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REVIEW

Molecular mechanisms of flavonoids in melanin synthesis and the potential for the prevention and treatment of melanoma

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Flavonoids are becoming popular nutraceuticals. Different flavonoids show similar or distinct biological effects on different tissues or cell types, which may limit or define their usefulness in cancer prevention and/or treatment application. This review focuses on a few selected flavonoids and discusses their functions in normal and transformed pigment cells, including cyanidin, apigenin, genistein, fisetin, EGCG, luteolin, baicalein, quercetin and kaempferol. Flavonoids exhibit melanogenic or anti-melanogenic effects mainly via transcriptional factor MiTF and/or the melanogenesis enzymes tyrosinase, DCT or TYRP-1. To identify a direct target has been a challenge as most studies were not able to discriminate whether the effect(s) of the flavonoid were from direct targeting or represented indirect effects. Flavonoids exhibit an anti-melanoma effect via inhibiting cell proliferation and invasion and inducing apoptosis. The mechanisms are also multi-fold, via ROS-scavenging, immune-modulation, cell cycle regulation and epigenetic modification including DNA methylation and histone deacetylation. In summary, although many flavonoid compounds are extremely promising nutraceuticals, their detailed molecular mechanism and their multi-target (simultaneously targeting multiple molecules) nature warrant further investigation before advancement to translational studies or clinical trials.

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1 Introduction

Flavonoids are a large group of polyphenolic compounds found in a wide range of vegetables and medicinal herbs; so far more than 5000 compounds have been identified [1]. These compounds exhibit a broad range of anti-tumor, anti-allergic, anti-inflammation, anti-fungal and anti-viral functions and have attracted much attention in the chemoprevention and cancer treatment fields. Because they are easily

available nutrients in regular diets and as they have exhibited high pharmaceutical potential in preclinical studies, many of these compounds have become popular as nutraceuticals. There have been many publications on flavonoids and their potential roles in the management of cancer; however, information about these compounds vis-a-vis the pathogenesis of melanoma have been limited [2]. As flavonoids show dramatic cell line and tissue specificities [3, 4], it is incumbent that we examine what has been done to date both mechanistically and preclinically before proceeding to translational studies of melanoma.

Flavonoids include several major groups of compounds with the shared core backbone structure of flavan: flavones, flavonols (3-hydroxyflavone), flavanols, isoflavones and anthocyanidins (Fig. 1A), all with different side group

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Abbreviations: **cAMP-PKA**, cyclic AMP-protein kinase A; **DTC**, dopachrome tautomerase; **TYRP-1**, tyrosine-related protein 1; **EGCG**, epigallocatechin gallate; **MiTF**, microphthalmia transcription factor; **NRF2**, nuclear factor erythroid 2 [NF-E2]-related factor 2; **ROS**, reactive oxygen species; **DNMT**, DNA methyltransferases; **NO**, nitric oxide

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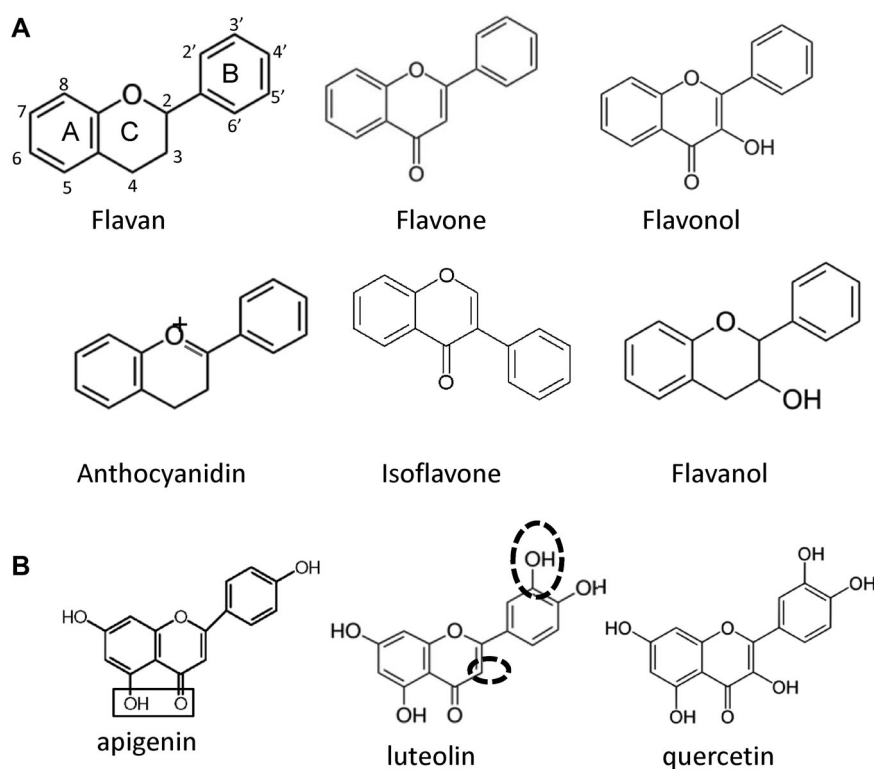


