JCOMS eISSN:2783-3127 https://journalofcoms.com

Journal of

Current Oncology and Medical Sciences

Vol. 3, No.4

Review Free Access

Molecular mechanisms associated with cutaneous melanoma biology, pathogenesis, and diagnosis

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Abstract

Introduction: Melanoma is considered the most lethal skin cancer, with poor prognosis in advanced stages. The 2018 World Health Organization (WHO) Classification classified melanoma into nine different subgroups depending on the cumulative sun damage, with its respective genetic alterations, which are necessary to investigate for targeted therapies. Nevertheless, the epigenetic alterations aren't included at all in the new molecular classification. It is understanding the molecular mechanisms associated with melanoma pathogenesis and its poor prognosis.

Methods: To analyze the molecular mechanisms implicated in melanoma carcinogenesis, we reviewed the most recent papers using PubMed database and Google Scholar, the search was carried out using the following medical subject headings (MeSH) in the search engine: "melanoma epigenetic mechanisms", "miRNAs and melanoma", immunology and melanoma", "melanoma pathogenesis", in combination with boolean connectors 'AND' and 'OR'. A total of 83 articles were reviewed, published between 2000 and 2022.

Conclusion: Given the importance of genetic and epigenetic mechanisms implicated in the prognosis and progression of cancer, this paper aims to review the literature about its respective regulators, and how they have a relationship between them in several metabolic, apoptotic, physiological, and biological processes. It is essential to understand the molecular and immunological mechanisms involved in melanoma pathogenesis and how the alteration of any of them leads to the genesis of cancer, to foster the development of novel targeted therapy strategies.

Keywords: Melanoma, Molecular mechanisms, Skin neoplasm, Genetic, Epigenetic

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Recieved: 2023.9.20, Accepted: 2023.11.25

Introduction

Skin cancer is the most common form of cancer in the world. It is categorized into melanoma and non-melanoma skin cancers. Melanoma only accounts for about 1% of all skin cancers, but is the most aggressive with a poor prognosis, it accounts 90% of all skin cancer deaths, and it's more frequent in patients between 25 and 40 years old (1). According to the "Surveillance, Epidemiology, and End Results" program (SEER), the rate of new cases of melanoma in the U.S. of the skin was 22.8 per 100,000 men and women per year, with a death rate of 2.2 per 100,000 based on data from 2014 to 2018. In 2019, an estimated 1,294,886 people were living with melanoma of the skin. Melanoma is associated with different risk factors; they can be divided in modifiable and non-modifiable risk factors (2).

The Cancer Genome Atlas (TCGA) classified tumors according to their genomic characteristics, the most prevalent mutated genes are BRAF, NRAS, NF1-loss and triple wild type (TWT). However, there are more mutations associated with tumorigenesis of melanoma, such as CDKN2A (25-35%), TP53 (15%), ARID2 (13.32%), IDH1, PPP6C, PTEN (14%), DDX3X, RAC1 (9.2%), MAP2K1/2 (10%), RB1, ATRX (9.11%), SETD2 (9.48%), SF3B1 (33%), TERT (14%), and ERBB2/4 (3.29%) (3,4).

Modifiable risk factors

Modifiable risk factors are related by a high occurrence of oncogenesis, some external factors such as ultraviolet A (320-400 nm) (5) and B (280-315 nm) exposure (6), Intermittent sun exposure has a relative risk of 1.61, sunburn has a relative risk of 2.03 (7), citrus consumption greater than 1.6 servings of citrus fruits daily increases melanoma risk, high Body Mass Index (BMI) is inversely correlated with telomerase length with the consequence of high risk of melanoma than people with normal BMI (8), immunosuppression states, such as Kidney transplants confers a relative risk of 3.6 developing melanoma. Nowadays, gut microbiome is recognized as a potentially modifiable risk factor associated with immunotherapy response (9).

