tumor-suppressor genes, and that sequence of events has many steps for intervention, with the aim of preventing, slowing down or reversing the process (Fig. 2). Therefore, targets for chemoprevention could be multiple and could vary from the initiation phase, to the promotion phase, and then to the progression phase [7]. Since flavonoids display a vast array of cellular effects, they can affect the overall process of carcinogenesis by several mechanisms (Fig. 2 and Table 3), including inhibition of DNA topoisomerase I/II activity [29,30], decrease or increase in reactive oxygen species [31], DNA oxidation and fragmentation [32–34], regulation of heat-shock-protein expressions [35], cell cycle arrest [36–39], modulation of survival/proliferation pathways [40–52], release of cytochrome *c* with subsequent activation of caspase-9 and caspase-3 [39,40,44,49,50,53,54], increased levels of caspase-8 and t-Bid [50,53], down-regulation of Bcl-2 and Bcl-x_L expression and enhanced expression of Bax and Bak [42–46,50], increased tissue (t) plasminogen (PA) and u-PA [55], decreased vascular endothelial growth factor (VEGF) [50,52,56–58] or metalloprotease (MMP) levels [51,52,59–65], increase in migration-inhibitory factor (MIF) [66], and modulation of nuclear factor κB (NF-κB) [41]. However, suppression of cell proliferation and induction of differentiation and apoptosis are important preventive approaches, and the induction of programmed cell death is currently considered as one relevant target in a preventive approach. In this context, apoptosis provides a physiological mechanism for the elimination of abnormal cells, and induced programmed cell death could have beneficial effects on carcinogenesis. It has been demonstrated that these phenolic compounds can trigger apoptosis [1,62], although the regulation and induction of the apoptotic process by these natural products remain to be elucidated.

In order to understand the signaling events leading to programmed cell death by dietary flavonoids, critical insights into the apoptosis induction activity of representative flavonoids in cancer cells are provided.

5. Flavonoids and apoptosis in cancer cells

5.1. Specificity and dose-dependent inhibitory effects

Flavonoids have been tested in normal and cancer cultured cells, and studies have shown that these natural compounds exerted apoptotic effects in a selective manner (i.e., by using the same concentrations, phenolic compounds induced apoptosis in cultured cancer cells, but not in their normal counterparts). Thus, EGCG, the major catechin in tea that has been largely studied compared to other tea compounds [63], induced a pronounced and specific growth-inhibitory effect on cancer cells, but not on their normal counterparts [64–67]. In this regard, EGCG has been reported to induce the apoptosis of different types of cancer, such as leukemia [68–70], prostatic cancer [64,71], gastric cancer [72], colon cancer [73,74], lung cancer [75], preadipocytes [76] and fibroblasts [65]. Hsu et al. [77] have described a differential effect of EGCG on cell proliferation in normal human primary epidermal keratinocytes after 24 h of exposure. The flavanol EGCG appeared to induce keratinocyte differentiation after 24 h of treatment at concentrations of 15–200 μmol/L, but when epidermal cells were grown for 15–25 days (aged keratinocytes) and incubated for 1 day with the catechin, cellular proliferation was stimulated at 200 μmol/L in keratinocytes cultured for 15 days and with 100 μmol/L EGCG in cells grown for 20 and 25 days. They suggested the use of this polyphenol in the treatment of wounds or certain skin conditions characterized by altered cellular activities or metabolism. In agreement, internucleosomal DNA fragments were detected after treatment with EGCG in A431 (human epidermoid carcinoma cells), HaCaT (human carcinoma keratinocytes), DU145 (human prostate carcinoma cell line) and L5178Y cell lines (mouse lymphoma cells), but not in NHEK cells (normal human epidermal keratinocytes) [78]. Additionally, EGCG seems to possess a dual mechanism of action, although it appears that this flavanol exerts its action in a selective manner. In normal keratinocytes, EGCG enhanced

