Published in final edited form as:
Cancer Lett. 2013 July 28; 335(2): 251–258. doi:10.1016/j.canlet.2013.02.056.

Emerging phytochemicals for prevention of melanoma invasion

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Abstract

Cutaneous malignant melanoma is the leading cause of death from skin diseases due to its propensity to metastasize. Once diagnosed with metastatic melanoma, most patients will die of their disease within 2 years. As suppression of metastases requires long-term interventions, potential anti-metastatic agents must not only be efficacious but also have low toxicity. Many phytochemicals used in traditional medicine have low toxicity and recent studies suggest that some are promising candidates for the prevention or treatment of metastatic melanoma. Here, we review the recent literature regarding phytochemicals that have shown inhibitory effects on melanoma cell migration or invasion.

Keywords

Cyclooxygenase-2; Melanoma; Phytochemicals; Prostaglandins; β-Catenin

1. Introduction – malignant melanoma

Malignant melanoma is the leading cause of death from skin disease due to its propensity to metastasize [1], and is increasing rapidly in children [2]. Although, melanoma is less common than other types of skin cancers, it causes the majority (75%) of skin cancer-related deaths [1,3]. If a disease is recognized and treated early, melanoma is curable, but as the disease progresses; its propensity to metastasize makes it difficult to treat. Once diagnosed with metastatic melanoma, most patients will die of their disease within 2 years [4]. According to a World Health Organization report, 48,000 melanoma-related deaths occur worldwide per year [5]. Recent advances in anti-melanoma therapeutics have led to improved survival of patients with metastatic melanoma but the prognosis remains poor for most patients [6,7]. Exposure of the skin to solar ultraviolet (UV) radiation has been implicated as a risk factor for both melanoma and non-melanoma skin cancers [5,8]. The contribution of chronic sun exposure to the risk of melanoma is controversial, however, and

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there are conflicting reports regarding the association between the development of melanoma and long-term sun exposure [9]. Other factors, such as frequent use of tanning salons, have been implicated in the development of malignant melanoma [10]. Individuals with fair skin, whose skin burns more easily, are at risk of melanoma. Although there have been campaigns to educate the general population regarding potential risk factors and the warning signs of the disease, the prevalence of melanoma continues to increase. The development of approaches that can be used to identify individuals who are at high risk of the disease is considered a high priority and there is an emerging body of knowledge concerning genetic predisposition to the disease. Concurrently, progress has been made in the identification of biomarkers that might be used to assess risk of progression or responsiveness to therapeutics. Progress in these areas is providing a framework for the analysis of the mechanisms of action of candidate anti-metastatic agents.

As it has been recognized that melanoma is the leading cause of death from skin diseases due, in large part, to its propensity to metastasize, attempts have been made to discover new and more effective agents which can inhibit the migration or invasion potential of melanoma cells. In this context, the studies on phytochemicals which are less or no toxic and have significant inhibitory effect on melanoma cell invasion gaining attention. In this mini review article we are providing information on those selective phytochemicals which have shown promising chemopreventive effect on melanoma cell migration. Additionally, the molecular mechanisms underlying these effects have also been discussed.

2. Pathogenesis of melanoma

Here, we provide a brief overview of the pathobiology of cutaneous melanoma of relevance to the design of the studies being used to evaluate the efficacy and mechanisms of action of the phytochemicals. Melanomas derive from melanocytes, which are of neural crest cell origin. Melanocytes are found in the cochlea of the inner ear, hair follicles, and the epidermal layer of the skin [11]. In the epidermis, melanocytes are found in the basal layer at a ratio of one melanocyte to five keratinocytes [12]. Fibroblasts also are scattered throughout the basal layer. Normally, the melanocytes are retained in the epidermis through interactions with the basal layer and keratinocytes that are regulated by the fibroblasts. The melanocytes are anchored by their attachment to both the basement membrane and adjacent keratinocytes. E-cadherin, which is found on the surfaces of both the melanocytes and the keratinocytes, serves as the molecular connection between these two cell types. Melanocytes produce and transport pigment-containing melanosomes to the keratinocytes and have appendages (dendrites) that facilitate the transport of the melanosomes [12]. However, in order to enter the cell cycle and divide the melanocytes must retract their connections with the basement membrane and the keratinocytes. After division, the unattached melanocytes move along the basement membrane and eventually re-establish their E-cadherin-mediated connections with keratinocytes. Once these connections are made they reform dendrites and connect to keratinocytes in more superficial layers of the epidermis [12].

