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Melanocytes and Melanoma Etiology

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Abstract

Melanocytes are pigment-producing cells originated from the neural crest. Epidermal melanocytes contribute to photoprotection and thermoregulation by packaging melanin pigment intomelanosomes and sending them to keratinocytes. There are still many gaps in the understanding of early events of melanocyte differentiation and advanced melanoma regarding genetic and molecular processes. In this review, the authors present a summary of the main aspects of the melanocyte biology and its relation with the melanoma etiology, which are of major importance for guiding novel therapies development and to well understand this disease.

Keywords

Melanocytes, Melanoma, Etiology

Subject Areas: **Dermatology, Oncology, Pathology**

1. Introduction

Melanoma is a malignant tumor originated from melanocytes, cellslocated in the basal layer of the epidermis and responsible for the production of melanin. Melanoma can occur in skin, mucosa, meninges and choroid plexus but may also appear in unusual places such as the gastrointestinal tract [1].

Melanomas can present aggressive and early metastasis, even in cases of initial lesions [1] [2]. Metastasis can occur by lymphatic route to the lymph nodes or through hematogenic route to distant organs, such as lungs, liver and brain [1].

It represents a serious public health issue worldwide, with an increasing incidence year after year [2] [3]. This increase is higher than that of any other kind of cancer, and the involvement of young people led melanoma to occupy an important position in cancer medicine [1] [2]. Although melanoma accounts for only 5% of skin cancers, it is the leading cause of death due to skin cancer [2].

The present work reviews general aspects of melanocytes biology and cutaneous melanoma etiology, which

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are required to understand this subject in depth.

2. Melanocytes

Most part of the available data about the pathways of melanocytes differentiation are from studies with birds melanocytes [4]. These cells are derived from multipotent cells from the neural crest, which are progenitors of several cell types, including neurons, glial cells, secreting cells of peripheral neuroendocrine system and connective tissue cells [3] [4].

Precursor cells of melanocytes, the melanoblasts, migrate from the neural crest to the skin during the first trimester of embryogenesis (between the 12th and 14th week of intrauterine life) [3] [4]. The progressive differentiation of melanoblasts in the skin during embryogenesis and in the neonatal period generates pigmented dendritic cells and mature melanocytes [5]. What characterizes the differentiated melanocyte is the presence of melanosomes, specialized cytoplasmic organelle in which melanin is synthesized. No specific markers for melanoblasts are known till date [4].

Melanocytes are located in the basal and spinous layers of the epidermis. They have an irregular and central nuclei, and a distended cytoplasm with extensions directed towards the epidermis surface. These extensions penetrate into the basal and spinous layer, transferring melanosomes to other cells. The melanin contained in melanosomes determines the skin color, having a protective function against ultraviolet (UV) radiation [4].

In the synthesis of melanin tyrosinase plays a key role: converting tyrosine to 3,4-dihydroxyphenylalanine (DOPA), subsequently on DOPA-quinone, and then to melanin (**Figure 1**). Tyrosinase currently has a major diagnostic importance, being used as a marker for identifying submicroscopic metastasis to sentinel lymph nodes through reverse transcription polymerase chain reaction (RT-PCR) [5] [6].

Tyrosinase is synthesized in the rough endoplasmic reticulum, and accumulated into vesicles in the Golgi complex. These vesicles are called pre-melanosomes, which initiates the melanin synthesis and, with the progressive accumulation of melanin, they are referred to as melanosomes [4]-[6]. Melanosomes migrate by the extensions of melanocytes and are transferred to other cells, tending to occupy a supranuclear position, by forming a hood that protects the genetic material of the nucleus from mutagenic effects of UV rays [4] [5] [7]. Note that the number of melanocytes is the same among different races, the color variation is due to the amount of melanin produced [6]. The color of hair, eyes and skin are determined by the proportion between the two forms of melanin: eumelanin (responsible for black and brown pigmentation) and pheomelanin (yellow and red pigmentation) [5]. The ratio between these two pigments depends on several genes [4].

