Chapter 9

An Update On Natural Compounds and Their Modern Formulations for the Management of Malignant Melanoma

Danciu Corina, Soica Codruta, Antal Diana,
Alexandra Popescu, Roxana Ghiulai,
Ioana Zinuca Pavel, Stefana Avram,
Minda Daliana and Cristina Dehelean

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67647

Abstract

The chapter includes a brief presentation of the types of skin cancer. The most aggressive type of skin cancer, melanoma, is discussed from the point of view of incidence, molecular, and immunohistochemical mechanism along with the most important biomarkers for identification. Recent studies containing active phytocompounds with chemopreventive activity pointing toward phytochemicals used for melanoma prevention and therapy are reviewed. Modern physicochemical formulations for the enhancement of bioavailability of some active phytochemicals with chemopreventive activity for malignant melanoma are discussed.

Keywords: malignant melanoma, mechanism, biomarkers, active phytocompounds, modern formulations

1. Introduction

Skin cancer, with its many forms, is one of the most prevalent diseases today. In the last decades, there was a rising incidence in the reported cases of skin cancer and is estimated that one out of three Caucasians may develop a type of skin cancer in their lifetime [1, 2]. There are three common types of skin cancer, namely basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. Basal and squamous cell carcinomas are also known as non-melanoma skin cancer (NMSC) [1, 3].

Melanoma and non-melanoma skin cancers represent the most common malignancies worldwide [2, 3]. Wehner et al. indicated that approximately 30% of white people are likely to develop NMSC in their lifetime as a result of increased exposure to ultraviolet radiation [4]. It is considered that exposure to high doses of ultraviolet B radiation can cause basal cell carcinoma, while squamous cell carcinoma can be triggered after a chronic exposure to UV [2]. Melanoma in the first place and squamous cell carcinoma in the second place are tumor types with a high risk of metastasis, while basal cell carcinoma rarely does. Usually, these types of cancer affect people with white skin, blue eyes, and red hair [5]. Regarding gender, men are more likely to develop a type of NMSC in their lifetime than women [1].

As already mentioned above, one of the most important risk factors for developing skin cancer is considered exposure to ultraviolet radiation (UV) [6]. There are three types of UV radiation depending on the wavelength: UVA, UVB, and UVC. UV radiation especially ultraviolet B radiation is known to cause oxidative stress, inflammation, DNA damage, and apoptotic cell death in the skin [6, 7]. UV exposure also determines immunosuppression and photoaging of the skin [8]. People who use indoor tanning especially at an early age (before 25 years) are exposed to a higher risk of developing a form of skin cancer in their lifetime [4]. Among UV exposure, ozone depletion, genetics, and immune suppression are also incriminated in the rising incidence of skin cancer [9]. Another factor considered responsible for skin cancer growth is long-term exposure to arsenic, a class I human carcinogen found in industrial, agricultural, and medicinal substances [10, 11]. PUVA therapy is a treatment used for severe skin diseases which consists of psoralens (P) and exposure to ultraviolet A radiation (UVA). PUVA may induce skin cancer by photomutagenicity and photoinduced immunosuppression. It is important to evaluate the risk-benefit ratio before starting with the PUVA therapy [10, 12, 13]. Exposure to ionizing radiation, inorganic metals, and polycyclic hydrocarbons are also risk factors for skin cancer development [10].

1.1. Basal cell carcinoma

Basal cell carcinoma (BCC) is considered the most common type of skin cancer affecting people worldwide [5, 3]. It develops in the basal layer of the skin, from keratinocyte stem cells. Usually BCC appears *de novo* on hair-bearing skin, more often in adults. The most frequent BCC is considered the nodular/nodulo-ulcerative type which is commonly found on the neck and head. It looks like a red or pink papule with raised edges, telangiectasias, pearly appearance, and central small ulcers. Nodular BCC develops slowly but in time it can invade the tissue around it producing significant damage [14].

Exposure to high doses and intermittent UV radiation can trigger BCC [5]. Most cases of BCC were reported in Australia and are mainly attributed to UV radiation [3]. UVB is considered the most harmful environmental factor that can induce DNA mutations after exposure. BCC can develop through alteration of p53 and PTCH1 tumor-suppressor genes.

In general, BCC is found on the arms, head, neck, or the back and is more prevalent in people with light hair, blue or green eyes, and freckles. Other factors reported to increase the risk of BCC are genetic conditions, ionized radiation, genodermatoses, exposure to chemical carcinogens, organic and inorganic solvents, and organophosphate compounds [14, 15]. In some cases

http://dx.doi.org/10.5772/67647

of superficial BCC, human papillomavirus (HPV) is considered to be a risk factor in cancer growth. Also, immunosuppression caused by other diseases is considered to have a role in NMSC development [14].

There are various types of treatment options for BCC. The therapeutic approach depends on the location and the size of the tumor. Also, it is important to know the morphology and histologic subtype of the tumor and whether or not it has spread to nearby tissues. The most common therapeutic option is surgical excision of the skin [14, 15]. In most of the cases, the method is applicable if the lesion is less than 2 cm in diameter and is found in the trunk or extremities. An advantage of surgical excision is that after surgery, the lesion can be histologically analyzed and the tumor subtype can be determined. Also, there are disadvantages of using this method, including the possibility of infection and the presence of scars [14]. When surgery is not possible due to tumor location or because it is locally advanced, radiation therapy is an option [15]. The method can be used when the tumor is located on the ears, nose, eyelids, and lips. Other therapeutical options include photodynamic therapy, cryosurgery [14, 16], and topical therapies with imiquimod, an immune response modifier or 5-fluorouracil, a pyrimidine analog [15]. It is important to properly treat BCC because it has a high tendency to reappear if it remains untreated. Even if treated, in 18% of the cases, BCC will reappear in a few years after treatment [14].

1.2. Squamous cell carcinoma

Squamous cell carcinoma (SCC) also arises from keratinocytes and is the second most common type of skin cancer [17, 18]. SCC can be localized in the epidermis, known as *in situ* SCC, or it can extend to the dermis or deeper in the skin, known as invasive SCC. UV exposure is the main risk factor incriminated for SCC development. Cumulative high doses of UV radiation are responsible for SCC evolution [17], also non-healing wounds or chronic lesions that were linked with previous cases of chronic immuno-inflammation are associated with SCC [5]. SCC is found at a higher prevalence at people who smoke, compared to non-smokers, probably as a result of the immunosuppressive effects caused by smoking. Viruses, such as human papillomavirus (HPV), human immunodeficiency virus (HIV), or human herpes virus (HHV), also increase the risk for SCC. Exposure to chemical agents, including arsenic and polycyclic aromatic hydrocarbons, can lead in time to SCC. People with genetic disorders, such as xeroderma pigmentosum and oculocutaneous albinism, are more likely to develop SCC [17].

This type of cancer is usually found in people older than 50 years and is located on the head and neck, being more frequent in males, than in females [17]. Usually, it does not metastasize, but when it does, the survival rate is poor, between 25 and 40% [18].

SCC treatment depends on the location of the tumor, the size, and whether or not it has metastasized. For high-risk tumors, Mohs micrographic surgery (microscopically oriented histographic surgery) is considered the best method, while for low-risk SCC, surgical excision is the most common therapeutic option. In order to have a low recurrence rate, safety margins must be also excised. They vary between 4 and 10 mm according to tumor size. Cryosurgery is another surgical method used for small tumors [17, 19]. Nonsurgical treatments are also effective therapies for SCC, namely radiation therapy, topical application of 5-fluorouracil

and imiquimod, and photodynamic therapy. There are studies, which indicate that non-steroidal anti-inflammatory drugs are useful in the prevention and treatment of SCC [17]. Other drugs used rather in the prevention than in the treatment of SCC are retinoids. The results of Tran et al. showed that the topical administration of retinoids neutralizes vitamin A depletion induced by exposure to UVB radiation and thus has a beneficial role on recovery [20]. Recurrence rate is around 5 years after treatment, so it is important that the patients are closely monitorized [17].

1.3. Melanoma

Melanoma is a melanocytic neoplasia, and it is thought to be the rarest form of skin cancer and the most aggressive one [1]. It is considered that melanocytic nevi are precursors for melanoma. They are benign nevi, usually found in body areas exposed to the sun and can undergo transformation due to several risk factors and then progress into dysplastic nevi, which can further turn into melanoma [21].

In order to establish whether or not a melanocytic nevus has a malignant transformation, five characteristics can be followed, also known as the ABCDE of melanoma. The first is ***A****symmetry* —usually a benign melanocytic nevus is symmetric; ***B****order*—the margins of the nevus are irregular; ***C****olor*—the pigmentation is not equable on the entire surface of the nevus; ***D****iameter* —the melanocytic nevus is bigger than 6 mm, ***E****volution* [1].

Exposure to UV radiation is considered one of the most important factors in melanoma development. Among it, genetic conditions, multiple nevi, and exposure to some chemical agents are incriminated as risk factors [22, 23]. People who have first-degree relatives with melanoma are exposed to a higher risk in developing this type of skin cancer. Immunosuppression is also thought to have a role in melanoma growth. Other risk factors include the exposure to some pesticides, such as parathion, carbaryl, maneb/mancozeb, and benomyl [23].

Commonly, melanoma appears on males older than 54 years and females older than 45 years and is mostly found on the trunk, face, and ears. In the last years, the number of melanomas found in women aged 15–24 has increased [23]. Indoor tanning is considered to have an important role in this higher incidence rate [24].

There are different forms of melanoma, the most common being melanoma of intermittently sun-exposed skin, acral (and mucosal) melanoma, lentigo maligna melanoma, and nodular melanoma. Melanoma of intermittently sun-exposed skin is generally found in Caucasian people and is related with BRAF mutations and melanocytic nevi. It affects more women than men, and is located in the lower extremities and the trunk [22]. Acral (and mucosal) melanomas can appear in areas of the body that are not frequently exposed to UV radiation such as the soles of the feet and the palms of the hands. This type is found in black people and is associated with chromosomal aberrations [5, 22]. Lentigo maligna (solar) melanoma develops after chronic exposure to UV radiation. It is found in people older than 60 years and is correlated with genomic aberrations and the lack of BRAF mutations. Nodular melanoma is a rapid growing tumor that can have pagetoid spread, commonly found in people older than 30 years [22].

Tumor thickness (mm)	Excisional margins (cm)
in situ melanoma	0.5
<1.0	1.0
1.0–4.0	2.0
>4.0	≥2.0

Melanoma has a high metastatic potential through lymphatic and vascular channels. Early diagnosis is very important because in the early stages it can be removed surgically. In order to reduce the risk of tumor recurrence, along with the tumoral tissue, part of the surrounding normal structure is also removed. The American Academy of Dermatology recommends the following excisional margins (cm) according to tumor thickness (mm) [1]:

When there is evidence of metastasis, systemic chemotherapy is used. Frequently used drugs are cisplatin, mitomycin, doxorubicin, and temozolomide. Immunotherapies are also an option for metastatic melanomas. This includes administration of interferon-alpha or interleukin 2 [1].

Patients with high-risk melanomas must be physically monitored every 3 months for the first 3 years, considered the period when metastases are more likely to appear and thereafter biannually or annually [22].

2. Melanoma: incidence, molecular, and immunohistochemical mechanism

2.1. Incidence and mortality of melanoma

From the total number of all skin cancer cases, melanoma represents less than 2%, nevertheless being the major cause of death from skin cancer. Despite this steadily increasing incidence of melanoma, the mortality caused by this pathology stabilized in the last 20 years, on the strength of advances in medical care. At present, the survival rate of 5 years exceeds 90%, being associated with the moment of diagnosis [25].

It was predicted that 73,870 patients would be diagnosed with this condition in 2015. The most affected segment was the white people of non-Hispanic origin. According to *American Cancer Society,* the incidence rates vary from 1 per 100,000 annual cases in blacks to 4 in Hispanics, and 25 in non-Hispanic whites. Although it can appear independently of age, usually the elders are more affected by melanoma, being diagnosed at an average age of 62 years [25]. By the age of 50, women are more affected than men; however, by age 65 the incidence is two times higher in men than in women, tripling by the age of 80. These disparities are pursuant to ultraviolet radiation, either solar or artificial. During 1930, the incidence of melanoma was 1 in 1500 American people, reaching 1 in 68 in 2002. Through the years 2002–2012, the incidence extended with 1.8% per year. This enhancement of new cases of melanoma is partially linked to the development of new, modern, diagnostic methods, and also to the rising of the average life expectancy [25, 26].

In the US, during the year 2015, there were estimated 73,870 new cases of melanoma from which 42,670 cases were in male and 31,200 in female. The total melanoma deaths in US, in 2015, reached 9940, whence 6640 cases in men and 3300 in women. In the State of California, the total number of new melanoma cases reaches the upper limit (8560 cases), being followed by Florida (5480 cases), while the minimum was observed in the District of Columbia (80 cases), followed by Alaska (100) [27].

In Europe, 100,000 new cases of melanoma were diagnosed during 2012, mostly in the central and eastern part. The incidence of melanoma deaths was high in these regions of Europe (36%); from a total of 22,000 deaths, 8000 cases were found in the Central and Eastern Europe. However, the most cases of melanoma in Europe were in the Nordic regions and the Netherlands [28].

A recent study has compared the incidence and mortality rate in 11 countries from Europe: Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Malta, Romania, Serbia, Slovenia, and Turkey. Regarding the distribution of incidence upon age and sex, it was observed that younger females and males had the same incidence rates, although it increased in middle-aged and older men in comparison with females from the same category. The incidence rate of melanoma was increased in most of the countries, the higher trend concerning the young people (25–49 years) being observed in Slovenia, though in men aged 50–69, the incidence was important in Slovenia, Slovakia, Bulgaria, and Serbia. After the age of 70, the rate was raised in men, independently on the level of the incidence in the country. In women, the rate of melanoma was increased in most regions of Europe, especially in Slovenia, whatever the age. For 50–69 aged females, the incidence was higher in Bulgaria and Romania, while for women older than 70 years, a greater incidence in Serbia and Slovakia was remarked. For this category of women, the incidence was also significant in Bulgaria, Croatia, and the Czech Republic. Regarding the melanoma mortality rates in the 11 countries of Europe previously mentioned, men were more affected than women in most countries. In younger groups, the mortality rate was non-significantly decreased, in both men and women. Men of middle and older age had a relevant mortality in Serbia. Also, the elders were affected in Turkey and Czech Republic. On the other hand, women aged 50–69 had elevated rates of mortality in most countries, particularly in Slovenia. The mortality increased with age, mainly in Serbia and Slovenia [29].

2.2. Molecular and immunohistochemical mechanism in melanoma

From the embryonic neural crest cells, throughout development, melanoblasts are first taken into dermis, and then these precursors migrate toward the epidermis to settle in its basal epithelial layer, on the basal lamina. Melanoblasts arise into dermis by 11 weeks. Melanin pigment is produced at around 12 weeks [30].