Figure 1. Skeleton structures of major flavonoids and luteolin, apigenin and quercetin. (A) Six main classes of flavonoids are listed, which are the focus of this review. Locations of three rings (A, B and C) are labelled on flavan which are the same for other compounds. (B) Structure comparison of luteolin, apigenin and quercetin. The different side groups are circled in luteolin structure. The boxed side groups in apigenin show a typical structure that is able to bind metal ions. Comparing these three popular compounds which sometimes show opposite effects on melanogenesis may provide some hints on how each side group functions biologically.

modifications [5, 6]. The main structure of flavan is comprised of three rings: A, B and C rings (Fig. 1A). The above major groups of flavonoids differ on their modification of side groups on these rings. These side groups play crucial roles in the function of these compounds as the side modification can produce very different activities. Table 1 lists the most commonly used flavonoids that have been tested in melanoma models and their major dietary sources. The most informative data have been derived from these compounds therefore this review will focus on these listed compounds and their possible molecular mechanisms of action in the pathogenesis of melanoma. However this is a limited list as there are many more flavonoids that have been studied in melanocytic cell lineage, including rutin, robinetin, rhamnetin, naringin, chrysin, ipriflavone, tangeritin and more, and some derivatives of these compounds [7–9].

Cutaneous melanomas arise from skin melanocytes, a cell type that is specialized in synthesizing melanin which contributes to skin color and protection against solar UV (ultraviolet) radiation. Skin color is an important part of beautification [10]; for example, a tan color has become desirable for white skinned individuals (Caucasians) while a lighter color has become more desirable for darker-skinned Asian individuals, especially women. Therefore the skin care industry has been seeking various methods to safely manipulate skin color. As a consequence, there are many studies using flavonoids as skin-whitening agents [11], as listed in Table 1. This review attempts to summarize the known effect of flavonoids in melanogenesis and melanomagenesis and their potential

molecular mechanisms. As is revealed in this short review, it is apparent that majority of the pigmentation and anti-melanoma studies have been performed in vitro and/or in B16 mouse melanoma cell lines, indicating that in vivo studies and studies with human cells are still needed to enable clinic use of these compounds.

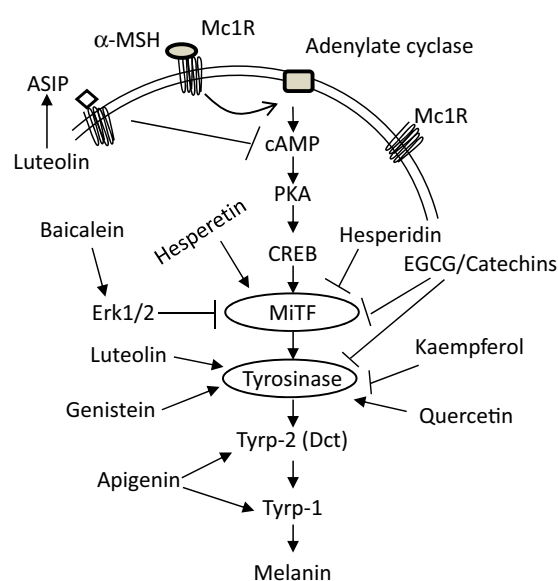
2 Flavonoids function in melanogenesis

As listed in Table 1, cyanidin, hesperetin, apigenin, genistein and fisetin all exhibited melanogenic effect, i.e., stimulated melanin synthesis. On the other hand, EGCG or other catechins, hesperidin, luteolin, baicalein and kaempferol all inhibited melanin synthesis. For quercetin, two studies showed stimulatory effect and one showed an inhibitory effect. We have listed cell lines (mouse or human) used in each study because the regulation of melanin syntheses may be different in human and mouse normal and malignant cells by these compounds. Indeed, caution when evaluating these compounds across species types needs to be the order of the day.