Nonmodifiable risk factors

Genetic syndromes such as Xeroderma pigmentosum, Neurofibromatosis, Charcot Marie Tooth, Familiar Atypical Multiple Mole and Melanoma Syndrome (FAMMM) and Amyotrophic Lateral Sclerosis (ALS) (8,10), familiar history (5-10% of melanomas occur in families with hereditary predisposition) (6), personal history of melanoma (32% higher risk of developing second primary malignancy) (11), race (Caucasians have a lifetime risk of 2-6%, African Americans 0.1% and Hispanics 0.58%) (11), people with blue eyes have a relative risk of 1.06 to 2.45 compared to those who have dark eyes, blonde hair 1.6-9.7 compared with dark hair (6), gender (in females are more common on the legs, in males are more common on the trunk) (8), giant congenital nevus >20 cm of diameter (the malignant transformation estimates between 4-40%) (6), atypical nevi (one has a relative risk of 1.45, two have 2.1, three have 3.03, four have 4.39 and five have 6.36 of transforming in melanoma) (10), high number of common nevi (>100 common nevi) is associated with almost 7 times higher risk of melanoma (7). Instead, people affected with vitiligo have a decreased risk of melanoma, while for melanoma patients, vitiligo is associated with better prognosis, with spontaneous regression of melanoma (12).

Genes involved in melanoma pathogenesis and prognosis

The mutations of BRAF (incidence of 45%), NRAS (15%), GNAQ, and GNA11 (80-90%) (involved in the G alpha signaling pathway) (13) are known to be responsible for the hyperactivity of mitogen-activated protein kinase (MAPK), which is involved in tumor proliferation and progression. ALK fusions are found in about 10-20% of Spitz nevi and 1% of Spitz melanomas (14). BRAF encodes a cytoplasmic serine/threonine kinase in the MAPK pathway (15). Their functions and mutations confer different molecular mechanisms associated with tumorigenesis (16). CDKN2A is a tumor suppressor gene, it encodes two transcripts (p16 and p14ARF, both needed to ubiquitination of p53), its mutation is observed in FAMMM. Another protein, CDK4 inhibits the binding of p16 leading to phosphorylation of RB (mutated in FAMMM and atypical nevi) (16), TERT encodes a reverse transcriptase that creates a template for telomer addition.

In addition, *ACD, TERF21P, TERF1, TERF2, TINF2 and POT1* are implicated in telomere maintenance and their mutations increase telomere length and fragility (16), BAP1 is a tumor suppressor gene that encodes deubiquitinating enzyme and a binding partner to BCRA1, implicated in chromatin modulation, transcriptional regulation, and DNA damage repair (16).

TP53, involved in the control of the progression of the cell cycle from G1 to S phase, its mutation is associated with high risk of melanoma (17), the loss of one copy of chromosome 3 is associated with high risk of metastasis and death (17).

The protein phosphatase 2 scaffold subunit A alpha (PPP2R1A) may mediate the survival and resistance of apoptosis of the type B malignant melanoma cell lines (18), Aurora B kinase (AURKB) is a chromosomal passenger protein regulating early mitotic stage transition from prophase to metaphase, which is overexpressed in melanoma (18). STAT3, a gene involved in cytokine signaling, regulates the expression of genes implicated in survival, cell cycle progression and angiogenesis (19). The nuclear factor-kappaB (NFkB) is a transcription factor that regulates a variety of mechanisms by its signal pathway, such as immune and inflammatory responses. Its activation is regulated by tumor necrosis factor-alpha (TNF-α), IL-1 and Toll-like receptors: TNFR, IL-1R, and TLR, however, NFkB can be activated by dysregulations of MAPK and PI3K signaling pathways, increasing the risk of proliferation and drug resistance (20). Those pigmentated subtypes showed a higher expression of microphthalmia-associated transcription factor (MITF) compared with non-pigmented melanomas. *MITF* encodes a melanocytic-lineage-specific transcription factor that regulates the differentiation, proliferation, and survival of melanocytes (16). Its mutation confers higher cell growth, increased synthesis of melanin pigment, and poor prognosis (3).

microRNAs (miRNAs) are non-coding RNAs and are important gene regulators. They are considered as a new potential therapeutic strategy and fundamental prognostic factor. miR-21-5p reduces cell proliferation and promotes apoptosis by increasing PDCD4, PTEN, and BTG2. miR-146a-5p is upregulated by BRAF and NRAS, promoting cell proliferation, cell migration and invasion (21).

Another molecular mechanism implicated in tumorigenesis of melanoma is the DNA methylation alterations. The DNA hypermethylation of PTEN, CDKN2A and RASSF1A have been reported in melanomas. Tellez C.S. et.al reported an elevated methylation status in their melanoma cell lines: ESR1 (50%), MGMT (50%), RARB2 (44%), RIL (82%), RASSF1A (69%), PAX7 (31%), PGRB (56%), PAX2 (38%), NKX2-3 (63%), OLIG2 (63%), HAND1 (63%), ECAD (88%), CDH13 (44%), and CDKN2A/p16 (6%) (22). (Table1)

Table 1. The 2018 World Health Organization (WHO) classification of cutaneous, mucosal, and uveal melanoma (23–31).