Differentiated melanocytes are hold tightly in the basement membrane, when they arrive in contact with keratinocytes. At higher epidermal layers, they are unable to survive, unless turned into nevi or melanomas. Keratinocytes play an important role in the maintenance of melanocytes homeostasis. The impairment of this homeostasis affects the epidermal melanin unit, giving rise to an unceasing proliferation of melanocytes. It is presumed that melanoma cells get rid of the keratinocytes control by several mechanisms: (a) down-regulation of some

http://dx.doi.org/10.5772/67647

molecules (like E-cadherin) that play a role in their communication and adhesion to keratinocytes; (b) up-regulation of certain molecules that participate in cell signaling significant for melanoma-melanoma and melanoma-fibroblast interplays, like N-cadherin, zonula occludens protein-1, and MCAM (*melanoma cell adhesion molecule*); and (c) impaired expression of several proteins that bind the extracellular matrix with the repercussions for the retention into the basement membrane [31, 32].

Recent studies revealed the up-regulation of CCN3 protein in melanocyte-keratinocyte coculture, influencing some important characteristics in the physiology of melanocytes. This matricellular protein is overexpressed in some malignant diseases being related to the evolution of prostate cancer, carcinoma of renal cells, and Ewing's sarcoma; moreover, its expression is linked to the differentiation of tumors in rhabdomyosarcoma and cartilage malignancies [33–35]. Depending on the type of cancer, CCN3 influence divergently the genesis of tumor. Therefore, in gliomas it manifests antiproliferative effects, as well as in chronic myeloid leukemia cells. By contrast, it stimulates the invasion of Ewing's sarcoma cells [36, 37]. Regarding the effects of CCN3 on melanocytes, it reduces their proliferation and is essential for the melanocyte network organization [38]. In addition to this, it enhances the adherence of melanocytes to the type-IV collagen from basement membrane. Studies *in vivo* show that CCN3 overexpression is associated with an extremely attenuated invasion of melanoma cells, through the inhibition of MMP (*matrix metalloproteinase*) expression [31, 39].

The progression of melanoma comprises five distinct phases. First of all, hyperplasia of melanocytes gives rise to nevi; these can be acquired or congenital. The second phase is characterized by dysplastic nevi along with cytological and architectural abnormality. The third one corresponds to radial growth phase (RGP) melanoma, distinguished by the presence of tumor cells within the epidermis, or their invasion through the superficial dermis, having a reduced ability to spread from the primary site. The last stage is the vertical growth phase (VGP), which is featured by the profound invasion of tumor cells into the dermis and hypodermic tissue, augmenting the systemic dissemination risk. Lastly, metastasis occurs, being the most advanced phase of this condition, as a result of the altered homeostasis in the skin. The impaired mechanisms of homeostatic control determine the development of melanoma, since these mechanisms control the proliferation of cells, as well as their differentiation and apoptosis [33, 41].

Throughout the development stages of melanoma, CCN3 protein expression is down-regulated. Immunohistochemical techniques performed on melanoma cells evidentiated the inversely proportional interdependence between CCN3 expression and tumor dimension. The transduction of CCN3 in progressive melanomas inhibited the activity of matrix metalloproteinases MMP 2 and 9, resulting in an intense reduction of tumor invasion. Hence, the absence of CCN3 expression in melanomas is related to an invasive phenotype [31].

Melanomas can appear from melanocytes that have the role in the production of melanin pigment, and also in its storage and distribution to keratinocytes. There are other melanomas that arise from nevi, particularly dysplastic or giant congenital nevi. Melanomas are divided into four clinic-histological types: superficial-spreading melanoma, nodular form, lentigo maligna, and acral lentiginous type. Different factors are involved in the development of melanoma, such as genetic triggers, methylation of DNA, acetylation, or milieu factors [40, 41].

Impairment in tumor-suppressor genes such as mutation, deletion, translocation, or activation of proto-oncogenes has a significant role in mutagenesis. Mutation or deletion of CDKN2A anti-oncogene and CDK4 proto-oncogene is related to the development of familial melanoma, which constitute 8–12% of total melanomas [42]. This type of melanoma is characterized by an abnormal regulation of cell cycle by cyclin-dependent enzymes. In this case, the 9p21 chromosome has a germline mutation in the locus of CDKN 2A gene, affecting two proteins p16 and p14 or Arf that have a suppressive role. The p16 protein has an inhibitory effect on cyclin D/CDK4 and CDK6, influencing therefore pRB (*retinoblastoma protein*) which, through liberation of E2F, change the G1 phase of cell cycle into S phase [43, 44]. It was discovered lately that E2F1 protein, as well, manifests a suppressor function through p53 or p73. Between 10 and 30% of familial melanoma cases are due to CDKN2A mutation. NRAS and p16 mutations are also incriminated in some isolated cases of melanoma. Mutation of CDKN2A can lead to multiple melanomas. This alteration seldom appears in actinic keratosis, skin carcinomas, and other types of cancers [45, 46].

The p14 protein, encoded by CDKN2A reading frame, affects the p53 protein through the influence on other protein, Mdm2. The p14 deprivation lowers the activity of p53 [47]. Studies taken on mice models have shown that p14 can determine apoptosis of oncogene-activated melanocytes [48, 49].

It is known that pigmentation of the skin that depends on the amount of melanin is in direct relation with the development of melanoma. It is 10 times more frequently in whites than in blacks; however, people affected by albinism are exceptionally affected. The highly polymorphic melanocortin 1-receptor gene (MC1R) situated on 16q24 chromosome controls sun sensitivity [50, 51]. It codifies the transmembrane receptor coupled with G protein. The pituitary polypeptide POMC (*pro-opiomelanocortin*) is cleaved to give rise to different peptide hormones, including MSH (*melanocyte-stimulating hormone*), that acts through this receptor, enhancing cAMP and therefore stimulating tyrosinase enzyme activity. As a fact, keratinocytes can also produce these molecules. The synthesis of dark eumelanin and yellow cysteine-abundant pheomelanin is augmented by MSH. It was observed that people with MC1R variations evolve to earlier melanoma [52]. New studies have revealed that MITF (*microphthalmia-associated transcription factor*) gene found on 3p14 chromosome has a role in the promotion of melanocyte cell cycle progression [53].

3. The most important biomarkers for the identification of malignant melanoma

In order to diagnose malignant melanoma, there are several methods and techniques that when combined can give a certain degree of surety to the diagnosis.

As previously discussed, the first step in diagnosing malignant melanoma is the visual inspection of the lesion and the assessment of the well-known **ABCDE** signs (**A**symmetry, irregular **B**orders, uneven **C**olor, large **D**iameter, **E**volution) [54]. These findings are combined with dermatoscopical inspection of the lesion in order to confirm or to infirm the naked eye observations. If these findings lead to a suspicion of malignancy, then most likely a wide-excision surgery is performed and upon the assessment, a sentinel lymph node biopsy is also performed

http://dx.doi.org/10.5772/67647

[55]. In order to establish a certain diagnosis, there are various sets of markers specific for this type of cancer, which enable the obtaining of a definite diagnosis and/or the prognosis and staging of the disease, which can be identified by several methods [56].

There are different types of biomarkers to investigate malignant melanoma such as the microscopical markers identified upon the examination of the tumor and the immunohistochemical biomarkers identified upon isolation from the tumor/tissue section. Another type of malignant melanoma markers is the serological biomarkers that are identified from the peripheral blood samples of the patients by various methods.

3.1. Microscopical biomarkers in malignant melanoma

Breslow thickness refers to the thickness of the tumor and was demonstrated to be the most important prognostic histopathological factor. This parameter is of great importance and is used to stage malignant melanoma [57].

Tumor ulceration is the loss of epidermal integrity of the melanoma and is used for staging malignant melanoma and was linked to rapid tumor growth and metastasis [58].

The mitotic rate refers to the number of mitoses per square millimeter. A high mitotic rate is correlated with high metabolic cell activity and predicts rapid tumor growth and metastasis. Also, increased mitotic rate was linked with decreased survival [59].

3.2. Immunohistochemical and serological biomarkers in malignant melanoma

Immunohistochemical biomarkers

Biomarker type	Significance/location	Specificity/involvement in prognosis
2.1 **HMB 45** (human melanoma black 45)	Monoclonal antibody for the premelanosome protein (Pmel antigen) [60]	Very specific for melanoma, especially for primary lesions, not being able to identify other types of tumors [61, 62]
2.2 **Melan A**	Antigen recognized by T cells 1 or MART-1 (membrane protein of melanosomes and endoplasmic reticulum found in melanocytes, melanoma, and endoplasmic reticulum) [63, 64]	Higher sensitivity for primary melanomas compared to metastatic ones [65]
2.3 **S 100**	Family of proteins expressed in Schwann cells, melanocytes, glial cells, is involved in many cellular functions such as the activation of cell processes along the Ca2+ signal-transduction pathway [66, 67]	Low specificity, used together with other biomarkers [65]
2.4 **Tyrosinase**	Oxidative enzyme involved in melanin production in melanocytes and melanoma; detected with T311 monoclonal IgG antibody [68]	Elevated sensitivity for primary melanoma than for later stage tumors [68]
2.5 **Ki-67**	Protein biomarker for cellular proliferation [69]	Adjunctive diagnostic biomarker [70]
2.6 **Nestin**	Filament protein associated with migration and metastasis [71]	Advanced stages of the disease (T3 and T4) [71]

Immunohistochemical biomarkers		
Biomarker type	**Significance/location**	**Specificity/involvement in prognosis**
2.7 **Vimentin**	Ectodermal, neural, and pancreatic progenitor cell marker, overexpression in cancer [72]	Tumor growth, invasion, and poor prognosis [72]
2.8 **Chondroitin sulfate proteoglycan 4**	Located in melanocytes, promotes cell adhesion, growth, migration, motility [65]	Invasion and metastasis [65]
2.9 **EFGR** (epidermal growth factor receptor)	BRAF transmembrane tyrosine kinase [73]	Progression and metastasis [74]
2.10 **P53** (cellular tumor antigen p53)	Tumor suppressor "guardian of the genome" (promotes cell cycle arrest, apoptosis, senescence, and DNA repair) [75, 76]	Inactivated or functionally impaired in melanoma [75]
Serological biomarkers		
3.1 **LDH** (lactate dehydrogenase)	Enzyme that converts lactate into pyruvate in anaerobic conditions [77]	High levels are correlated with metastasis, decreased survival rate, negative outcome after treatment [77]
3.2 **CRP** (C-reactive protein)	Nonspecific marker of inflammation, infection, cancer [65]	Predictor for disease progression in high levels [65]
3.3 **MIA** (melanoma-inhibiting activity)	Protein associated with *in vitro* increased invasiveness, extravasation, and metastasis [77]	Elevated levels, marker for advanced stage (III and IV) and poorer prognosis [77]
3.4 **VEGF** (vascular endothelial growth factor)	Protein that stimulates vasculogenesis and angiogenesis [78]	Elevated levels linked to poor progression-free survival [78]
3.5 **BRAF**	Raf kinases family involved in RAF-MEK-ERK signal transduction pathway (cell growth, proliferation, and differentiation) [79]	BRAF mutation, BRAF-V600E, associated with increased tumor thickness, ulceration, and reduced survival [80]
3.6 **miRNA** (MicroRNAs)	RNA molecules involved in proliferation, differentiation, stress responses, apoptosis [77, 78]	Disease progression and risk of recurrence [77, 78]
3.7 **GM3**	Ganglioside (glycosphingolipid) involved in cell proliferation, differentiation, apoptosis, embryogenesis, and oncogenesis [81]	Low levels (early stage) linked to invasive proliferation; higher levels (late stage) linked to cell migration and invasion [81]
3.8 **OPN** (osteopontin)	Glycol-phosphoprotein implicated in reduction of apoptosis, promotion of tumor growth, tumor-promoting stromal cell in the bone marrow [82]	Rapid melanoma growth, angiogenesis, and aggressive metastasis [83]
3.9 **IL-8** (interleukin-8)	Chemokine produced by malignant cells [82]	Associated with tumor burden, stage of the disease, survival, and response to therapy [82]
3.10 **YKL-40**	Heparin secreted during the late stages of cell differentiation [84]	Overexpression associated with poor survival [84]
3.11 **CTC** (circulating tumor cells)	Cells from melanoma metastatic tumors [77]	Linked to inferior survival; predictive marker for selecting the systemic therapy [77]
3.12 **Melanoma-initiating cells**	Cancer stem cells [85]	Can lead the specific targeted systemic therapy [85]

http://dx.doi.org/10.5772/67647

4. Recent studies concerning active phytocompounds targeting malignant melanoma

Although synthesized to serve the plant in modulating its interaction with the environment, many natural compounds act as drugs when used by humans by binding to various enzymes and receptors. In the area of anticancer medications, it has been shown that about 75% of the medications are naturally derived or inspired [86]. Phytocompounds hold an important potential in the chemoprevention and treatment of melanoma, including metastasis [87, 88]. The main advantage of natural products relies in their multi-target activity, with one compound being able to interact with multiple proteins and receptors. Anti-melanoma activities have been pointed out for both phytocompounds from nutraceuticals (polyphenols, polysaccharides, and carotenoids), as well as for highly active secondary metabolite classes like the alkaloids or the terpenes. Additionally, there are a number of crude extracts or enriched fractions with anti-melanoma actions, for which the active compound(s) could not be singled out yet. A promising field in the actual usage of plant-derived compounds is their synergistic activity when administered in combination with synthetic anticancer medications [89].

4.1. Polyphenols

The potential of polyphenolic compounds (**Figure 1**) as anti-melanoma agents was recognized as early as 1995, when Menon and coworkers found that orally administered curcumin, catechin, and rutin significantly prolonged the life span of animals with experimental melanoma [90]. The anticancer potential of these food constituents is supported by epidemiological studies [91]. Their general mechanisms of action may be summarized as follows: antioxidative, photoprotectant, induction of apoptosis, and inhibition of angiogenesis. Antioxidative capacities are seminal for the protective activity against UV-induced oxidative stress [92]. The ability to act as UV filters is related to the physiologic function of certain flavonoids present in epicuticular waxes protecting the plant epidermis against UV radiation [93]. Apoptosis is mainly promoted by polyphenols via regulation of Bcl-2 family proteins and caspase-3 activation, while the anti-migratory effects are mainly obtained by down-regulation of MMPs expression.

