Coll. Antropol. **34** (2010) Suppl. 2: 303–306
Review

# The Role of Apoptosis in the Pathogenesis of Malignant Melanoma

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**A B S T R A C T**

*Malignant melanoma genesis is a very complex process that involves a sequence of pathogenetic cellular events. Mutation of various genes and numerous other cellular mechanisms play an important role in the course of malignant melanocyte alteration and their malignant transformation from naevi into melanoma. Apoptosis is an active, genetically controlled process of programmed cell death, which leads to cell destruction and cell death without involvement of surrounding cells or inflammatory response. In this process, disrupted mechanisms of cell regulation and apoptosis take place in malignant melanoma cells, thus leading to their uncontrolled proliferation and melanocyte growth. Apoptosis is a process that involves two major pathways, the intrinsic and extrinsic apoptotic pathway, which interlace at certain points and ultimately result in apoptosis. It can be said that molecular events regulating cell survival, nomal growth arrest, apoptosis and cell differentiation, contribute to the overall pathogenesis of malignant cell growth. It is presumed that in the future, understanding of molecular aberrations and cellular processes, such as cell signaling, cell cycle regulation and cell apoptosis, will be essential for better patient monitoring and rational design of effective treatment.*

***Key words:*** *apoptosis, melanoma, pathogenesis, caspase, oncogene*

## Introduction

It is well-known that the development of all skin carcinomas, as well as malignant melanoma, is a complex multiphase process. Regarding many unknown terms in molecular pathogenic events, those carcinogenesis mechanisms are still reviewed.

Cell division as well as cell death play the main role in the precise control of cell count and tissue size. Apoptosis is an active, genetically controlled process of programmed cell death which leads to cell destruction, and during which there is no surrounding cells or inflammatory response involvement[1,2]. It is important to notice the importance of molecular events that regulate the apoptosis, cell survival, delay of cell growth and cell differentiation regarding carcinogenesis process[3–5]. It is well-known that the malignant tumor cells have defect mechanisms of regulation and apoptosis, whereupon cell proliferation is uncontrolled. Meanwhile, proliferation balance and apoptosis can implicate the risk of malignant transformation and consequently development of malignant melanoma[6,7]. Despite that, it is noticed that acting on cell apoptosis and renewal of disrupted cell apoptotic and regulating processes could be a promissing way of skin carcinoma treatment, including malignant melanoma[8].

The apoptosis itself as a cell death process includes number of pathogenetic events. That process includes two apoptotic pathways, intrinsic and extrinsic pathway, which converge in many points, and finally lead to cell death[9]. In that complex process numerous enzymes are included such as caspases. Caspases are proteases with cysteine tail, which cleave proteins behind the aspartic acid end, leading to termination of apoptosis. Twelve caspases have so far been identified, among which there are initiator and effective caspases. Initiator caspases (e.g. 8 and 9) cleave inactive pro-forms of effector caspases, thereby activating them, while effector caspases (e.g. 3, 6 and 7) subsequently cleave other cell proteins and trigger apoptotic cell death.

Development of malignant melanoma is very complex process. It is known that melanocytes, except in benign and malignant neoplasms, reside within the basal layer of the epidermis, while its dendrites come into contact

Received for publication January 10, 2010

with keratinocytes as far as the mid stratum spinosum[10]. It is presumed that keratinocytes participate in cell adhesion and control the growth of melanocytes and expression of surface receptors. Melanoma arises from multiplication of transformed melanocytes in the epidermis, avoiding keratinocyte control. Multiplication of melanocytes can lead to cell hyperplasia, dysplasia, malignant melanoma, invasion and metastases[11]. Melanogenesis encompasses numerous molecular events, where genetic mutations play a great role[8]. Mutations of various genes are important in that process, for example, NRAS or BRAF gene and CDKN2A or PTEN gene.

The role of FasL molecule, expressed on the cell surface of melanocytes, which is involved in avoiding the imunologic cells action, is also important in melanogenesis. It is noticed that once malignant transformation of melanocytes takes place, they express FasL, which allows melanoma to avoid the immune response. It is also well-known that UV radiation decreases FasL expression on melanocytes. As a result, cell surface interactions between FasL and Fas on adjacent cells are not possible, apoptosis can be inhibited, resulting in multiplication of transformed cells and carcinogenesis[8]. In the process of carcinogenesis there are number of cell transformation stages: initiation/induction, promotion, premalignant progression and malignant conversion[6]. It is shown that the onset (initiation) of carcinogenesis is a result of genetic impairment caused by exogenous agent (for example UV radiation) that changes the pathway of cell proliferative control and/or differentiation. Afterwards, expansion of driven cell population or clone arises (promotion), and it is believed that it is a product of epigenetic effects. Progression and conversion are marked by cells with high level of genetic instability, chromosomal abnormalities, expression of surface molecules and oncogenic activities[12].