Pigmentation is a very complex biochemical process involving more than 300 loci in mice, according to International Federation of Pigment Research Society website (<http://www.espcr.org/micemut/>). Most of these loci have corresponding orthologues with human genes. Figure 2 lists the major pathway showing a few key genes in this process. Mainly, upon stimulation by the α -melanocytes stimulating hormone (α -MSH), MC1R (Melanocortin Receptor 1) transmits

Table 1. An overview of flavonoids: sources and functions

Category	Major dietary sources	Compounds	Pigment synthesis	Reference	Reference cell line	Function in melanoma prevention/treatment	Reference
Anthocyanidin	Berries, grapes	Cyanidin	Increase	[103]	Human	Anti-proliferation	[123]
Flavanol	Chocolate, cherry, green tea	EGCG/Epicatechin	Inhibition	[19, 20]	Human, mouse	Anti-metastasis, anti-proliferation, apoptosis, immunomodulation	[95, 124, 125]
Flavanone	Citrus fruits	Hesperetin	Increase	[21, 126]	Human, mouse	No known effect	[8]
		Hesperidin	Inhibition	[18]	Human, mouse	No report	
Flavone	Celery, parsley	Apigenin	Increase	[27, 127]	Mouse	Anti-proliferation, anti-metastasis	[98]
		Luteolin	Inhibition	[23]	Mouse	Anti-proliferation, anti-metastasis	[128, 129]
		Baicalein	Inhibition	[22]	Mouse	Anti-proliferation	[48, 130]
Flavonol	Baikai skullcap (medicinal herb), Onions, apples, Kale, Leek, broccoli	Quercetin	Increase	[25, 131, 132]	Human	Anti-proliferation, anti-metastasis	[98]
		Fisetin	Increase	[7, 133]	Mouse	Anti-proliferation, anti-metastasis	[107, 108, 134]
	Strawberry		Increase		Human, mouse		
		Kaempferol	Inhibition	[26]	Mouse	Anti-proliferation (G2/M arrest)	[102]
		Daidzein	No effect	[24]	Mouse, human	Anti-proliferation, anti-metastasis	[24, 135]
Isoflavone	Soybean	Genistein	Increase	[24]	Mouse, human	Anti-proliferation	[135, 136]

**Figure 2.** Molecular mechanisms of flavonoids on melanin synthesis. The current understanding of melanin synthesis follows the cAMP-PKA-MITF-tyrosinase scheme, in which MITF serves as the master transcriptional factor activating tyrosinase, DCT and TYRP-1, and receives signals from MC1R. Inhibitory or stimulatory effects of each compound are listed in the scheme with references discussed in the text.

a signal to cAMP-PKA (cyclic AMP and Protein Kinase A) [12], whose activation leads to enhanced expression of the melanocytes master transcriptional factor MITF (Microphthalmia Transcription Factor) which in turn activates expression of the major melanogenic enzymes tyrosinase, dopachrome tautomerase (DTC, also known as tyrosine-related protein 2, TYRP-2) and tyrosine-related protein 1 (TYRP-1) via binding to E boxes on their promoters [13–16]. Agouti signaling protein (ASIP) antagonizes the functions of α -MSH and inhibits the melanin synthesis pathway (Fig. 2) [17]. This schema is oversimplified as melanins exist as two classes: eumelanin and pheomelanin and their synthesis share some regulatory features but differ in others. Most of melanogenesis effects of flavonoids have been targeted to this simplified scheme. As shown in Fig. 2, hesperidin and catechins (including EGCG) inhibited MITF protein accumulation [18, 19]; EGCG in addition inhibited tyrosinase accumulation [19, 20]. Hesperetin which stimulated melanogenesis, on the other hand, enhanced MITF accumulation; the upstream signal was not investigated [21]. Baicalein, a depigmenting agent, inhibited MITF accumulation via ERK1/2- phosphorylation mediated degradation [22]. Luteolin, genistein, kaempferol and quercetin all targeted tyrosinase directly or indirectly [23–26]. Apigenin did not target tyrosinase, rather it targeted TYRP-2/DCT and TYRP-1, perhaps via p38 mitogen activating protein kinase [27].