Intensely researched polyphenols in the context on melanoma include flavonoids (quercetin, apigenin, silymarin, genistein, catechins, anthocyanins, biflavonoids), curcumin, and resveratrol [94]. Quercetin (**1**), an abundant dietary flavonol, acts via all above-cited mechanisms. It decreases the viability and promotes the apoptosis of ultraviolet UVB-irradiated B16F10 melanoma cells by several mechanisms: it increases the levels of reactive oxygen species, depolarizes mitochondrial membrane potential, induces the imbalance of calcium homeostasis, attenuates MEK-ERK signaling, and changes the ratio of Bcl-2, Bax, and Bim expression in favor of cell death elicitation [95]. It offers photoprotection against melanogenesis through a regulatory effect on the Nrf2-ARE pathway [96]. Furthermore, quercetin inhibits melanoma cell migration in a setup of experimentally activated receptor tyrosine kinase c-Met, known to be involved in the acquisition of metastatic phenotypes [97].

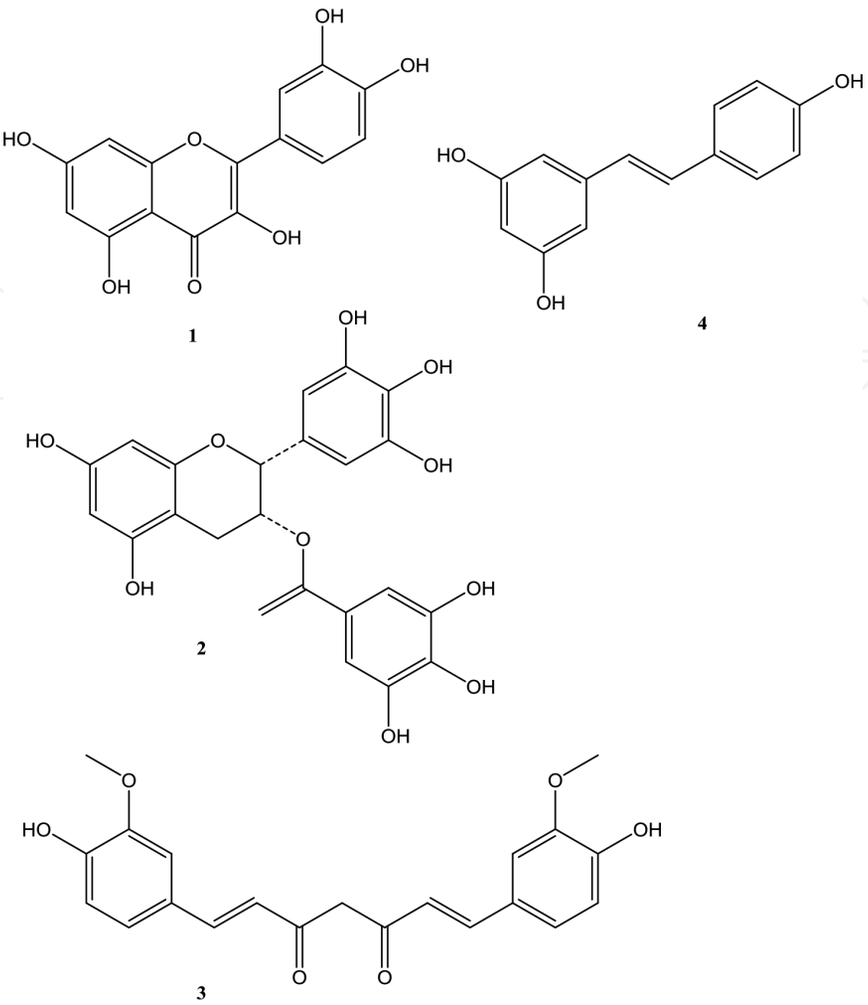

Figure 1. Chemical structure of representative polyphenols targeting malignant melanoma. 1: quercetin, 2: (–)-epigallocatechin-3-gallate, 3: curcumin; 4: resveratrol.

Apigenin, a flavone from the point of view of chemical structure, offers an effective photoprotection against UVA- and UVB-induced skin carcinogenesis [98], fights metastasis by preventing the activation of MMP-2, MMP-9, VEGF, and Twist1 genes through down-regulation of STAT2 signaling [99], promotes apoptosis via caspase-3 and cleaved poly(ADP-ribose) polymerase [100]. In addition, apigenin blocks the COX-2 pathway and increases terminal differentiation in the epidermis [101].

Silymarin, a complex of flavonolignans from the achenes of milk thistle, is a natural product known especially for its hepatoprotective, anti-oxidative and anti-inflammatory effects [102]. When applied locally, silymarin was able to inhibit carcinogenesis induced by UV-radiation and to decrease tumor multiplicity and growth. It produces cell cycle arrest in melanoma cells via inhibition of kinase activities of (MEK)-1/2 and RSK-2 [103], induces apoptosis and counteracts metastasis via down-regulation of MMPs expression [104]. Silymarin is a prominent inhibitor of Wnt/β-catenin translocation in melanoma cells, beside few other phytocompounds

http://dx.doi.org/10.5772/67647

like the flavonoid fisetin, the triterpene lupeol, and the alkaloid tryptanthrin; aberrant Wnt signaling occurs in one-third of melanomas [105].

Catechins are flavanols devoid of the 3-oxo group specific to other flavonoids; they may especially be found in woody plants like wine and the tea plant. Catechins as well as their esters with gallic acid, in particular epigallocatechin-3-gallate (EGCG, **2**), are the subject of intense research in cancer prevention and treatment. In the field of anti-melanoma activities, the chemopreventive effect of ECGC is mainly achieved by a photoprotective effect exerted through interleukin 12 induction followed by DNA repair, inhibition of angiogenesis, and the stimulation of cytolytic T cells [106]. The proapoptotic effects are exerted by EGCG through down-regulation of inhibitory proteins such as Bcl-2, D1, and cdk2, as well as the up-regulation of the proapoptosis protein Bax. Important tumor-suppressor proteins (p16, p21, and p2) are induced by EGCG [107, 108]. More recently, the discussed polyphenol was shown to bind to the cell surface receptor 67LR, overexpressed in melanoma [109]. As an agonist of 67LR, EGCG is able to suppress melanoma tumor growth by activating a specific signaling pathway (cAMP/PKA/PP2A) via regulation of miRNA-let-7b expression in melanoma cells [110]. Moreover, EGCG displays an excellent anti-metastatic profile, reducing the number of lung metastases in mice [111]. Molecular targets in this regard are COX-2, PGE2 receptors, the impairment of epithelial-to-mesenchymal transition [112], reduction of angiogenesis promoters and down-regulation of MMP2 activity and ERK1/2 pathway [113].

Curcumin (**3**), an orange-colored constituent of turmeric rhizomes (*Curcuma* sp.), is an extensively investigated compound with anti-inflammatory and anticancer activities. In human melanoma cell lines (A375 and C8161), it reduces cell viability, has an antiproliferative effect arresting mitosis at the transition of G2/M phase, suppresses cell invasion, and induces autophagy [114]. The anti-metastatic effect was demonstrated *in vivo* using C57BL/6 mice; the down-regulation of metalloproteinases and collagenases activities, modulation of integrin receptors [115] and interference with the STAT3 pathway support this observation [116].

Resveratrol (**4**), a stilbene found mainly in grapes and *Polygonum cuspidatum*, inhibits melanoma proliferation *in vitro*, arresting cell divisions at the G1/S transition and induces apoptosis targeting Bcl-2-associated X protein, B-cell lymphoma 2, and caspases -9 and -3 [117]. Through the suppression of β-catenin and STAT3-pathway, resveratrol reduces the levels of survivin, a protein which is essential for the survival of melanoma cells. Furthermore, the compound has anti-migratory activities mediated by the deactivation of the proto-oncogenic Akt [118]. The low bioavailability of resveratrol triggered the obtainment of several analogs, as well as the preference for the use of the natural pterostilbene having a longer half-life [87].

4.2. Polysaccharides

The potential of polysaccharide fractions obtained from terrestrial and marine organisms in cancer treatment receives an increasing attention. These compounds are potent immunomodulators, leading to the release of cytokines which may play important roles in the defense

against cancer [119]. Their main mechanism of action includes activation of natural killer cells, induction of inflammatory caspases, and down-regulation of MMPs expression, but as a general feature their mechanism of action is much less known than in the case of plant polyphenols. An important advantage of polysaccharides is their very low toxicity.

The sulfated and partially acetylated fucoidans from brown algae *Coccophora langsdorfii* and *Fucus evanescens*, consisting of (1→3)- and (1→4)-linked α-l-fucopyranose residues, produced a significant inhibition of colony formation of SK-MEL-5 and SK-MEL-28 melanoma cells [120].

Beta-glucans are constituents of cell walls in certain fungi, algae, and vascular plants such as oat or barley. Oral administration of yeast-derived beta-glucan to mice inoculated with B16 murine melanoma cells significantly reduced tumor weight, pulmonary metastasis, and survival rate. These effects are mediated by the activation of NK cells. An important finding was that beta-glucan supplementation was devoid of hematopoietic toxicity, unlike chemotherapeutic drugs such as 5-fluorouracil [121]. Low-molecular-weight beta-glucan prepared from oat decreased the cell viability of cancer cell lines Me45 and A431, while normal keratinocytes were not affected. The cytotoxic effect was mediated by induction of caspase-12 expression. This natural product had the advantage of a good solubility in water and a low viscosity, in comparison with other glucans [122]. Polysaccharides from higher plants, like raspberry fruits, have as well been shown to inhibit melanoma growth, and to have a synergistic activity with docetaxel *in vivo*, reducing at the same time liver and kidney injuries inflicted by the latter [123]. Pectins from corn demonstrated their ability to inhibit cancer cell growth and metastasis by modulating specific markers such as galectin-3, VEGF, MMP-2 and MMP-9, and NF-κB [124].

4.3. Volatile oils

Volatile oils are complex mixtures of monoterpenes, sesquiterpes, aromatic compounds, and their derivatives, which are produced by plants in order to protect themselves against pathogens, herbivores, or, on the contrary, to act as signals for pollinators and disseminators [125]. In the search for innovative strategies in melanoma treatment, these natural products have as well been investigated. The main mechanisms of action known so far are represented by induction of apoptosis (via increasing the expression of p53 and caspase-3) and reduction of angiogenesis with down-regulation of MMPs.

The volatile oil of the tea tree plant (*Melaleuca alternifolia*) and its main component terpinen-4-ol (5) were evaluated in an experimental setting of human melanoma M14 WT cells and M14 adriamycin-resistant cells. Concentration as low as 0.005–0.03% proved to induce apoptosis, and conversely the resistant cell variant was more sensitive [126]. More recently, Greay and coworkers [127] applied a topical formulation containing 10% tea tree oil and found a significant delay in the growth of established subcutaneous B16-F10 melanoma, while systemic toxicity was absent. The volatile oil of *Tridax procumbens*, a common annual

http://dx.doi.org/10.5772/67647

Asteraceae in most tropical regions, displayed anti-metastatic effects by the inhibition of lung nodule formation by B16F-10 cells in C57BL/6 mice. Furthermore, it induced apoptosis increasing the expression of p53 and caspase-3 and reduced angiogenesis [128]. *Curcuma zedoaria* volatile oil suppressed as well melanoma growth and lung metastasis, with the down-regulation of MMPs [129].

4.4. Alkaloids

Alkaloids are nitrogen-containing plant metabolites with usually very intense pharmacologic effects at even low dosages. Consequently, their safety profile is very different from the one of compounds discussed above. The main compounds from this class, which are able to target melanoma, include berberine, harmine, paclitaxel, and glycoalkaloids from Solanaceae. While the number of reports on the anti-melanoma potential of alkaloids is rather scarce in comparison with that of polyphenols, the mechanisms of action are well studied. The anti-migratory properties of alkaloids in malignant melanoma have been the subject of a comprehensive review [87]. Alkaloids may induce apoptosis via the inhibition of the Ras-signaling cascade at various downstream points, blocking both PI3K/AKT and MAP/ERK pathways. Metastasis is mainly counteracted by blocking the COX pathway and angiogenesis. NF-κB inhibition is as well a target of anti-melanoma alkaloids.

Berberine, an isoquinoline alkaloid (**6**), occurs in several medicinal plants of the Berberidaceae, Ranunculaceae, and Papaveraceae families [130]. While best known for antimicrobial, anti-inflammatory, anti-hyperglycemic, and anti-hyperlipidemic effects [131], berberine is one of the most active alkaloids in melanoma. It has been shown to inhibit kinases like PI3K, ERK, and GSK3β [132]. Moreover, in combination with doxorubicin, it intensely reduces proliferation and increases apoptosis, effect demonstrated both *in vitro* and *in vivo* [133]. Berberine inhibits the migration of melanoma cells via the reduction of COX-2/PGE2 receptors expression [134] and has anti-angiogenetic properties [135]. Harmine is an indole alkaloid contained by only a few plant species, including *Peganum harmala* and *Banisteriopsis caapi*. While its ability to bind monoamino-oxidase A and to elicit psychoactive effects is known since several decades, the potential anticancer properties of this compound have only been explored in recent years. In melanoma, it displays important anti-migratory effects through the inhibition of NF-κB and other transcription factors (CREB, ATF-2) [136]; it reduces lung metastasis in C57BL/6 mice and down-regulates the expression of pro-metastatic genes (MMP-9, ERK, and VEGF) [137].

Paclitaxel is a pseudoalkaloid, first isolated from the bark of the Pacific yew (*Taxus brevifolia*), approved for the clinical treatment of several solid tumors with poor prognosis including melanoma [138]. It stabilizes microtubules, disrupting the dynamic equilibrium between free and polymerized tubulin; as a consequence, cell division is arrested in the G2/M transition of mitosis [139]. Additionally, paclitaxel has antiangiogenic effects, which are potentiated by COX-2 inhibitors [140]. Disadvantages of this compound include its extreme hydrophobicity, which could be overcome in modern nanoformulations, as well as drug resistance to paclitaxel

due to the induction of the efflux protein P-gp and unusual expression of class III isotype of β-tubulin [139].

Glycoalkaloids from Solanaceae, including α-solanine and solamargine, displayed anti-melanoma effects in recent reports. Interestingly, solamargine inhibited selectively the growth of WM239 and WM115 melanoma cells, in comparison to benign WM35 cells. Cellular necrosis occurred upon permeabilization of the lysosomal membrane, which triggered, in turn, the extrinsic pathway of mitochondrial destruction. The intrinsic apoptosis pathway was as well disrupted by this compound [141]. α-Solanine displays anti-proliferative effects, induces apoptosis, and suppresses melanoma cell invasion, mainly via the reduction of MMP-2 and MMP-9 activities. The potential of this compound in the treatment of metastatic melanoma is further substantiated by the inhibition of JNK and PI3K kinases as well as the reduction of NF-κB activity [142]. Two chemical structures of representative natural compounds targeting melanoma, other than polyphenols, are presented in **Figure 2**.

