GREEN SYNTHESIZED SILVER NANOPARTICLES VIA PLANT EXTRACTS AS ANTI-MELANOMA AGENTS

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GREEN SYNTHESIZED SILVER NANOPARTICLES VIA PLANT EXTRACTS AS ANTI-MELANOMA AGENTS (Abstract): Melanoma is one of the most common and aggressive type of cancer. The actual treatment includes excisional surgery, electrochemotherapy and pharmacotherapy. The pharmaceutical treatment has major drawbacks such as severe side effects and development of drug resistance, fact that orientated the research towards the formulation of new drug delivery systems. Nanocarriers represent an innovative and alternative tool which significantly improves the bioavailability and site-specific targeting of pharmaceutical agents. Due to their biological activities, flexibility and reliability, silver nanoparticles (AgNPs) have gained high attention. The green synthesis of AgNPs, mediated by a plant extract, is an eco-friendly, cheap and effective method. Green synthesized AgNPs have been reported to develop cytotoxic effects against different tumor cell lines. With respect to melanoma, few studies have been conducted until now, but with promising results. The antitumor activity depends on the size and shape of AgNPs and relies on the synergistic effect between phytochemicals and nanocarriers. Overall, green synthesized AgNPs represent a promising strategy for melanoma treatment. **Keywords**: ANTI-MELANOMA, SILVER NANOPARTICLES, GREEN SYNTHESIS, PLANT EXTRACTS.

Melanoma is the most dreadful form of skin cancer and one of the most aggressive cancers in its metastatic forms. Lately, the incidence of melanoma in the developed countries has grown more rapidly than any other type of cancer, especially in the population older than 50 years (1). There are two types of factors that are incriminated for a high risk in the development of skin cancer: genetic and environmental factors (exposure to UV radiation) (2). Other major risk factors for melanoma development include family history, fair pigmentation phenotypes, high numbers of melanocytic nevi (over 50) or the presence of dysplastic nevi, exposure to arsenic and other chemicals (polycyclic hydrocarbons, polyvinylchloride, asbestos) and immunosuppressive drugs (azathioprine, cyclosporine A), viral infections (papillomavirus infections) and tobacco consumption (2, 3). Several molecular mechanisms have been associated with melanoma pathogenesis. They are mainly related to

the oncogenic driver mutations in the major signaling pathways that are involved in apoptosis, proliferation and differentiation of melanocytes, such as ERK/MAP-kinase and PI_3-kinase pathways (tab. I) (4).

TABLE I

Molecular changes associated with melanoma pathogenesis

Molecular event	Outcome
Mutations of BRAF and NRAS genes	activation of ERK/MEK pathways uncontrolled cell proliferation tumor development and growth
Activation of PI3K/AKT	activation of anti-apoptotic β-catenin inhibition of pro-apoptotic BAD melanoma proliferation and metastasis
Inhibition of CDKN2A	activation of CDK4 cell proliferation melanoma uncontrolled growth
Mutations/chromosomal duplications of C-KIT	increase of KIT protein expression alteration of melanocyte proliferation tumorigenesis development
MC1R polymorphisms	increase of susceptibility to melanoma impairment of melanin production increase of UV-induced DNA-mutagenesis
Increase of N-cadherin and VE-cadherin expressions	activation of PI3K-AKT pathway metastasis

BAD, Bcl-2-associated death promoter; BRAF, gene that encodes the protein B-RAF, involved in cell growth; NRAS, gene that encodes the protein N-RAS, involved in the regulation of cell division; CDKN2A, cyclin-dependent kinase inhibitor 2A; CDK4, cyclin-dependent kinase 4; C-KIT, receptor tyrosine kinase protein encoded by the KIT gene; ERK/MEK, extracellular signal-regulated kinase/mitogen-activated protein kinase; MC1R, melanocortin 1 receptor; N-cadherin, VE-cadherin, members of type-1 transmembrane proteins; PI3K, phosphatidylinositol 3-kinase/protein kinase B; AKT, serine/threonine kinase known as protein kinase B.

The excisional surgery, electrochemotherapy, pharmacotherapy and immunotherapy are the main therapeutic approaches in melanoma. The modern pharmacotherapeutics are selected after tumor genotyping process and they mainly include BRAF inhibitors (dabrafenib, vemuranifib), MEK inhibitors (cobimetinib, selumetinib, trimetinib), KIT inhibitors (imatinib) and agents targeting the endoplasmic reticulum stress-associated pathways (ruthenium-derived compounds) (4). These compounds possess a rate of response of approximately 50% and develop a rapid effect. The main disadvantage is the resistance of the melanomic cells which appears after seven months of treatment. Besides, these compounds induce cutaneous toxicities (1, 2).