Note that even though luteolin increased tyrosinase protein accumulation in B16 melanoma cells, this compound

in the end inhibited melanin synthesis and several studies suggest that it is a skin-whitening agent [23, 28]. This may be because luteolin actually inhibited tyrosinase activity and the upstream α -MSH mediated cAMP signaling [28]. Results from our lab have revealed that luteolin dramatically up-regulated ASIP (Agouti-signaling protein) at mRNA level (17.0 fold of increase as compared to untreated control cells) in human A375 melanoma cells (data not shown). This action may reflect a novel layer of regulation by luteolin for the melanogenesis pathway, but will require extensive studies to validate. As shown in Fig. 2, ASIP binds to MC1R and inhibits α -MSH-mediated cAMP/PKA activation and hence inhibits downstream melanin synthesis [17], ASIP polymorphisms are associated with human pigmentation phenotypes and melanoma risk [29, 30]. To date, ASIP is not known to regulate pigmentation via an autocrine route; a previous study showed ASIP expression at the mRNA and protein level in melanoma cell lines [31], which is consistent with our unpublished results, suggesting this protein may have the potential to exhibit autocrine function.

Of importance is that although some compounds have similar structures, they show drastic differences on melanogenesis regulation. For example, in comparing apigenin and luteolin, there is only one extra hydroxyl group in luteolin (Fig. 1B), yet, apigenin stimulated, while luteolin inhibited, melanin synthesis. It is speculated that the extra hydroxyl group in luteolin played a crucial role in determining some specificities of this compound. Also, compared to luteolin, quercetin has an extra hydroxyl group on the C ring, which apparently also results in different cellular functions (Fig. 1B and Table 1). This differential function of structurally similar flavonoids is not only observed in the melanogenesis pathway, it has also been observed in cardiovascular and cancer-related pathways as well [32].

3 Flavonoids function in melanoma prevention, treatment and metastasis prevention

Flavonoids have been widely used as experimental chemoprevention and chemotherapy agents in many different cancer types including breast, prostate, pancreas, bladder, lung and colon cancer [33–35]. Epidemiological studies show that estimated dietary intake of total flavonoids (most of the time it is not specified) is usually (but not always) inversely correlated with cancer risk [36–38]. Carefully designed cancer prevention trials (including melanoma) are currently lacking. Below we summarize the potential molecular mechanisms of flavonoids and their activities in anti-oxidant, anti-inflammation and immune modulation, anti-proliferation, anti-angiogenesis, apoptosis induction and potential epigenetic modification, with most studies executed in vitro, a few in mouse; and some were epidemiological observations.

3.1 Flavonoids as reactive oxygen species (ROS) scavenger for melanoma

Numerous studies have showed that many flavonoids are potent antioxidants, therefore they may serve as effective scavengers of reactive oxygen species [39, 40]. Because excessive ROS cause many problems including DNA, lipid and protein damage and aberrant cellular signaling, flavonoids are apparently protective agents in such conditions.

A number of in vitro assays have been developed to measure the radical scavenger activities in vitro, including 2,2-diphenyl-1-picrylhydrazyl radical (DPPH assay), ferric reducing anti-oxidant power (FRAP assay), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation (TEAC assay), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical (ABTS(-+) assay) (ABTS assay), Folin-Ciocalteu reducing capacity (FC assay), electrochemical total reducing capacity and hypoxanthine/xanthine oxidase system coupled with nitroblue tetrazolium (NBT) reduction (NBT/XO) [28, 41, 42]. All flavonoids listed in Table 1 showed some degrees of free radical scavenger activities, with luteolin and quercetin among the most potent antioxidants in the category [4, 43]. For example, luteolin showed dose-dependent anti-oxidant activity in DPPH and NBT/XO assays in a cell-free system, as well as in B16 cells by H2DCF-DA (dihydrodichlorofluorescein diacetyl)-based intracellular ROS assays [28].