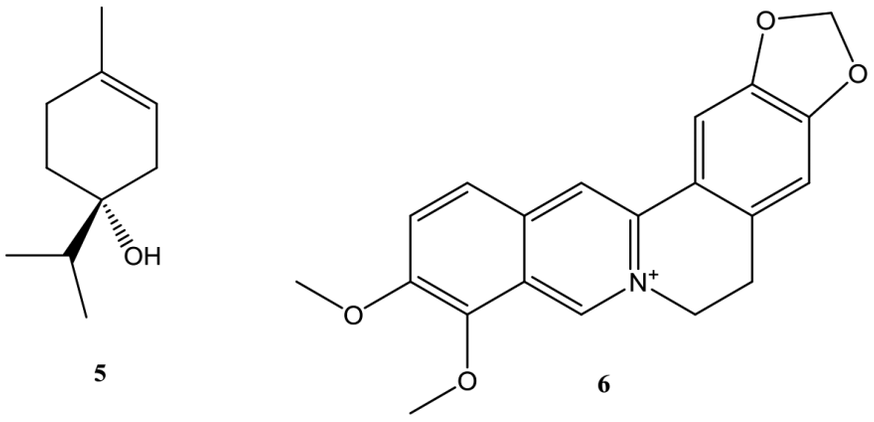

Figure 2. Chemical structures of representative natural compounds targeting melanoma, other than polyphenols. 5: Terpinen-4-ol, the major monoterpene in tea tree volatile oil, 6: Berberine, an intensely researched alkaloid.

5. Modern physicochemical formulations for the enhancement of bioavailability of selected active phytochemicals with chemopreventive activity for malignant melanoma

Formulation development can be regarded as a determining aspect in the delivery of antineoplastic natural compounds due to their physicochemical properties, in particular water solubility, which limit their bioavailability [143]. A variety of formulation strategies have been reported highlighting the main challenges that arise in the development of optimized drug delivery in the anticancer field [144], including skin cancer. The most studied natural anticancer compounds in terms of bioavailability improvement are different types of polyphenols (curcumin, silymarin, and resveratrol) and alkaloids (paclitaxel). In **Tables 1–4**, we aim to summarize the main reported attempts to increase the bioavailability of the above-mentioned compounds through various modulations of their physicochemical parameters as well as modern pharmaceutical formulations.

Curcumin formulations	Components	Method of application	Indications/remarks	Ref.
Curcumin + piperine	-curcumin -piperine	Oral administration to male Wistar rats (epilepsy model) (2 g/kg curcumin, +20 mg/kg piperine)	20-fold higher curcumin bioavailability compared to curcumin alone (piperine decrease hepatic and intestinal glucuronidation)	[145]
Curcuminoids (curcumin, demethoxycurcumin, bis-demethoxycurcumin) + Sesamin Ferulic acid Naringenin Xanthohumol (simultaneous administration) Curcuminoids micellar solubilized + Sesamin Ferulic acid Naringenin Xanthohumol; Curcuminoids micellar solubilized	2:2:1:1:1 (*w/w):* -Curcumin powder: (82% curcumin +16% demethoxycurcumin +2% bis-demethoxycurcumin) -sesamin -ferulic acid -naringenin -xanthohumol -95.1% Tween-80 -1.4% curcumin -1.4% sesamin -0.7% naringenin -0.7% ferulic acid -0.7% xanthohumol -7% curcuminoid powder (=6% curcumin) -93% Tween-80	Oral capsule administered to healthy humans (98 mg curcuminoids, single dose)	8-fold increase in bioavailability; 73-fold increase in bioavailability; 88-fold increase in bioavailability	[146]
Liquid micelles of curcumin Micronized powder of curcumin Native curcumin powder	- 7% curcumin powder (equivalent to 6% curcumin) -93% Tween-80 -25 % curcumin powder -58.3% triacetin -16.7% panodan -82% curcumin -16% dimethoxy-curcumin - 2% bis-dimethoxy-curcumin	Oral administration to healthy humans (500 mg curcuminoids, single dose)	277-, 114-, 185-fold increase in bioavailability in women/men/all subjects compared to native curcumin powder; 14-, 5-, 9-fold increase in bioavailability in women/men/all subjects compared to native curcumin powder; Women absorbed curcumin much more efficiently than men	[147]
Curcumin nanosuspension	-10% curcumin - 2% polyvinyl alcohol -88% water	Oral capsules administered to male Wistar rats, 100 mg/kg	Increased oral absorption and bioavailability -> significant increased concentration in selective organs; -direct uptake from the gastrointestinal tract; -increased permeability induced by surfactants;	[148]

Curcumin formulations	Components	Method of application	Indications/remarks	Ref.
			-increased resistance to degradation and elimination.	
Sustained-release formulation of curcumin	*MicroActive® Curcumin* (patented formulation): -25% micronized curcuminoids -polyglycerol esters of fatty acids -medium-chain triglycerides -hydroxypropyl-methylcellulose -sodium alginate -microcrystalline cellulose	Oral capsules administered to healthy humans, 500 mg curcumin (single dose)	9.7-fold increase in bioavailability (test period: 12 h)	[149]
Curcumin + phosphatidylcholine	*Meriva®*, (patented complex of curcumin with soy phosphatidylcholine): -20% curcuminoid mixture - 75% curcumin - 15% demethoxycurcumin - 10% bisdemethoxycurcumin -40% soy phosphatidylcholine -40% microcrystalline cellulose	Oral administration to male Wistar albino rats, 340 mg/kg	Reduced curcumin concentration in the gastrointestinal mucosa; Significant increased plasma/liver levels (compared to an unformulated curcumin); *Indications:* Chemoprophylaxis intervention studies targeting sites other than the gastrointestinal tract	[150]
Curcumin + PVP (polyvinylpyrrolidone) + cellulosic derivatives +antioxidant agents	*CurcuWIN*, -minimum 20% curcuminoids - PVP (polyvinylpyrrolidone) - cellulosic derivatives - antioxidant agents	Randomized, double-blind, crossover human study in healthy volunteers (hard gel capsules, 376 mg curcuminoids)	-45.9-fold increase in curcumin oral absorption compared to unformulated standard curcumin, 5.8-fold increase over a phytosome formulation, 34.9-fold increase over (curcumin + volatile oils from turmeric rhizome) formulation	[151]
Lipid based nanoparticles: phospholipid vesicles/lipid-nanospheres	*Phospholipid vesicles:* -DMPC/SA/PEG-DSPE/curcumin (molar ratio 10/1/0.06/1.9): DMPC = 1,2-Dimyristoyl-sn-glycero-3-phosphocholine; SA = l-glutamic acid, N-(3-carboxy-1-oxopropyl)-, 1,5-dihexadecyl ester);	IV administration to male Wistar rats (2 mg/kg)	Selectively delivered to macrophages in spleen and bone marrow; Potential intravenous delivery system for antioxidant and anti-inflammatory therapies	[152]

Curcumin formulations	Components	Method of application	Indications/remarks	Ref.
	PEG-DSPE = (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[monomethoxy poly(ethylene glycol) (5000)] -*t*-butyl alcohol/benzene, (1/1,*v*/*v*) -physiological saline 70 mg/mL; *Lipid-nanospheres:* -curcumin + soybean oil (10 mg/mL) -DMPC/SA/PEG-DSPE (10/1/0.06, molar ratio) + soybean oil (280 mg/mL) -2.5% glycerin solution			
Liposomal curcumin	-10:1 (*w*/*w*)-total lipids:curcumin; *Lipids*: -1,2-dimyristoyl-sn-glycero-3-phosphocholine/1,2-dimyristoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt) or a pegylated version of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine/cholesterol/1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy-(polyethylene glycol)-2000]	IV administration to female athymic *nu*/*nu* mice (human colorectal tumor xenografts in nude mice models) (liposomal curcumin 40 mg/kg, oxaliplatin 5 mg/kg); *in vitro* studies (LoVo and Colo205 cells)	Equivalent or greater growth-inhibitory and apoptotic effects with oxaliplatin both *in vitro* and *in vivo* in colorectal cancer	[153]
Self nanoemulsion of curcumin and silymarin	-15 mg curcumin -25 mg silymarin - 1 g oil phase (glycerin monooleate; Tween 20:PEG 400, 1:8:1 ratio) - deionized water:oil phase, 5:1 (self-nanoemulsifying method)	Stability study	Higher stability of silymarin and curcumin in comparison with a conventional emulsion Efficient drug delivery system	[154]

Table 1. Curcumin formulations.

http://dx.doi.org/10.5772/67647

Silymarin formulations	Components	Method of application	Indications/remarks	Ref.
Self-microemulsifying drug delivery system (SMEDDS)	-10% ethyl linoleate -30% Cremophor EL -60% ethyl alcohol (% *w/w*)	Oral administration to male mongrel dogs (50 mg/kg, single dose)	3 months stability; Faster release; 2.2-fold enhanced oral absorption, increased bioavailability; *Indication:* Oral administration of silymarin	[155]
Semisolid dispersion binary system using Gelucire 44/14 as carrier	Silymarin:Gelucire 1:1 and 1:3 (*w/w)*	Oral administration to New Zealand albino rabbits (280 mg/kg)	Linear increase of silymarin solubility and dissolution degree depending on the career concentration -> significant superior pharmacokinetic profile	[156]
Solid dispersion of silymarin with a hydrophilic polymer (HPMC E15 LV)	-silymarin powder -78.7 % silymarin -20.3 % silybin -isosilybin) -HPMC E 15LV (1:1, 1:3, and 1:5 (*w/w*)) (kneading, spray drying and co-precipitation method of preparation)	Influence of preparation methodology of solid dispersions of silymarin	Co-precipitation method revealed the best soluble and stable amorphous solid dispersion; The best results: 1:1 for the kneading method and 1:3 for other methods; Increased silymarin dissolution: co-precipitation (2.5-fold) > spray drying (1.9-fold) > kneading (1.5-fold); Mean dissolution time: 8.3 min for co-precipitation, 14.2 min for spray drying and 17.4 min for kneading	[157]
Silymarin loaded chitosan nanoparticles	-40 mg chitosan -10 mg silymarin - 2% acetic acid solution - 1mL ethanol -10 mg sodium tripolyphosphate -1M NaOH 1M/0.1M HCl (for pH adjustment) -5% D-mannitol solution (ionic gelation method of preparation)	Oral administration to Swiss Albino mice (CCl_4 hepatotoxicity model), 50 mg/kg	Sustained release Improved pharmacological activity	[158]
Solid dispersion and silica nanoparticles of silymarin with slow-release matrix material and release enhancer	- silymarin solid dispersion: -silymarin:povidone K30:soy bean lecithin: acrylic resin IV: 1–3:0.3–0.8:0.2–0.5 (*w:w*) -silymarin solid dispersion:	Oral administration in Beagle dogs	Continuous controlled release for 72 h; Half-life 2.3 times longer	[159]

Silymarin formulations	Components	Method of application	Indications/remarks	Ref.
	silymarin-loaded silica nanoparticles:slow-release matrix material:release enhancer: 0.5–1.25:0.1–0.3:0.1–0.3 (*w*/*w*)			
Silymarin glyceryl monooleate/ Poloxamer 407 liquid crystalline matrices	-20 g glyceryl monooleate -2.4 g poloxamer 407 -silymarin 2%, 4%, 8% (melting/ congealing method of preparation)	Oral capsules administered to Beagle dogs (14.4 mg/kg silybin)	*In vitro* limited release of silymarin; 3.46-fold enhancement in oral bioavailability in comparison with Legalon® (as a result of the liquid crystalline phase formed in the digestive tract)	[160]
Silymarin-loaded liquid proliposome	- 1.0 g silymarin -10.0 g soy lecithin - 5.0 g cholesterol - 0.5 g sodium oleate -100.0 mL propylene glycol simple dissolving process)	Oral administration to Kunming mice (hepatitis experimental models ; 75, 150, or 300 mg/kg)	Improved bioavailability and hepatoprotection; Efficient oral drug-delivery system for silymarin	[161]
Liposomal lecithin-based carrier system of phytosomal silymarin	-lecithin:cholesterol 6:1 (maximum entrapment-55%) (film hydration method of preparation)	*In vitro* study on Chang liver cells; Oral administration to Wistar rats	3.5-fold enhanced oral bioavailability; Higher hepatoprotective and anti-inflammatory effects in comparison with a silymarin suspension	[162]
Bile salt (sodium deoxycholate) containing liposomes of silymarin	-ethanol:dichloromethane (12/13, *v*/*v*) -liposomes 20 mg/mL different methods of preparation: thin-film dispersion, reversed-phase evaporation, supercritical fluid technology)	Oral administration to male Wistar rats (20 mg/kg)	Supercritical fluid technology -> adequate for liposomes preparation (the best stability of liposomes, the highest entrapment efficiency and drug loading, the smallest particle dimension); Enhanced *in vitro* drug release; Increased oral bioavailability	[163]

Table 2. Silymarin formulations.