The process of melanocyte proliferation is complex. Under normal circumstances, neighboring keratinocytes and fibroblasts secrete growth factors, which act in a paracrine manner to lead the melanocytes through a very controlled process of local detachment, proliferation, migration and re-adhesion to neighboring regulatory cells [12]. These paracrine growth factors bind to surface receptors on melanocytes called receptor tyrosine kinases (RTKs), which once bound by the ligands activate the intracellular mitogen-activated protein kinase (MAPK) pathway [11]. Activation of the MAPK pathway leads to melanocyte proliferation, which includes activation of phosphatidylinositol 3-kinase (PI3K) and Akt cell survival signals [13]. To become a melanoma, a melanocyte has to become both independent of, and unresponsive to, the regulatory fibroblasts and keratinocytes [11]. If the

pathways that regulate these interactions are activated in an aberrant manner, then the melanocyte may escape the control of its neighboring regulatory cells and be on its way towards becoming a melanoma. Abnormal activation can reflect at least two different events. The melanocytes may begin producing their own growth factors in an autocrine manner or enzymes involved in the signaling pathways may become constitutively activated.

Significant progress has been made in identification of the specific genomic mutations that occur frequently in melanoma cells and the impact of these mutations on the function of the encoded proteins and cellular activities. Importantly, these studies have revealed that the mutations that control the progression to malignancy differ among the various subtypes of melanomas. For example, most of the melanomas that develop in sun-exposed areas have a mutation of the *BRAF* gene [14,15], whereas melanomas that develop on mucosal and acral surfaces, which are thought to be unrelated to sun exposure, have a mutation of the *KIT* gene [16]. Knowledge and categorization of these specific mutations has allowed for a greater understanding of melanoma pathogenesis as well as for the development of drugs that specifically target aberrant processes related to the specific mutations.

Mutations of the *BRAF* gene mutation are present in greater than 50% of cutaneous melanomas. More than 95% of these *BRAF* mutations result in the substitution of a glutamic acid in place of a valine amino acid at position 600 of the encoded protein [17]. This abnormal $BRAF^{V600E}$ protein results in continuous activation of MAPK replacing the normal regulation of MAPK activation and deactivation *via* phosphorylation and dephosphorylation. Thus, the $BRAF^{V600E}$ mutation is considered to be an activating mutation [17]. Although not sufficient to result in a carcinoma on its own, this mutation in combination with another, such as loss of PTEN or p16, results in melanoma [11]. Interestingly, the $BRAF^{V600E}$ mutation is also frequently found in normal nevi [18], which gives further credence to the concept that multiple mutations must occur if the melanocyte is to progress down the continuum from a normal cell to melanoma.

Genomic mutations that affect the *N-RAS* gene occur in 15–25% of melanomas [19]. Most often this mutation is a substitution of an arginine for a glutamine at position 61 in the encoded protein [11]. $N\text{-}RAS^{Q61R}$ is an abnormal RAS protein. The RAS protein is a guanosine-5-triphosphate (GTP)ase, which usually cleaves bound GTP. $N\text{-}RAS^{Q61R}$ is unable to cleave GTP and is therefore always bound to GTP, which makes it continuously active [13]. Although a completely different genetic mutation affecting a completely different protein, the $N\text{-}RAS^{Q61R}$ also results in constitutive activation of the MAPK pathway. It also stimulates Akt, which promotes cell proliferation and survival. As noted above mutation of the *KIT* gene, which encodes an RTK, is frequently noted in melanomas on mucosal and acral surfaces [16]. This mutation renders the RTK active independently of the usual stimulus of the binding of the appropriate receptor ligand. Again, this results in stimulation of the MAPK pathway. As previously mentioned, activating aberrations in the PI3K pathway results in increased Akt, which in turn promotes abnormal cell survival [20]. This effect can be achieved either by activating mutations of members of this pathway or by loss of the suppressor gene *PTEN* [21]. We have touched on the most common abnormalities genetic abnormalities in melanoma but certainly there are other irregularities that are associated with melanoma, including those affecting p53 [22] or other RTKs [23]. The complexity of the cellular functions in melanocytes and their regulation suggests that there are a multitude of aberrant molecular activities that could drive or contribute to the development and metastases of melanoma.

