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Botanicals for the prevention and treatment of cutaneous melanoma

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Summary

Cutaneous melanoma, a cancer of melanocytes, when detected at later stages is arguably one of the most lethal cancers and the cause of more years of lost life than any other cancer among young adults. There is no standard therapy for advanced-stage melanoma and the median survival time for patients with metastatic melanoma is <1 yr. An urgent need for novel strategies against melanoma has directed research towards the development of new chemotherapeutic and biologic agents that can target the tumor by several different mechanisms. Recently, several dietary agents are being investigated for their role in the prevention and treatment of various forms of cancer and may represent the future modality of the treatment. Here, we have reviewed emerging data on botanicals that are showing promise for their potential inhibitory effect against cutaneous melanoma.

Keywords

botanicals; melanoma

Melanoma

Skin cancer is the most common form of cancer in the USA, and is categorized into two major groups: melanoma and nonmelanoma skin cancers. The latter group comprises primarily basal cell and squamous cell carcinomas, which are fast growing, rarely fatal tumors. It is estimated that about 1 200 000 nonmelanoma skin cancers develop annually in the USA. In contrast, melanoma represents only about 5% of all diagnosed cancers in the USA, 15% of which prove to be fatal (Ricotti et al., 2009). The worldwide incidence of melanoma continues to rise in spite of public health initiatives that primarily promoted sun protection. It was estimated that 114 900 new cases of melanoma will be diagnosed in the USA in the year 2010, of which 68 130 will be invasive. On a gender-based analysis, about 38 870 men and 29 260 women will be diagnosed with invasive melanoma for the same year and about 8 700 people are expected to die from the disease (Jemal et al. 2010).

The risk factors associated with the development of malignant melanoma are multifactorial, with both genetic and environmental factors playing a role in its pathogenesis. Individuals with fair skin color, blond or red hair, who burn easily, are more at risk than others. Although melanoma is seen more with increasing age, it is the most frequent cancer in women aged 25–29 yr, and the second most frequent cancer afflicting women aged 30–34. Recently, tanning salons have been implicated in the development of malignant melanoma (Mackie et al., 2009). There is some evidence that repeated acute sun exposure during

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childhood or adolescence resulting in blistering sunburns is associated with increased risk for melanoma in the adult life (Ricotti et al., 2009). The effect of chronic sun exposure, however, is more controversial, with conflicting data regarding the association between long-term chronic sun exposure and melanoma development (Pfahlberg et al., 2001).

Melanoma prevention

Despite a steady increase in its incidence during the past three decades, melanoma has not attracted the same level of attention by researchers and funding agencies as cancers of prostate, breast, colon and lung. As a result it has become a major public health problem in many countries. Surgery remains the cornerstone of curative treatment in earlier stages (Mackie et al., 2009). Metastatic disease is incurable in most affected people, because generally melanoma does not respond to most systemic treatments. The increase in the incidence of melanoma, its resistance to chemotherapy, together with its high potential to metastasize has emphasized the importance of its prevention.

Chemoprevention is an under-explored approach that could significantly decrease the morbidity and mortality from this deadly cancer by slowing the process of melanoma carcinogenesis. Originally referred to as the use of agents, natural or synthetic, to reverse, suppress or prevent premalignant lesions from progressing to invasive cancer, the definition now embraces the term ‘delay’, providing new opportunities to seek intervention in the process of disease progression (Francis et al., 2006). Francis et al. have identified different forms of melanoma chemoprevention with primary chemoprevention occurring in healthy individuals and involving the use of safety measures, avoidance of excessive sun exposure, etc. Secondary chemoprevention, on the other hand, aims to prevent premalignant melanoma precursors from becoming melanoma. Lastly, tertiary chemoprevention seeks to prevent recurrence of melanoma in patients treated with no current signs of the disease (Francis et al., 2006).

Because of the poor survival rate in advanced disease and the limited efficacy of present standard therapy, there has been an increased interest in the use of adjuvant therapy in patients with a high risk of recurrence. To date, several biological agents have been tested. Early, randomized controlled trials of Bacillus Calmette-Guerin, levamisole, *Corynebacterium parvum*, chemotherapy, isolated limb perfusion, radiotherapy, transfer factor, megestrol acetate and vitamin A have yielded largely negative results. Current studies are focusing on vaccines and the interferons (IFN) (Molife and Hancock, 2002). The two biologic therapies that appear most active against melanoma are IFN-α and interleukin (IL)-2. Indeed in some centers, IFN has been accepted as a standard therapy for melanoma patients, but controversy on its use continues (Molife and Hancock, 2002).

Pharmacological and nutriceutical agents that are mechanistically linked to inhibiting events in melanoma carcinogenesis can be potential candidates for the prevention and treatment of this disease. A variety of agents, exerting effects through different mechanisms, are being studied through in vitro, animal and human models with varying results. A significant inverse association between vitamin A intake and cutaneous melanoma risk was reported in a case-controlled study conducted in Italy (Naldi et al., 2004). There is increasing evidence that plants provide a broad spectrum of potential drug substances for cancer therapy with multifaceted effects and targets. In this context, several naturally occurring dietary agents such as genistein and resveratrol are being assessed in clinical trials for their efficacy against melanoma (Mackie et al., 2009). Here, we have summarized recent evidence regarding the ability of botanicals to inhibit the development of melanoma (Table 1). As the roles of vitamins A and D in melanoma have been the subject of several extensive reviews (Egan, 2009; Gandini et al., 2009; Kast, 2008; Niles, 2003), we have omitted these from our

discussion and have focused only on those botanicals that have not been discussed in detail in the literature in the context of cutaneous melanoma.

Genistein

Genistein (4′5,7-trihydroxyisoflavone), a flavonoid present in human diet, derived mainly from soybeans, is also found in other legumes, including peas, lentils and beans. Genistein occurs as the glycoside genistin and is metabolized to the aglycone genistein in the lower gut. Genistein is partly absorbed without previous cleavage and does not have to be hydrolyzed to become biologically active. The many biological functions of genistein reported to date include antioxidant, antimicrobial, phytoestrogenic, and tyrosine kinase inhibitor activities. Recently, genistein has received considerable attention as epidemiologic evidence indicated that consumption of soybean-containing diets was associated with a lower incidence of certain human cancers in Asian populations (Kiguchi et al., 1990). Studies demonstrated that genistein protected against DNA damage induced by hydroxyl radicals, generated from UV photolysis of hydrogen peroxide. Genistein was shown, in association with inhibition of topoisomerase II and tyrosine kinases, to suppress growth and increase melanin content in several different human melanoma cell lines (Kiguchi et al., 1990). The up-regulation of the cyclin-dependent kinase inhibitor (cdki) p27(KIP1) by genistein is likely responsible for the inhibition of cyclin-dependent kinase (cdk)-2, whereas p21(CIP1) is dispensable for genistein-induced G2 arrest (Casagrande and Darbon, 2000). Genistein-induced growth inhibition of melanoma cells might depend on cellular p53 content. Transfection studies showed that high levels of p53 make melanoma cells resistant to the growth inhibitory action of genistein. Induction of differentiation of melanoma cells by genistein is also regulated by cellular p53, as cells lacking p53 responded readily to genistein-induced dendrite-like structure formation (Rauth et al., 1997). It is thought that genistein-induced cellular differentiation occurs through stabilization of protein-linked DNA strand breakage (Constantinou and Huberman, 1995; Yan et al., 1999). A correlation between the growth inhibitory activity of genistein and miR-27a has been reported. Functional assays revealed that the levels of miR-27a and its target gene ZBTB10 were significantly altered in genistein-treated melanoma cells (Sun et al., 2009).