http://dx.doi.org/10.5772/67647

Paclitaxel formulations	Components	Method of application	Indications/Remarks	Ref.
Paclitaxel (microemulsion/Taxol®)+ silymarin	-Taxol® -paclitaxel microemulsion -silymarin *(insufficient data)*	Oral administration to rats: -Taxol® +10–20 mg/kg silymarin; -paclitaxel microemulsion +20 mg/kg silymarin	Silymarin association increased the oral bioavailability of paclitaxel; The highest absolute bioavailability (19%) -> for silymarin + paclitaxel microemulsion; Faster absorption for paclitaxel microemulsion compared to Taxol®	[164]
Paclitaxel (nanoemulsion) + curcumin	-paclitaxel nanoemulsion -curcumin *(insufficient data)*	Oral administration to SKOV3 tumor-bearing *nu/nu* mice (50 mg/kg curcumin +20 mg/kg paclitaxel)	5.2-fold increased oral relative bioavailability and anti-tumor activity of paclitaxel (3.2-fold enhanced accumulation in the tumor tissue)	[165]
Paclitaxel (Genetaxyl®) + cyclosporin A	Genetaxyl® (paclitaxel 6 mg/mL) + cyclosporin A; Genetaxyl®: -20% (*v/v*) Cremophor EL Genaxol®: -50% (*v/v*) Cremophor EL	Oral administration to cancer patients (60/120/180 mg/m$^{2)}$ +oral cyclosporin A, 10 mg/kg IV: 175 mg/m^2, 3 h infusion	Less than proportional increase in systemic exposure with increasing doses; Limited paclitaxel bioavailability by micellar entrapment; Cremophor EL ↓ concentration: -↓ median time to peak concentration; -↑ absorption of paclitaxel; -↑ metabolism of paclitaxel	[166]
Paclitaxel + Cremophor EL	Taxol®: Paclitaxel in Cremophor EL – dehydrated ethanol (1:1, *v/v)*	Intraperitoneal administration to cancer patients	↓ Plasma concentration and bioavailability; Prolonged peritoneal activity	[167]
Crystalline nanosuspension	-paclitaxel (20 mg/mL): -0.1% (*w/w*) Cremophor EL in phosphate saline	IV administration to tumor bearing xenograft female SCID-beige mice (20 mg/kg)	Weaker antiproliferative effects than the Cremophor standard formulations	[168]
Self micro emulsifying oily formulations (SMEOFs) +cyclosporine A	*Paclitaxel 1.5% (% w/v):* -paclitaxel 1.5 % -vitamin E 5.00%	-Oral administration to wild-type and P-glycoprotein knockout mice [169]; -oral administration to humans [170]	Satisfying oral bioavailability -> the systemic exposure and oral bioavailability of paclitaxel was comparable to Taxol® (40%);	[169], [170]

http://dx.doi.org/10.5772/67647

Paclitaxel formulations	Components	Method of application	Indications/Remarks	Ref.
	-TPGS 29.95% (d-alpha-tocopheryl polyethylene glycol 1000 succinate) -tyloxapol 33.05% -ethanol 30.5% *Paclitaxel 3% (% w/v):* -paclitaxel 3.0% -vitamin E 5.0% -TPGS 29.45% -tyloxapol 32.55% -ethanol 30%		Higher dose of paclitaxel->less effective uptake (saturation of the first-pass metabolism); SMEOF = suitable vehicle for oral administration of paclitaxel associated with cyclosporine A	
Supersaturable self-emulsifying drug delivery system of paclitaxel (S-SEDDS) containing HPMC (precipitation inhibitor); +cyclosporine A	*-lack of data*	Oral administration to male Sprague-Dawley rats (10 mg/kg paclitaxel; 5 mg/kg cyclosporine A)	5-fold higher oral bioavailability, 10-fold higher maximum concentration for S-SEDDS formulation compared to Taxol®; Association of cyclosporin A=> further increase in oral bioavailability; Slow crystallization of paclitaxel (*in vitro* dilution)	[171]
Solid-nanoemulsion preconcentrate (surfactant-co-surfactant system based on Tween 20 and PEG)	*Nanoemulsion preconcentrate:* -50 mg paclitaxel - 1 mL of different combinations of oil, surfactant, and cosurfactant; Optimized formulation: oil:surfactant:cosurfactant (μL) - 175:350:175; *Nanocarrier:* -Oil: -propylene glycol monocaprylate/ glycerol monooleate, 4:1 (*w/w*), -Surfactant: -polyoxyethylene 20 sorbitan monooleate/ polyoxyl 15 hydroxystearate, 1:1 (*w/w*),	Oral administration to Wistar albino rats (10 mg/kg)	Increased oral bioavailability; High cumulative *in vitro* drug release; Significant antiproliferative effect on breast cancer cell line MCF7	[172]

Paclitaxel formulations	Components	Method of application	Indications/Remarks	Ref.
	-Cosurfactant: -diethylene glycol monoethyl ether/ polyethylene glycol 300, 1:1(*w/w*)			
Niosomal formulation: Niosomes with nonionic surfactants	*Organic phase:* -Span 40 (M) – 0.0475 -cholesterol (M) – 0.0475 -DCP (dicetyl phosphate) (M) – 0.005 -paclitaxel (mM) – 0.234 -chloroform (Ml) – 10; *Water phase:* -ultrapure water (mL) q.s. 10 (niosomes prepared through film hydration method)	*In vitro* studies	Diffusion controlled release; Low toxicologic profile; Gastrointestinal stability Suitable formulation for oral drug delivery	[173]
Paclitaxel-loaded pegylated ethosomes	-paclitaxel -cholesterol -PEG3350 -phosphatidylcholine (1:6.5:9:28, *w/w*) -deionized water and ethanol 99.4% (67.5:32.5, *v/v*) (pegylated ethosomes prepared by reverse phase evaporation technique)	*In vitro* studies	Longer half life, slower release rate; 4.5-fold increase in cytotoxicity on human melanoma SK-MEL-3 cell line, in comparison with the free drug; Possible alternative to the conventional therapy	[174]
Paclitaxel-loaded lipid nanocapsules	(mg/g): - 1.9 paclitaxel - 51.9 Solutol®HS15 -842.6 water - 90.7 Captex® 8000 - 8.1 Lipoid® S100-3 - 4.8 NaCl powder	Oral administration to male Sprague-Dawley rats (10 mg/kg)	3-fold higher oral bioavailability (same results for Taxol®-verapamil (=P-glycoprotein inhibitor) association); Potential formulation to augment the oral bioavailability of the drug, avoiding verapamil association	[175]
Solid lipid nanoparticles surface-modified with hydroxypropyl-β-cyclodextrin (HPCD)	- 100 mg stearic acid - 5 mg paclitaxel -0.25 mL ethanol - 75 mg lecithin - 75 mg poloxamer 188	Oral administration to male Sprague-Dawley male rats (25 mg/kg)	Oral administration: -fast release of paclitaxel (nearly complete release); -high cellular uptake by	[176]

Paclitaxel formulations	Components	Method of application	Indications/Remarks	Ref.
	- 3 mL distilled water -HPCD (modified hot sonication method of preparation)		Caco-2 cells; -high concentrations in the lymph nodes (increased bioavailability); Role of HPCD in reducing the nanoparticle size and enhancing the solubility and dissolution of the drug; HPCD may inhibit P-glycoprotein; Alternative formulation for oral administration of the drug	
Solid lipid nanoparticles (=specific colloidal carriers)	-paclitaxel: stearic acid+DPPG.NA – 1:10, 1:15, 1:20 (micro-emulsification technique)	Female Swiss Albino mice (7.5 mg/kg)	Increased anticancer effect; Increased specificity on breast cancer cells (for 1:20 formulation); Release profile delayed with the enhancement of lipid concentration; Entrapment efficiency, particle size and zeta potential value raised as lipid concentration was augmented	[177]
Solid lipid nanoparticles modified with 2-hydroxypropyl-β-cyclodextrin	- 5 mg paclitaxel - 100 mg stearic acid -0.25 mL ethanol - 75 mg lecithin - 75 mg poloxamer 188 - 400 mg HPCD (hot-melted sonication technique)	Intratumoral administration in female BALB/c nude mice for evaluation of antitumor activity (10 mg/kg) IV administration to male Sprague Dawley rats for pharmacokinetic study (5 mg/kg)	*IV delivery:* -diminished burst release; -low nephrotoxicity; -prolonged antiproliferative activity, increased absorption; *Intratumoral administration:* -high antitumor efficiency (sustained release, high cellular uptake); -reduced toxicity to normal organs; Potential therapeutic formulation for breast cancer with reduced nephrotoxicity	[178]
Biocompatible and biodegradable polymeric nanoparticles	*Organic phase:* -1 mg paclitaxel	*In vitro* studies	Initial burst release followed by 7-days sustained release;	[179]

Paclitaxel formulations	Components	Method of application	Indications/Remarks	Ref.
	- PLGA – 2%, 3%, 4% (*w*/*v*) (Poly(lactic-co-glycolic acid)) -10 mL acetone; *Aqueous phase:* -PVA (polyvinyl alcohol) 1%, 3%, 5% (*w*/*v*) (emulsification-solvent diffusion method)		Higher antiproliferative activity on Caco-2-cell-line when compared to blank nanoparticles but similar to paclitaxel solution	
Stabilized Pluronic micelles	-*lack of data* (solvent/evaporation technique)	Oral/IV administration in rats/mice	Prolonged gastrointestinal release; High oral absorption; Optimized pharmacokinetic profile after IV administration; *Indication*: delivery vehicles (carriers) for oral or intravenous paclitaxel administration	[180]

Table 3. Paclitaxel formulations.

Resveratrol formulations	Components	Method of application	Indications/remarks	Ref.
Liposomal formulation	-DPPC (dipalmitoyl-phosphatidylcholine) -PEG_{2000}-DSPE –poly(ethyleneglycol)-distearoylphosphatidyl ethanolamine) -cholesterol dissolved in ethanol, 1.85:0.15:1 (molar ratio) -resveratrol (maximum incorporation): ~5 mg/μmol of total lipid (equivalent to 10 mg/μmol total lipids added).	IV administration to nude Balb/c female mice (murine tumor model); 5 mg/kg	Inhibition of tumor growth by 70%; Improved chemical stability (prevention of inactivating cis-trans isomerization)	[181]
β-cyclodextrin-based nanosponges (BNS) formulation	-1:5 and 1:10 (*w/w*) resveratrol:BNS; -1:2 and 1:4 (molar ratio) cyclodextrin:cross- linker (carbonyldiimidazole)/BNS	*In vitro* studies	Increased release and stability (in comparison with plain drug); Improved cytotoxicity on HCPC-I cell; Good penetration in pigskin; Better accumulation in rabbit mucosa. *Indication*: buccal and topical administration	[182]
Monodisperse cyano-functionalized porous polymeric microspheres	- 4 g cyano-functionalized porous particles -0.2 *wt*% Tween 20 aqueous solution (86g) -0.2 g resveratrol - 10 g ethanol; *Cyano-functionalized porous particles:* *a. Seed dispersion:* -0.50 g polystyrene - 40 g 0.25 *wt*% sodium lauryl sulfate in water/EtOH (5/1, *w/w*) solution (=SE solution); *b. CD (Chlorododecane) swelling:* -0.5 g 1-CD - 10 g SE solution; *c. The second monomer swelling:* -7 g/5 g/2 g styrene -1 g/3 g/6 g divinylbenzene - 2 g acrylonitrile -10 g toluene/heptane (porogen): 0/100, 30/70,	*In vitro* studies	Antioxidant activity preserved for 5 weeks	[183]

http://dx.doi.org/10.5772/67647

Resveratrol formulations	Components	Method of application	Indications/remarks	Ref.
	60/40, 100/0 *wt%* -0.1 g benzoyl peroxide - 40 g SE solution; *d. Stabilization:* -50 g polyvinyl alcohol 5% solution (seeded polymerization technique)			
Encapsulation within yeast cell	*Lack of data*	*In vitro* studies	Increased DPPH-radical scavenging activity; Good stability, enhanced bioavailability (increased solubility, sustained release)	[184]
Zinc pectinate beads	Zinc acetate = cross-linking agent; -cross-linking solution pH 1.5 -zinc acetate concentration 5% (% *w/v*) -crosslinking time 0.5 h -drying at room temperature -pectin concentration 5% (% *w/v*) -pectin to resveratrol ratio of 3:1 (ionotropic gelation method)	*In vitro* studies	Better delayed release for zinc-pectinate beads than calcium-pectinate beads; Encapsulation of a great amount of resveratrol in zinc-pectinate beads	[185]
Zinc-pectin-chitosan composite microparticles	-pectin:resveratrol, 3:1 -1% chitosan	*In vivo* studies on rats	Specific drug release in the colon	[186]
Resveratrol+quercetin+curcumin	-quercetin -resveratrol -curcumin (applied alone/in combination, at 50 μM)	*In vitro* studies	Increased oral absorption of resveratrol and curcumin, without interfering with the absorption of quercetin	[187]

Table 4. Resveratrol formulations.

http://dx.doi.org/10.5772/67647

Author details

Danciu Corina[1]*, Soica Codruta[2], Antal Diana[3], Alexandra Popescu[4], Roxana Ghiulai[2], Ioana Zinuca Pavel[1], Stefana Avram[1], Minda Daliana[1] and Cristina Dehelean[5]

*Address all correspondence to: corina.danciu@umft.ro

1 Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania

2 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania

3 Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania

4 Department of Physical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania

5 Department of Toxicology, Faculty of Pharmacy, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania

References

[1] Almeida CA, Barry SA. Cancer basic science and clinical aspects, 1st ed. West Sussex, UK: Wiley-Blackwell; 2010, pp. 262–293.

[2] Leiter U, Eigentler T, Garbe C. Epidemiology of skin cancer. Adv Exp Med Biol. 2014; **810**:120–140.

[3] Lomas A, Leonardi-Bee J, Bath-Hextall F. A systematic review of worldwide incidence of nonmelanoma skin cancer. Br J Dermatol. 2012;**166**:1069–1080.

[4] Wehner MR, Shive ML, Chren MM, Han J, Qureshi AA, Linos E. Indoor tanning and non-melanoma skin cancer: systematic review and meta-analysis. BMJ. 2012;**345**:e5909. doi:10.1136/bmj.e5909.

[5] Feller L, Khammissa RA, Kramer B, Altini M, Lemmer J. Basal cell carcinoma, squamous cell carcinoma and melanoma of the head and face. Head Face Med. 2016;**12**(1):11. doi:10.1186/s13005-016-0106-0.

[6] Reichrath J, Rass K. Ultraviolet damage, DNA repair and vitamin D in nonmelanoma skin cancer and in malignant melanoma: an update. Adv Exp Med Biol. 2014;**810**:208–233.

[7] Ichihashi M, Ueda M, Budiyanto A, Bito T, Oka M, Fukunaga M, Tsuru K, Horikawa T. UV-induced skin damage. Toxicology. 2003;**189**(1–2):21–39.

[8] Zhan JY, Wang XF, Liu YH, Zhang ZB, Wang L, Chen JN, Huang S, Zeng HF, Lai XP. Andrographolide sodium bisulfate prevents UV-induced skin photoaging through inhibiting oxidative stress and inflammation. Mediators Inflamm. 2016;**2016**:3271451. doi:10.1155/2016/3271451.

[9] Leiter U, Garbe C. Epidemiology of melanoma and non-melanoma skin cancer: the role of sunlight. Adv Exp Med Biol. 2008;**624**:89–103. doi:10.1007/978-0-387-77574-6_8.

[10] Gonzales M, Erdei E, Berwick M. Epidemiology of skin cancer. In: Nouri K, editor. Skin cancer. New York, USA: McGraw-Hill; 2008, pp. 32–38. doi:10.1036/0071472568.

[11] Muenyi CS, Ljungman M, States JC. Arsenic disruption of DNA damage responses-potential role in carcinogenesis and chemotherapy. Biomolecules. 2015;**5**(4):2184–2193. doi:10.3390/biom5042184.

[12] Maiorino A, De Simone C, Perino F, Caldarola G, Peris K. Melanoma and non-melanoma skin cancer in psoriatic patients treated with high-dose phototherapy. J Dermatolog Treat. 2016;**27**(5):443-7. doi: 10.3109/09546634.2015.1133882. [Epub 2016 Jan 28].

[13] Archier E, Devaux S, Castela E, Gallini A, Aubin F, Le Maître M, Aractingi S, Bachelez H, Cribier B, Joly P, Jullien D, Misery L, Paul C, Ortonne JP, Richard MA. Carcinogenic risks of psoralen UV-A therapy and narrowband UV-B therapy in chronic plaque psoriasis: a systematic literature review. J Eur Acad Dermatol Venereol. 2012;**26**(Suppl 3):22–31. doi:10.1111/j.1468-3083.2012.04520.x.