Melanomas typically associated with Cumulative Solar Damage	Melanomas not consistently associated with Cumulative Solar Damage	
Pathway I. Superficial spreading melanoma/low-CSD melanoma	Pathway IV. Spitz melanoma	Nodular melanoma
Pathway II. Lentigo maligna melanoma/high-CSD melanoma	Pathway V. Acral melanoma	
Pathway III. Desmoplastic melanoma	Pathway VI. Mucosal melanoma	
	Pathway VII. Melanomas arising in congenital nevi	
	Pathway VIII. Melanomas arising in blue nevi	
	Pathway IX. Uveal melanoma	

The 2018 World Health Organization (WHO) classification of cutaneous, mucosal, and uveal melanoma is based on its arising sun-exposure skin, the role of ultraviolet (UV) radiation, precursors, and driving and/or recurrent genomic changes (23–31). In general terms, melanoma can be divided into two groups: UV-related and UV-unrelated melanomas.

UV-related group is more frequent in white population, it arises from epithelium associated-melanocytes in cutaneous sites with cumulative sun damage (CSD), which includes pathways I-III, while UV-unrelated group is more frequent in non-white population, it arises from non-epithelium associated-melanocytes regardless of CSD and it is associated with IV-X pathways (32).

Pathway I. Superficial spreading melanoma/low-CSD melanoma

Pathway I is the route by which melanocytes acquire the genetic aberrations necessary to become melanoma, however, it is associated with lower CSD. This pathway contributes to the appearance of superficial spreading melanoma. Superficial spreading melanoma is the most common form of melanoma. This kind of melanoma is particularly localized in parts of the body with intermittent sun exposure like in vacation or weekends. In men, its most frequent localization is in the back while in women is the back of the legs or calf region. They typically express BRAF V600E mutations, TERT, and NRAS mutations in less proportion (33).

Pathway II. Lentigo maligna melanoma/high-CSD melanoma

Pathways II and III are the pathways necessary to transform melanocytes in melanoma, however, in contrast with pathway I, these two types of pathways are associated with high CSD. Through pathway II, melanocytes acquire various genetic mutations, including NF1, BRAF V600K, NRAS, KIT, CCND1, MITF and TP53 which are associated with high CSD, and leads to lentigo maligna melanoma (LMM) transformation. LM is a melanoma subtype considered a melanoma in situ; it represents about 4-15% of all melanomas. The most frequent site of this subtype is in head and neck (78.3%). They can be presented as an amelanotic/hypomelanotic macule or patch, especially in fair-skinned individuals on chronically sun-damaged skin. There's described a sex-related preferential location of LM, developing on the right side of the face in males and on the left side in females (24,25).

Pathway III. Desmoplastic melanoma

As mentioned above, pathway III is associated with an extremely high mutation burden with high CSD. Desmoplastic melanoma (DM) arises from this pathway. DM is a rare variant of cutaneous melanoma; it accounts for about 1% of all melanomas. They're commonly amelanotic or sparsely pigmented and are typically endophytic (33). The genetic alterations associated with this kind of melanoma is the inactivation of NF1 and RAS mutations, which results in the activation of MAPK pathway, however, the genetic mutations and the genesis of this type of melanoma is not yet completely known (33).

Pathway IV. Spitz melanoma

Previously to WHO classification, Spitz melanoma (SM) was classified based on the cytomorphologic features in spitzoid melanomas. Nowadays, SMs are classified based on their morphologic and genomic alterations such as HRAS, ALK, NTRK1, MAP3K8, BRAF, and CDKN2A mutations, in contrast with its counterpart Spitz Nevi (SN). SMs are rare, they represent about 1-2% of all melanocytic lesions. The mean age of diagnosis in SM is 22 years old. They can be localized in any part of the body but is more frequent in lower extremities (40-50%), trunk (20%), upper limbs (15%), and head/neck (5%). SM are elevated lesions, mostly of them are larger than 1 cm in diameter and can have pink to black coloration. The majority are asymmetrical, with coloration variety, present shiny white lines, and polymorphous vascular patterns. (26).