Studies indicate that genistein and daidzein, another isoflavonoid present in dietary soybean, have differential effects on the viability of melanoma cells (Russo et al., 2006). Darbon et al. showed that genistein treatment resulted in arrest of melanoma cells in the G2 phase of the cell cycle, whereas daidzein, which lacks a hydroxyl group, induced accumulation of cells in the G1 phase. Genistein exerted this effect by impairing the Cdc25C-dependent tyrosine dephosphorylation of cdk-1, in addition to activating the checkpoint kinase (Chk)-2 (Darbon et al., 2000). A similar study showed that genistein treatment induced melanoma cells to acquire dendrite-like structures and increased the production of melanin. In contrast, daidzein only retarded the growth of these cells and failed to induce differentiation, suggesting that genistein and daidzein can inhibit certain malignant phenotypes of melanoma via different mechanisms (Wang et al., 2002).

Dietary supplementation with genistein and daidzein reduced tumor size and number as well as lung metastasis in mice injected with melanoma cells (Li et al., 1999). One study showed that growth of solid tumors was inhibited by 50% when mice were fed genistein for 1 week before and after inoculation with melanoma cells. Interestingly, plasma genistein concentrations at the time of tumor removal were similar to levels reported in humans consuming diets high in soybeans or soybean products (Record et al., 1997). Administration of genistein intraperitoneally reduced tumor-induced angiogenesis in syngeneic mice implanted with melanoma cells (Farina et al., 2006). Moreover, genistein inhibited nodule formation in the lungs of tumor-bearing mice and reduced the lung collagen hydroxyproline

content and serum sialic acid level, a marker of metastasis. In contrast, the same study found no significant effect on the reduction of lung metastasis with daidzein (Menon et al., 1998).

Impaired extracellular signaling by genistein might prevent cancer cells from invading or establishing metastasis through suppression of adhesion-induced protein tyrosine phosphorylation (Yan and Han, 1997). Genistein at physiologic concentrations enhanced cisplatin-induced inhibition of cell growth and apoptosis in human melanoma cell lines (Tamura et al., 2003). Quantification of the blood volume present in tumor tissue, enabling estimation of the degree of vascularization, was employed to compare the antitumor and the antiangiogenic effects of genistein, alone or combined with cyclophosphamide therapy. Tumor cells entrapped in alginate beads were injected subcutaneously into mice and the quantification of alginate implant vascularization was performed with labeled mouse albumin injected intravenously. The results indicated a higher antiangiogenic rather than cytostatic effect of genistein in the mouse tumor model (Wietrzyk et al., 2001). Another study evaluated the antitumor effect of genistein, alone or in combination with cyclophosphamide, in mice bearing transplantable subcutaneously growing tumors, by estimating the number of lung colonies and primary tumor recurrence. A reduced number of lung colonies in mice bearing lung tumor implants were observed in the genistein-treated group and this reduction in tumor number was more pronounced with the combination. However, even though no lung metastases in the control mice bearing the melanoma tumor were observed, primary tumor recurrence was highest in the untreated group and lowest in the group treated with genistein alone (Wietrzyk et al., 2000).

Long-term psoralen plus ultraviolet A radiation (PUVA) therapy has been associated with an increased risk of squamous cell carcinoma and malignant melanoma (Shyong et al., 2002). Application of genistein significantly decreased PUVA-induced skin thickening, erythema, ulceration and epidermal inflammation in mice and was associated with inhibition of poly(ADP)ribose polymerase (PARP) cleavage, caspase-3 activation and a decrease in proliferating cell nuclear antigen (PCNA)-positive cells in the suprabasal layers of the epidermis (Shyong et al., 2002). It has been suggested that genistein modulates immune responses and increases host resistance to melanoma tumors in mice not due to a direct effect of serum levels of genistein and/or its metabolites on cellular proliferation but through an increase in cytotoxic T-cell activity. Genistein exerts differential effects on IL activity and IL-2-stimulated natural killer cell activity is enhanced by genistein (Guo et al., 2001). Moreover, genistein exhibited inhibitory effects against IL-5 and IL-3 bioactivities, but did not inhibit GM-CSF and IL-6 bioactivities (Yun et al., 2000).

A phase II clinical trial is underway that will determine the effect of genistein together with IL-2 in patients with metastatic melanoma or renal clear cell carcinoma. The rationale for the trial is based on the assumption that genistein may stop the growth of tumor cells through its antiangiogenic effect, whereas IL-2 may stimulate the immune system to kill tumor cells. The primary outcome of the study would determine the differences in peak and duration of the expansion of circulating $CD4^+$, $CD8^+$, and $CD4^+$, $CD25^+$, and $CD56^+$ cells, whereas the secondary outcome would measure the differences in peripheral blood mononuclear cell gene expression and the overall safety and toxicity of the regimen (http://www.clinicaltrials.gov).

(–)-Epigallocatechin gallate (EGCG)

Green tea derived from dried unfermented leaves of *Camellia sinensis* (family Theaceae) has been associated with numerous biological activities including antimutagenic, antibacterial, hypocholesterolemic, antioxidant, antitumor and cancer-preventive activities. In green tea extracts, the major dry mass constituent is the family of catechins, which includes (+)-gallocatechin, (–)-epicatechin, (–)-epigallocatechin (EGC), (–)-epicatechin

gallate (ECG) and (–)-epigallocatechin gallate (EGCG). Several studies have been performed to determine the effect of these compounds on the growth and viability of melanoma cells (Valcic et al., 1996). Nihal et al. (2005) showed that EGCG treatment of melanoma cell lines resulted in decreased cell proliferation, as assessed by Ki-67 and PCNA protein levels. EGCG-induced cell cycle arrest and apoptosis of melanoma cells was mediated via modulations in the cki-cyclin-cdk network and Bcl2 protein family. Furthermore, at similar EGCG concentration, normal melanocytes were not affected. Other studies have compared the effects of EGC, ECG and EGCG on the viability, density and doubling time of cell lines derived from melanoma metastasized to lymph nodes or distant organs. The cytotoxic effect and growth inhibition of all melanoma cell lines tested was most pronounced with EGCG, indicating that the anticancer action of the various catechins may vary with the type of malignancy (Ravindranath et al., 2009).