Table 3
Molecular effects of flavonoids on the overall process of carcinogenesis

	Effect	System studied	References
Initiation			
Quercetin	Decreased 8-oxo-dOG	HepG2	[32]
Genistein	Increased CYP levels	HepG2	[31]
Myricetin	Decreased DNA oxidation, topoisomerase II inhibition	Rat hepatocytes (primary culture)	[33]
Daidzein	Topoisomerase II inhibition	V79 (lung, hamster)	[30]
Promotion			
Cranberry extract	DNA laddering	HT-29, Caco-2	[34]
Tea	Cell cycle arrest (G_2/M)	LNCaP, DU145	[36]
EGCG	Cell cycle arrest	YCU-H891, YCU-H861	[44]
	Increased caspase-9 and Bax		
	Decreased Bcl-2, Bcl-x_L, p-EGFR, p-STAT-3 and p-ERK		
	Increased Fas, Bax and p53	HepG2	[45]
	Decreased PI-3K and AKT; increased ERK1/2	LNCaP	[48]
	Cell cycle arrest, DNA laddering	HT-29	[49]
	Increased caspase-3 and caspase-9		
	Decreased p-EGFR, p-HER2, p-AKT and p-ERK		
Quercetin	Cell cycle arrest (G_2/M, G_0/G_1)	CNE2	[39]
	Increased caspase-3, caspase-7 and Bax		
	Increased caspase-3	Neurons (primary culture)	[40]
	Decreased AKT and ERK1/2; JNK not affected		
	Cell cycle arrest in G_1, apoptosis induction	Mouse thymocytes	[37]
	Decreased NF-κB, increased c-Jun		
Luteolin	DNA laddering; increased caspase-3/7, caspase-8/10 and caspase-9	H4IIE	[53]
	Increased caspase-3, caspase-8, caspase-7 and PARP	HLF	[50]
	Decreased Bcl-x_L		
	Increased translocation Bax/Bak and JNK	HepG2	[46]
Apigenin	Cell cycle arrest (G_2/M)	MCF-7	[38]
	Increased cytochrome *c* release, caspase-3 and caspase-9	HL-60	[54]
	Increased caspase-3, caspase-8, caspase-7 and PARP	HLF	[50]
Genistein	Increased p38; decreased ERK1/2; JNK not affected	MCF-10F	[47]
	Decreased AKT, NF-κB and p-EGFR	MDA-MB-231	[41]
	Decreased Bcl-x_L, AKT and EGFR	A549	[42]
Tectorigenin	Cell cycle arrest, DNA laddering	HL-60	[43]
	Decreased Bcl-2 and p-EGFR		
Progression			
Pomegranate	Decreased VEGF	MCF-7	[58]
	Increased MIF	MCF-10F	[61]
Strawberry	Decreased VEGF and NF-êB	HaCat	[57]
Catechin, epicatechin	Increased mRNA, t-PA and u-PA; decreased fibrinolytic activity	HUVEC	[55]
Green tea (EGCG)	Decreased VEGF	NHK	[56]
	Decreased tube formation	HUVEC	[59]
	Decreased migration, MMP-2 and MMP-9		
	Decreased migration, MMP-2 and MMP-9	U87	[60]
Quercetin	Decreased FAK, MMP-2 and MMP-9	MiaPaCa2	[51]
	Blockade EGFR signaling pathway		
	Decreased VEGF and EGF		[52]
Luteolin	Decreased FAK, MMP-2 and MMP-9	MiaPaCa2	[51]
	Decreased VEGF and STAT-3	HLF	[41]
	Blockade EGFR signaling pathway		

proliferation at 0.5 μmol/L and did not affect cell growth at 50 μmol/L (cells were treated every 2 days for 5 days), but it decreased cell proliferation at both concentrations in a dose-dependent fashion in squamous carcinoma cells [66]. In this regard, EGCG could exert a dose-dependent inhibitory effect on cell proliferation: low concentrations of flavanol (1 μmol/L) induced an antiapoptotic response, while higher concentrations (50 μmol/L) caused a proapoptotic effect in neuroblastoma SH-SY5Y cells [74].

Similarly, one of the major flavonoids found in the human diet, quercetin [79], which is extensively metabolized during absorption in the small intestine and in the liver [80], exerts a dose-dependent inhibitory effect on cell proliferation. Van der Woude et al. [81] have described a biphasic effect of quercetin on cell proliferation in colon carcinoma (HCT-116 and HT-29) and mammary adenocarcinoma (MCF-7) cell lines after 24 h of exposure. Quercetin inhibited colon (HCT-116 and HT-29) cell proliferation (30 and 80 μmol/L, respectively), while MCF-7 cell viability did not decrease at concentrations assayed (up to 100 μmol/L). A dual effect of quercetin has also been reported in a human squamous SCC-25 carcinoma cell line:

growth stimulation after 72 h of exposure at concentrations of 1 and 10 μmol/L, and growth inhibition with 100 μmol/L quercetin [82]. In this context, in H4IIIE rat hepatoma cells, quercetin protected against H_2O_2-induced cytotoxicity, DNA strand breaks and apoptosis at a concentration of 10 μmol/L, and this effect was attributed to the antioxidant activity of the polyphenol [83]. However, higher concentrations of this flavonoid (50 μmol/L) induced cytotoxicity, oligonucleosomal DNA fragmentation and activation of caspase-2, caspase-3 and caspase-9 in the same cell line [83]. These data suggest that cytoprotective concentrations of some flavonoids are lower by a factor of 5–10 than their DNA-damaging and proapoptotic concentrations [83]. Accordingly, it has been proposed that low concentrations of quercetin may activate the mitogen-activated protein kinase (MAPK) pathway [extracellular regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38], leading to the expression of survival genes (*c-fos* and *c-jun*) and defensive genes (*glutathione-S-transferase*), which evokes survival and protective mechanisms, whereas higher concentrations of quercetin activate the caspase pathway, leading to apoptosis [40,84]. Nevertheless, it seems that the flavonoid quercetin exerts this action in a selective manner, since it has been shown that quercetin (25 and 50 μmol/L) significantly inhibited the growth of the highly aggressive PC-3 cell line and the moderately aggressive DU145 prostate cancer cell line, but it did not affect the poorly aggressive LNCaP prostate cancer cells or the normal BG-9 fibroblast cell line [85]. In this regard, quercetin induced the apoptosis of four human cancer cell lines (squamous HSC-2 cell carcinoma, HSC-3 cells, submandibular gland HSG carcinoma cells and promyelotic HL60 leukemia), but not of three normal human oral cells (gingival HGF fibroblasts, pulp HPC cells and periodontal ligament HPLF fibroblasts) [86].

Genistein is one of the major phytoestrogens in soybeans and other legumes, and has a structure similar to that of estrogens. Small intestine enzymes metabolize genistein, and the isoflavonoid and its metabolites have been detected in plasma, prostatic fluid, breast aspirate, cyst fluid, urine and feces [87].