The detailed molecular mechanisms of flavonoids remain to be clarified but can be summarized into three major categories:

- (i) As chelators for redox-potent transition metal ions, which include Cd^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Cr^{3+} and Zn^{2+} [44, 45]. These metals cause an ROS increase via different mechanisms and some are potent carcinogens. The metal binding sites for flavonoids are usually adjacent hydroxyl and/or ketone side groups. For example, the potential metal binding site for apigenin is between the 5-OH group of A ring and the ketone group on C ring (Fig. 1B, boxed).
- (ii) Reacting directly with free radicals via their free hydroxyl group(s) and quench these activities [42]. For example, quercetin scavenges superoxide free radicals mainly function through 3'4'-dihydroxy groups on the B ring [46].
- (iii) Modulating multiple cellular anti-oxidant systems which re-establish redox balance in cells after oxidative stress.

These functions are not mutually exclusive. In a previous review we summarized the source of ROS in melanoma [47], including mitochondria, NADPH oxidases, nitric oxidases, lipoxygenase, cyclooxygenase 2 (COX-2) and melanosomes. These ROS sources are regulated by major redox transcriptional regulators NRF2 (nuclear factor erythroid 2 [NF-E2]-related factor 2), and the AP-1 family members [48–50], among other factors [51]. NRF2 is an important target for flavonoids as it is also the master transcriptional factor for redox regulation [52]. Luteolin was initially found to be a

NRF2 inhibitor in lung carcinoma A549 cells [53]; however, in colorectal and prostate cancers and in neuronal cells luteolin activated NRF2 [54–56]. This compound also enhanced NRF2 translocation into the nucleus where it functions as a transcriptional activator in neuronal cells [57]. Furthermore, luteolin inhibited Cr(VI)-induced malignant cell transformation of human lung epithelial cells by targeting multiple ROS mediated cell signaling pathways [58]. Luteolin inhibited NRF2 target glutathione S-transferase in SK-Mel-28 human melanoma cells [59]; we found that luteolin inhibited NRF2 protein accumulation at 30 μ M but stimulated NRF2 accumulation at 8 μ M in SK-Mel-28 cells (Liu-Smith et al., unpublished data). These studies suggest that luteolin exhibits different effects on the same target gene in different cell lines, or even opposite effects on the same target at different concentrations. On the other hand, apigenin showed more consistent effects in different cell lines or in different studies: apigenin stimulates NRF2 activities in prostate cancer, mouse skin epidermal JB6 P+ cells, hepatocellular carcinoma HEPG2-C8 cells and primary hepatocytes [56, 60–63], via MAPK pathway, epigenetic modification of NRF2 promoter, or PI3K pathway. Quercetin shows similar NRF2-enhancing effect as apigenin, with the end results of activating NRF2-regulated anti-oxidant genes including heme-oxygenase 1, NAD(P)H Dehydrogenase, Quinone 1(NQO1) and genes for glutathione synthesis [64–67]. Genistein and EGCG also induced NRF2 in different cellular background for invoking a protective anti-oxidant mechanism [68–70]. For other targets, baicalein enhanced Cox-2 expression [71], but luteolin, apigenin, genistein suppressed its expression or function [72–75]. Luteolin, quercetin and apigenin also exhibit AP-1 inhibitory effects [76–78].

Mitochondria and ROS-generating enzymes can also be targets for flavonoids; however, published data show that flavonoids serve either as ROS scavengers or ROS stimulators. In A375 cells apigenin directly targeted and compromised the oxidative phosphorylation system in mitochondria and induced ROS levels which led to cell death [79]. Similarly, baicalein also induced ROS in B16 cells, possibly via 12-lipoxygenase [48]; and quercetin increased ROS levels in DB-1 melanoma cells via inhibiting bio-reduction capacity, namely the glutathione-S transferase and NQO1 levels [80]. On the other hand, luteolin directly inhibited xanthine oxidase activity in a dose-dependent manner and reduced cellular ROS levels in B16 cells [28]. As all antioxidants have the potential to be converted into pro-oxidants, it is not surprising to see these conflicting results. Our own experiments with luteolin showed dose-dependent differential stimulating and inhibitory results on NRF2 (described above) accumulation in the same cell line, we speculate that some of the flavonoids may require a specific dose range to act as antioxidants, or else they may stimulate ROS production. Despite much evidence that flavonoids serve as ROS scavengers, the anti-oxidant property is not the only mechanism for their protective roles for human cells [81]. Next we will discuss their other cellular roles.