Liu et al. (2001) examined the antimetastatic effects of EGCG on melanoma cells both in vitro and in the mouse model. The efficacy of EGCG against colony formation, cell migration and invasion was associated with decreased phosphorylation of focal adhesion kinase (FAK) and downregulation of matrix metalloproteinases (MMPs). A combination of EGCG and dacarbazine was more effective than EGCG alone in reducing the number of pulmonary metastases and primary tumor growths, and increased the survival rate of melanoma-bearing mice (Liu et al., 2001). The effect of polyphenols on the growth and metastatic potential of melanoma cells was evaluated in in vivo studies. Intraperitoneal administration of quercetin, apigenin, EGCG, resveratrol, and the anti-estrogen tamoxifen to syngeneic mice resulted in significant, dose-dependent delay of tumor growth, without toxicity. Again, EGCG was found to be the most potent, with apigenin, quercetin and tamoxifen showing similar results followed by resveratrol (Caltagirone et al., 2000). MMPs, angiogenic growth factors and their receptors have been targeted in a number of clinical trials due to their crucial role in metastasis. EGCG, a potent inhibitor of MMP-2 and MMP-9, was shown to selectively suppress the migration of tumor-associated endothelial cells as well as endothelial progenitor cells with no effect on normal endothelial cells, indicating its antiangiogenic potential (Ohga et al., 2009). Stromal cells are involved in key metastatic processes of melanoma and other solid tumors. EGCG can modulate the behavior of stromal fibroblasts, affecting their adhesion and migration through multiple mechanisms. It was shown that fibroblast adhesion to fibronectin and fibrinogen is inhibited by EGCG through binding to these compounds and is mediated through its effect on integrin $\alpha 2\beta 1$. In addition, EGCG decreased the intracellular H_2O_2 levels, thereby altering the tonic balance required for cell adhesion to collagen (Ohga et al., 2009). EGCG significantly upregulated the expression of E-cadherin associated with decreased invasion and metastasis (Wu et al., 2008) and enhanced tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis in melanoma cells (Shen et al., 2009; Tanaka et al., 2010). The inhibitory effect of EGCG on adhesion of melanoma cells to laminin is included in the mechanism(s) of previously reported metastasis inhibition (Suzuki and Isemura, 2001). It was further noted that the 67-kDa laminin receptor (67LR) conferred EGCG responsiveness to tumor cells. It was through the eukaryotic translation elongation factor 1A and 67LR that EGCG induced the dephosphorylation of myosin phosphatase, targeting subunit 1 at the threonine residue, and activated myosin phosphatase, resulting in growth inhibition of tumor cells (Umeda et al., 2008). As the concentration of EGCG required to elicit anticancer effects is much higher than the peak plasma concentration obtained after drinking an equivalent of two to three cups of green tea, combination regimens have been tried in several in vitro and in vivo studies. One such study investigated the effect of All-trans-retinoic acid (ATRA) in combination with EGCG on subcutaneous tumor growth in mice inoculated with melanoma cells. The combined EGCG and ATRA treatment significantly suppressed melanoma tumor growth in mice and increased the expression of 67LR in the tumor tissue. Attenuated 67LR expression in melanoma cells upon RNAi-mediated silencing

of the retinoic acid receptor alpha further demonstrated that ATRA enhanced the antitumor effect of EGCG through upregulation of 67LR (Lee et al., 2010b). To enhance bioavailability, a library of methylated EGCG compounds was prepared by coupling a solid-supported aldehyde with a ketone and an acid. Although in general these compounds did not inhibit melanoma growth significantly, the growth-inhibitory effect of one of these compounds, 7-OMe, has been found to be as potent as EGCG in several melanoma cell lines (Tanaka et al., 2010).

Epigenetic regulation of gene transcription by histone deacetylase (HDAC) inhibitors is gaining momentum as a novel cancer therapy. A combination study used SAHA-suberoylanilidine hydroxamic acid Zolinza (vorinostat), an FDA-approved HDAC inhibitor, in combination with EGCG. Relative to monotherapy, the combination treatment resulted in significantly greater inhibition of cell proliferation and increased apoptosis. This was associated with activation of p21(CIP1), p27(KIP1) and caspases –3, –7 and –9, and pro-apoptotic protein Bax as well as downregulation of cdks –2 and –4, cyclin A, and antiapoptotic Bcl2 (Nihal et al., 2010). The effect of the immunomodulatory cytokine IFN-α2b, commonly used in melanoma treatment with marginal efficacy and high toxicity, has also been studied in conjunction with EGCG. The combination was more effective than either agent alone with marked decrease in cell proliferation and colony formation ability, and induction of apoptosis. This was accompanied by an increase in Fas protein levels, Fas-ligand-mediated apoptosis and a decrease in nuclear factor kappa B (NFκB)/p65 activity. Athymic nude mice implanted with melanoma tumors showed a decrease in tumor growth and protein levels of proliferation marker PCNA in the combination group, signifying that EGCG could impart therapeutic advantage if used in conjunction with IFN (Nihal et al., 2009).

A multimodality treatment regimen using DNA vaccination in combination with EGCG was found to be effective in inhibiting melanoma growth. The combination treatment led to an enhanced tumor-specific T-cell immune response, resulting in a higher cure rate than either therapy alone. Importantly, combined DNA vaccination and oral EGCG treatment provided long-term antitumor protection in cured mice, suggesting that combining immunotherapy with a tumor-killing cancer drug may be an effective anticancer strategy (Kang et al., 2007). The synergistic activity of EGCG with hinokitiol (beta-thujaplicin) on melanogenesis was examined in another study. The results showed that EGCG in synergy with hinokitiol significantly inhibited melanin synthesis and reduced protein levels of microphthalmia-associated transcription factor (MITF) and tyrosinase, the rate-limiting melanogenic enzyme (Kim et al., 2004).

Evidence in the literature suggests a role of UV exposure in melanoma pathogenesis and recent studies have delineated the mechanisms through which EGCG may prevent UV-induced immunosuppression and subsequent photocarcinogenesis. A study performed in IL-12 knockout mice showed that topical treatment with EGCG prevented UV-induced suppression of contact hypersensitivity in wild-type mice but had no effect in the knockout mice. Injection of anti-IL-12 monoclonal antibody to wild-type mice blocked the preventive effect of EGCG, suggesting that prevention of UV-induced immunosuppression by EGCG is mediated through IL-12-dependent DNA repair. EGCG-mediated repair of UV-induced DNA damage was more efficient in the skin of wild-type mice as shown by a reduced number of cyclobutane pyrimidine dimer (CPD)-positive cells and decreased migration of CPD-positive antigen-presenting cells from the skin to draining lymph nodes (Meeran et al., 2006). The study further showed that EGCG failed to repair UV-induced CPDs in DNA repair-deficient cells from XPA patients, indicating the involvement of nucleotide excision repair mechanism in EGCG-mediated DNA repair (Meeran et al., 2006). However, even

with considerable evidence from cell culture and animal studies, at present there are no clinical trials underway to assess the efficacy of EGCG against melanoma.