ERK inhibitors such as GDC-0994 and SCH 772984 are currently in clinical trials. Another therapeutic strategy is related to compounds that target non-coding RNAs such as antisense oligopeptides. The monoclonal antibodies (ipilimumab, pembrolizumab, nivolumab) are recommended in the late stage of metastatic melanoma, advanced or unresectable melanomas but they can lead to autoimmune side effects (4). The severe side effects of the conventional chemotherapeutic or immunotherapeutic agents and the development of drug resistance are the major drawbacks of melanoma pharmacotherapy. The identification of drug delivery systems that can ensure site-specific targeting and optimal bioavailability is an important approach in the development of a successful treatment. Nanostructured materials have attracted considerable attention due to their pleiotropic applications in various fields, such as: electronics, medicine, cosmetics, mechanical engineering, applied physics and chemistry, environmental area, and biotechnology (5, 6, 7). In drug delivery field, nanocarriers represent an important tool to develop formulations that can solve some issues affecting the therapeutic efficacy of antitumor agents, such as poor bioavailability, low therapeutic index, lack of targeting, nonspecific bio-distribution, poor water solubility, and low chemical stability (8, 9). The noble metal nanoparticles are of interest due to their exceptional magnetic, optical, and electronic properties (10). AgNPs and gold nanoparticles (AuNPs) are among the most frequently used nanocarriers due to their flexibility and reliability (11). They also have biological activities and promising results have been reported in antitumor assays. In this review we focused on the potential applications of plant extract synthesized AgNPs in melanoma treatment.

Silver nanoparticles: synthesis and properties

In the nano-sized form (1-100 nm), Ag has unique optical, electronic, thermal, chemical, and biological properties, as well as a high electrical conductivity compared to its macroscopic form (12, 13, 14). The spectrum of AgNPs applications is very wide including pharmaceutical industry (diagnostics, therapeutics, drug delivery), orthopedics, medical device coatings (catheters, dentures, surgical masks), wound dressings, food industry, textile industry, cosmetics, catalysis, optoelectronics, photonics, electronics, and production of household, healthcare-related products and antimicrobial nanopaints (8, 14, 15). The synthesis of AgNPs can be accomplished by physical, chemical, and biological (green synthesis) methods. The physical methods include evaporation-condensation, laser ablation, arc-discharge, energy ball milling technique, and direct current magnetron sputtering (13, 16). Although the physical approaches allow the synthesis of shape-controlled AgNPs, they are highly energy consuming (5). The chemical methods include chemical reduction by using organic and inorganic reducing agents, microemulsion techniques, microwave-assisted synthesis, electrochemical techniques, irradiation-assisted techniques, and pyrolysis (13, 16). The main disadvantage of the chemical approaches is the toxicity of reactants and organic solvents (12).

Both microorganisms (bacteria, fungi and Actinomycetes) and plant extracts have been used in the synthesis of AgNPs (12). There is a growing interest for the green synthesized AgNPs. The green synthesis is eco-friendly, cheap, and gives high yields. Plants are easily available and safe to handle. In addition, plants contain a broad

spectrum of metabolites (polyphenols, terpenoids, alkaloids, proteins, carbohydrates) that might act as reducing agents more efficiently than microorganisms (15, 16). These plant metabolites can ensure the stability and biocompatibility of AgNPs (12). Over 200 plants belonging to different families were screened for green synthesis of metallic nanoparticles (Ag, Au, Fe, and Cu nanoparticles). Among them, AgNPs were the most intensively studied (13). The green synthesis of AgNPs involves a plant extract and a solution of Ag salt. The reaction takes place at room temperature and atmospheric pressure and it is completed within a short period of time (12). The green synthesized AgNPs showed better antimicrobial, antioxidant, and antitumor properties compared to bulk Ag, being very promising for the development of new therapeutic agents (11).

Potential application of green synthesized AgNPs via plant extracts in melanoma treatment

AgNPs showed promising effects against a wide variety of tumor cell lines: U251 human glioblastoma, MDA-MB-231, MCF-7, and T47D human breast adenocarcinoma, HCT15, HT29, and Caco-2 human colon cancer, A549 human alveolar adenocarcinoma, HepG2 hepatocellular adenocarcinoma (11, 14, 17), HeLa human cervical cancer (18), Hep2 human epidermoid larynx carcinoma (19), A375 human melanoma, and B16F10 mouse murine melanoma (14) cell lines. Various plant extracts were used to synthesize AgNPs with antitumor properties. Some examples include extracts isolated from *Vitex negundo* (Chinese chaste tree), *Piper nigrum* (black pepper), *Euphorbia nivulia* (leafy milk hedge), *Annona squamosa* (11), *Origanum vulgare* (oregano), *O. heracleoticum*, *Alpinia katsumadai*, *Ficus sp*., *Mentha arvensis* (corn mint), *Taxus sp*., *Panax ginseng* (true ginseng), *Coriandrum sativum* (coriander) (20). Antitumor efficacy of AgNPs is mediated mainly by upregulation of apoptosis and autophagy, cell cycle arrest, activation of caspases, induction of mitochondrial dysfunction, endoplasmic reticulum stress, oxidative stress, and DNA damage (14). Only a few studies have investigated the antitumor effects of green synthesized AgNPs in melanoma. In these studies, AgNPs were obtained using hydroalcoholic and aqueous extracts from several plant species (tab. II).