Compared with other skin cells such as keratinocytes and fibroblasts, melanocytes have a limited capacity of proliferation [6] [8]. It is very difficult to stimulate the proliferation of human melanocytes *in vitro*, and in normal skin (*in vivo*) there are scarce melanocytes in division. However, the number of melanocytes in the epidermis increases after one to two weeks of exposure to sunlight [6] [7]. Furthermore, apoptosis of melanocytes has not been described yet, and a possible explanation for this is the high concentration of anti-apoptotic proteins

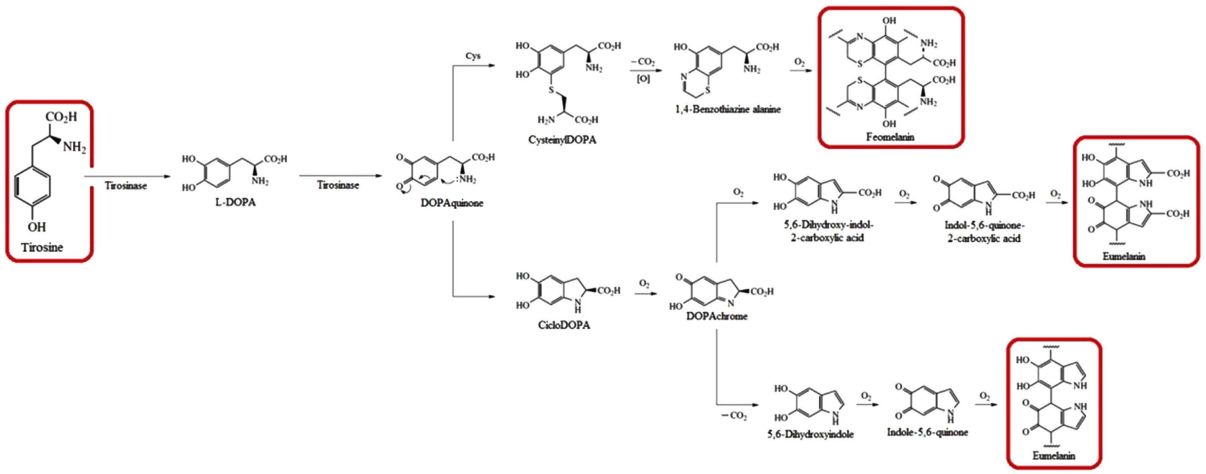

Figure 1. Melanin synthesis pathways and intermediates products.

such as Bcl-2 [8]. These data indicate that melanocytes have long life and are very stable [6] [8].

3. Etiology of Melanoma

Epidemiological studies suggest a multifactorial pathogenesis for melanoma, as it has been reported in other tumors [1] [7]. We present below the main etiological factors related to the development of melanoma.

Ultraviolet Radiation. One of the main risk factors for the development of melanoma is the exposition to type B UV radiation [1] [4] [7] [9]. There are epidemiological and experimental evidence supporting this causality (**Table 1**). UV rays can be related to the onset of skin cancer by: (1) suppressing the immune response of the skin, (2) damaging the DNA molecules of melanocytes, stimulating (3) the production of melanin and (4) the division melanocytes [1] [5] [7].

The risk for developing melanoma is higher in people with light skin, blond or red hair, people with a history of sunburn and who do not tan easily [2] [6] [9]. The incidence of melanoma in white people is inversely related to the latitude they live. The world's highest incidence of melanoma is in Australia, a subtropical country, with a Celtic population. In this population there was no miscegenation between the Celts and the native population, the Aborigines; unlike what happened in Brazil, where miscegenation between Europeans, Indians and Africans is remarkable, especially in the state of Bahia [7].

Because of changes in the solar rays angle of incidence and in the absorption of solar radiation by the atmosphere, the intensity of UV radiation changes through the day [7] [9]. Type B UV radiation has a higher incidence between 10:00 and 16:00 o'clock and type A UV radiation is stable throughout the day. Of the UV rays that reach the earth, 95% are UVA and UVB are only 5% [4] [5] [9]. UVA radiation penetrates up to deeper layers of the skin, causing inhibition of DNA synthesis, production of pyrimidine dimers and depletion of Langehans cells in the epidermis [7]. UVB radiation, in turn, penetrates only through superficial layers of the skin and, although it is the smallest portion of UV radiation, UVB is the most carcinogenic. The short wavelength UVC radiation is completely absorbed by the ozone in the atmosphere [5] [7] [9].

The intensity of UVA radiation that reaches the basal layer of the epidermis is 700 times greater than that of UVB radiation [7] [9]. Thus, during normal exposure to the sun, the amount of UVA radiation that reaches the skin is so significant that becomes as immunosuppressant and carcinogenic as UVB [5] [7] [9].