A biphasic effect on cell proliferation has also been demonstrated for genistein. It has been suggested that estrogen receptor (ER) might be involved in the slight stimulation of proliferation in breast cancer cells [88]. Accordingly, it has been reported that genistein at concentrations lower than 1 μmol/L stimulated the growth of estrogen-dependent breast cancer cells when cultured under estrogen-free conditions, while concentrations higher than 5 μmol/L inhibited estrogen-stimulated cell proliferation; it acted as an ER antagonist at concentrations higher than 5 μmol/L and, at 10 μmol/L, induced apoptosis in MCF7 breast cancer cells after 24 h of treatment [89,90]. In this regard, high doses of genistein (111 μmol/L) induced apoptosis in Caco-2Bbe cells [91]; in contrast, when a much lower concentration of the isoflavonoid was tested (3.7 μmol/L), increased cell proliferation, without affecting cell number or caspase-3 activity, was found [91]. Genistein (20 μmol/L) inhibited the proliferation of LNCaP prostate cancer cells and enhanced apoptosis [92]. However, it has also been demonstrated that this isoflavonoid exerted these actions in a selective manner, and when apoptosis induction was evaluated, programmed cell death was detected in the highly aggressive PC-3 cells (50 μmol/L, 1–3 days), but not in the nontumorigenic prostate epithelial CRL-2221 cells, after the same treatment with genistein [93]. Thus, genistein treatment (50 μmol/L for 3 days) significantly inhibited the growth of PC-3 cells (69.6%), but the percentage of inhibition was smaller in CRL-2221 cells (20.8%) [93].

5.2. Induction of apoptosis

Programmed cell death is characterized by morphological and biochemical changes in cells (e.g., DNA fragmentation) [94,95]. The flavanol EGCG induces apoptosis at high doses (20–500 μmol/L) in numerous cancer cell lines (i.e., human breast cancer [96], lung cancer [75], gastric cancer [72], colon cancer [73,74,96], prostate cancer [64,71], melanoma [96] and mouse leukemia [69]). In this regard, programmed cell death was induced after exposing WI38VA cancerous fibroblasts to 200 μmol/L EGCG for 8 h [65] and, similarly, concentrations above 100 μmol/L evoked apoptosis in a mouse leukemia cell line (L1210) [69]. Horie et al. [72] have described that this flavanol induced apoptosis in MKN-45 gastric cells cultured with 100 μmol/L for 24 or 48 h, as determined by the appearance of DNA laddering. This feature was also observed in human lymphoid leukemia MOLT4B cells [68], in a human prostate cancer cell line (DU145) after treatment with 100 μmol/L EGCG during 72 h [71] and in HT-29 human colon adenocarcinoma cells (concentrations higher than 100 μmol/L) [73]. In addition to DNA fragmentation, the condensation of chromatin and nuclear fragments was shown in human colon carcinoma LoVo cells incubated with EGCG at 220 μmol/L for 24 h, although the cell membrane was kept intact [74]. In this regard, dose-dependent changes in cell morphology were also observed with phase-contrast microscopy after cell treatment with EGCG in the oral squamous SSC-25 carcinoma cell line [97].

Recent studies have shown that quercetin has antiproliferative effects [1,2,98] and can induce death by an apoptotic mechanism in numerous types of cancer such as leukemia [99–101], breast cancer [102], ovarian cancer [103], lung cancer [104], hepatoma [105], oral cancer [39] and colon cancer [98,106]. In this regard, several studies have demonstrated that induction of apoptosis by quercetin ranges in micromolar concentrations (29–150 μmol/L) in different cancer cell cultures such as human colon cancer [98,107], breast cancer [102], prostatic cancer [108,109], leukemia [99,101], lung cancer cells [104], murine hepatoma [105] and melanoma [110]. Moreover, this flavonol has also been shown to induce morphological alterations and DNA fragmentation in leukemia [111], human preneoplastic

colonocytes, different human hepatocarcinoma cell lines [62,105], rat pancreatic carcinoma cells (BSp73AS) [112] and mouse thymocytes [37].

Genistein has been reported to inhibit the growth of several cancer cells through the modulation of genes that are intimately related to the regulation of apoptosis, but also to other processes such as cell growth or signal transduction pathways, since this isoflavonoid is an inhibitor of protein tyrosine kinases [113]. Genistein can induce programmed cell death in breast cancer [114,115], prostate cancer [93,116,117], head and neck squamous cell carcinoma [118,119], stomach cancer [120] and lung cancer cells [113].

Moreover, genistein concentrations that induce apoptotic death have been previously reported to range between approximately 30 and 200 μmol/L in different cancer cell lines from breast cancer [114,115], prostate cancer [93,117], lung cancer [113], head and neck squamous call carcinoma [118,119], leukemia [29,121], liver cancer [122] and colon cancer [91]. Genistein has also been shown to induce morphological alterations and DNA fragmentation in LNCaP human prostate cancer cells treated with 75 or 150 μmol/L isoflavonoid for 24 hours [117], and similar data have been reported in several neuroblastoma cell lines after treatment with 1, 10 and 50 μg/ml for 6 h in a dose-dependent fashion [123]. DNA laddering was also observed in breast MCF-7 cancer cells after 24 h of treatment with 50 μmol/L genistein [115], in PC-3 human prostate cancer cell line with 50 μmol/L isoflavonoid (1–3 days) [93] and in different colon cancer cell lines with 60–150 μmol/L after 1 h of treatment [29].