Pathway V. Acral melanoma

Acral melanomas arise on the non-hair bearing skin, especially in the lower extremities (78%), comprises about 2-3% of all melanomas. They have a high number of structural chromosomal changes and lower frequencies of BRAF mutations (10-23%), KIT mutations (3-29%), amplification of CCND1 and CDK4, and deletion/mutations in CDKN2A, PTEN, NF1 and hTERT (27). They have a high expression of melanoma markers, such as S-100 (95%), SOX10 (100%), Melan-A (70%), and HMB-45 (80%) observed in immunohistochemistry (IHC) (27). The most characteristic alteration on the signal pathways is the mitogen activated protein kinase (MAPK), the most frequent of these are BRAF mutations at position 600 (V600E, V600K, V600D and V600R). The MAPK

pathway contributes to many aspects of the oncogenic behavior of melanoma cells including uncontrolled proliferation by enhanced Cyclin D1 (CCND1) expression and suppression of p27, immune escape by inhibiting expression of major histocompatibility complex-I (MHC-I), and invasion by regulation of integrins and cytoskeleton proteins. In addition, TERT promoter mutations, were observed in about 5-10%, which can be associated with melanoma progression (27).

Pathway VI. Mucosal melanoma

Primary mucosal melanomas (MM) are derived from neural crest cells that migrate to several sites, they can be found in the respiratory, gastrointestinal, and genitourinary tract, it represent about 0.8-3.7% of all melanomas. They are associated with aggressive and less favorable prognoses. C-KIT is overexpressed (80%), BRAF mutations are less common (<10%) and SF3B1 mutations (12%) cause directly aberrant gene transcripts which lead to mRNA degradation or abnormal protein function in MM. There are some specific risk factors such as tobacco, and formaldehyde (associated with oral and sinonasal MM), and human immunodeficiency virus (HIV) infection (associated with rectal MM) (28,29).

Pathway VII. Melanoma arising from congenital melanocytic nevi

Congenital melanocytic nevi (CMN) are hamartomas of the neuroectoderm, they are seen in about 1-6% of all birth, and they are caused by genetic mosaicism. Large/giant CMN occur in 1/20,000-50,000 births. They can be classified by its size in small (<1.5 cm), medium (1.5-20 cm), and large (>20 cm). BRAF mutations are mostly presented in small nevi, and NRAS mutations in large/giant CMN. Melanoma risk is difficult to quantify, but there is a high risk in lesions that lie across the spine or those who has numerous satellite lesions (10-15% of risk) (30). Large/giant CMN have a risk of 2% to transformation to malignant melanoma (31).

Pathway VIII. Melanoma arising from blue nevi

As mentioned, pathway VIII is an UV-unrelated group. This type of pathway is associated with chromosomal aberrations added to a precursor lesion, blue nevi. Blue Nevis are uncommon lesions. They express GNAQ and GNA11 mutations, and infrequently in PLCB4 or CYSLTR2, EIF1AX, SF3B1 and BAP1 mutations. In addition, the gain of chromosomal arms 1q, 4p, 6p and losses of 1p and 4q have been identified (33).

Pathway IX. Uveal melanoma

The eye is an immune-privileged organ, so, intraocular environment is considered an immunosuppressive environment, where melanoma can proliferate, invade, and progress to metastasis. Uveal melanoma (UM) is a rare disease, and it has been demonstrated that it is different from its cutaneous counterpart. More than 90% involve choroid, 6% are confined to the ciliary body and 4% to the iris. They represent the most frequent intraocular primary tumor in adults (34). They are usually unilateral and associated with light-colored eyes, congenital ocular melanocytosis, melanocytoma and the BAP1-tumor predisposition syndrome. About 85% of UMs carry GNAQ and GNA11 mutations, 10% LI29 CYSTLTR2 and D630 PLCB4 mutations (35).

Pathway X. Nodular melanoma

Nodular melanomas arise from any of the pathways mentioned above, that's why they have heterogeneous epidemiologic and genomic features. They are characterized to be nodular or papular at the clinical examination, with homogeneous or heterogeneous pigment. BRAF and NRAS mutations have been demonstrated in these kinds of tumors, however, its genomic alterations are still unknown (33).

Nowadays, there is a molecular classification of melanoma, with prognostic importance, however it has not yet been added to the current WHO classification.