Both UVA and UVB radiation induce an error in the repair mechanisms of DNA, causing mutations that can lead to cancer development. The major mechanism is the induction of pyrimidine dimers in the DNA which if not repaired leads to mistakes in transcription [7] [9]. The factors that influence the mutagenicity are: (1) the quality of the radiation, (2) the intensity of the radiation dose, (3) its speed of action, (4) the ability to repair DNA and (5) individual factors [5] [7].

There are evidences that resistance to apoptosis induced by UV radiation, has a role in the genesis of melanoma [5] [10]-[13]. The ability to repair damaged DNA decreases by approximately 15% in the age group between 20 and 50 years. It is induced by one nucleotide, resulting in part from the activation of the tumor suppressor protein p53 [7] [9] [10] [12] [13].

Another data associated with an increased incidence of melanoma is the rate of reduction in the ozone layer (from 3% to 7% since 1969), causing the amount of UVB radiation which reaches the Earth also to increase. Studies using theoretical models indicate that there is an increase from 1% to 2% of UVB exposure for each 1% reduction in the quantity of ozone in the atmosphere [7]. Therefore, the reduction of the ozone layer, favoring greater exposure to UVB radiation increases the risk to develop melanoma and other skin cancer in a predisposed populations [7] [11] [13].

Nevus X Melanomas. Another very important aspect in the etiology of melanoma is the presence of inherited

Table 1. Evidences that reinforces the role of UV radiation in the oncogenesis of melanoma.

Patients with xeroderma pigmentosum have a defect in the repair of pyrimidine dimers and have a higher risk for developing melanoma. This is a necessary step, but not sufficient to cause melanoma.
Patients with albinism have a high risk for skin cancer in general, but a low risk for melanoma.
Black people have a low incidence of melanoma. This protection is afforded by eumelanin.
Intermittent exposure to sunlight, particularly when leading to sunburn, are closely associated with the development of melanoma.
Excessive sun exposure leads to DNA damage with greater frequency.

or acquired nevi [2] [14]. In a study with 426 patients with melanoma and 416 controls, Bataille evaluated the number and type of melanocytic nevi found in both groups. It was observed a 28.7-fold higher risk for the occurrence of melanoma in patients with four or more atypical nevi and a 7.7-fold higher risk when there was more than 100 nevi larger than 2mm in diameter [14].

Melanoma can originate *de novo*, from a dysplastic nevus, or even from benign nevi, including congenital nevi [2] [14]. This term does not mean the lesion recurrence but one lesion that has already appears as melanoma [14]-[18].

Skender-Kalmenas found that from 289 melanomas smaller than 1mm in thickness, 51% of cases were associated with nevi, of those 56% were dysplastic, 41% acquired and without atypia, and 3% were inherited [19]. This study showed that frequently the precursor lesions are masked by exuberant proliferation of malignant neoplasm. Thus, in melanoma of low thickness, the chance of missing the lesion that gave rise to them is lower. It is also important to note that about 50% of melanomas present themselves without any identifiable precursor lesion.

Other Risk Factors. Using multivariate analysis to determine risk factors, Rigel [20] found six independent variables that influence the development of melanoma:

1) Family history for melanoma;
2) Individuals with white skin, blue or green eyes, blond or red hair;
3) Presence of sunburn scars on the back;
4) History of three or more severe sunburns prior to 20 years old;
5) History of three or more years of summer work outdoors;
6) Presence of actinic keratosis.

If a person has one case of melanoma in the family the relative risk for developing this tumour is 2.3, and if there are two cases it goes up to 5 [20] [21]. This information should be passed on to family and melanoma patients in order to do an early diagnostic screening in family members.

Cellular Factors. The normal melanocytes do not grow in culture, having an ability to form colonies on average of 0.9%. Melanoma cells at the early stages form colonies in 10% and melanoma in vertical growth phase do it in 30% [5] [22].

The *insulin-like growth factor* (IGF-1) and insulin are mitogens for melanocytes in all stages of tumor growth [12] [22]. Melanomas in earlier vertical growth stage do not survive without IGF-1 or insulin. On the other hand, mechanisms of self-stimulation can be observed in metastatic cells, as they survive without external stimulating factors [17] [22].