The improved understanding of the role of these specific mutations as well as their impact on the cellular functions raised interest in the development of therapeutic strategies that specifically inhibit the abnormally functioning cellular proteins [17]. These drugs are used to

treat known carcinogenic lesions and do not fall into the category of chemopreventive agents. They do not address the specific genetic mutations but rather their known irregular protein products and resulting aberrant cellular functions. The first agent used for this purpose was sorafenib, which inhibits RAF kinase. Unfortunately, phase II trials of this drug showed poor efficacy. Subsequently, two agents targeting BRAF kinase were developed. These are vemurafenib, also known as PLX4032, and GSK2118436 [24,25]. The results of the clinical trials of these two drugs have been exciting. Vemurafenib has been tested in phase I, II and III trials, and it appears that 80% of patients with the BRAF mutation experience tumor regression [25]. The main issue with these BRAF kinase inhibitors is that the melanomas eventually stop responding to the drug. In fact, the median progression-free interval for patients treated with either of these BRAF kinase inhibiting agents was only 6–7 months [25]. Investigations into the mechanisms by which melanomas become resistant to these therapies have revealed a myriad of additional mutations that allow for continued malignant progression despite the halt of the abnormal BRAF kinase function [17]. Thus, so far there has been no clear cut solution to the development of resistance. It is widely speculated that use of these drugs as a component of a combination of therapies may hinder the development of resistance, but this has not yet been tested [17]. Other clinically evaluated agents include the KIT inhibitors imatinib and sunitinib, which were developed to treat patients with gastrointestinal stromal tumors, and nilotinib and dasatanib, which are KIT inhibitors specifically developed to treat melanoma patients with KIT mutations [16]. Many more agents are undergoing preclinical evaluation. Ultimately, targeting of specific abnormal cellular proteins seems quite promising for the treatment of melanoma. It is likely that these targeted medications will play an important role in the future as part of combination or multiple modality therapies for melanoma.

3. Factors affecting melanoma metastasis, cell migration or invasion

3.1. Cyclooxygenase-2 (COX-2) and prostaglandins

UV radiation is a recognized risk factor for the development of skin cancers, including melanoma [8]. Exposure of the skin to UV radiation induces an increase in the expression levels of cyclooxy-genase-2 (COX-2), a rate-limiting enzyme that catalyzes the conversion of arachidonic acid to prostaglandin (PG) metabolites. Two COX isoforms with distinct physiologic functions have been identified. COX-1 is expressed constitutively in many tissues and has an important role in the maintenance of homeostasis. In contrast, COX-2 is an inducible enzyme and plays a crucial role in tumor progression, invasion and metastasis [26]. The enhanced expression of COX-2 in skin exposed to UV radiation has been identified as a risk factor for the development of skin cancer [27,28]. Prostaglandin metabolites play a central role in orchestrating the multiple events involved in tumor progression, invasion and metastasis. PGE_2 is the major and most effective of the metabolites and exerts its effects through its G protein-coupled receptors: EP1, EP2, EP3 and EP4. It has been implicated in angiogenesis, compromised host immunity and enhanced invasion and metastasis [27,28]. Because of its important role in tumor invasion and metastasis, COX-2 is considered as a promising target for cancer therapy [29]. Therefore, the search of novel and non-toxic inhibitors of COX-2 as well as the inhibitors of PGE_2 may provide a better option for the treatment of malignant melanoma.

3.2. Epithelial-to-mesenchymal transition (EMT)

The epithelial-to-mesenchymal transition (EMT) has been shown to play a major role in the invasion and metastasis of epithelial tumors. EMT can render tumor cells migratory and invasive, as well as enhancing intravasation and extravasation [30]. During EMT, cells can change from an epithelial to a mesenchymal state. Cells lose their characteristic epithelial traits and instead acquire properties of mesenchymal cells. This process is primarily

coordinated by the disappearance or loss of epithelial biomarkers, such as E-cadherin and certain cytokeratins, with the concomitant appearance or gain of mesenchymal biomarkers, such as vimentin, fibronectin and N-cadherin, that promote cell invasion and metastasis [30]. E-cadherin is required for the formation of stable adher-ens junctions and thus the maintenance of an epithelial phenotype. Loss of E-cadherin expression is one of the most common indicators of EMT onset [31,32], and reduced expression of E-cadherin has been reported in various cancers and is associated with tumor metastasis [33]. The expression of N-cadherin, a marker of mesen-chymal stage, is associated with an increased invasive potential of cancer cells [34,35]. The transcription factors, Snail and Slug, have been identified as repressors of E-cadherin in both *in vitro* and *in vivo* models [36,37]. Upregulation of Twist is associated with malignant transformation of melanoma and overexpression of Twist has been shown to result in an increase of N-cadherin, which leads to a decrease in the expression of E-cadherin [36].