Resveratrol

Resveratrol (trans-3,5,4′-trihydroxystilbene) was first isolated in 1940 as a constituent of the roots of white hellebore (*Veratrum grandiflorum* O. Loes), and has since been found in various plants, including grapes, berries and peanuts. Besides cardioprotective effects, resveratrol exhibits anticancer properties, as indicated by its ability to suppress proliferation of a wide variety of tumor cells. In this context, several studies have shown that resveratrol induced cell-cycle disruption and apoptosis in chemoresistant melanoma cells (Gatouillat et al., 2010). In addition, resveratrol-mediated apoptosis was significantly marked in melanoma cells when associated with induction of ERK1/2 mitogen-activated kinase (MAPK) phosphorylation (Niles et al., 2003). Inhibition of melanoma cell proliferation by resveratrol was associated with upregulation of quinone reductase 2 and the p53 tumor suppressor gene (Hsieh et al., 2005). Nonetheless, Yang and Meyskens (2005) contend that although resveratrol inhibits anchorage-independent growth through alterations in activated activator protein-1 (AP-1) transcription signaling and reduces intracellular reactive oxygen species levels, it does not, even at high doses, cause apoptosis or cell cycle arrest in melanoma cells.

The number and position of hydroxy substituents seemed to play an important role in the inhibitory effects of stilbenes. Resveratrol and the structurally related molecule 4-hydroxystilbene induced growth inhibition, apoptosis, S-phase arrest, and upregulation of cyclins A, E and B1 in melanoma cells. However, it was observed that the presence of two extra hydroxyl groups in reseveratrol diminished its efficiency to inhibit cell growth and arrest cell cycle in the S-phase with respect to 4-hydroxystilbene (Larrosa et al., 2003). The depigmenting effect of oxyresveratrol is mediated through reversible inhibition of tyrosinase activity rather than suppression of the expression and synthesis of the enzyme (Kim et al., 2002). The methylated analogs of resveratrol possess significantly more antiproliferative activity than the parent compound (Cardile et al., 2007). The resveratrol analog piceatannol (3,5,3′,4′-tetrahydroxy-trans-stilbene), present in grapes and wine, is a protein kinase inhibitor that modifies multiple cellular targets exerting immunosuppressive, antileukemic and antitumorigenic activities in several cell lines and animal models. It is a potent inducer of apoptosis in melanoma cells (Larrosa et al., 2004). Furthermore, various 4′-resveratrol esters produced using decarbonylative Heck coupling are being studied for their potential as anti-melanoma agents. The acetate and the palmitate analogs have demonstrated selective activity against melanoma cells over normal dermal fibroblasts (Wong et al., 2010). Striking in vivo effects were observed with hexahydroxystilbene (M8), which inhibited tumor as well as metastasis growth of human melanoma in two different animal models, alone and in combination with dacarbazine (Szekeres et al., 2010).

Resveratrol potentiates the apoptotic effects of cytokines, chemotherapeutic agents and gamma-radiation. The PI3K/AKT, NFjB and COX-2 pathways, involved in the radioprotective response, are highly active in melanoma cells. Suppression of COX-2 and PI3K/AKT substantially increased G2/M arrest and decreased clonogenic survival of gamma-irradiated melanomas, predominantly via a necrotic mechanism (Fulda and Debatin, 2004). Alternatively, resveratrol sensitized melanoma cells for TRAIL-induced apoptosis through p53-independent induction of p21-mediated cell cycle arrest associated with survivin depletion (Fulda and Debatin, 2004). The upregulation of transcription factors STAT3 and NFjB, which control the expression of antiapoptotic genes including cFLIP and Bcl-xL, is thought to contribute to TRAIL-resistance in melanoma cells. It was shown that resveratrol decreased STAT3 and NFκB activation, while activating the JNK/cJun pathway that suppressed expression of cFLIP and Bcl-xL proteins and increased sensitivity to

exogenous TRAIL in DR5-positive melanomas. An initial increase in the surface expression of DR5 receptor by either gamma-irradiation or arsenite, and subsequent downregulation of antiapoptotic cFLIP and Bcl-xL by treatment with resveratrol has been proposed as an effective approach to reactivate apoptotic death pathways in TRAIL-resistant human melanomas (Ivanov et al., 2008). Resveratrol also upregulated TRAIL promoter activity and induced TRAIL surface expression in some melanoma cell lines, resulting in rapid development of apoptosis. However, for melanoma lines exhibiting suppressed translocation of TRAIL to the cell surface, a necrotic mechanism of cell death was primarily involved in radiation response. Hence, surface expression of TRAIL induced by resveratrol appears to be a decisive event, one which determines an apoptotic versus a necrotic response of melanoma cells to sequential treatment (Johnson et al., 2008).

Increased nuclear localization of apurinic/apyrimidinic endonuclease-1/redox factor-1 (Ref-1), a multifunctional protein involved in DNA repair and redox regulation of many transcription factors, is present in nevi and malignant melanoma biopsies. In vitro studies have shown that Ref-1 overexpression protected melanoma cells from cisplatin- or H_2O_2-induced apoptosis and is associated with increased NFκB transcriptional activities. Using three-dimensional molecular structure modeling and virtual screening, it was shown that resveratrol docked into a druggable pocket of the Ref-1 protein, inhibited Ref-1-activated AP-1 DNA-binding activities as well as Ref-1 endonuclease activities and rendered melanoma cells more sensitive to dacarbazine treatment (Yang et al., 2005). It has further been proposed that nitric oxide (NO) initiates progression of human melanoma via a feedback loop mediated by Ref-1 that is inhibited by resveratrol. Significant decreases in AP-1/JunD, MMP-1, Bcl-2, and iNOS protein levels were observed after exposure to resveratrol (Yang et al., 2008).

Although melanoma cells show varying degrees of sensitivity to antitumor compounds, treatment with resveratrol markedly impaired proliferation of both temozolomide-sensitive and temozolomide-resistant melanoma cell lines (Fuggetta et al., 2004). Resveratrol, at subtoxic doses, potentiated doxorubicin cytotoxicity. When administered to mice, resveratrol inhibited tumor growth and prolonged survival, supporting a potential use of resveratrol alone or in combination with other chemotherapeutic agents in the management of chemoresistant tumors (Gatouillat et al., 2010).