The *fibroblast growth factor* (FGF-β) is a heparin ligand found in normal cells such as fibroblasts and keratinocytes. The normal melanocytes do not produce FGF-β, but extracts from melanomas and nevi contain this factor [11] [22]. The ability of melanoma to proliferate in the absence of exogenous growth factors have been attributed to the role of autocrine FGF-β and other stimulating factors constitutively produced by neoplastic cells. It seems that the most important factor to the growth of melanoma is FGF-β [12] [17] [22].

The *platelet derived growth factor* (PDGF) is produced by many tumors, such as osteosarcomas, sarcomas, gliomas, bladder carcinomas, breast and lung cancer. Melanoma cells produce PDGF in two isoforms, PDGF-A and PDGF-B (c-cys oncogene). PDGF stimulates the formation of stroma and blood vessels [17] [22] [23].

The *tumor growth factor* α (TGF-α) is an homologous of the *epidermal growth factor* (EGF) and has the same receptor. Both of them have similar biological activities [11] [22] [23]. TGF-α is expressed by 60% of the melanoma cells, what does not occur with EGF. This factor has been described as responsible for the paraneoplastic syndromes associated with melanoma (fever and melanosis) [23].

The *tumor growth factor* β (TGF-β) produced by melanoma cells has the capacity to inhibit *natural killercells* of the peripheral blood, which may present a potential immunosuppressive effect related to the development of melanoma [12] [17] [24]. TGF-β is a negative growth factor for various cell types and also for melanocytes, butmelanoma cells are resistant to TGF-β. This cytokine inhibits the cell-mediated immune response and probably has a role in the progression of melanoma [24] [25].

Interleukin 6 (IL-6) derived from dermal fibroblasts has shown growth inhibition of earlier melanoma cells but it has no effect on advanced melanoma [11] [26]. *Interleukin* 8 (IL-8) is a member of the α-chemokines whose expression is related to the ability of melanomas to metastasize. Elevated IL-8 serum levels were identified in 37.5% of patients with metastatic melanoma [22] [27].

Interleukin 10 (IL-10) is a cytokine with immunosuppressive effect produced by T-helper type 2 cells. It is an

effective inhibitor of cellular immune response [12] [22]. Elevated IL-10 serum levels were identified in patients with melanoma and vary considerably according to the stage of the disease: 3% for stage I, 6% for stage II, 35% for stage III, and 73% for stage IV [28].

Molecular factors. The identification and characterization of genes are critical to understand the genesis of melanoma and to enable future development of therapies.

Rearrangements or deletions of the p-arm of chromosome 9 are the most frequent aberrations detected in subjects with melanoma (46%) [5] [29]. These mutations are found in atypical nevi and early melanomas [11] [21] [30]. Recently, the p16 gene was also found in this region, and it is frequently deleted or mutated in patients with melanoma [5] [12] [31].

This finding was a milestone in researches on the etiology of melanoma. The p16 gene is known to inhibit cyclin dependent kinase 4 (CDK4) and 6 (CDK6) in phosphorylation of retinoblastoma, causing a disruption in the control of cell growth. The p16 gene (9p21) seems to be critical in the development of melanoma, although not specific. Currently, it is established that the p16 mutation is a common event in the progression of sporadic and familial melanoma [1] [11] [32]. Upon the occurrence of multiple melanoma from nevi, the causative factor has been attributed as the occurrence of mutations in the CDKN2A gene which encodes the tumor suppressor proteins p16 and p19 [5] [32].

Aberrations in chromosome 1 are the most frequently found in patients with advanced melanoma. The terminal portion 1p has a tumor suppressor gene, which is critically involved in the progression of melanoma. Changes in the chromosome 6 are the second most frequent chromosomal aberration in melanoma patients, found in over 50% of patients [5] [21] [29]. Duplication or multiplication of chromosome 7 had also been found. This duplication has been associated with overexpression of the receptor of epidermal growth factor, located on chromosome 7p12-13. Rearrangement of chromosome 10 was found in atypical nevi and early stages melanoma, in the region 10q24-26. The rearrangement or deletion of chromosome 11 is also described in more than a half of patients with melanoma [12] [29].

4. Conclusion

The set of these data about all the molecular alterations that result in the development of melanoma have opened perspectives in the molecular target of carcinogenesis. Although extensive epidemiologic evidence points to solar UV as the major risk factor for melanoma, there is a significant gap in our knowledge about how this carcinogen factor interacts with the skin at the microenvironmental and molecular level.

Conflict of Interest Disclosure

The authors declare that there is no conflict of interests regarding the publication of this article.

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