3.3. Wnt/β-catenin signaling

Various studies have implicated Wnt regulation of β-catenin signaling in melanoma progression and metastasis [38]. A key regulatory component of this pathway is the level of β-catenin in the cytosol, which determines the activation of Wnt-responsive genes. In the absence of Wnt stimulation, β-catenin is continuously degraded by the proteasome [39,40]. This degradation of β-catenin depends upon its phosphorylation, which involves association of the β-catenin with a multiprotein complex composed of the tumor suppressor protein adenomatous polyposis coli (APC), Axin and glycogen synthase kinase-3 (GSK) [41]. Non-phosphorylated β-catenin accumulates in the cytoplasm, when activated it enters the nucleus and interacts with T-cell factor transcription factors to control various target genes that are involved in cellular proliferation and invasion. Accumulation of nuclear β-catenin has been correlated with late stages of tumor progression and metastasis [42]. In the canonical model of Wnt signaling, β-catenin is phosphorylated at certain key residues by GSK-3β and casein kinase 1α (CK1α) leading to its ubiquitination and subsequent degradation [43]. Like cancers of other organs, the regulation of β-catenin is lost in melanoma [44,45]. This then leads to nuclear accumulation of β-catenin and subsequent stimulation of downstream target genes, which includes the genes of cell proliferation (e.g., cyclins and c-myc) and cell invasion (e.g., matrix metalloproteinases) [46]. In contrast, some studies have shown that activation of β-catenin blocks invasion in melanoma. Arozarena et al. [47] have demonstrated that this opposing effect of β-catenin is mediated through microphthalmia-associated transcription factor (Mitf), a melanoma-specific protein and a down-stream target of β-catenin. Mitf has been shown to have a role in tumor cell proliferation and differentiation, and has been suggested that it can redirect β-catenin transcriptional activity away from canonical Wnt signaling-regulated genes toward Mitf-specific target genes regulated by β-catenin [48]. Therefore, investigation of the role β-catenin signaling is important for the development of strategies to prevent melanoma metastasis.

4. Phytochemicals for prevention of melanoma cell migration or invasion

The idea of interrupting the process of carcinogenesis by the use of either natural or synthetic external substances was introduced in the 1970s by Dr. Sporn, who coined the term, “chemoprevention” [49]. Since that time, the meaning of the term has been expanded to include interruption of tumor initiation, promotion or progression. Primary chemoprevention refers to the use of an agent that prevents carcinogenesis in a healthy patient who would have otherwise gone onto develop a cancer. Secondary chemoprevention refers to preventing the full transition to malignancy in a patient that already has developed a pre-malignant lesion. Tertiary chemoprevention refers to the use of an agent that prevents a second primary cancer or metastasis in a patient who has had a first malignancy that had been treated [50]. Chemoprevention strategies for melanoma have been under investigation

for some time, and the effects of some selected botanicals on melanoma growth *in vitro* and *in vivo* has been reviewed recently [51]. Here, we summarize the results of the most recent studies of the effects of phytochemicals on melanoma invasion with a focus on those phytochemicals that offer some hope for prevention of this early stage of metastasis. New promising phytochemicals are summarized with their structures, sources and active ingredients in Table 1.

4.1. Grape seed proanthocyanidins

Grape seeds, which are enriched in proanthocyanidins [52], are separated during the industrial production of grape juice and wine. The grape seed proanthocyanidins (GSPs) are composed of dimers, trimers and highly polymerized oligomers of monomeric catechins [52]. They have been shown to be act as anti-skin carcinogenesis agents and function as potent antioxidants and anti-inflammatory agents in various tumor models [53,54]. The investigations by Vaid et al. [55] revealed that the treatment of metastasis-specific human melanoma cell lines (A375 and Hs294t) with GSPs inhibits their migration and that this effect is associated with inhibition of COX-2 expression and PGE_2 production (Fig. 1). Similar effects were evident when the melanoma cells were treated with celecoxib, a potent inhibitor of COX-2. Treatment of melanoma cells with 12-O-tetradecanoylphorbol-13-acetate (TPA), a tumor promoter, promotes COX-2 expression and enhances cell migration. This TPA-induced cell migration was blocked by treatment of the cells with GSPs. These observations support the evidence that inhibition of melanoma cell migration by GSPs requires the inhibition of COX-2 expression. COX-2 is a downstream target of the nuclear factor kappa B (NF-κB) pathway. Vaid et al. [55] demonstrated that treatment of melanoma cells with GSPs results in inactivation of NF-κB, and down-regulates the levels of the IKKα subunit which is responsible for NF-κB activation. Caffeic acid phenethyl ester, an inhibitor of NF-κB, also inhibits the migration of melanoma cells. Further, inhibition of melanoma cell migration by GSPs is associated with the inhibition of extracellular signal regulated protein kinase (ERK1/2) phosphorylation. The inhibition of MEK with UO126, a MEK inhibitor, blocked the migration of the melanoma cells. These findings suggest a possible involvement of the mitogen-activated protein kinase (MAPK) pathway, which is an upstream regulator of NF-κB and COX-2, in inhibition of melanoma cell migration by GSPs.