The concept of ‘vasculogenic mimicry’ is used to describe the unique ability of highly invasive tumor cells to form capillary-like structures and matrix-rich patterned network in three-dimensional cultures that mimic embryonic vasculogenic network. The formation of capillary-like structures has been linked to reactive oxygen species levels. Antioxidants, including resveratrol, dramatically decreased the levels of vascular endothelial growth factor (VEGF) and its receptors in melanoma cells (Vartanian et al., 2007). The antiangiogenic potential of resveratrol stereoisomers has been linked, at least in part, to their differential capacity to affect $\alpha5\beta3$ integrin functions. The trans isomer was found to be significantly more potent than the cis isoform when compared for their effect on the angiogenic process and endothelial $\alpha5\beta3$ function. Cell culture studies showed that the trans form inhibited endothelial cell proliferation and prevented endothelial cell sprouting in fibrin gel, invasion in collagen gel, and morphogenesis in matrigel. In animal studies, trans-resveratrol inhibited murine melanoma tumor growth and neovascularization (Belleri et al., 2008).

Niles et al., (2006) have reported that mice fed on a diet containing resveratrol did not show any significant inhibition of melanoma xenograft tumor growth, possibly due to rapid metabolism of the compound. Pharmacokinetic studies in several animal models have shown that the highest concentration of resveratrol in the plasma, after intravenous or oral administration is reached within the first 5 min, with the *trans* form being predominant in

the plasma or tissues. Oral administration of this form decreased hepatic metastatic invasion of melanoma cells inoculated intrasplenically in mice. This was associated with inhibition of vascular adhesion molecule-1 expression in the hepatic sinusoidal endothelium. Interestingly, oral administration of the trans-resveratrol did not inhibit growth of melanoma cells inoculated into the footpad of mice (Asensi et al., 2002). Resveratrol treatment has been shown to inhibit tumor growth in animal models of uveal melanoma. In vitro data demonstrated that tumor inhibition was associated with a decrease in cell viability, resulting at least in part from an increase in apoptosis through the mitochondrial pathway and the eventual activation of caspase-3 (Van Ginkel et al., 2008).

Thus, up to now resveratrol studies have yielded mixed results, both negative and positive, with many researchers finding no effect and others observing a strong response with the agent especially in cell culture studies. It has been suggested that the difficulty in translating in vitro results to in vivo models can be largely attributed to the inability of achieving and maintaining elevated levels of resveratrol in the blood (Osmond et al., 2010). It was further recommended that methods of drug delivery in animal models be focused on maintaining elevated serum concentrations for an adequate period of time by continuous infusions, or using resveratrol analogs with longer half-lives (Osmond et al., 2010). A phase I clinical trial studying the effects of resveratrol in healthy adult participants has recently been completed. The primary objective was to measure CYP enzyme activity. Secondary objectives included the study of phase II enzymes as assessed by GST activity and GST-pi levels in blood lymphocytes and serum bilirubin level. The results will help establish the suitability of the compound for further studies (http://www.Clinicaltrials.gov).

Curcumin

Curcumin (diferuloyl methane), the major pigment from the rhizome of *Curcuma longa* L., has been widely studied for its tumor-inhibiting properties. More recently, attention was directed on dietary phytochemicals such as curcumin in an attempt to repair photodamaged skin as a means of preventing degeneration into solar-induced skin cancers. It is thought that the protective effect of curcumin against oxidative stress and inflammation and its ability to block multiple targets may prove its utility in photoaging skin and photocarcinogenesis (Heng, 2010). Studies show that curcumin induced melanoma cells to undergo apoptosis and cell cycle arrest. This was associated with downregulation of iNOS and DNA-dependent protein kinase catalytic subunit expression, and upregulation of p53, p21(CIP1), p27(KIP1) and Chk-2 (Zheng et al., 2004). In addition, curcumin induced apoptosis in melanoma cells through a Fas receptor/caspase-8 pathway independent of p53. Curcumin suppressed the apoptotic inhibitor, XIAP and blocked the NFκB cell survival pathway, resulting in decreased expression of NFκB-target genes COX-2 and cyclin D1 (Marin et al., 2007; Bush et al., 2001). It was further observed that curcumin induced ER stress in melanoma cells through inhibition of proteasomal activity and accumulation of cytosolic calcium by inhibiting the Ca^{2+}-adenosine triphosphatase (ATP) pump, resulting in activation of caspases, p23 cleavage and downregulation of the antiapoptotic Mcl-1 protein (Bakhshi et al., 2008). Gene silencing of ATP-binding cassette transporter ABCA1 by siRNA sensitizes melanoma cells to the apoptotic effect of curcumin through reduced basal levels of active NFκB (Bachmeier et al., 2009). In addition, C6 ceramide sensitized melanoma cells to curcumin-induced cell death and apoptosis at least in part through augmentation of the intrinsic apoptotic pathway (Yu et al., 2010). A more recent study showed that curcumin in combination with tamoxifen induced apoptosis and autophagy in melanoma cells. Importantly, non-cancerous cells were unaffected by the combination treatment (Chatterjee and Pandey, 2011). Curcumin-induced anti-proliferative and proapoptotic effects are independent of the B-Raf/ERK MAPK and the AKT pathway (Siwak et al., 2005). However, despite its proapoptotic effects, curcumin reportedly inhibited the ability of IFN-α, IFN-γ,

and IL-2 to phosphorylate STAT1 and STAT5 proteins (Bill et al., 2009). This suggested that curcumin may adversely affect the responsiveness of immune effector cells to clinically relevant cytokines with antitumor properties (Siwak et al., 2005).

Curcumin was shown to suppress melanogenesis in stimulated melanoma cells through downregulation of melanogenesis-related proteins such as MITF, tyrosinase and tyrosinase-related proteins 1 and 2 (Lee et al., 2010a). The antimetastatic activity of curcumin is linked to the modulation of integrin receptors, collagenase activity and E-cadherin. In cell adhesion assays, curcumin-treated cells showed diminished binding to extracellular matrix proteins such as fibronectin, vitronectin and collagen IV, as well as decreased expression of $\alpha 5\beta 1$ and $\alpha 5\beta 3$ integrin receptors. In addition, curcumin inhibited MMP-2, FAK and collagenase activity and enhanced the expression of antimetastatic proteins including tissue inhibitor metalloproteinase-2, nonmetastatic gene 23, and E-cadherin (Banerji et al., 2004; Ray et al., 2003).