Although sun exposure probably plays a leading or supporting role in melanogenesis, the aetiology of melanoma is probably variable and multifactorial[3]. It is significant that molecular events which regulate cell survival, delay of cell growth, apoptosis and cell differentiation have an important role in the process of malignant cell growth[4,13].

Therefore, it is attempted to act on that pathogenic mechanisms, as potential therapeutic aims.

## Genetic and Other Factors in Pathogenesis of Malignant Melanoma

The important factors in melanogenesis are genetic mutations, especially CDKN2A, PTEN, NRAS or BRAF gene mutations[8]. Mutations in NRAS or BRAF gene can cause abnormal constitutive activation serine-threonine kinases in ERK-MAPK pathway of melanoma cells growth stimulation[14]. It has been shown by *in vitro* analysis that melanoma cells growth is induced by activation of NRAS and BRAF genes[15]. Mutations in CDKN2A or PTEN genes are another step in melanogenesis. Mutations in CDKN2A gene have been implicated in 25–40% of hereditary melanomas, while mutations in PTEN gene have been found in 25–50% of nonhereditary melanomas[16]. It is stated that especially the mutations in CDKN2A gene increase probability of development malignant melanoma. CDKN2A encodes two protein products, p16 and p14 ARF (alternate reading frame), which are both negative regulators of cell cycle progression. The net effect of a CDKN2A mutation with loss of p14ARF function is decreased p53 function with enchanced growth of altered cells. Therefore, mutation in CDKN2A gene prevents recruitment of ARF. Immortality of cells in lack of ARF or p53 has been often shown in several studies[8,16].

Common mutations that effect CDKN2A locus in melanomas typically target $p16^{INK4A}$, suggesting a significant role of this protein in melanocyte cell cycle control maintenance. It seems that this gene is destroyed in about 50% of melanomas and inactivated by point mutations in about 9% of tumors[17].

Conduction of genetic studies is significant because of the effort of implementing gene therapy that is still studied. There are efforts to turn down various/these genes by impeding with RNA, using specific small interfering RNA (siRNA) or short hairpin RNA (shRNA). Such investigations have shown functional success in tissue cultures as well as tumor tissues, and in mice models. Therefore, siRNA could represent a promissing approach to skin cancer treatment in the early stage[8].

It is impotant to emphasize cell cycle expression and apoptotic regulating proteins and telomerases in melanocytic lesions[18]. Studies have shown that the main role in apoptosis initiation and cell cycle control pertains to p53 protein which is known tumor supressor[3,18–20]. Dysplastic nevi show significantly higher expression of p53 protein in contrast to malignant melanomas, which suggests the importance to discover other factors in melanoma progression[18]. However, it has also been reported that p53 gene is not often mutated in melanomas[1,2]. Nevertheless, most genotoxic substances participate in apoptosis induction through p53, so it is assumed that melanoma cells are sensitive to DNA damage[3,18].

Capacity of cell proliferation (therefore melanocytes), cancerogenesis and cell immortality are controlled by the enzyme telomerase. It has be shown that the activation of telomerase is significant in pathogenesis of numerous malignant tumors in humans[18].Telomerase is a DNA polymerase involved in the formation of telomeres and the maintenance of telomere sequences during replication. The role of telomeres is to protect the chromosomes from degradation and aberrant recombinations during replication in order to postpone apoptosis of the cell[18]. Increased activity of telomerases is related to progression of dysplastic nevus into malignant melanoma. This fact represents an evidence that telomerase has an active anti-apoptotic role.

Several studies have shown that melanogenesis is associated with decrease of other anti-apoptotic proteins such as bcl-x and increase of bcl-2 protein expression. However, several results indicate that in all tissues of

mealnocytic origin there is a decrease of anti-apoptotic proteins or detectable level of bcl-2[21,22].

Development, differentiation and maintenance of melanocytes is regulated by MITF factor (microphtalmia--associated transcription factor). In melanoma with poor prognosis MITF amplification has been often perceived with poor response on chemotherapy. *In vitro* analyses have prooven that excessive expression of MITF changes melanocytes, which indicates that MITF is an oncogen. According to that MITF could be potential therapeutic target[8].

In later stage of disease dissemination, invasion and metastases are result of changes in cell adhesion molecules, including cadherines and integrines[8]. Cadherines are important adhesion cell molecules, ensuring communication with actin cytoskeleton and inducing signal by β-catenine that is linked to WNT (wingless-type mammary tumor integration-site family) pathway. Changes in cadherine expression affect melanoma cell interaction with keratinocytes and alternate β-catenine signaling. On the other hand, integrines mediate cell contact with components of extracellular matrix, for example caminin, collagen and fibronectin. Melanoma growth is associated with expression of α5β3 integrines increasing the expression of antiapoptotic bcl-2[23].