The NF-κB is a transcription factor that regulates a broad spectrum of biologic processes, including, inflammation, cell proliferation and apoptosis. Importantly, NF-κB has been implicated in the development of inflammation-induced cancer and has been identified as an important regulator of EMT in several types of cancer [56]. It has been demonstrated that treatment of melanoma cells with GSPs resulted in the suppression of mesenchymal biomarkers, such as vimentin, fibronectin and N-cadherin, while restoring the levels of epithelial biomarkers such as, E-cadherin, desmoglein 2, keratin-8 and -18 in melanoma cells [55]. This suggests that GSPs have the ability to reverse the EMT process in melanoma cells. Thus, this may be one mechanism by which GSPs reduce the invasiveness of melanoma cells and inhibit melanoma cell migration.

4.2. Green tea catechins

Green tea catechins/polyphenols have been shown to have anti-carcinogenic activities in various tumor models, including skin cancers [57,58]. Their anti-invasive effect on melanoma cells appears to be similar to that of grape seed proanthocyanidins [55]. (–)-Epigallocatechin-3-gallate (EGCG) is considered to be the major and most effective component of tea catechins. It has significantly greater anti-invasive activity in melanoma cells than other monomer catechins, such as (–)-epicatehin, gallocatechin, (–)-epicatechin-gallate and (–)-epigallocatechin, as determined using Boyden chamber assay. In this assay,

the inhibitory effects of green tea catechins on the migration of melanoma cells were in the order of: EGCG > (–)-epigallocatechin > (–)-epicatechin-3-gallate > (–)-gallocatechin >(–)-epicatechin [59]. The inhibition of melanoma cell migration by EGCG was associated with inhibition of COX-2 expression and PGE_2 production (Fig. 1). This inhibitory effect of EGCG on cell migration was not due to its inhibitory effect on cell viability or induction of cell death. Moreover, EGCG did not affect the migration ability of normal human epidermal melanocytes. EGCG has been found to inhibit TPA-induced migration of melanoma cells. As TPA promotes COX-2 expression and subsequently enhances cell migration [60], these observations suggest that inhibition of melanoma cell migration by EGCG is mediated through the inhibition of COX-2 expression. PGE_2 exerts its biologic functions through four G protein-coupled receptors, EP1, EP2, EP3 and EP4 [61], that can stimulate cell survival signals as well as invasive potential of cancer cells [62]. PGE_2 has been shown to promote melanoma cell migration, and this effect of PGE_2 is associated with the activation of PGE_2 receptors [60]. Singh and Katiyar [59] found that the inhibitory effect of EGCG on melanoma cell migration was mediated through an inhibitory effect on the PGE_2 receptors. Thus, EGCG acts by decreasing the expression of both COX-2 and the PGE_2 receptors, thereby affecting the levels of both the ligand (PGE_2) and receptor (EP). The ability to target different aspects of aberrant activation of the same pathway is a desirable feature of agents in development for chemotherapeutics and chemoprevention of melanoma, which has such a complex pathogenesis. As NF-κB is an upstream regulator of COX-2, the effect of EGCG on the levels of NF-κB/p65 in melanoma cells was tested. EGCG was found to inhibit the activation of NF-κB/p65 in a dose-dependent manner. Caffeic acid phenethyl ester, an inhibitor of NF-κB, inhibits melanoma cell migration. These observations suggest that the inhibitory effect of EGCG on melanoma cell migration is mediated, at least in part, through the downregulation of COX-2 and NF-κB. As described above, NF-κB is an important regulator of EMT in several types of cancer [56]. Singh and Katiyar [59] found that treatment of melanoma cells with EGCG resulted in suppression of mesenchymal biomarkers while restoring the levels of epithelial biomarkers. These observations suggest that EGCG has the ability to reverse EMT and that this may be one of the possible mechanisms through which EGCG reduces the invasiveness of melanoma cells and inhibits migration of melanoma cells.