3.2 Flavonoids function in anti-inflammation and immune-modulation in melanoma

There is strong evidence that inflammation and immune suppression play important roles in melanoma etiology, progression, and even prognosis: (i) the major environmental risk factor Ultra-Violet (UV) radiation causes skin immune suppression [82], (ii) melanoma tumors contain large amount of infiltrated immune cells [83] and (iii) BRAF inhibitor-mediated immunosuppression is a reason for therapeutic failure [84]. Also inflammation exhibits an intrinsic correlation with oxidative stress which is highly elevated in melanoma [85].

For all these aspects, the anti-inflammatory properties of flavonoids in preclinical have shown a potentially important impact on melanoma etiology, prevention and treatment outcomes. Briefly, flavonoids modulate inflammatory effects through a few key mediators in melanoma and skin tissues: AP-1 family transcriptional factors [86], NF κ B [87], STAT3 [88] and nitric oxidases (mainly iNOS and nNOS) [89, 90]. AP-1 and NF κ B are able to up-regulate cytokine expression such as IL-8 [91, 92]; as stated above, AP-1 can be inhibited by luteolin, quercetin and apigenin [76–78]. Luteolin was shown to promote proteasome-mediated degradation of STAT3 and thus blocked the inflammatory signals from cytokines such as IL6 and IL10 [93]. Quercetin, on the other hand impaired STAT3 nuclear localization via altering its phosphorylation [94]. EGCG prevents UV-induced immunosuppression via a mechanism that involves production of IL-12. In IL-12 knockout mice or mice injected with anti-IL-12 antibodies, EGCG lost its ability to inhibit UV-induced immune suppression [95]. Nitric oxide (NO) plays an important role in the melanoma inflammatory microenvironment which promotes tumor growth and metastasis; iNOS-expression in melanoma is negatively correlated with patient survival [96]; and nNOS is up-regulated in melanoma and is a potential target for melanoma therapy [90]. Paracrine NO production led to decreased CXC chemokine ligand 10 (CXCL10) levels which resulted in less inflammatory tumor microenvironment in melanoma patients and WM1727A, A375 and SB2 melanoma cell lines [96]. Flavonoids exhibit complex reactions with NO. In cell free system flavonoids have NO-scavenger activity but may generate superoxide at the same time [97]; under oxidative stress flavonoids may play an anti-inflammation role via inhibiting iNOS or inhibiting the NF κ B pathway [97]. Thus it is likely that the impact of flavonoids on NO levels (perhaps also ROS levels) is dependent on the flavonoid type, concentration and cellular conditions such as expression levels of iNOS.

3.3 Flavonoids anti-proliferative, apoptotic induction and anti-metastatic activities

Flavonoids exhibit anti-proliferative and anti-apoptotic effects via HGF/SF-Met signaling, MAPK pathway, cell cycle

regulation, differentiation induction and PI3K-AKT pathway. Like their function in melanogenesis, different flavonoids exhibit different effects on their cellular targets-which are quite diverse.

Flavonoids inhibited xenografted B16-BL6 mouse melanoma growth in the decreasing order of effectiveness: EGCG, apigenin, quercetin, with the latter two compounds showing similar effects as tamoxifen [98]. EGCG inhibited colony formation in soft-agar [99], possibly by inhibiting cyclin D1, CDK2 and PCNA (Proliferating Cell Nuclear Antigen) while inducing p21^{waf1/cip1} and p27^{kip1}, and promoting apoptosis [100]. EGCG reduced MITF protein accumulation via ERK1/2-independent mechanism [19], which may also contribute to its anti-proliferation effect because MITF is generally a melanoma survival gene [101]. Similarly, apigenin induced G2/M cell cycle arrest via inhibiting CDK1 activity [102], which may contribute to its anti-melanoma activity *in vivo* on xenografted B16 cells [98]. Cyanidin glucopyranoside induced B16 differentiation via up-regulating cAMP, tyrosinase expression, and the differentiation marker MART-1 [103]. Both EGCG and quercetin inhibited HGF/SF-Met signaling, a key regulator of melanoma migration and invasion [104, 105]. Fisetin inhibited 451Lu cell proliferation via disrupting the β -catenin/MITF signaling pathway [106]; also inhibited melanoma cell invasion and metastasis through inhibiting the epithelial to mesenchymal transition in a three-dimensional skin model and in a xenografted mice model [107, 108]. Combination treatment of xenografted A375 and SK-Mel-28 tumors with fisetin and RAF inhibitor Sorafenib showed greater reduction in tumor growth than single compounds or control mice due to multiple mechanisms, including induction of apoptosis, inhibition of proliferation and angiogenesis, and inhibition of the MAPK and PI3K pathways [109].