I. BRAF-mutant: about 60% presents CDKN2A mutation, TP53 mutation (10%), ARID2 mutated (15%), PPP6C mutated (10%), PDL1 and MITF amplification (36).

II. RAS-mutant: CDKN2A mutated (about 70%), CCDN1 amplification (10%), TP53 mutation (20%), ARID2 mutation (15%) and PPP6C mutation (15%) (36).

III. NF1-mutant: CDKN2A mutation (70%), RB1 mutation (10%), TP53 mutation (30%), and ARID2 mutation (30%) (36).

IV. Triple Wildtype: CDKN2A mutation (40%), CDK4 amplification (15%), CCDN1 amplification (10%), and MDM2 amplification (15%) (36).

Tumorigenesis in melanoma cells is regulated by multiple signaling pathways, modulated by genetic and epigenetic mechanisms, with a straight interrelation between them (3,4,13–22,36–38) (Figure 1).

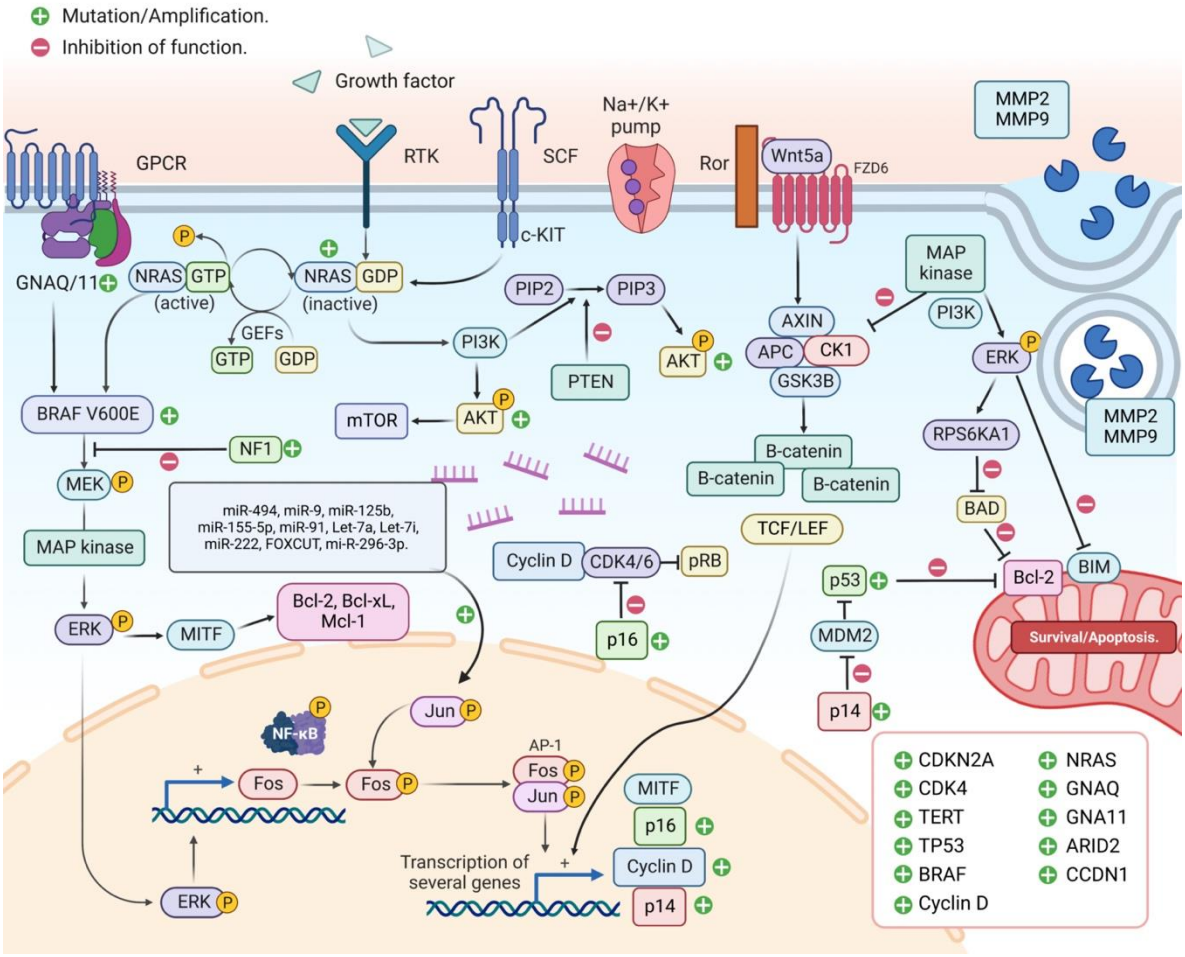

Figure 1. Genetic and epigenetic mechanisms of malignancy in melanoma.