Liu et al. [63] also reported the anti-metastatic effect of green tea catechins against melanoma cells using *in vitro* and *in vivo* models. They reported that EGCG inhibited: (i) B16-F3m cell migration and invasion using an *in vitro* Transwell assay; (ii) the spread of B16-F3m cells on fibronectin, laminin, collagen, and Matrigel in a dose-dependent manner; and (iii) the tyrosine phosphorylation of focal adhesion kinase and the activity of MMP-9. In an athymic nude mouse model, EGCG reduced lung metastases in mice bearing B16-F3m melanomas. A combination of EGCG and dacarbazine was more effective than EGCG alone in reducing the pulmonary metastasis and primary tumor growths. Ohga et al. [64] have demonstrated that EGCG inhibits the migration of tumor-associated endothelial cells as well as endothelial progenitor cells indicating its anti-angiogenic action. EGCG inhibited invasion and metastasis of melanoma cells by increasing the expression of E-cadherin [65]. Taniguchi et al. [66] found that the oral administration of EGCG inhibit metastasis of B16 melanoma cell lines (B16-F10 and BL6) in both experimental and spontaneous systems.

4.3. Silymarin

Silymarin is a flavanoid isolated from the milk thistle plant (*Silybum marianum* L Gaertn.). It is composed primarily of silibinin (≈90%) with small amounts of other silibinin stereoisomers, such as isosilybin and dihydrosilybin [67]. Silibinin is the major active constituent of silymarin and the anti-carcinogenic properties of silymarin and silibinin are almost identical. Silymarin has been shown to inhibit UV radiation-induced skin squamous

cell carcinoma in animal models [68,69] but its chemopreventive effect on melanoma growth and invasion has not been explored. Very recently, Vaid et al. [70] have reported that silymarin inhibits the invasion or cell migration of melanoma cells, and that this is associated with the inactivation of β-catenin signaling pathway. It has been shown that phosphorylation of β-catenin at critical target residues such as at Ser^{45}, $Ser^{33/37}$ and Thr^{41} by GSK-3β and CK1α within the cytosolic destruction complex leads to degradation of β-catenin and thus reduces its nuclear accumulation [43]. In this study, the treatment of melanoma cells with silymarin enhances the expression of GSK-3β and CK1α, and β-catenin is phosphorylated at critical target residues. This then lead to degradation of β-catenin within the degradation complex resulting in its reduced nuclear accumulation.

Diverse molecular events are integrated in the progression and metastasis of cancer cells. Oncogenic activation of β-catenin occurs primarily as a consequence of its stabilization by escaping ubiqui-tin-mediated proteasomal degradation. A major regulator of β-catenin stability and activity is β-TrCP. In this study, Vaid et al. sought to determine whether the inactivation of β-catenin in melanoma cells by silymarin is affected by expression of its regulator, the β-TrCP [70]. It was observed that silymarin enhanced the binding of β-TrCP to the phosphorylated forms of β-catenin, which suggests β-TrCP-mediated ubiquitination and degradation/inactivation of β-catenin [39,70]. Thus, this finding supports the hypothesis that silymarin inhibits melanoma cell migration by targeting β-catenin. In an attempt to further verify the role of silymarin on prevention of invasive potential of melanoma cells through inactivation of β-catenin signaling, the authors used two distinct melanoma cell lines, namely Mel1241 and Mel1011. The two cell lines differ in the status of constitutive activation of Wnt/β-catenin signaling. Mel1241 melanoma cells, which have constitutively active Wnt/β-catenin, are highly invasive. Treatment of Mel1241 cells with silymarin resulted in significant inhibition of cell migration which was associated with a reduction in the nuclear accumulation of β-catenin and a reduction in the levels of the matrix metalloproteinases MMP-2 and MMP-9 which are the downstream targets of β-catenin signaling. Interestingly, under identical conditions, these effects of silymarin were not found in the Mel1011 cell line, which lacks constitutively active β-catenin. The overall possible mechanism of action of silymarin on melanoma cell migration is shown in Fig. 1. This new insight into the anti-melanoma cell migration activity of silymarin could serve as the basis for chemoprevention or therapy of malignant melanoma in human patients.

4.4. Berberine

Berberine is an isoquinoline alkaloid found in the roots, rhizome and stem bark of a number of medicinal plants, *e.g., Berberis vulgaris* (barberry), *Berberis aquifolium* (Oregon grape), *Berberis aristata* (tree turmeric) and *Tinospora cordifolia* [60]. The potential medicinal value of berberine is indicated by its use in the Indian Ayurvedic [71], Unani and Chinese systems of medicine. Berberine possesses anti-carcinogenic properties and appears to exhibit insignificant toxicity in normal epidermal keratinocytes [72]. Singh et al. [60] have assessed the chemotherapeutic effects of berberine on the migration of human melanoma cells. For this purpose, two melanoma cancer cell lines (A375 and Hs294t) that exhibit meta-static characteristics were selected. As COX-2 over expression and abundant production of PGs have been linked with tumor progression, invasion and metastasis [26], screening for potential COX-2 as well as PGE_2 inhibitors may prove an effective strategy in identifying agents that can be used to prevent or treat malignant melanoma. Treatment of the melanoma cells with berberine inhibited the migration of cells in a dose-dependent manner and this was found to be associated with inhibition of COX-2 expression and PGE_2 production (Fig. 1). The inhibition of cell migration by berberine was not associated with its inhibitory effects on cell viability or induction of apoptosis. Similar effects were noted when the melanoma cancer cells were transfected with COX-2 siRNA. TPA-induced cell migration was also