Overall, whether flavonoids directly target the affected genes is not clear; what is clear is that all these compounds, more or less, show an anti-proliferation and/or anti-metastatic effect against melanoma (Table 1). Recent development in nanotechnology have made flavonoids much more effective in targeting melanoma cells both *in vitro* and *in vivo* [110–112]; therefore in the near future we may witness clinical use of these promising natural compounds. However, whether individual compounds delivered by nanoparticles needs to be assessed as drugs first is an issue that has not yet been addressed by regulatory bodies.

3.4 Flavonoids in epigenetic modification: histone acetylation and DNA methylation

The diverse targets of flavonoids may be directly related to their diverse structures [113]. However, there may be a substantial contribution for the epigenetic modification function of flavonoids. Increasing evidence suggests that many flavonoids are able to regulate gene expression via epigenetic approaches including histone modification, DNA methylation and miRNA/lncRNA (microRNA and long non-coding

Table 2. Known functions of flavonoids in epigenetics

Flavonoids	Targets	Reference
EGCG/Epicatechin	DNMT, HDAC3, HAT	[137–139]
Hesperetin	DNMT	[140]
Apigenin	DNMT, HDAC	[140, 141]
Luteolin	DNMT	[140]
Baicalein	DNMT	[142]
Quercetin	DNMT1, HDAC, HAT	[143, 144]
Kaempferol	HDAC	[145]
Daidzein	DNMT	[140, 146]
Genistein	DNMT	[118, 140, 146, 147]

DNMT, DNA methyl Transferase; HDAC, Histone Deacetylase; HAT, Histone acetyl transferase.

RNA) [114]. These epigenetic modifications may affect much diversified target genes. Histones can be modified by acetylation and methylation via histone acetyl transferase (HAT), histone deacetylase (HDAC), histone methyl transferase (HMT) and histone demethylase (HDM) [115]. DNA can be modified by methylation via DNA methyl transferases [114]; how DNA is de-methylated is still not clear and is under intensive investigation [116]. The epigenetic modification functions of most flavonoids from Table 1 are listed in Table 2, with most data obtained from cell types other than melanoma. Only limited studies were performed in melanoma cell lines. DNA methyl transferases (DNMTs), HDACs and HAT are common targets of flavonoids in melanoma, and these enzymes affect the expression of tumor suppressors such as p21^{CIP1} and p16^{INK4A} [117, 118]. In several human melanoma cell lines, green tea polyphenols (mixture of epicatechin monomers) showed significant inhibitory effect on HDAC activities and class I HDAC proteins, and promoted HAT activity, resulting in proliferation inhibition and cell killing [117, 119]. This mechanism may explain a previous observation that EGCG up-regulated p16^{INK4A}, p27^{KIP1} and p21^{CIP1} protein levels in A375 and Hs294t melanoma cells [100], as it was well known that these tumor suppressor genes were subjected to epigenetic silencing in melanoma cells [120–122].

4 Conclusions

Flavonoids are widely available from food and herbs, and have the potential to become therapeutic agents with minimum toxicity. However, not many (if any) clinical trials have been done to establish the profile of flavonoids and the toxicity curve at the doses required to prevent or treat cancer in humans, more *in vivo* studies and human trials are needed to explore their clinical activities. Although it is difficult to pinpoint each compound's intracellular target, their overall effectiveness should be noted. Lack of specificity may be because they are able to simultaneously target many different genes, but that may be the exact reason for their functional versatility. Also, like other chemical compounds, flavonoids are subjected to metabolism and the metabolites may also

be active components in vivo, rendering it even more difficult to identify a single target. Investigators and the public should respect this diversity of action and not to be limited by the “targeted therapy” mantra in the exploration of clinical usefulness of nutraceuticals.

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These authors state no conflict of interests.

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