Recently a comprehensive review of several possible pathways of progression of melanocytes into melanoma has been published, but the question remains why immune system can't efficiently inhibit carcinogenesis[24]. This question remains unsolved despite the fact that several tumor antigens have been discovered in human melanomas that could initiate host immune response to melanoma, such as antigens coded by carcinoma development genes (for example, various MAGE genes), or coded by differentiation genes (Melan-a/Mart-1, gp 100/pMel, 17TRP-1, TRP-2 and others), or antigens which are a result of point mutations (for example, CDK4)[25].

It has been reported that patients with melanoma usually develop T-cell response to melanoma, but T-cells quickly become uneffective. This is generally a consequence of local immunosuppression at the sight of tumor, for example development of T-cell anergy and gaining tumor resistance to apoptosis. Local immunosuppression also may be accountable for failure of vaccination by tumor-specific antigens in most of patients[25].

## Future of Molecular Knowledge and Therapeutical Effects in Malignant Melanoma

In patients with malignant melanoma it is important to perform an operative procedure as soon as possible to prevent potential spreading of the tumor. Other forms of treatment in advanced phase of disease include chemotherapy, irradiation, interferon therapy and other. Cellular apoptosis is the main mechanism by which mentioned methods perform their anticarcinogeneous role, including cytotoxic chemotherapy, irradiation, hormonal therapy and new biological medications[26]. Ground on which carcinomatous cells, incuding melanomas, are resistant to many of these methods of treatment is survival signaling, which bypasses apoptotic cell death, and represents a therapeutic target[27–29].

It is important to understand of the complexity of clinical variety of melanoma and emphasize the idea that melanoma is, like other tumors, not just a solitary disease, but a heterogeneous group of disorders that develop from complex molecular changes. Knowledge of the molecular aberrations and important cellular processes, such as network of cell signaling, regulation of cell cycle and cell death, is important for better diagnosing, more accurate prognosis evaluation and rational plan of efficient treatment[9].

Despite various possibilities of treatment, there is a need for more simple and effective modes of treatment in the future, including biological response modificators and gene therapy[8]. In this manner, cell apoptotic pathways and apoptotic death could represent a crucial physiological mechanism of tissue homeostasis and growth regulation. Such new knowledge should be used in development of new medications based on apoptosis, with goal of cell protection, especially in cases of weakened cell mortality, like in malignant tumors[3].

Although there are already many various therapeutic possibilities in malignant melanoma, success is still not optimal, especially in the treatment of metastases and advanced stages of tumor development. Discovery of several mutations of genes in patients with familiar melanoma (for example PTEN), and several genes which have not been previously implicated in melanogenesis, is important in developing new therapeutic strategies[8,23]. There are many expectations in the future for method of turning down the genes by RNA interfering, and that could be the new and promissing approach. Such methods of *in vivo* siRNA or shRNA delivery should still be improved and should find a precise target genes, so that the therapy would be more effective[8]. However, it is expected that the development of flexible and firm modern approaches for such integrated genome analyses would be essential for continuous development of human genetics[9]. Recent advances in understanding molecular aberrations that make a foundation in melanoma oncogenesis are probably going to improve diagnosis, prognosis and treatment of melanoma.

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## ULOGA APOPTOZE U PATOGENEZI MALIGNOG MELANOMA

## S A Ž E T A K

Nastanak malignog melanoma je vrlo složen proces koji uključuje niz patogenetkih staničnih događaja. U procesu maligne promjene melanocita i njihove zloćudne transformacije iz nevusa u melanom važnu ulogu ima mutacija različitih gena te niz drugih staničnih mehanizama. Apoptoza je aktivni, genetski kontroliran proces programirane stanične smrti koja dovodi do destrukcije i smrti stanica bez uključivanja okolnih stanica ili upalnog odgovora. Pritom se u stanicama malignog melanoma odvijaju poremećeni mehanizmi regulacije i apoptoze stanica, što dovodi do njihovog nekontroliranog umnažanja i rasta melanocita. Apoptoza je proces koji uključuje dva glavna puta, unutarnji i vanjski apoptotički put, koji se isprepleću u pojedinim fazama te u konačnici dovode do pojave apoptoze. Može se reći da molekularni događaji koji reguliraju stanično preživljavanje, zastoj normalnog rasta, apoptozu i diferencijaciju stanica, doprinose ukupnoj patogenezi malignog rasta stanica. Smatra se da bi u budućnosti upoznavanje molekularnih poremećaja i staničnih procesa, kao procesa stanične signalizacije, regulacije staničnog ciklusa i stanične apoptoze, bilo ključno za bolje praćenje bolesnika i racionalni plan za što uspješnije liječenja.