Recently, various curcumin-related compounds have been synthesized and tested in order to select new antitumor agents displaying stronger and more selective growth inhibition activity. In vitro studies show that the compound α,β-unsaturated ketone D6 was more effective in inhibiting melanoma growth when compared to curcumin (Pisano et al., 2010). The synthetic curcuminoid derivatives were further analyzed in in vivo studies for their role in inhibition of tumor-specific angiogenesis. Intraperitoneal administration of tetrahydrocurcumin, salicyl curcumin and curcumin III reduced the number of tumor-directed capillaries induced by injecting mice with melanoma cells. Treatment with these curcuminoids reduced serum NO as well as tumor-necrosis factor (TNF)-α levels in these animals, possibly through decreased production by the activated macrophages (Leyon and Kuttan, 2003). The small molecule curcumin analog FLLL32 was developed to improve STAT3-specific inhibitors for melanoma therapy. FLLL32 induced caspase-dependent apoptosis in melanoma cells via reduced STAT3 phosphorylation at the tyrosine residue but retained the cellular response to cytokines with antitumor activity. Notably, FLLL32 did not reduce the function or viability of immune cells from normal donors. In peripheral blood mononuclear cells, FLLL32 inhibited IL-6-induced STAT3 phosphorylation but did not reduce signaling in response to immunostimulatory cytokines such as IFN-c and IL-2 (Bill et al., 2009). Although increasingly being recognized as a potential chemotherapeutic agent, the clinical application of curcumin is hindered by its poor water solubility and fast degradation. A study investigated amphiphilic block copolymer micelles of poly(ethylene oxide)-b-poly(epsilon-caprolactone) as vehicles for solubilization, stabilization and controlled delivery of curcumin. These micelles were able to solubilize curcumin effectively, protect the encapsulated curcumin from hydrolytical degradation in physiological matrix, and control the release of curcumin over several days (Ma et al., 2008).

Curcumin was shown to be cytotoxic to melanoma cells resistant to doxorubicin. Animals receiving a combination therapy of curcumin and immune preparation of soluble protein exhibited an enhancement of their humoral immune response with subsequent increase in the median survival time (Odot et al., 2004). The effect of oral administration of polyphenolic compounds on inhibition of lung metastasis was compared in mice inoculated with melanoma cells. The maximal effect observed was with curcumin and catechin. Consequent to inhibition of lung tumor nodules, there was an increase in the average life span, with lung collagen hydroxyproline and serum sialic acid levels significantly lower in treated animals (Menon et al., 1995). In vitro studies showed that curcumin and catechin inhibited the invasion of melanoma cells by inhibition of MMPs, thereby inhibiting lung metastasis (Menon et al., 1999). Studies further showed that curcumin suppressed osteopontin-induced cell migration, tumor growth and NFjB-mediated MMP-2 activation

(Philip and Kundu, 2003). Phosphatase of regenerating liver-3 (PRL-3) has been suggested as a potential target for anticancer drugs based on its involvement in tumor metastasis. In a spontaneous metastatic tumor model, curcumin selectively downregulated the expression of PRL-3 but not PRL-1 or -2, in a p53-independent manner. Curcumin inhibited phosphorylation of Src kinase and STAT3 partly through PRL-3 downregulation and prevented melanoma cells from invading the draining lymph nodes (Wang et al., 2009). At present, several clinical trials are investigating the effect of curcumin in various cancerous and non cancerous conditions; however, no studies are underway to examine the effect of this compound against melanoma in human populations.

Fisetin

Fisetin (3,7,3′,4′-tetrahydroxyflavone) belongs to the flavonol subgroup of flavonoids together with quercetin, myricetin and kaempferol and is found in several fruits and vegetables including strawberries, apples, persimmons and onions (Kimira et al., 1998). Fisetin was originally identified in a screen for compounds that could prevent oxidative stress-induced nerve cell death (Ishige et al., 2001). Further studies showed that fisetin also possessed neurotrophic activity, promoting nerve cell differentiation via activation of the Ras-ERK cascade (Sagara et al., 2004). Oral administration of fisetin was shown to enhance learning and memory in mice (Maher et al., 2006). Fisetin not only has direct antioxidant activity but can also increase the intracellular levels of glutathione, the major intracellular antioxidant. In addition, it has anti-inflammatory activity and has been shown to inhibit the activity of 5-lipoxygenase in microglia cells, thereby reducing the production of lipid peroxides and their proinflammatory byproducts (Syed et al., 2008). Emerging data suggests that in addition to its neuroprotective effects, fisetin possesses anticancer properties. Fisetin induced apoptosis in various cancer cell lines through activation of the caspases and inhibition of cdks (Syed et al., 2008). Furthermore, inhibition of uPA by fisetin in the advancing capillary vessels surrounding the tumor may be responsible for reducing angiogenesis and consequently tumor growth (Jankun et al., 2006). We recently showed that treatment of human melanoma cells with fisetin resulted in decreased cell viability with G1-phase arrest and disruption of Wnt/β-catenin signaling. This was accompanied with a decrease in the expression of Wnt protein and its co-receptors and a parallel increase in the protein expression of endogenous Wnt inhibitors Dickkopf and WIF-1 (Syed et al., 2011). Increased cytosolic levels of Axin and β-TrCP and decreased phosphorylation of GSK3-β correlated to decreased β-catenin stabilization in fisetin-treated melanoma cells. Fisetin-mediated interference with the functional cooperation between β-catenin and the transcription factor LEF/TCF-2 resulted in down-regulation of positively regulated TCF targets such as c-myc, Brn-2 and MITF. Retention of MITF expression in human primary melanomas, including non-pigmented ones, has led to its use as a diagnostic tool in melanoma. It has been suggested that targeting MITF in combination with B-RAF or cdk inhibitors may offer a rational therapeutic avenue into melanoma, recognized as a highly chemoresistant neoplasm (Garraway et al., 2005). Transfection studies demonstrated that in addition to the disruption of the canonical Wnt pathway, fisetin was able to override the proliferative effect of MITF and induce growth repression in human melanoma cells. Intraperitoneal administration of fisetin to mice resulted in inhibition of human melanoma tumor development associated with a significant decrease in endogenous MITF protein levels (Syed et al., 2011). These results suggest that fisetin can be developed as an effective agent against melanoma due to its potential inhibitory effect on β-catenin/MITF signaling.

Silymarin

Silymarin, a flavanolignan, extracted from the fruits and seeds of milk thistle (*Silybum marianum* L. Gaertn.), has been shown to possess a protective effect against photocarcinogenesis. Mechanistic studies conducted in mouse models indicated that

silymarin possesses antioxidant, anti-inflammatory and immunomodulatory properties responsible for its efficacy against photocarcinogenesis (Vaid and Katiyar, 2010). Studies performed in UV-irradiated human melanoma cells showed that silymarin protected against UV-induced apoptosis partially through activation of the human deacetylase SIRT1, which is involved in cell survival. Furthermore, modulation of the cell cycle with increase in the G2/M phase possibly provides time for efficient DNA repair in silymarin-treated cells (Li et al., 2007b). Silymarin can reduce UV-induced apoptosis by activating AKT and MAPK pathways (Li et al., 2006). Nonetheless, it is unclear whether the protection afforded by silymarin in melanoma cells is of any benefit in the prevention of melanoma. One study showed that silymarin enhanced the cytotoxic effect of anti-Fas agonistic antibody on human melanoma cells (Li et al., 2007a). However, a more recent study showed that silibinin, the major active constituent of silymarin, actually prevented mitomycin C-induced apoptosis in human melanoma cells through suppression of the mitochondria-mediated intrinsic apoptosis pathway, but not the extrinsic pathway (Jiang et al., 2009). Further studies are needed to understand the role of silymarin in the prevention of melanoma carcinogenesis.