5.3. Molecular targets on caspase processing

The observed antiproliferative properties of flavonoids suggest that these compounds may induce apoptosis by modulating different key targets involved in both apoptotic pathways. However, little is known regarding the precise mechanism of flavonoid-induced apoptosis, and only recently has interest started to focus on flavonoids' potential to interact with intracellular signaling pathways. Many genes participate in the regulation of the apoptotic process, and activation of caspases is a central effector mechanism. Nakagawa et al. [70] have reported a decreased viability of T-cell acute lymphoblastic leukemia Jurkat cells and a concomitant increase in cellular caspase-3 activity with EGCG (12.5–50 μmol/L); similar data were reported recently after the incubation of 3T3-L1 preadipocytes with 100–400 μmol/L flavonoid for 24 and 48 h [76]. EGCG (20–400 μmol/L) decreased the proliferation of four different gastric carcinoma cell lines (MKN-1, MKN-45, MKN-74 and KATO-III) in a dose-dependent manner [72]. In the same study, in MKN-45 cells, 200 μmol/L EGCG for 6–48 h increased the activity and levels of caspase-3 and caspase-9, while caspase-8 activity showed an induction weaker than those of the other two caspases, and its protein levels remained almost unchanged when compared to untreated cells [72]. Mitochondrial and cytosolic pathways were both activated in normal $CD14^+$ monocytes with 10–50 μmol/L EGCG (24 h) [67] and in human HLE hepatoma cells after 24 h of incubation with the flavanol at 10–100 μmol/L [124]. In normal monocytes, EGCG induced apoptosis in a dose-dependent manner, and even the lowest concentration tested (10 μmol/L) provoked programmed cell death [67]. Another study [125] has reported the activation of caspase-3 in oral carcinoma cells at concentrations equal to or higher than 50 μmol/L (50, 100 and 200 μmol/L for 24 h of treatment). Apoptosis markers, such as decreased mitochondrial membrane potential, increased mitochondrial release of cytochrome *c* and enhanced caspase-3 and caspase-9 levels, were demonstrated in colon HT-29 cancer cells after 12 h of treatment (100, 250 and 500 μmol/L) [73]. Chung et al. [71] have shown an apoptotic effect in DU145 cells (human prostate cancer cell line) after 27 h of treatment (100 μmol/L) by inducing mitochondrial depolarization. EGCG also led to the cleavage of caspase-8 and caspase-3 in HLF hepatoma cells after 24 h of treatment with the flavanol (150 μmol/L) [50]. Moreover, EGCG-induced apoptosis, but not growth inhibition, has been demonstrated in H661 lung cancer cells and Ha-*ras*-gene-transformed human bronchial cells; this feature was completely or partially blocked with the inclusion of catalase into the medium [63,75].

As mentioned above for EGCG, little is known regarding the mechanism of quercetin-induced apoptosis. Activation of caspase-3 and caspase-9, cleavage of poly ADP-ribose polymerase (PARP) and release of cytochrome *c* by quercetin have been reported in a study carried out in HL-60 leukemic cells with a concentration of 60 μmol/L after 12 h of incubation [54] and, accordingly, the mitochondrial pathway was also activated in colon Caco-2 cancer cells after 48 h of incubation with the flavonoid (5–50 μmol/L) [98]. Quercetin induced the cleavage of caspase-3, caspase-7, caspase-9 and PARP in a dose-dependent manner (29–58 μmol/L) after 14 h of treatment in human A549 lung cancer cells [104]. Similarly, activation of caspase-3, accompanied by the release of cytochrome *c* and decreased mitochondrial membrane potential, was reported in pancreatic BSp73AS (6h) and MiaPaCa-2 (24 h) cancer cells after incubation with 100 μmol/L quercetin [112]. Mitochondrial membrane potential also decreased and caspase-3 increased in human thymoma HPB-ALL cells after 12 h of treatment with 50 μmol/L quercetin [126].

Concerning the molecular mechanisms of action of genistein-induced apoptosis, the activation of caspase-3 by 111 μmol/L genistein after 24 h in a human colon adenocarcinoma cell line (Caco-2Bbe) has been reported [91]. Moreover, this isoflavonoid induced a time-dependent and dose-dependent cytotoxic effect on human DU145 (androgen-independent) and LNCaP (androgen-sensitive) prostatic cancer cells after incubation with 10, 30, 50 or 70 μmol/L isoflavonoid for 24 or 48 h, and that decreased cell viability was due to both necrosis and apoptosis, which were also detected in both cell lines after 48 h of treatment with

higher concentrations of genistein (50 and 70 μmol/L) [116]; reduced cell viability was concomitant with increased caspase-3 activity, independently of androgen sensitivity [116]. Similarly, Kumi-Diaka and Butler [127] have reported an enhanced caspase-3 activity with 15, 30, 45 and 60 μmol/L genistein (24 h) in TM4 testicular cells, together with necrosis (up to 5%), at the highest concentration tested (60 μmol/L). In a human breast cancer cell line (MCF-7), PARP cleavage was detected after 2–6 days of incubation with 50 μmol/L genistein [115], while in MDA-MB-231 breast cancer cells, caspase-3 activation and PARP cleavage were observed after 48–72 h of genistein treatment (30 μmol/L) [114]. Additionally, activation of caspase-3 and caspase-9 with mitochondrial depolarization at 15, 30 or 60 μmol/L genistein (24 h of exposure) has been described in T-lymphoma cells [128].