[14] Nouri K, Ballard CJ, Patel AR, Anadolu-Brasie R. Basal cell carcinoma. In: Nouri K, editor. Skin cancer. New York, USA: McGraw-Hill; 2008, pp. 61–85. doi:10.1036/0071472568.

[15] Alter M, Hillen U, Leiter U, Sachse M, Gutzmer R. Current diagnosis and treatment of basal cell carcinoma. J Dtsch Dermatol Ges. 2015;**13**(9):863–75. doi:10.1111/ddg.12798.

[16] Griffin LL, Ali FR, Lear JT. Non-melanoma skin cancer. Clin Med (Lond). 2016;**16**(1):62–65. doi:10.7861/clinmedicine.16-1-62.

[17] Anadolu-Brasie R, Patel AR, Patel SS, Singh A, Nouri K. Squamous cell carcinoma of the skin. In: Nouri K, editor. Skin cancer. New York, USA: McGraw-Hill; 2008, pp. 86–114. doi:10.1036/0071472568.

[18] van der Pols JC. Epidemiology of basal cell and squamous cell carcinoma of the skin. In: Dummer R, Pittelkow MR, Iwatsuki K, Green A, Elwan NM, editors. Skin cancer-a worldwide perspective. Verlag-Berlin-Heidelberg, Germany: Springer; 2011, pp. 3–12. doi:10.1007/978-3-642-05072-5.

[19] Bahner JD, Bordeaux JS. Non-melanoma skin cancers: photodynamic therapy, cryotherapy, 5-fluorouracil, imiquimod, diclofenac, or what? Facts and controversies. Clin Dermatol. 2013;**31**(6):792–798. doi:10.1016/j.clindermatol.2013.08.020.

[20] Tran C, Sorg O, Carraux P, Didierjean L, Saurat JH. Topical delivery of retinoids counteracts the UVB-induced epidermal vitamin A depletion in hairless mouse. Photochem Photobiol. 2001;**73**(4):425–431.

[21] Bosserhoff A, Strizzi L. Introduction. In: Bosserhoff A, editor. Melanoma development-molecular biology, genetics and clinical application. Wien and New York: Springer; 2011, pp. 1–7. doi:10.1007/978-3-7091-0371-5.

[22] Barnhill RL, Mihm MC, Elgart G. Malignant melanoma. In: Nouri K, editor. Skin cancer. New York, USA: McGraw-Hill; 2008, pp. 140–167. doi:10.1036/0071472568.

[23] Berwick M. Melanoma epidemiology. In: Bosserhoff A, editor. Melanoma development-molecular biology, genetics and clinical application. Wien and New York: Springer; 2011, pp. 35–57. doi:10.1007/978-3-7091-0371-5.

[24] Lazovich D, Isaksson Vogel R, Weinstock MA, Nelson HH, Ahmed RL, Berwick M. Association between indoor tanning and melanoma in younger men and women. JAMA Dermatol. 2016. doi:10.1001/jamadermatol.2015.2938. [Epub ahead of print].

[25] Wolf Horrell Erin M., Wilson Kalin, D'Orazio John A. Melanoma: epidemiology, risk factors, and the role of adaptive pigmentation. In: Murph M, editor. Melanoma: current clinical management and future therapeutics. Rijeka, Croatia: InTech; 2015, pp. 1–19, ISBN 978-953-51-2036-0, doi:10.5772/58994.

[26] Rigel, D.S. The effect of sunscreen on melanoma risk. Dermatol Clin. 2002;**20**(4):601–606.

[27] Cancer facts & figures. Atlanta: American Cancer Society Inc.; 2015. https://www.cancer.org

[28] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;**136**(5):E359–E386. doi:10.1002/ijc.29210.

[29] Barbaric J, Sekerija M, Agius D, Coza D, Dimitrova N, Demetroiu A, Diba CS, Eser S, Gavric Z, Zakelj MP, Zivkovic S, Zvolsky M, Bray F, Coebergh JW, Znoar A. Disparities in melanoma incidence and mortality in South-Eastern Europe: increasing incidence and divergent mortality patterns. Is progress around the corner? EJC. 2016;**55**:47–55. doi:10.1016/j.ejca.2015.11.019.

[30] Parichy DM, Reedy MV, Erickson CA. Regulation of melanoblast migration and differentiation. In: Nordland JJ, Boissy RE, Hearing VJ, King RA, Ortonne JP, editors. The pigmentary system: physiology and pathophysiology, 2nd ed. Oxford University Press; 2006, pp. 108–139. doi:10.1002/9780470987100.ch5.

[31] Kalabis MF, Martinez G, Telson SM, Liu ZJ, Balint K, Juhasz I, Elder DE, Perbal B, Herlyn M. Downregulation of CCN3 expression as a potential mechanism for melanoma progression. Oncogene. 2008;**27**:2552–2560. doi:10.1038/sj.onc.1210896.

[32] Haass NK, Smalley KS, Li L, Herlyn M. Adhesion, migration and communication in melanocytes and melanoma. Pigment Cell Res. 2005;**18**:150–159. doi:10.1111/j.1600-0749.2005.00235.x.

[33] Maillard M, Cadot B, Ball RY, Sethia K, Edwards DR, Perbal B, Tatoud R. Differential expression of the ccn3 (nov) protooncogene in human prostate cell lines and tissues. Mol Pathol. 2001;**54**:275–280. doi:10.1136/mp.54.4.275.

[34] Glukhova L, Angevin E, Lavialle C, Cadot B, Terrier-Lacombe MJ, Perbal B, Bernheim A, Goguel AF. Patterns of specific genomic alterations associated with poor prognosis in highgrade renal cell carcinomas. Cancer Genet Cytogenet. 2001;**130**:105–110. doi:10.1016/S0165-4608(01)00477-0.

[35] Manara MC, Perbal B, Benini S, Strammiello R, Cerisano V, Perdichizzi S, Serra M, Astolfi A, Bertoni F, Alami J, Yeger H, Picci P, Scotlandi K. The expression of CCN3 (nov) gene in musculoskeletal tumors. Am J Pathol. 2002;**160**:849–859. doi:10.1016/S0002-9440(10)64908-5.

[36] Gupta N, Wang H, McLeod TL, Naus CC, Kyurkchiev S, Advani S, Yu J, Perbal B, Weichselbaum RR. Inhibition of glioma cell growth and tumorigenic potential by CCN3 (NOV). Mol Pathol. 2001;**54**:293–299. doi:10.1136/mp.54.5.293.

[37] McCallum L, Price S, Planque N, Perbal B, Pierce A, Whetton AD, Irvine AE. A novel mechanism for BCR-ABL action: stimulated secretion of CCN3 is involved in growth and differentiation regulation. Blood. 2006;**108:**1716–1723. doi:10.1182/blood-2006-04-016113.

[38] Fukunaga-Kalabis M, Martinez G, Liu ZJ, Kalabis J, Mrass P, Weninger W, Firth SM, Planque M, Perbal B, Herlyn M. CCN3 controls 3D spatial localization of melanocytes in the human skin through DDR1. J Cell Biol. 2006;**175**:563–569. doi:10.1083/jcb.200602132.

[39] Haass NK, Smalley KS, Herlyn M. The role of altered cell–cell communication in melanoma progression. J Mol Histol. 2004;**35**:309–318. doi:10.1023/B:HIJO.0000032362.35354.bb.

[40] Weber B, Stresemann C, Brueckner B, Lyko F. Methylation of human microRNA genes in normal and neoplastic cells. Cell Cycle. 2007;**6**:1001–1005.

[41] Esteller M. Epigenetics in cancer. Eng J Med. 2008;**358**:1148–1159. doi:10.1056/NEJMra072067.

[42] Polsky D, Cordon Carlo C. Oncogenes in melanoma. Oncogene. 2003;**22**:3087–3091. doi:10.1038/sj.onc.1206449.

[43] Zigmund M, Nikuseva-Martic T, Cacic M, Pecina-Slaus N. New insights on genetics of malignant melanoma. Lijec Vjesn. 2005;**127**:89–93.

[44] Hashemi J, Platz A, Ueno T, Stierner V, Ringborn V, Hansson J. CDKN2A germ-line mutations in individuals with multiple cutaneous melanomas. Cancer Res. 2000;**60**: 6864–6867.

[45] Wang J, Shen WH, Jin YJ, Brand-Rauf PW, Yin Y. A molecular link between E2F-1 and the MAPK cascade. J Biol Chem. 2007;**282**:18521–18531.

[46] Eskandapour M, Hashemi J, Kanter L, Ringborg U, Platz A, Hansson J. Frequency of UV-inducible NRAS mutations in melanomas of patients with germline CDKN2A mutations. J Natl Cancer Inst. 2005;**95**:790–798.

[47] Boukamp P. UV-induced skin cancer: similarities – variations. JDDG. 2005;**3**:493–503. doi:10.1111/j.1610-0387.2005.05037.x.

[48] Chin L. The genetics of malignant melanoma: lessons from mouse and man. Nature Rev. 2003;**3**:559–570.

[49] HA L, Ichikawa T, Anver M, Dickins R, Lowe S, Sharpless NE, Krimpenfort P, DePinho RA, Bennett DC, Sviderskaya EV, Merlino G. ARF functions as a melanoma tumor suppressor by inducing p53-independent senescence. PNAS. 2002;**104**:10968–10973. doi:10.1073/pnas.0611638104.

[50] Rees JL. The genetics of sun sensitivity in humans. Am J Hum Genet. 2004;**75**:739–751. doi:10.1086/425285.

[51] Duffy DL, Box NF, Chen W, Palmer JS, Montgomery GW, James MR, Hairward NK, Martin NG, Sturm RA. Interactive effects of MC1R and OCA2 on melanoma risk phenotypes. Hum Mol Genet. 2004;**13**:447–461. doi:10.1093/hmg/ddh043.

[52] Van der Velden PA, Sandkuijl LA, Bergman W, Pavel S, Van mourik L, Frants RR, Gruis NA. Melanocortin-1 receptor variant R151C modifies melanoma risk in Dutch families with melanoma. Am J Hum Genet. 2001;**69**:774–779.

[53] Gao L, Zhao H, Cornelius LA. The molecular mechanisms of melanoma tumorigeneis: an update. G Ital Dermatol Venereol. 2007;**142**:71–82.

[54] Abbasi NR, Shaw HM, Rigel DS, et al. Early diagnosis of cutaneous melanoma: revisiting the ABCD criteria. JAMA. 2004;**292**(22):2771–2776. doi:10.1001/jama.292.22.2771.

[55] Morton DL, Thompson JF, Cochran AJ, Mozzillo N, Elashoff R, et al. Sentinel-node biopsy or nodal observation in melanoma. N Engl J Med. 2006;**355**:1307–1317. doi:10.1056/NEJMoa060992.

[56] Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009;**27**:6199–6206. doi:10.1200/JCO.2009.23.4799.

[57] Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg. 1970;**172**:902–908.

[58] Grande Sarpa H, Reinke K, Shaikh L, Leong SP, Miller JR, et al. Prognostic significance of extent of ulceration in primary cutaneous melanoma. Am J Surg Pathol. 2006;**30**:1396–1400.

[59] Francken AB, Shaw HM, Thompson JF. The prognostic importance of tumor mitotic rate confirmed in 1,317 patients with primary cutaneous melanoma and long follow-up. Ann. Surg. Oncol. 2004;**11**:426–433.

[60] Gown AM, Vogel AM, Hoak D, Gough F, McNutt MA. Monoclonal antibodies specific for melanocytic tumors distinguish subpopulations of melanocytes. Am J Pathol. 1986;**123**(2):195–203.

[61] Fernando SS, Johnson S, Bate J. Immunohistochemical analysis of cutaneous malignant melanoma: comparison of S-100 protein, HMB-45 monoclonal antibody and NKI/C3 monoclonal antibody. Pathology. 1994;**26**(1):16–19.

[62] Ordonez NG, Ji XL, Hickey RC. Comparison of HMB-45 monoclonal antibody and S-100 protein in the immunohistochemical diagnosis of melanoma. Am J Clin Pathol. 1988;**90**:385–390.

[63] Chen YT, Stockert E, Jungbluth A, et al. Serological analysis of Melan-A(MART-1), a melanocyte-specific protein homogeneously expressed in human melanomas. Proc Natl Acad Sci U S A. 1996;**93**(12):5915–5919.

[64] Kawakami Y, Eliyahu S, Delgado CH, et al. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci U S A. 1994;**91**:3515–3519.

[65] Weinstein D, Leininger J, Hamby C, Safai B. Diagnostic and prognostic biomarkers in melanoma. J Clin Aesthet Dermatol. 2014;**7**(6):13–24.

[66] Bonfrer JM, Korse CM, Nieweg OE, Rankin EM. The luminescence immunoassay S-100: a sensitive test to measure circulating S-100B: its prognostic value in malignant melanoma. Br J Cancer. 1998;**77**(12):2210–2214.

[67] Nakajima T, Watanabe S, Sato Y, et al. Immunohistochemical demonstration of S100 protein in malignant melanoma and pigmented nevus, and its diagnostic application. Cancer. 1982;**50**:912–918.

[68] Hofbauer GF, Kamarashev J, Geertsen R, et al. Tyrosinase immunoreactivity in formalin-fixed, paraffin-embedded primary and metastatic melanoma: frequency and distribution. J Cutan Pathol. 1998;**25**:204–249.

[69] Gerdes J, Lemke H, Baisch H, et al. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol. 1984;**133**:1710–1715.

[70] Gimotty PA, Van Belle P, Elder DE, Murry T, et al. Biologic and prognostic significance of dermal Ki67 expression, mitoses, and tumorigenicity in thin invasive cutaneous melanoma. JCO. 2005;**23**(31):8048–8056. doi:10.1200/JCO.2005.02.0735.

[71] Akiyama M, Matsuda Y, Ishiwata T, Naito Z, Kawana S. Nestin is highly expressed in advanced-stage melanomas and neurotized nevi. Oncol Rep. 2013;**29**(4):1595–1599.

[72] Lai S, Piras F, Mura E, Spiga S, et al. Nestin and vimentin intermediate filaments expression in cutaneous melanoma. Ital J Anat Embryol. 2011;**116**(2):96. doi:10.13128/IJAE-10066.

[73] Gross A, Niemetz-Rahn A, Nonnenmacher A, Tucholski J, et al. Expression and activity of EGFR in human cutaneous melanoma cell lines and influence of vemurafenib on the EGFR pathway. Target Oncol. 2015;**10**(1):77-84. doi:10.1007/s11523-014-0318-9.

[74] Boone B, Jacobs K, Ferdinande L, Taildeman J, Lambert J, Peeters M, Bracke M, Pauwels P. Brochez L. EGFR in melanoma: clinical significance and potential therapeutic target. J Cutan Pathol. 2011;**38**:492–502. doi:10.1111/j.1600-0560.2011.01673.x.