Lupeol

Lupeol is a triterpene widely distributed in various plant families. The mango pulp, carrot root, cucumber, soybean and melon seeds are rich sources of lupeol. Earlier studies showed that lupeol present in alcoholic extracts of *Taraxacum* plants inhibited cell growth and induced melanogenesis in melanoma cells (Hata et al., 2000). Indeed, several lupane triterpenes promote melano-genesis, a hallmark of melanoma cell differentiation. Structure–activity relationship studies have further demonstrated that the keto function at C-3 of the lupane skeleton plays an important role in the melanogenic activity of lupane triterpenes. Conversely, the carbonyl group at C-17 is essential for their apoptosis-inducing activity against human cancer cells via inhibition of topoisomerase I (Hata et al., 2006). cAMP-elevating agents including alpha-melanocyte stimulating hormone, forskolin and dibutyryl cAMP, enhance lupeol-induced differentiation. It was shown that lupeol-induced melanoma cell differentiation occurred through activation of p38 MAPK, downstream of protein kinase A and that the structural differences at C-3 of lupane triterpenes played an important role in the activation of p38 MAPK (Hata et al., 2003). Melanoma cell differentiation induced by lupeol separates into two stages: morphological and functional. Lupeol attenuates actin stress fiber assembly in melanoma cells, resulting in dendritic formation in the cells, followed by increased expression of tyrosinase, MITF, Rab27a and myosin-Va proteins required for melanosome transport (Ogiwara and Hata, 2009). It has further been suggested that lupeol suppresses the migration of malignant melanoma cells through disassembly of the actin cytoskeleton (Hata et al., 2005).

Studies by Saleem et al. showed that lupeol inhibited the growth of highly aggressive human metastatic melanoma cells in vitro and in vivo through induction of apoptosis and exerted only a marginal effect on normal human melanocytes. Lupeol-induced apoptosis was accompanied by downregulation of Bcl2, upregulation of Bax, activation of caspase-3 and induction of PARP cleavage. The cell cycle arrest was associated with decreased expression of cyclin D1, cyclin D2 and cdk-2 and increased expression of p21(CIP1) proteins. Animal studies showed that lupeol significantly reduced melanoma tumor growth and modulated the expression of proliferation markers PCNA and Ki-67, apoptotic markers, and cell cycle regulatory molecules in tumor xenografts (Saleem et al., 2008). It was observed that lupeol specifically targeted melanoma cells that harbored the constitutive Wnt/β-catenin signaling pathway and the growth inhibition correlated with decrease in the expression of Wnt target genes c-myc, cyclin D1 and invasion marker osteopontin (Tarapore et al., 2010). Furthermore, lupeol restricted the translocation of β-catenin from the cytoplasm to the

nucleus and decreased the growth of melanoma cells with constitutive Wnt/β-catenin signaling implanted in athymic nude mice. However, some issues have been raised on the basis of a recent study which suggests that the presence of active Wnt/β-catenin signaling in melanoma is correlated with an improved outcome. The role of this pathway in melanoma and other cancers is more complex than previously recognized and well-designed studies are needed to ascertain the efficacy of lupeol in melanoma.

Miscellaneous agents

The cancer-protective property of some fruits and vegetables is related partly to their isoprenoid constituents. A screening study showed that isoprenoids extracted from different plant families were able to suppress the growth and proliferation of cancer cells including melanoma with varying degrees of potency (Tatman and Mo, 2002). Perillyl alcohol, a monoterpene isolated from the essential oils of lavendin, peppermint and several other plants, has been shown to inhibit the growth of melanoma cells through modulation of small G-protein activity (Tatman and Mo, 2002). However, only a limited number of studies have examined the efficacy of this compound in melanoma. A study in TPras transgenic mice demonstrated that topical application of perillyl alcohol delayed the appearance of melanoma tumors (Lluria-Prevatt et al., 2002). Nonetheless, a double-blind, randomized, phase II trial of topical perillyl alcohol in individuals with sun-damaged skin detected only a modest protective effect, probably due to inadequate delivery to the epidermis (Stratton et al., 2010).

Other agents that have been examined for their cytotoxic effect on melanoma cells include exo-biopolymer from rice bran (Kim et al., 2007), garlic extract (Hakimzadeh et al., 2010) and isothiocyanates derived from wasabi (Fuke et al., 2006). In addition, flavonoids such as naringenin, hesperitin (Lentini et al., 2007) and chrysin from acacia honey (Pichichero et al., 2011) have been evaluated. In most cases very few studies have been performed on these compounds, and although many of them appear to be potential candidates against melanoma, to elucidate completely the effective molecules and their mechanisms, more studies are needed in cell culture and animal models.

Conclusion

It is becoming clear that plants and plant-based ingredients can provide a broad spectrum of potential drug substances for cancer therapy with multifaceted effects and targets. Strategies devoted solely to protecting against UV radiation have, at best, had a modest impact on the development of melanoma. In this context, several of the candidate melanoma chemopreventive/therapeutic agents that belong to the phytochemical category are being investigated for their potential to stop the increasing trend of the disease. Although the preclinical data looks promising, epidemiological studies have yet to prove their efficacy. The success of chemopreventive strategies will be facilitated by the identification, development, and validation of early and reliable biomarkers of carcinogenesis, which will help establish the usefulness of these agents against melanoma carcinogenesis. Such agents alone or in combination with other agents could then be used for the inhibition of melanoma.