5.4. Molecular targets in Bcl-2 family proteins

Previous reports have documented that the ratio between proapoptotic and antiapoptotic Bcl-2 proteins determines, in part, the susceptibility of cells to death signals [46,129]. At 100 μmol/L, the green tea constituent EGCG has been shown to inhibit antiapoptotic Bcl-2 proteins such as Bcl-2 and Bcl-x_L [130]. In this regard, EGCG decreased both protein and mRNA levels of Bcl-2 and Bcl-x_L after 24 h of treatment with 10–100 μmol/L flavonoid. Bid levels were also diminished at 50 and 100 μmol/L concentrations, whereas Fas, Bax, XIAP and c-IAP1/2 remained unchanged [124]. In this study, in a time course experiment, 100 μmol/L EGCG evoked a decrease of Bcl-2 and Bcl-x_L after 3 and 6 h of exposure, and this reduction persisted for longer times (12 and 24 h) for both antiapoptotic proteins, but Fas and Bax remained unaffected at any checked time [124]. Similarly, Roy et al. [131] have observed caspase-3 cleavage, PARP and increased levels of Apaf-1, released cytochrome *c* and Bax/Bcl-2 ratio in ER-negative human MDA-MB-468 breast carcinoma cells after 48 and 72 h of incubation with 20, 40 and 60 μg/ml flavonoid. On the other hand, it has been demonstrated that EGCG-induced apoptosis is not related to members of the Bcl-2 family, as flavanol (100 μmol/L for 72 h) did not alter the expressions of Bcl-2, Bcl-x_L and Bad in human prostate cancer DU145 cells [71]. EGCG also led to an apoptotic effect in human monocytic leukemia U937 cells after 6 h of treatment (200 μmol/L) by promoting binding to Fas and subsequent activation of caspase-8 [132]. Accordingly, EGCG induced apoptosis through a Fas-mediated pathway, since it enhanced Fas/APO-1 and its ligands (membrane-bound Fas ligand and soluble Fas ligand) after the treatment of a human hepatoma cell line (HepG2) with 50, 100 and 200 μmol/L EGCG for 6, 12, 24 and 48 h [45].

Quercetin down-regulates the antiapoptotic Bcl-2 proteins Bcl-x_L and Bcl-2 and up-regulates the proapoptotic proteins Bcl-2, Bax and caspase-3 in prostatic PC-3 carcinoma cells at 50 and 100 μmol/L (24 h of treatment) [108]. Accordingly, increased levels of total Bad and Bax have been previously observed in A549 cells, while Bcl-2 decreased and Bcl-x_L increased in a dose-dependent fashion after 14 h of treatment (29–58 μmol/L) [104]. Moreover, cleaved caspase-3 and PARP were increased, Bcl-2 was significantly decreased and Bax remained unchanged in colon cancer cells (HT-29 and SW480) with 50 μmol/L quercetin after 72 h of exposure [133]. Similarly, the cleavage of caspase-3 and PARP, and increased levels of Bax were demonstrated in human leukemia cells (Jurkat T) after 12 h of incubation with 50 μmol/L flavonoid [99]. Quercetin also led to an antiproliferative effect in Ishikawa cells (endometrial cancer cell line) after 7 days of treatment (10 and 100 μmol/L) without increasing *bcl-2* or *bax* gene expression [52]. An apoptotic effect with unchanged levels of Bcl-x_L — but decreased Bcl-2 expression, enhanced caspase-3 activity and lost mitochondrial transmembrane potential — has also been reported in murine melanoma B16-BL6 cells at 10^{-4} g/ml quercetin after 24 h of exposure [110].

In genistein-treated rat neurons (primary culture, 100 μmol/L), DNA fragmentation and levels of cleaved PARP increased, while Bcl-2 and Bcl-x_L remained unchanged after 15 min or 1, 3, 6 or 24 h of treatment [134]. Baxa and Yoshimura [135] have demonstrated increased caspase-3 activity at 6, 8 and 24 h of exposure in 92316T lymphoma cells (60 μmol/L), whereas Bcl-2 and Bcl-x_L did not change after incubation with 15, 30 or 60 μmol/L for 24 h, and cIAP-1 decreased at concentrations above 15 μmol/L in a dose-dependent manner (24 h). Accordingly, Bcl-2 phosphorylation increased at 24–48 h (150 μmol/L) in MCF-7 breast cancer cells, while Bax expression was not elevated during the same incubation period [136]. On the contrary, apoptosis was observed in MCF-7 cells exposed to 25 or 50 μmol/L genistein for 24 h together with an increased expression of both Bcl-2 and Bax proteins, which suggested that elevated Bcl-2 protein and mRNA levels might neutralize the proapoptotic effect of Bax and that the mechanism of genistein-induced apoptosis might rely on the stress pathway rather than on the *bcl-2* gene family [137]. A different susceptibility to genistein-induced apoptosis has been reported for MCF-7 compared to MDA-MB-231 breast cancer cells [115]. Bax remained unchanged in both cell lines during exposure to the isoflavonoid (50 μmol/L genistein for 18, 24, 48, 72 or 144 h), but Bcl-2 increased slightly in MDA-MB-231 cells, although in MCF-7 cells, Bcl-2 decreased at 18 and 24 h of incubation and increased at 48 h of incubation. Therefore, in MCF-7 cells, the Bax/Bcl-2 ratio initially increased, but after 48 h of treatment, it decreased, suggesting that MCF-7 cells are more sensitive to induction of apoptosis by genistein than are MDA-MB-231 cells [115]. On the other hand, after treatment of MDA-MB-231 breast cancer cells with 30 μmol/L genistein for 24, 48 or 72 h, Li et al. [114] showed up-regulation of Bax and down-regulation of Bcl-2 expressions, and a comparable increase in Bax/Bcl-2 ratio was described in H460 and H322 lung cancer cells after

exposure to 30 or 50 μmol/L of the isoflavonoid for 24–72 h [113]. Genistein induced apoptosis in colon cancer HT-29 cells, prompting an increase in Bax and a slight decrease in Bcl-2 of both mRNA expression and proteins levels at 30, 60 and 120 μmol/L isoflavonoid (72 h of exposure) [138]. Similarly, genistein (25 or 50 μmol/L) also led to apoptosis since DNA fragmentation and cleaved PARP were demonstrated in the HN4 cell line (head and neck squamous call carcinoma) after 3–5 days of treatment, together with up-regulation of Bax and down-regulation of Bcl-2 [118,119].