[75] Surget S, Khoury MP, Bourdon JC. Uncovering the role of p53 splice variants in human malignancy: a clinical perspective. OncoTargets Therapy. 2013;**7**:57–68. doi:10.2147/OTT.S53876.

[76] Houben R, Hesbacher S, Schmid CP, et al. High-level expression of wild-type p53 in melanoma cells is frequently associated with inactivity in p53 reporter gene assays. PLoS One. 2011;**6**(7):e22096. doi:10.1371/journal.pone.0022096.

[77] Palmer SR, Erickson LA, Ichetovkin I, Knauer DJ, Markovic SN. Circulating serologic and molecular biomarkers in malignant melanoma. Mayo Clinic Proc. 2011;**86**(10):981–990. doi:10.4065/mcp.2011.0287.

[78] Verykiou S, Ellis RA, Lovat PE. Established and emerging biomarkers in cutaneous malignant melanoma. Healthcare. 2014;**2**(1):60–73. doi:10.3390/healthcare2010060.

[79] Wan PTC, Garnett MJ, Roe SM, Lee S, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004;**116**(6):855–867. doi:10.1016/S0092-8674(04)00215-6.

[80] Hugdahl E, Kalvenes MB, Puntervoll HE, Ladstein RG, Akslen LA. BRAF-V600E expression in primary nodular melanoma is associated with aggressive tumor features and reduced survival. Br J Cancer. 2016. doi:10.1038/bjc.2016.44.

[81] Wang P, Guan P, Xu S, Wang Z, et al. Emerging GM3 regulated biomarkers in malignant melanoma, recent advances in the biology, therapy and management of melanoma. In: Dr. Lester Davids, Editor, Melanoma — Epidemiology, Genetics and Risk Factors. Rijeka, Croatia: InTech; 2013. doi:10.5772/54941.

[82] Karagiannis P, Fittall M, Karagiannis SN. Evaluating biomarkers in melanoma. Front Oncol. 2014;**4**:383. doi:10.3389/fonc.2014.00383.

[83] Kumar S, Sharma P, Kumar D, Chakraborty G, Gorain M, Kundu GC. Functional characterization of stromal osteopontin in melanoma progression and metastasis. PLoS One. 2013;8(7):e69116. doi:10.1371/journal.pone.0069116.

[84] Gogas H, Eggermont AMM, Hauschild A, et al. Biomarkers in melanoma. Ann Oncol. 2009;**20**(Suppl 6):vi8–vi13. doi:10.1093/annonc/mdp251.

[85] Boiko AD, Razorenova OV, van de Rijn M, et al. Human melanoma initiating cells express neural crest nerve growth factor receptor CD271. Nature. 2010;**466**(7302):133–137. doi:10.1038/nature09161.

[86] Cragg GM., Newman DJ. Natural products: a continuing source of novel drug leads. Biochim Biophys Acta. 2013;**1830**(6):3670–3695.

[87] AlQathama A, Prieto JM. Natural products with therapeutic potential in melanoma metastasis. Nat Prod Rep. 2015, **32**(8):1170–1182.

[88] Pal HC, Hunt KM, Diamond A, Elmets CA, Afaq F. Phytochemicals for the management of melanoma. Mini Rev Med Chem. 2016;**16**:1–27. doi:10.2174/ 1389557516666160211120157.

[89] Chinembiri TN, du Plessis LH, Gerber M, Hamman JH, du Plessis J. Review of natural compounds for potential skin cancer treatment. Molecules. 2014;**19**:11679–11721.

[90] Menon LG, Kuttan R, Kuttan G. Inhibition of lung metastasis in mice induced by B16F10 melanoma cells by polyphenolic compounds. Cancer Lett. 1995;**95**(1–2):221–225.

[91] Steinmetz KA, Potter JD. Vegetables, fruit and cancer. II. Mechanisms. Cancer Causes Control. 1991;**2**(6):427–442.

[92] Solovchenko A., Merzlyak M. Optical properties and contribution of cuticle to UV protection in plants: experiments with apple fruit. Photochem Photobiol Sci. 2003;**2**:861–866.

[93] Liu-Smith F., Meyskens F. Molecular mechanisms of flavonoids in melanin synthesis and the potential for the prevention and treatment of melanoma. Mol Nutr Food Res. 2016. doi:10.1002/mnfr.201500822.

[94] Dzialo M, Mierziak J, Korzun U, Preisner M, Szopa J, Kulma A. The potential of plant phenolics in prevention and therapy of skin disorders. Int J Mol Sci. 2016;**17**(2). doi:10.3390/ijms17020160.

[95] Rafiq RA, Quadri A, Nazir LA, Peerzada K, Ganai BA, Tasduq SA. A potent inhibitor of phosphoinositide 3-kinase (PI3K) and mitogen activated protein (MAP) kinase signalling, quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone) promotes cell death in ultraviolet (UV)-B-irradiated B16F10Melanoma cells. PLoS One. 2015;**10**(7):e0131253.

[96] Chaiprasongsuk A., Onkoksoong T, Pluemsamran T, Limsaengurai S, Panich U. Photoprotection by dietary phenolics against melanogenesis induced by UVA through Nrf2-dependentantioxidantresponses. Redox Biol. 2016;**8**:79–90.

[97] Cao HH, Cheng CY, Su T, Fu XQ, Guo H, Li T, Tse AK, Kwan HY, Yu H, Yu ZL. Quercetin inhibits HGF/c-Met signaling and HGF-stimulated melanoma cell migration and invasion. Mol Cancer. 2015;**14**:103.

[98] Saewan N., Jimtaisong A. Photoprotection of natural flavonoids. J Appl Pharm Sci. 2013;**3**(9):129–141.

[99] Cao HH, Chu JH, Kwan HY, Su T, Yu H, Cheng CY, Fu XQ, Guo H, Li T, Tse AK, Chou GX, Mo HB, Yu ZL. Inhibition of the STAT3 signaling pathway contributes to apigenin-mediated anti-metastatic effect in melanoma. Sci Rep. 2016;**6**:21731. doi:10.1038/srep21731.

[100] Hasnat MA, Pervin M, Lim JH, Lim BO. Apigenin attenuates melanoma cell migration by inducing anoikis through integrin and focal adhesion kinase inhibition. Molecules. 2015;**20**(12):21157–21166. doi:10.3390/molecules201219752.

[101] Kiraly AJ, Soliman E, Jenkins A, Van Dross RT. Apigenin inhibits COX-2, PGE2, and EP1 and also initiates terminal differentiation in the epidermis of tumor bearing mice. Prostaglandins Leukot Essent Fatty Acids. 2016;**104**:44–53. doi:10.1016/j.plefa.2015.11.006.

[102] Ghosh A., Ghosh T, Jain S. Silymarin – a review on the pharmacodynamics and bioavailability enhancement approaches. J Pharm Sci Technol. 2010;**2**(10):348–355.

[103] Lee MH, Huang Z, Kim DJ, Kim SH, Kim MO, Lee SY, Xie H, Park SJ, Kim JY, Kundu JK, Bode A, Surh YJ, Dong Z. Direct targeting of MEK1/2 and RSK2 by silybin induces cell-cycle arrest and inhibits melanoma cell growth. Cancer Prev Res (Phila). 2013;**6**(5):455–465. doi:10.1158/1940-6207.

[104] Vaid M, Singh T, Prasad R, Katiyar SK. Silymarin inhibits melanoma cell growth both in vitro and in vivo by targeting cell cycle regulators, angiogenic biomarkers and induction of apoptosis. Mol Carcinog. 2015;**54**(11):1328–1339.

[105] Gajos-Michniewicz A, Czyz M. Modulation of WNT/β-catenin pathway in melanoma by biologically active components derived from plants. Fitoterapia. 2016;**109**:283–292.

[106] Katiyar S, Elmets CA, Katiyar SK. Green tea and skin cancer: photoimmunology, angiogenesis and DNA repair. J Nutr Biochem. 2007;**18**(5):287–296.

[107] Nihal M, Ahmad N, Mukhtar H, Wood GS. Anti-proliferative and proapoptotic effects of (-)-epigallocatechin-3-gallate on human melanoma: possible implications for the chemoprevention of melanoma. Int J Cancer. 2005;**114**:513–521.

[108] Chung SY, Hong W, Guang Xun L, Zhihong Y, Fei G, Huanyu J. Review: cancer prevention by tea: evidence from laboratory studies. Pharm Res. 2011;**64:**113–122.

[109] Umeda D, Yano S, Yamada K, Tachibana H. Green tea polyphenol epigallocatechin-3-gallate signaling pathway through 67-kDa laminin receptor. J Biol Chem 2008;**283**:3050–3058.

[110] Yamada S, Tsukamoto S, Huang Y, Makio A, Kumazoe M, Yamashita S, Tachibana H. Epigallocatechin-3-O-gallate up-regulates microRNA-let-7b expression by activating 67-kDa laminin receptor signaling in melanoma cells. Sci Rep. 2016;**6**:19225.

[111] Takahashi A, Watanabe T, Mondal A, Suzuki K, Kurusu-Kanno M, Li Z, Yamazaki T, Fujiki H, Suganuma M. Mechanism-based inhibition of cancer metastasis with (-)-epigallocatechin gallate. Biochem Biophys Res Commun. 2014;**443**(1):1–6. doi:10.1016/j.bbrc.2013.10.094.

[112] Singh T, Katiyar SK. Green tea catechins reduce invasive potential of human melanoma cells by targeting COX-2, PGE2 receptors and epithelial-to-mesenchymal transition. PLoS One. 2011;**6**(10):e25224. doi:10.1371/journal.pone.0025224.

[113] Chang CW, Hsieh YH, Yang WE, Yang SF, Chen Y, Hu DN. Epigallocatechingallate inhibits migration of human uveal melanoma cells via downregulation of matrix metalloproteinase-2 activity and ERK1/2 pathway. Biomed Res Int. 2014;**2014**:141582. doi:10.1155/2014/141582. [Epub 12 Aug 2014].

[114] Zhao G, Han X, Zheng S, Li Z, Sha Y, Ni J, Sun Z, Qiao S, Song Z. Curcumin induces autophagy, inhibits proliferation and invasion by downregulating AKT/mTOR signaling pathway in human melanoma cells. Oncol Rep. 2016;**35**(2):1065–1074.

[115] Ray S, Chattopadhyay N, Mitra A, Siddiqi M, Chatterjee A. Curcumin exhibits antimetastatic properties by modulating integrin receptors, collagenase activity, and expression of Nm23 and E-cadherin. J Environ Pathol Toxicol Oncol. 2003;**22**(1):49–58.

[116] Zhang YP, Li YQ, Lv YT, Wang JM. Effect of curcumin on the proliferation, apoptosis, migration, and invasion of human melanoma A375 cells. Genet Mol Res. 2015;**14**(1):1056–67.

[117] Wu Z, Liu B, E C, Liu J, Zhang Q, Liu J, Chen N, Chen R, Zhu R. Resveratrol inhibits the proliferation of human melanoma cells by inducing G1/S cell cycle arrest and apoptosis. Mol Med Rep. 2015;**11**(1):400–404. doi:10.3892/mmr.2014.2716.

[118] Bhattacharya S, Darjatmoko SR, Polans AS. Resveratrol modulates the malignant properties of cutaneous melanoma through changes in the activation and attenuation of the antiapoptotic protooncogenic protein Akt/PKB. Melanoma Res. 2011;**21**(3):180–187.

[119] Chan GC, Chan WK, Sze DM. The effects of beta-glucan on human immune and cancer cells. J Hematol Oncol. 2009;**2**:25. doi:10.1186/1756-8722-2-25.

[120] Imbs TI, Ermakova SP, Malyarenko Vishchuk OS, Isakov VV, Zvyagintseva TN. Structural elucidation of polysaccharide fractions from the brown alga Coccophora langsdorfii and in vitro investigation of their anticancer activity. Carbohydr Polym. 2016;**135**:162–168.

[121] Vetvicka V, Vetvickova J. Glucan supplementation has strong anti-melanoma effects: role of NK cells. Anticancer Res. 2015;**35**(10):5287–5292.

[122] Choromanska A, Kulbacka J, Rembialkowska N, Pilat J, Oledzki R, Harasym J, Saczko J. Anticancer properties of low molecular weight oat beta-glucan – an in vitro study. Int J Biol Macromol. 2015;**80**:23-28.

[123] Yang YJ, Xu HM, Suo YR. Raspberry pulp polysaccharides inhibit tumor growth via immunopotentiation and enhance docetaxel chemotherapy against malignant melanoma in vivo. Food Funct. 2015;**6**(9):3022–3034.

[124] Jayaram S, Kapoor S, Dharmesh SM. Pectic polysaccharide from corn (Zea mays L.) effectively inhibited multi-step mediated cancer cell growth and metastasis. Chem Biol Interact. 2015;**235**:63–75.

[125] Raguso RA, Pichersky E. A day in the life of a linalool molecule: chemical communication in a plant-pollinator system. Part 1: linalool biosynthesis in flowering plants. Plant Spec Biol. 1999;**14**:95–120.

[126] Calcabrini A, Stringaro A, Toccacieli L, Meschini S, Marra M, Colone M, Salvatore G, Mondello F, Arancia G, Molinari A. Terpinen-4-ol, the main component of *Melaleuca alternifolia* (tea tree) oil inhibits the in vitro growth of human melanoma cells. J Invest Dermatol. 2004;**122**(2):349–360.

[127] Greay SJ, Ireland DJ, Kissick HT, Heenan PJ, Carson CF, Riley TV, Beilharz MW. Inhibition of established subcutaneous murine tumor growth with topical *Melaleuca alternifolia* (tea tree) oil. Cancer Chemother Pharmacol. 2010;**66**(6):1095–1102.

[128] Manjamalai A, Kumar MJ, Grace VM. Essential oil of Tridax procumbens L. induces apoptosis and suppresses angiogenesis and lung metastasis of the B16F-10 cell line in C57BL/6 mice. Asian Pac J Cancer Prev. 2012;**13**(11):5887–5895.

[129] Chen W, Lu Y, Gao M, Wu J, Wang A, Shi R. Anti-angiogenesis effect of essential oil from *Curcuma zedoaria* in vitro and in vivo. J Ethnopharmacol. 2011;**133**(1):220–226.

[130] Mantena SK, Sharma SD, Katiyar SK. Berberine inhibits growth, induces G1 arrest and apoptosis in human epidermoid carcinoma A431 cells by regulating Cdki-Cdk-cyclin cascade, disruption of mitochondrial membrane potential and cleavage of caspase 3 and PARP. Carcinogenesis. 2006;**27**(10):2018–2027. [Epub 18 Apr 2006].