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Table 1

Summary of the effects of botanical agents in melanoma carcinogenesis

Botanicals	In vitro studies	In vivo studies	Clinical trials	References
Genistein	Protects against UV-induced DNA damage; induces G2 phase arrest, impairs Cdc25C-dependent cdk1 dephosphorylation, activates Chk-2; inhibits cdk2, upregulates p27; increases melanin; induces cell differentiation; suppresses adhesion-induced protein tyrosine phosphorylation; enhances cisplatin-induced cell growth inhibition and apoptosis	Reduces primary tumor size/number, decreases lung metastasis; reduces lung collagen hydroxyproline and serum sialic acid levels; decreases angiogenesis; potentiates cyclophosphamide-mediated reduction in primary tumor recurrence, lung metastasis; decreases PUVA-induced skin thickening, erythema, ulceration, inflammation, inhibits PARP cleavage, caspase-3 activation, decreases PCNA positive epidermal cells; enhances IL-2-stimulated NK activity, inhibits IL-5/IL-3 activity	Phase II clinical trial, monitored effect of genistein on tumor growth and IL-2-stimulated immune response	Kiguchi et al. (1990; Darbon et al. (2000); Casagrande and Darbon (2000); Constantinou and Huberman (1995); Yan et al. (1999); Yan and Han (1997); Tamura et al. (2003); Li et al. (1999); Farina et al. (2006); Menon et al. (1998); Wietrzyk et al. (2001); Wietrzyk et al. (2000); Shyong et al. (2002)
EGCG	Decreases cell proliferation, Ki-67 and PCNA; induces cell cycle arrest and apoptosis; decreases colony formation, cell migration and invasion, decreases FAK phosphorylation, inhibits MMPs; decreases migration of tumor-associated endothelial cells; inhibits fibroblast adhesion to fibronectin, and fibrinogen, decreases H_2O_2 levels; upregulates E-cadherin, potentiates vorinostat-mediated inhibition of cell proliferation and induction of apoptosis; with IFN-α2b decreases cell proliferation, colony formation, induces Fas-ligand mediated apoptosis, decrease NFκB/p65 activity; with hinokitiol inhibits melanin synthesis, decreases MITF and tyrosinase	Potentiates dacarbazine in reducing tumor growth, pulmonary metastases, increasing survival rate; enhances antitumor effect of ATRA through 67LR; with IFN-α2b significantly decreases tumor growth, decreases PCNA; with DNA vaccine enhances tumor-specific T-cell immune response; prevents UV-induced immunosuppression through IL-12-dependent DNA repair	–	Nihal et al. (2005); Liu et al. (2001); Ohga et al. (2009); Ohga et al. (2009); Wu et al. (2008); Umeda et al. (2008); Lee et al. (2010b); Nihal et al. (2010); Nihal et al. (2009); Kang et al. (2007); Kim et al. (2004); Meeran et al. (2006)
Resveratrol	Induces cell-cycle disruption and apoptosis; upregulates quinone reductase 2 and p53; potentiates apoptotic effects of cytokines, chemotherapeutic agents and γ-radiation, sensitizes toTRAIL-induced apoptosis through p53-independent induction of p21-mediated cell cycle arrest; increases sensitivity to exogenous TRAIL by decreasing STAT3 and NFκB and activating JNK/cJun pathway; inhibits Ref-1-activated AP-1 DNA-binding, endonuclease activities, sensitizes to dacarbazine, decreases AP-1/JunD, MMP-1, Bcl-2, and iNOS; antiangiogenic, decreases VEGF, prevents endothelial cell sprouting, invasion, and morphogenesis	Inhibits neovascularization, tumor growth; decreases hepatic metastasis with downregulation of VCAM-1; potentiates doxorubicin cytotoxicity, inhibits tumor growth, prolongs survival	Phase I clinical trial, monitored effect on CYPs, phase II enzymes and serum bilirubin levels	Gatouillat et al. (2010); Hsieh et al. (2005); Fulda and Debatin (2004); Ivanov et al. (2008); Yang et al. (2005); Yang et al. (2008); Belleri et al. (2008); Vartanian et al. (2007); Asensi et al. (2002)
Curcumin	Induces cell cycle arrest and apoptosis through p53 dependent/independent pathway, decreases iNOS and DNA-dependent protein kinase catalytic subunit expression, upregulates p53, p21, p27and Chk-2; suppresses XIAP and NFκB, decreases COX-2 and cyclin D1; induces ER stress, inhibits proteasomal activity and Ca-ATPase pump, activates caspase, p23 cleavage, downregulates Mcl-1; with ABCA1 silencing enhances apoptosis, decreases NFκB; with C6 ceramide increases apoptosis; inhibits IFN/IL-2 mediated phosphorylation of STAT1 and STAT5; suppresses melanogenesis, decreases MITF, tyrosinase, TRP1 and 2;	Inhibits metastasis, reduces lung tumor nodules, increases average life span, decreases lung collagen hydroxyproline and serum sialic acid levels; immune preparation enhances humoral response, increases survival; decreases PRL-3, inhibits Src kinase and STAT3 phosphorylation, prevents lymph node invasion	–	(Zheng et al. (2004); Marin et al. (2007; Bush et al. (2001); Bakhshi et al. (2008); Bachmeier et al. (2009); Yu et al.); Siwak et al. (2005); Lee et al. (2010a); Banerji et al. (2004); Ray et al. (2003); Odot et al. (2004); Menon et al. (1995); Philip and Kundu, 2003); Wang et al. (2009)

Botanicals	In vitro studies	In vivo studies	Clinical trials	References
	inhibits metastasis, decreases fibronectin, vitronectin, and collagen IV binding, decreases $\alpha5\beta1$ and $\alpha5\beta3$, inhibits MMP-2, FAK and collagenase activity, increases TIMP-2, nonmetastatic gene 23, and E-cadherin; suppresses osteopontin-induced cell migration, and tumor growth; potentiates doxorubicin cytotoxicity			
Fisetin	Decreases cell viability with G1-phase arrest, disrupts Wnt/β-catenin signaling, decreases Wnt 5A, LRP6 and Fzd, increases GSK3β activity, Dickkopf, WIF-1, Axin and β-TrCP, decreases cytosolic and nuclear β-catenin, decreases TCF-2, c-myc, Brn-2 and MITF	Inhibits tumor growth, decreases MITF, cdk2 and Bcl-2	–	Syed et al. (2011)
Silymarin	Protects against UV-induced apoptosis, activates SIRT1, increases G2/M phase; activates AKT and MAPK pathways; prevents mitomycin C-induced apoptosis through suppression of intrinsic apoptosis pathway; enhances cytotoxicity of anti-Fas antibody		–	Li et al. (2007b); Li et al. (2006); Jiang et al. (2009); Li et al. (2007a)
Lupeol	Induces melanogenesis; increases differentiation potentiated by cAMP-elevating agents, activation of p38MAPK; increases expression of tyrosinase, MITF, Rab27a, and myosin-Va; inhibits cell growth, induces cell cycle arrest, decreases cyclin D1, D2 and cdk2, increases p21, induces apoptosis, downregulates Bcl2, upregulates Bax, activates caspase-3 and induces PARP cleavage; restricts nuclear translocation of β-catenin	Inhibits tumor growth, modulates proliferation markers PCNA and Ki-67, apoptotic markers, and cell cycle regulatory molecules; specifically targets melanoma cells with constitutive Wnt/β-catenin signaling, decreases expression of Wnt target genes c-myc, cyclin D1 and osteopontin	–	Hata et al. (2003); Ogiwara and Hata (2009); Saleem et al. (2008); Tarapore et al. (2010)