5.5. Synergism with natural and synthetic compounds

Flavonoids can interact synergistically with other polyphenols present in vegetables and fruits, and also with conventional synthetic drugs in the treatment of cancer to induce apoptosis. Accordingly, EGCG synergistically enhances apoptosis-inducing activity by combined treatment with other chemopreventive agents such as drugs (sulindac, tamoxifen, etc.) or other natural compounds. In this regard, the synergistic induction of apoptosis with four different doses of epicatechin (10, 50, 100 and 200 μmol/L), in conjunction with 75 or 100 μmol/L EGCG in the human lung cancer PC-9 cell line, has been shown [139]. Moreover, two preventive agents, sulindac and tamoxifen, significantly and synergistically enhanced the apoptosis induced by EGCG in PC-9 cells [139]. Sulindac is a popular agent used for the suppression of colon adenoma formation in familial adenomatous polyposis patients, but its usage is restricted because of its side effects. In this study, sulindac at concentrations up to 100 μmol/L did not induce apoptosis of PC-9 cells, whereas 10 μmol/L sulindac, together with 75 μmol/L EGCG, induced apoptosis (eightfold). Similarly, cotreatment with tamoxifen (10 and 20 μmol/L) and EGCG (75 μmol/L), compared with either tamoxifen or EGCG alone, induced programmed cell death in a higher percentage. The synergistic potential of sulindac was more powerful than that induced by tamoxifen or epicatechin [139]. Additionally, synergistic effect was also demonstrated by two naturally agents combined, EGCG and curcumin (1:1), which showed an antiproliferative effect in human normal, premalignant and malignant human oral epithelial cells that was more effective than that of either compound alone [140].

Resveratrol and quercetin additively activated caspase-3 and cytochrome *c* release in a human pancreatic cancer cell line (BSp73AS) [112], and quercetin, together with ellagic acid (at 5 and 10 μmol/L each), also synergistically induced apoptosis in human leukemia MOLT-4 cells [100]. Moreover, quercetin has been shown to synergistically enhance the antiproliferative effect of cisplatin (a drug widely used in the treatment of head and neck cancer) in human laryngeal Hep2 cells [141]. The combination of quercetin (40 μmol/L) and cisplatin (2.5 μg/ml) for 24 h induced apoptosis via the inhibition of protein kinase B (AKT) phosphorylation, release of mitochondrial cytochrome *c*, cleavage of caspase-9, increase in caspase-8 activity and Bax protein levels, and decrease in Bcl-2 and Bcl-x_L protein expressions [141]. Similarly, cisplatin (10 μg/ml), combined with quercetin (15 μg/ml), showed an enhanced proapoptotic effect in HeLa cells through strong activation of caspase-3 and inhibition of heat shock protein (Hsp72) [35].

Genistein can also interact synergistically with other polyphenols. A cooperative action of soy isoflavones (genistein, biochanin A and daidzein) has been demonstrated in four different human bladder cancer cell lines (RT4, J82, HT1376 and T24) for inducing apoptosis when compared to the administration of a single compound (at 50 μg/ml each for 48–72 h) [122]. Moreover, the combination of genistein (2.5 μg/ml) and the black tea polyphenol thearubigin (0.0625 μg/ml) for 60 h significantly inhibited the cell growth of PC-3 human prostatic cancer cells and induced cell cycle arrest, while no effect was observed when thearubigin was administered alone [142]. Additionally, genistein can also interact with conventional drugs used for the treatment of cancer by synergistically enhancing the antiproliferative effect of cisplatin on human pancreatic BxPC-3 cells [143]. The pretreatment of cells with genistein (25 μmol/L) followed by cisplatin (0.5 μg/ml) for 24 h enhanced cisplatin-induced apoptosis through a decrease in Bcl-2 and Bcl-x_L [143]. Similarly, cisplatin (1 or 10 μg/ml), in combination with genistein (20 μmol/L), showed an enhanced apoptotic effect after 24 h of treatment in five melanoma cell lines (MeWo, p22, p39, HM3KO and A101D), reduced Bcl-2 and Bcl-x_L protein levels and increased Apaf-1 [144]. 5-Fluorouracil (5-FU) is one of the drugs most widely used for different cancers, but its chemoresistance is a big obstacle in chemotherapy. A combined incubation of genistein (100 μmol/L) and 5-FU (50 μmol/L) for 3, 6, 12 or 24 h led to a synergistic cell death effect, accompanied by increased cleavage of PARP in colon HT-29 cancer cells [145].

5.6. New research strategies and regulation of signaling pathways

Accumulating evidence suggests that flavonoids may exert regulatory activities in cells through actions at different signaling transduction pathways [cyclin-dependent kinases (CDKs), caspases, Bcl-2 family members, epidermal growth factor (EGF)/epidermal growth factor receptor (EGFR), phosphatidylinositol-3-kinase/AKT, MAPK, NF-κB, etc.], which may affect cellular function by modulating genes or phosphorylating proteins and may be beneficial in cancer. New technologies such as DNA microarray allow us to measure a high number of genes in one analysis and can be used for finding new genes that are affected by food components, in particular flavonoids, which can be useful for a better understanding of the mechanisms involved in the cancer-preventive actions of these compounds. In this regard, EGCG could exert a dose-dependent inhibitory effect on cell proliferation, as it has been proposed in different reports using gene array methodology. Low concentrations of flavanol (1 μmol/L) induced an antiapop-