Molecular mechanisms implicated in pathogenesis

Melanoma biology

Melanocytes are a heterogeneous group of cells, derived from the neural crest. They produce the protective skin-darkening pigment melanin in epidermis, hair, and iris, which is responsible of the protection of DNA from UV-mediated damage (39). Nowadays it has been observed that melanocytes are essential not only for UV-mediated damage protection since they have been found in the inner ear, nervous system, and heart (40).

Cutaneous melanoma is the most aggressive skin cancer; it derives from melanocytes. It accounts about 90% of melanomas including mucosal and uveal melanomas and represents about 1% of all skin cancers. The biology of the tumor is associated with the microenvironment, it has been demonstrated the hypoxic and acidity of microenvironment as an important role in melanoma biology (37). Like other solid cancers, melanomas need to increase glucose uptake, to support the high proliferation rate by upregulating glucose transporters and carbonic anhydrase, with the generation of L-lactate by the Warburg Effect, and the actively exporting protons in the extracellular microenvironment (41). The acidity generated by protons exported inhibits the proliferation of CD4+ and CD9+ Cytotoxic T Lymphocytes (CTL), Dendritic cells and Natural Killer cells (NK's), and activates proteolytic enzymes, such as matrix

metalloproteinases 2 (MMP2), cathepsin B, and cathepsin L, which are responsible for the degradation of extracellular matrix and the potential ability of invasion and metastasis. Hypoxic environment is common in advanced cancers, with local destruction, and necrosis, which activates hypoxia-inducible factors (HIF), such as HIF1, HIF2, and HIF3, promoting adaptation of cancer and stromal cells. In addition, tumor cells secret proteins, lipids, and nucleic acids by extracellular vesicles, which can dysregulate the physiological functions of extracellular matrix and cells (37). Currently, it has been described nanosized vesicles (30-120 nm), known as Exo, are involved in angiogenesis, tumor growth, and metastasis, by transporting active molecules, such as interleukins, vascular endothelial growth factor (VEGF), MMP-2, MMP-9 (42,43), and miRNAs, which are small non-coding RNAs (miR-494 (44), miR-9 (45), miR-125b (45,46), miR-155-5p (47), miR-91, Let-7a, Let-7i, miR-222 (46), FOXC promoter upstream transcript (FOXCUT) and miRNA has-miR-296-3p (38)). Mexo, an important protein needed to vehiculate molecules, can enter through the lymphatic vessels and their role is the formation of the pre-metastatic niche (PMN), by inducing members of LOX family (LOXL2 and LOXL4) and recruiting CD11b+ Ly6CmedLy6G+ myeloid cells and Cd4+ CD25 hiFOXP3+ Tregs, which secrete anti-inflammatory and pro-angiogenic factors, associated with the poor immunological response (37).

Acidosis plays an additional role by the dedifferentiation of cancer cells, to an immature phenotype, commonly known as Cancer Stem-like Cells (CSC), with the ability to self-renew and keep them in a quiescent state responsible for chemotherapy and radiotherapy resistance (37). These types of cells are immature and poor differentiated cells, so, they can be identified as the high expression of dedifferentiated surface cell markers and a low differentiated surface cell marker. The markers commonly associated with an immature melanocyte state are CD271 (known as p75) and CD133, drug resistance (ATP-binding cassette transporters, ABCs) and a high activity of ALDH1A1 and ALDH1A3 (48).

Nowadays, it is demonstrated that lipid metabolism is implicated in promoting melanoma progression. Carnitine palmitoyltransferase 2 (CPT2), phospholipase D3 (PLD3), inositol triphosphate protein kinase B (ITPKB), and inositol triphosphate receptor 3 (ITRP3), genes that encode lipid metabolism proteins, are significantly upregulated genes in melanomas compared with benign nevi, and their expression is associated with melanoma pathogenesis. However, the role of this kind of proteins in melanoma pathogenesis is still unclear (41).