Flavonoids were the main constituents of plant extracts that serve as bio reducing and capping agents in the green synthesis of AgNPs. The cytotoxic effects were variable depending on the plant species, chemical composition of extracts, concentration and size of AgNPs. It is well-known the fact that the physicochemical properties of AgNPs (particle shape, size, surface chemistry) but also their concentrations are important parameters that influence the antitumor activity (11). Generally, low concentrations of AgNPs induce antiproliferative effects and smaller nanoparticles cause stronger cytotoxic effects (13).

A potent antimelanoma activity was determined for AgNPs synthesized using extracts from the roots of *Gelsemium sempervirens* (IC_{50}=50 μg/mL) and *Phytolacca decandra* (IC_{50}=78 μg/mL) (21). Also, at 100 μg/mL, AgNPs obtained using extracts from peels and leaves of *Citrus maxima* exhibited a significant cytotoxicity (more than 75%) in B16F10 mouse melanoma cells (27). Several mechanisms of AgNPs-induced cytotoxicity in melanoma cell lines have been described,

such as: induction of apoptosis, up-regulation of autophagy, DNA breakage, chromatin condensation, activation of caspase-3 and endonucleases, as well as cell cycle arrest. Caspase-3 plays an important role in the cascade of reactions that induces apoptosis. Endonucleases are enzymes involved in the DNA fragmentation at the internucleosomal linker regions (21, 27).

It has been suggested that the antiproliferative activity of green synthesized AgNPs is based on the synergism between plant constituents and Ag (21). Numerous studies proved the chemo preventive and antitumoral potential of different flavonoids and flavonoid-rich extracts in melanoma. Flavonoids act by targeting different pathways, immune and inflammatory responses involved in melanoma pathogenesis. Thus, flavonoids like apigenin inhibit the signal transducer and activator of transcription (STAT) and AKT pro-survival signaling and activate ERK pro-apoptotic signaling pathways. Kaempferol has an inhibitory activity on ribosomal S6 kinase and stress activated protein kinase (MSK1), which were found to be overexpressed in human squamous cell carcinomas (28). Also, triterpenoids such as oleanolic acid from *Phytolaccca decandra* showed cytotoxic effects in melanoma cells. Oleanolic acid displays antiproliferative effects by suppression of epidermal growth factor receptor (EGFR) activity and inducing mitochondria-dependent caspase 3-mediated apoptosis in A375 human skin melanoma cells (29). It is obvious that the constituents of plant extracts can functionalize silver nanoparticles enhancing their antitumor activity.

TABLE II

The anti-melanoma effects of green synthesized AgNPs

Plant species	Extract	Reducing and capping agents	AgNPs		Cell line	IC_{50} (µg/mL)	Outcome	Ref.
			Size (nm)	Shape				
Phytolacca decandra, Phytolaccaceae, pokeweed	ethanolic, 70%, v/v	flavonoids triterpenes	90.87	spherical	A375	78	antiproliferative activity, apoptosis, activation of caspase 3, cell cycle arrest, DNA fragmentation	21, 22
Gelsemium sempervirens, Gelsemiaceae, yellow jessamine	ethanolic, 70%, v/v	flavonoids alkaloids	112.2	spherical	A375	50		21, 23
Hydrastis canadensis, Ranunculaceae, orangeroot	ethanolic, 70%, v/v	flavonoids alkaloids	111.3	spherical	A375	80		21, 24
Thuja occidentalis, Cupressaceae, northern white cedar	ethanolic, 70%, v/v	flavonoids terpenes	122.8	spherical	A375	120		21,25
Butea monosperma, Fabaceae, flame of forest	aqueous	chalcones flavonoids	20-80	spherical	B16F10	-	no cytotoxicity	26
Citrus maxima, Rutaceae, pomelo	aqueous	flavonoids polyphenolic acids tannins terpenes	2-50	spherical	B16F10	-	oxidative stress, apoptosis, cell cycle arrest	27

CONCLUSIONS

Although the available data fail to define the whole picture of bioactivity of green synthesized AgNPs in melanoma, the results of up to date studies are very promising. The identification of plant extracts having potent intrinsic antitumor properties and being able to generate easily AgNPs with high stability and biocompatibility could be one of the successful approaches to develop new anti-melanoma agents. Also, more research is needed to evaluate the overall impact of AgNPs on human health.

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