totic response, while higher concentrations (50 μmol/L) caused a proapoptotic effect on neuroblastoma SH-SY5Y cells [146]. EGCG for 6 h (50 μmol/L) increased the mRNA expression of proapoptotic genes such as *bax* and *gadd45* (cell cycle inhibitor) and decreased the antiapoptotic *bcl-2* and Bcl-x_L mRNAs. A low EGCG concentration (1 μmol/L) induced a decrease in the expression of *bax* and *caspase-6* genes, evoking survival and protective mechanisms [146]. EGCG at 25 μmol/L in Ha-*ras*-transformed human bronchial epithelial 21BES cells inhibited cell growth and induced cell death and changes in the expression of genes related to the apoptotic process (laminin, fibronectin, components of tumor growth factor-β signaling pathway, etc.) [147]. Similarly, it has been reported that EGCG (200 μmol/L for 7 h) caused changes in genes connected with apoptosis and tumor suppression, and also altered oncogenes in PC-9 lung cancer cells [148]; in more detail, EGCG down-regulated the expression of the NF-κB-inducing kinase (*NIK*) gene, the death-associated protein kinase 1 (*DAPK1*) gene and the tyrosine protein kinase (*SKY*) gene, and up-regulated the expression of the retinoblastoma-binding protein (*RBQ1*) gene. Moreover, treatment with EGCG modulated ERK, AKT, Bcl-2 and Bax expressions differently in normal and skin cancer cells: levels of phosphorylated ERK and AKT increased at 0.5 μmol/L but not at 50 μmol/L EGCG, and Bcl-2/Bax ratio also rose in normal keratinocytes, whereas in squamous carcinoma cells, both phosphorylation of ERK and AKT and the Bcl-2/Bax ratio decreased [66].

Quercetin can also potentially interact with different intracellular signaling pathways to induce apoptosis (i.e., cell cycle arrest, inhibition of cell survival/proliferation pathways, etc.). Several reports, in which gene array methodology was used, have tried to identify the cellular targets of quercetin. In HT-29 colon cancer cells after 24 h of exposure to 150 μmol/L quercetin, inhibition of proliferation, increase in caspase-3 activity and alteration of proteins involved in growth and differentiation were observed [149]. Herzog et al. [107] have reported that quercetin (150 μmol/L for 24 h) regulated several heat shock proteins, annexins and cytoskeletal caspase substrates in human preneoplastic colonocytes (NCOL-1). Moreover, quercetin (25 μmol/L) inhibited the expression of specific oncogenes and genes controlling the cell cycle in the PC-3 prostate cancer cell line [85]. Similarly, treatment of Caco-2 cells with 5 or 50 μmol/L quercetin for 48 h caused the down-regulation of cell cycle genes and several oncogenes (*akt-1*, *erb-2* and *c-myc*), changes in genes involved in apoptosis (increased caspase-1 and Apaf-1), cell adhesion, cell–cell interaction, metabolism, transcription and signal transduction [98]. In a recent study, it has been demonstrated that quercetin at low (10 μmol/L) or high concentrations (100 μmol/L) induced gene expression changes at two exposures (24 and 48 h) in CO115 colon cancer cells by modulating genes involved in cell cycle arrest (up-regulation of *p21* and down-regulation of *cdk4*), apoptosis (up-regulation of *Bax* and *caspase-8*) and phase I/II metabolism [150].

Li and Sarkar [151] reported that 50 μmol/L genistein down-regulated the expression of specific oncogenes and genes controlling cell cycle, signal transduction, cell proliferation, protein phosphorylation and transcription in the PC-3 prostate cancer cell line. On the other hand, genistein up-regulated genes mainly related to signal transduction, protein dephosphorylation, heat shock response, inactivation of MAPK and EGFR signaling, apoptosis and cell cycle arrest [151].

5.7. Dose–effect relationship

Flavonoids can induce apoptosis, as seen previously, but concentrations of EGCG needed to significantly down-regulate antiapoptotic proteins and induce programmed cell death in vitro are much higher than the physiological concentration achieved by ordinary tea consumption. Consumption of six or seven cups of green tea per day (corresponding to approximately 30 mg/kg/day EGCG) will generate a plasma EGCG concentration of approximately 1 μmol/L [152]. However, higher plasma EGCG concentrations can be achieved by taking EGCG supplements [153]. In this context, in a phase II trial, green tea has been explored in humans with androgen-independent prostate carcinoma for its antineoplastic effects [154]. Patients were instructed to have 6 g/day green tea orally in six divided doses for 6 months. Although green tea showed limited activity and side effects related to caffeine, as previously reported in a phase I trial in adults with solid tumors [155], ingestion of green tea was considered to be safe for at least 6 months [154,155].

It is also remarkable that published data on quercetin pharmacokinetics in humans suggest that a dietary supplement of 1–2 g of quercetin may result in plasma concentrations between 10 and 50 μmol/L [83] and, as it has been demonstrated, these concentrations induce apoptosis and down-regulate antiapoptotic proteins in vitro. In this context, it is worthy to mention that high plasma genistein concentrations are difficult to achieve and sustain. It has been reported that the plasma genistein level of individuals on high-soy diets is 1–4 μmol/L [9]. However, in a phase I trial, the administration of two different preparations of unconjugated soy isoflavones (which contained 43% and 90% genistein, respectively) generated a maximal plasma concentration of 16.3 μmol/L in humans with prostate cancer, and it showed antimetastasic activity and no toxicity [156]. Moreover, Mulholland et al. [157] studied, in an initial phase I trial, a synthetic water-soluble prodrug of quercetin (3′(*N*-carboxymethyl)carbomyl-3,4′,5,7-tetrahydroxyflavone), QC12, which resolved the problem of using dimethyl sulphoxide as a vehicle for the flavonoid and suggested its potential usage in clinical investigation. Recently, a quercetin derivative in phase II has been shown to be active in advanced adenocarcinoma of the esophagus by inhibiting biological pathways [158].