blocked by the treatment of melanoma cells with berberine. These melanoma cells (A375 and Hs294t) overexpress two PGE_2 receptors (EP2 and EP4). The expression of EP2 and EP4 was decreased when cells were treated with berberine *in vitro*. These observations suggest that inhibition of the EP2 and EP4 levels by berberine may contribute to the inhibition of cell migration. Treatment of melanoma cells with an EP4 agonist enhanced cell migration and EP4 agonist-induced cell migration was inhibited by the treatment of cells with berberine further suggesting that berberine inhibits melanoma cancer cell migration by targeting PGE_2 receptor. Berberine also reduced the activity of NF-κB and the levels of other proteins of NF-κB family in melanoma cells. Together, the results of this comprehensive *in vitro* study suggest that the inhibitory effect of berberine on melanoma cell migration is mediated by downregulation of COX-2, PGE_2, PGE_2 receptors and NF-κB [60]. Similar effects of berberine on melanoma cell lines have been reported by Kim et al. [73]. These investigators found that berberine inhibited the metastatic potential of melanoma cells by reducing ERK activity and the protein levels of COX-2 through berberine-induced AMP-activated protein kinase activation. These results were confirmed using the specific MEK inhibitor, PD98059, and a COX-2 inhibitor, celecoxib. These new information suggest the potential of berberine against melanoma cell invasion and need to be further studied for the prevention or treatment of melanoma metastasis.

4.5. Other phytochemicals

The potential of some other phytochemicals to inhibit melanoma invasion has been investigated in some limited studies. Lupeol is a triterpene that is widely distributed in plants; and found in mango pulp, carrot, melon seeds and soybeans. Hata et al. [74] reported that treatment with lupeol inhibits the migration of melanoma cells *in vitro* by targeting the actin cytoskeleton of the cells. Curcumin, a pigment product of the rhizome of *Curcuma longa* L., has been widely investigated for its anti-carcinogenic properties in various tumor models. Menon et al. [75] have shown that curcumin inhibited the invasive potential of melanoma cells by inhibiting the expression levels of MMPs. Curcumin also has been shown to inhibit osteopontin-induced cell migration and NF-κB-mediated MMP-2 activation [76]. Wang et al. have demonstrated that curcumin inhibited melanoma cell invasion to the draining lymph nodes through inhibition of phosphorylation of Src kinase and the STAT3 pathway [77]. Yan et al. have shown that genistein inhibits invasive and metastatic potential of B16-BL6 melanoma cells by targeting protein tyrosine kinase [78,79]. These studies also suggest that genistein might repair extracellular matrix signaling and subsequently results in prevention of cancer cell invasion.

5. Conclusion

The incidence of cutaneous melanoma is increasing in children as well as adults but the present therapeutic options are limited. Alternative strategies for the prevention and treatment of malignant melanoma must be developed and investigated. Natural phytochemicals are promising options as these bioactive plant products have medicinal values and many have been used over a considerable period of time for the treatment of various diseases. Further *in vitro* and *in vivo* studies of these phytochemicals, such as green tea catechins, proanthocyanidins, silymarin and berberine, and identification of their definitive molecular targets would help in the design of more effective therapies. The use of phytochemicals in combination with already available therapeutic drugs would offer an enhanced ability to simultaneously target the multiple signaling pathways involved in the development and metastasis of melanoma. In addition, it may be possible to use phytochemicals on a long-term basis to inhibit malignant melanoma in individuals at high risk of this devastating disease.

Acknowledgments

The work reported from Dr. Katiyar's laboratory was supported by Veterans Administration Merit Review Award (1I01BX001410) and National Institutes of Health/NCI (CA166883). The content of this article does not necessarily reflect the views or policies of the funding sources. We thank Dr. Fiona Hunter for her assistance in editing the manuscript.