[131] Barbagallo CM, Cefalu AB, Noto D, Averna MR. Role of nutraceuticals in hypolipidemic therapy. Front Cardiovasc Med. 2015;**2**:22.

[132] Song YC, Lee Y, Kim HM, Hyun MY, Lim YY, Song KY, Kim BJ. Berberine regulates melanin synthesis by activating PI3K/AKT, ERK and GSK3β in B16F10 melanoma cells. Int J Mol Med. 2015;**35**(4):1011–1016.

[133] Mittal A, Tabasum S, Singh RP. Berberine in combination with doxorubicin suppresses growth of murine melanoma B16F10 cells in culture and xenograft Phytomedicine. 2014;**21**(3):340–347.

[134] Singh T, Vaid M, Katiyar N, Sharma S, Katiyar SK. Berberine, an isoquinoline alkaloid, inhibits melanoma cancer cell migration by reducing the expressions of cyclooxygenase-2, prostaglandin E2 and prostaglandin E2 receptors. Carcinogenesis. 2011;**32**(1):86–92.

[135] Hamsa TP, Kuttan G. Antiangiogenic activity of berberine is mediated through the downregulation of hypoxia-inducible factor-1, VEGF, and proinflammatory mediators. Drug Chem Toxicol. 2012;**35**(1):57–70.

[136] Hamsa TP, Kuttan G. Harmine inhibits tumor specific neo-vessel formation by regulating VEGF, MMP, TIMP and pro-inflammatory mediators both in vivo and in vitro. Eur J Pharmacol. 2010;**649**(1–3):64–73.

[137] Hamsa TP, Kuttan G. Studies on anti-metastatic and anti-invasive effects of harmine using highly metastatic murine B16F-10 melanoma cells. J Environ Pathol Toxicol Oncol. 2011;**30**(2):123–137.

[138] Kundranda MN, Niu J. Albumin-bound paclitaxel in solid tumors: clinical development and future directions. Drug Des Devel Ther. 2015;**9**:3767–3777.

[139] Yue Q-X, Liu X, Guo D-A. Microtubule-binding natural products for cancer therapy. Planta Med. 2010;**76**:1037–1043.

[140] Merchan JR, Jayaram DR, Supko JG, He X, Bubley GJ, Sukhatme VP. Increased endothelial uptake of paclitaxel as a potential mechanism for its antiangiogenic effects: potentiation by Cox-2 inhibition. Int J Cancer. 2005;**113**(3):490–498.

[141] Al Sinani SS, Eltayeb EA, Coomber BL, Adham SA. Solamargine triggers cellular necrosis selectively in different types of human melanoma cancer cells through extrinsic lysosomal mitochondrial death pathway. Cancer Cell Int. 2016;**16**:11.

[142] Lu MK, Shih YW, Chang Chien TT, Fang LH, Huang HC, Chen PS. α-Solanine inhibits human melanoma cell migration and invasion by reducing matrix metalloproteinase-2/9 activities. Biol Pharm Bull. 2010;**33**(10):1685–1691.

[143] Abirami A, Mohamed Halith S, Pillai KK, Anbalagan C. Herbal nanoparticle for anticancer potential- a review. World J Pharm Pharm Sci. 2014;**3**:2123–2132.

[144] Srinivas NR. Formulation development for cancer compounds – biopharmaceutical issues and perspectives. Asian J Pharm. 2009;90–93. doi:10.4103/0973-8398.55044.

[145] Sharma V, Nehru B, Munshi A, Jyothy A. Antioxidant potential of curcumin against oxidative insult induced by pentylenetetrazol in epileptic rats. Methods Find Exper Clin Pharm. 2010;**2**(4):227. doi:10.1358/mf.2010.32.4.1452090.

[146] Kocher A, Schiborr C, Behnam D, Frank J. The oral bioavailability of curcuminoids in healthy humans is markedly enhanced by micellar solubilisation but not further improved by simultaneous ingestion of sesamin, ferulic acid, naringenin and xanthohumol. J Funct Foods. 2015;**14**:183–191.

[147] Schiborr C, Kocher A, Behnam D, Jandasek J, Toelstede S, Frank J. The oral bioavailability of curcumin from micronized powder and liquid micelles is significantly increased in healthy humans and differs between sexes. Mol Nutr Food Res. 2014;**58**:516–527. doi:10.1002/mnfr.201300724.

[148] Ravichandran R, Pharmacokinetic study of nanoparticulate curcumin: oral formulation for enhanced bioavailability. J Biomater Nanobiotechnol. 2013;**4**:291–299. doi:10.4236/jbnb.2013.43037.

[149] Madhavi D, Kagan D. Bioavailability of a sustained release formulation of curcumin. Integr Med (Encinitas). 2014;**13**:24–30.

[150] Marczylo TH, Verschoyle RD, Cooke DN, Morazzoni P, Steward WP, Gescher AJ. Comparison of systemic availability of curcumin with that of curcumin formulated with phosphatidylcholine. Cancer Chemother Pharmacol. 2007;**60**:171–177.

[151] Jäger R, Lowery RP, Calvanese AV, Joy JM, Purpura M, Wilson JM. Comparative absorption of curcumin formulations. Nutr J. 2014;**13**:11. doi:10.1186/1475-2891-13-11.

[152] Sou K, Inenaga S, Takeoka S, Tsuchida E. Loading of curcumin into macrophages using lipid-based nanoparticles. Int J Pharm. 2008;**352**:287–293.

[153] Li L, Ahmed B, Mehta K, Kurzrock R. Liposomal curcumin with and without oxaliplatin: effects on cell growth, apoptosis, and angiogenesis in colorectal cancer. Mol Cancer Ther. 2007;**6**:1276–1282.

[154] Rachmadi UW, Permatasari D, Rahma A, Rachmawati H. Self-nanoemulsion containing combination of curcumin and silymarin: formulation and characterization. Res Dev Nanotechnol Indones. 2015;**2**:37–48.

[155] Li X, Yuan Q, Huang Y, Zhou Y, Liu Y. Development of silymarin self-microemulsifying drug delivery system with enhanced oral bioavailability. AAPS PharmSciTech. 2010;**11**:672–678. doi:10.1208/s12249-010-9432-x.

[156] Hussein A, El-Menshawe S, Afouna M. Enhancement of the in-vitro dissolution and in-vivo oral bioavailability of silymarin from liquid-filled hard gelatin capsules of semi-solid dispersion using Gelucire 44/14 as a carrier. Pharmazie. 2012;**67**:209–214.

[157] Sonali D, Tejal S, Vaishali T, Tejal G. Silymarin-solid dispersions: characterization and influence of preparation methods on dissolution. Acta Pharm. 2010;**60**:427–443. doi:10.2478/v10007-010-0038-3.

[158] Gupta S, Kumar Singh S, Girotra P. Targeting silymarin for improved hepatoprotective activity through chitosan nanoparticles. Int J Pharm Investig 2014;**4**:156–163. doi:10.4103/2230-973X.143113.

[159] Xu X, Yu J, Tong S, Zhu Y, Cao X. Formulation of silymarin with high efficacy and prolonged action and the preparation method thereof. US Patent 20110201680 A1, 2011.

[160] Lian R, Lu Y, Qi J, Tan Y, Niu M, Guan P, Hu F, Wu W. Silymarin glyceryl monooleate/poloxamer 407 liquid crystalline matrices: physical characterization and enhanced oral bioavailability. AAPS PharmSciTech. 2011;**12**:1234–40. doi:10.1208/s12249-011-9666-2.

[161] Wang M, Xie T, Chang Z, Wang L, Xie X, Kou Y, Xu H, Gao X. A new type of liquid silymarin proliposome containing bile salts: its preparation and improved hepato-protective effects. PLoS One. 2015;**10**:e0143625. doi:10.1371/journal.pone.0143625. eCollection 2015.

[162] Kumar N, Rai A, Reddy ND, Raj PV, Jain P, Deshpande P, Mathew G, Kutty NG, Udupa N, Rao CM. Silymarin liposomes improves oral bioavailability of silybin besides targeting hepatocytes, and immune cells. Pharmacol Rep. 2014;**66**:788–798. doi:10.1016/j.pharep.2014.04.007.

[163] Yang G, Zhao Y, Zhang Y, Dang B, Liu Y, Feng N. Enhanced oral bioavailability of silymarin using liposomes containing a bile salt: preparation by supercritical fluid technology and evaluation in vitro and in vivo. 2015;**2015**:6633–6644.

[164] Park JH, Park JH, Hur HJ, Woo JS, Lee HJ. Effects of silymarin and formulation on the oral bioavailability of paclitaxel in rats. Eur J Pharm Sci. 2012;**45**:296–301. doi:10.1016/j.ejps.2011.11.021.

[165] Ganta S, Devalapally H, Amiji M. Curcumin enhances oral bioavailability and anti-tumor therapeutic efficacy of paclitaxel upon administration in nanoemulsion formulation. J Pharm Sci. 2010;**99**:4630–4641. doi:10.1002/jps.22157.

[166] Chu Z, Chen JS, Liau CT, Wang HM, Lin YC, Yang MH, Chen PM, Gardner ER, Figg WD, Sparreboom A. Oral bioavailability of a novel paclitaxel formulation (Genetaxyl) administered with cyclosporin A in cancer patients. Anticancer Drugs. 2008;**19**:275–281.

[167] Gelderblom H, Verweij J, van Zomeren DM, Buijs D, Ouwens L, Nooter K, Stoter G, Sparreboom A. Influence of Cremophor El on the bioavailability of intraperitoneal paclitaxel. Clin Cancer Res 2002;**8**:1237–1241.

[168] Chiang P-C, Gould S, Nannini M, Qin A, Deng Y, Arrazate A, Kam KR, Ran Y, Wong H. Nanosuspension delivery of paclitaxel to xenograft mice can alter drug disposition and anti-tumor activity. Nanoscale Res Lett. 2014;**9**(1):156.

[169] Oostendorp RL, Buckle T, Lambert G, Garrigue JS, Beijnen JH, Schellens JH, van Tellingen O. Paclitaxel in self-micro emulsifying formulations: oral bioavailability study in mice. Invest New Drugs. 2010;**29**:768–776.

[170] Veltkamp SA, Thijssen B, Garrigue JS, Lambert G, Lallemand F, Binlich F, Huitema ADR, Nuijen B, Nol A, Beijnen JH, Schellens JHM. A novel self-microemulsifying formulation of paclitaxel for oral administration to patients with advanced cancer. Br J Cancer. 2006;**95**:729–734.

[171] Gao P, Rush BD, Pfund WP, Huang T, Bauer JM, Morozowich W, Kuo MS, Hageman MJ. Development of a supersaturable SEDDS (S-SEDDS) formulation of paclitaxel with improved oral bioavailability. J Pharm Sci. 2003;**92**:2386–2398.

[172] Ahmad J, Mir SR, Kohli K, Chuttani K, Mishra AK, Panda AK, Amin S. Solid-nanoemulsion preconcentrate for oral delivery of paclitaxel: formulation design, biodistribution, and γ scintigraphy imaging. Biomed Res Int. 2014;**2014**:984756. doi:10.1155/2014/984756.

[173] Bayindir ZS, Yuksel N. Characterization of niosomes prepared with various nonionic surfactants for paclitaxel oral delivery. J Pharm Sci. 2010;**99**:2049–2060. doi:10.1002/jps.21944.

[174] Eskolaky EB, Ardjmand M, Akbarzadeh A. Evaluation of anti-cancer properties of pegylated ethosomal paclitaxel on human melanoma cell line SKMEL- 3. Trop J Pharm Res. 2015;**14**:1421–1425.

[175] Peltier S, Oger JM, Lagarce F, Couet W, Benoît JP. Enhanced oral paclitaxel bioavailability after administration of paclitaxel-loaded lipid nanocapsules. Pharm Res. 2006;**23**:1243–1250.

[176] Baek JS, So JW, Shin SC, Cho CW. Solid lipid nanoparticles of paclitaxel strengthened by hydroxypropyl-β-cyclodextrin as an oral delivery system. Int J Mol Med. 2012;**30**:953–959. doi:10.3892/ijmm.2012.1086.

[177] Patil SM, Joshi HP. Colloidal drug delivery system for tumor specificity of paclitaxel in mice. Der Pharm Lett. 2012;**4**:961–967.

[178] Baek JS, Kim J-H, Park J-S, Cho CW. Modification of paclitaxel-loaded solid lipid nanoparticles with 2-hydroxypropyl-β-cyclodextrin enhances absorption and reduces nephrotoxicity associated with intravenous injection. Int J Nanomed. 2015;**10**:5397–5405.

[179] Aygül G, Yerlikaya F, Caban S, Vural I, Çapan Y. Formulation and *in vitro* evaluation of paclitaxel loaded nanoparticles, Hacettepe Univ J Faculty Pharm. 2013;**33**:25–40.

[180] Yoncheva K, Calleja P, Agüeros M, Petrov P, Miladinova I, Tsvetanov C, Irache JM. Stabilized micelles as delivery vehicles for paclitaxel. Int J Pharm. 2012;**436**:258–264. doi:10.1016/j.ijpharm.2012.06.030.

[181] Coimbra M, Isacchi, B, van Bloois L, Torano JS, Ket A, Wu XJ, Broere F, Metselaar JM, Rijcken CJF, Storm G, Bilia R, Schiffelers RM. Improving solubility and chemical stability of natural compounds for medicinal use by incorporation into liposomes. Int J Pharm. 2011;**416**(2):433–442.

[182] Ansari KA, Vavia PR, Trotta F, Cavalli R. Cyclodextrin-based nanosponges for delivery of resveratrol: *in vitro* characterisation, stability, cytotoxicity and permeation study. AAPS PharmSciTech. 2011;**12**(1):279–286.

[183] Nam JB, Ryu JH, Kim JW, Chang IS, Suh KD. Stabilization of resveratrol immobilized in monodisperse cyano-functionalized porous polymeric microspheres. Polymer. 2005;**46** (21):8956–8963.

[184] Shi G, Rao L, Yu H, Xiang H, Yang H, Ji R. Stabilization and encapsulation of photosensitive resveratrol within yeast cell. Int J Pharm. 2008;**349**(1–2):83–93.

[185] Das S, Ng KY, Ho PC. Formulation and optimization of zinc-pectinate beads for the controlled delivery of resveratrol. AAPS PharmSciTech. 2010;**11**(2):729–742.

[186] Das S, Chaudhury A, Ng KY. Preparation and evaluation of zinc-pectinchitosan composite particles for drug delivery to the colon: role of chitosan in modifying in vitro and *in vivo* drug release. Int J Pharm. 2011;**406**(1–2):11–20.

[187] Lund KC, Pantuso T. Combination effects of quercetin, resveratrol and curcumin on in vitro intestinal absorption. J Restorat Med. 2014;**3**:112–120.

IntechOpen