It is noteworthy that most studies of flavonoids, and polyphenols in general, aimed to evaluate the protective

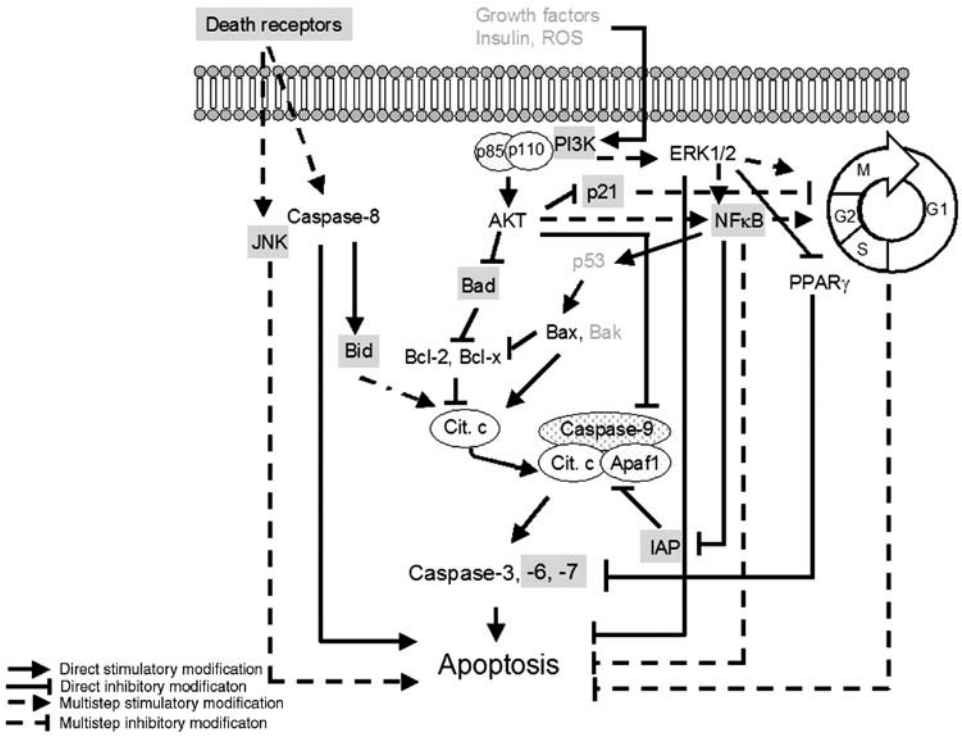


Fig. 3. Flavonoids interact with a wide range of molecules involved in apoptosis and cell proliferation pathways by affecting their expression or activity. The diagram summarizes the components of these two pathways, which have been reported to be influenced by flavonoids, but this does not imply that they are direct targets for these dietary compounds. Black letter denotes changes common to all selected flavonoids, while black letter in gray square shows changes observed for two polyphenols. Gray letter denotes targets involved in signaling pathways but not reported to be regulated for any selected flavonoid in this review.

effect of these compounds against diseases, but their potential toxicity or negative effects have been scarcely studied. As an example, flavonoids can inhibit thyroid peroxidase and interfere with thyroid hormone biosynthesis, as it was shown in rats fed a diet supplemented with genistein [159]. These effects are very important in cases of iodine deficiency and for babies exposed to particularly high doses of isoflavones through soy feeding. Nevertheless, it is remarkable to mention that a clinical study in humans failed to show significant effects on thyroid hormones after consumption of isoflavones.

6. Conclusion

Flavonoids are potent bioactive molecules that possess anticarcinogenic effects since they can interfere with the initiation, development and progression of cancer by the modulation of cellular proliferation, differentiation, apoptosis, angiogenesis and metastasis. Moreover, the chemopreventive effect of dietary flavonoids is quite specific, and cancer cell lines seem to be more sensitive to polyphenol actions than are normal cells. However, some of the apparently contradictory results that have been reported from different laboratories point out the importance of experimental conditions (dose, cell type, culture conditions and treatment length) for the interpretation of the results of in vitro studies because biological outcome can be affected (effects opposite to the expected ones; toxicity).

Flavonoids have emerged as potential chemopreventive candidates for cancer treatment, especially by their ability to induce apoptosis. However, because of the complexity and interrelationships of transduction pathways, the mechanisms for inducing the apoptosis of these polyphenols may overlap with other signaling cascades; thus, programmed cell death can be promoted through the modulation of different proteins in other pathways that can contribute to cell death (Fig. 3). Flavonoids examined in this review possess common effects, namely, induction of apoptosis involving the release of cytochrome *c* from mitochondria, activation of caspases and down-regulation or up-regulation of Bcl-2 family members, but also induction of cell cycle arrest and inhibition of survival/proliferation signals (AKT, MAPK, NF-κB and EGF) (Fig. 3). Moreover, examples of interaction between polyphenols and/or conventional synthetic drugs used in cancer treatment have been described, providing an interesting approach to combination therapy.

Although results from in vitro experiments cannot be directly extrapolated to clinical effects, they constitute a valuable tool for elucidating the pathways involved in the overall carcinogenesis process. More studies are needed to clearly understand the mechanisms of action of flavonoids

as modulators of cell apoptosis, which is crucial for the evaluation of their potential as anticancer agents.

Acknowledgments

This work was supported by grant AGL2004-302 from the Spanish Ministry of Science and Technology (CICYT) and grant 200570M050 from the Comunidad de Madrid (CAM).

S. Ramos has a Ramón y Cajal contract from the Spanish Ministry of Science and Technology.

The author thanks Dr. Laura Bravo, Dr. Luis Goya, Dr. Ma. Angeles Martin and Ana Belen Granado-Serrano for the critical review of this manuscript.

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