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Abbreviations

COX-2	cyclooxygenase-2
EMT	epithelial–mesenchymal transition
PG	prostaglandin
GSPs	grape seed proanthocyanidins
MAPK	mitogen-activated protein kinases
MMP	matrix metalloproteinases
NF-κB	nuclear factor-kappaB
UV	ultraviolet

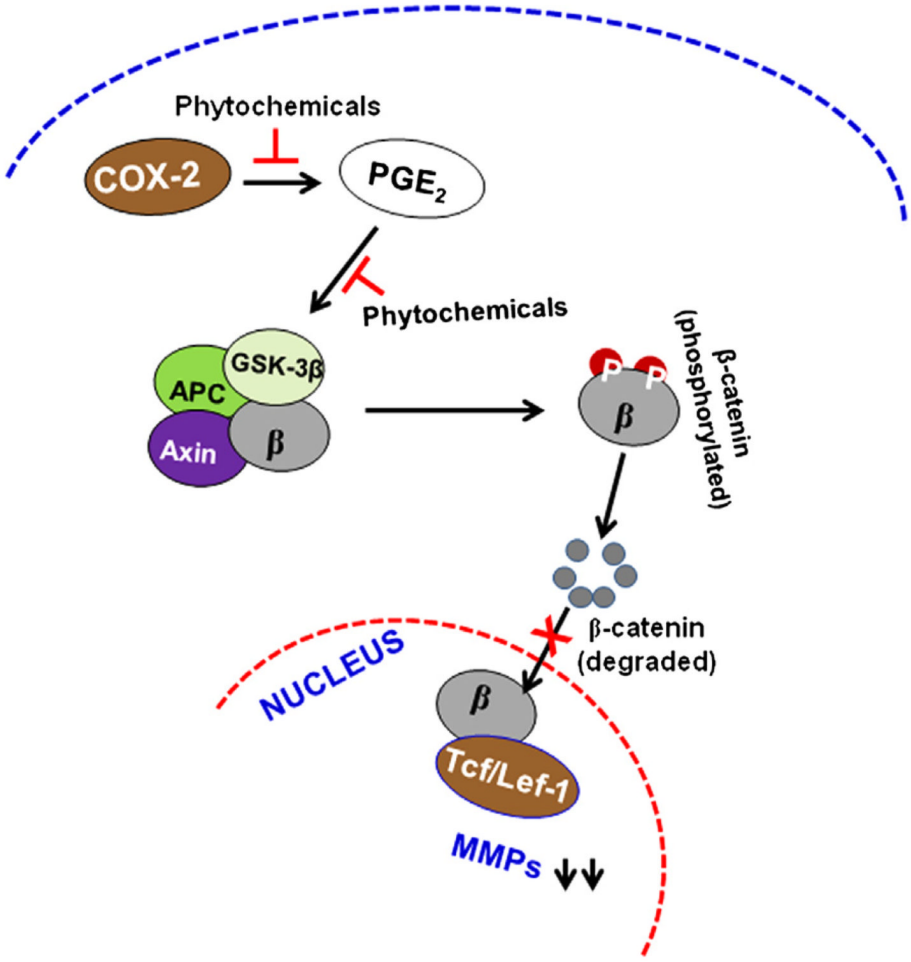
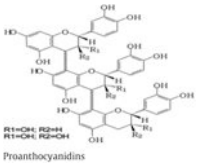
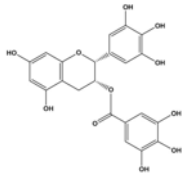
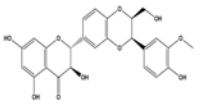
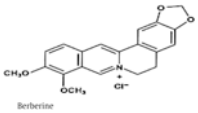


Fig. 1.

Schematic diagram summarizes the mechanism of action of phytochemicals on the migration and invasion potential of melanoma cells. Phytochemicals may target the endogenous expression of COX-2 and production of PGE_2 which leads to degradation of β-catenin. Degradation of β-catenin leads to inhibition of migration of melanoma cells.

Table 1

Selected phytochemicals, their structures, sources, and active ingredients, which possess anti-carcinogenic and anti-invasive potential against melanoma cells.

Phytochemicals and structures	Source	Active ingredients	References
 Proanthocyanidins	Grape seeds, pine bark, red wine, cranberry 	Dimers, trimers, tetramers and oligomers of monomeric catechins	[27,51–53]
 (−)-Epigallocatechin-3-gallate	Tea leaves & buds 	Monomeric catechins, epicatechins and their derivatives	[55–57,61–64]
 Silymarin/silibinin	Milk thistle buds and flowers 	Flavanolignan, silibinin	[66–68]
 Berberine	Roots and rhizomes of berberine plants	Isoquinoline alkaloid	[58,70,71]