Microenvironment

Melanoma is one of the most immunogenic tumors, so its microenvironment has a high concentration of infiltrating immune cells, however, most of them, are inhibitory immune populations, including T regulatory (T reg) cells, tissue-associated macrophages (TAMs) and myeloid-derived immunosuppressive cells (MDSCs) (27). Melanoma, like most of the tumors, manipulates immune defenses by intrinsic and extrinsic pathways (2). These pathways are known as “hallmarks” of cancer(49). At the same time that melanoma acquires chromosomal alterations, it also acquires different characteristics in contrast with melanocytes, which include resisting cell death, deregulating cellular metabolism, sustaining proliferative signaling (50), evading growth suppressors, avoiding immune destruction, enabling replicative immortality, tumor-promoting inflammation, activating invasion and metastasis (51), inducing or accessing vasculature, unlocking phenotypic plasticity (52), no mutational epigenetic reprogramming (53,54) and senescence (55). Immune evasion is necessary for tumor growth and progression. The microenvironment is the most important component of its immune response protection (56,57).

The most frequent inflammatory cells in the melanoma microenvironment are CD163+ histiocytes, CD3+ T lymphocytes, CD68+ histiocytes, cytotoxic CD8+ T lymphocytes, CD4+ regulatory T cells (58,59) and, CD20+ B lymphocytes. The low expression of p16 protein expression, low density of CD3+, and CD8+ cells is associated with poor prognosis by immunosuppressive statement, and with melanoma immune escape (27). CD4+ regulatory T cell is an important subtype of T cell in charge of downregulating the intensive inflammation, by secreting immunosuppressive cytokines (IL-10, IL-35, and TGF-B), inducing cytolysis by CD8+ T cells, targeting dendritic cells, and disrupting the immune

function of the cells. CD4+ regulatory T cells are increased in the tumor microenvironment (58,59), lymph nodes (60), and peripheral blood, so they are involved in melanoma progression and metastasis (2). Melanoma cells induce differentiation of myeloid cells in the bone marrow, into MDSC (61–63). MDSCs differentiate into TAMs, which are subdivided into M1 and M2 phenotypes. M1 phenotype is an anti-tumor subtype of TAM, while M2 phenotype promotes tumor progression and invasion (64,65).

The immune system has an efficient recognition of tumor cells, by presenting melanoma antigens to T cells, which can expand and become effector melanoma-specific T cells. Two immune checkpoints can upregulate or downregulate the immune stimulation: cytotoxic T lymphocyte antigen 4 (CTLA-4), a coinhibitory molecule on T cells that inhibits cells activation by ligation with CD86 and CD80; and programmed death 1 (PD-1), another immune checkpoint, that can be inhibited by programmed death 1 ligand (PD-L1 and PD-L2) expressed in tumor cells (66,67). PD-1/PD-L1 acts as a negative regulator of immune response. Healthy cells express PD-L1 in their membrane surfaces, which interacts with PD-1 receptors in T lymphocytes and prevents T lymphocyte activation. This immune protection mechanism is observed in surrounding healthy cells in an infection site (66,67); however, this physiological mechanism is used by tumor cells to evade immune response (68), which is upregulated by HIF-1, AP-1, and NF-KB transcription factors (69).

In addition to PD-1, CTLA-4 is the second most frequently known immune suppressive checkpoint regulator, its function is associated with immune suppressive activities by inhibiting T cell activation. CTLA-4 outcompetes CD28 for the ligands, CD80/CD86, in consequence, T cells become anergic (70).

Diagnosis

The diagnostic approach starts with dermoscopic evaluation, it's necessary to describe the skin lesion with the mnemotechnic ABCDE (Asymmetry (the most common criterion: 84.5%), Border, Color (the multicomponent pattern is the most characteristic and most common patient associated with melanoma), Diameter and Evolution) as seen in Figure 2. Dermoscopy is a fundamental for early diagnosis and in the preoperative estimate of the Breslow index (71), however there are some characteristics in the visual examination that it's necessary to be considered before the examination: we can recognize different dermoscopic structures with their different accuracy, such as atypical pigment network (Sensitivity: 21-100%; Specificity: 46-88.5%), angulated lines (Sensitivity: 16.7%; Specificity: 91.7%), negative network (Sensitivity: 22-34.6%; Specificity: 77.2-95%), atypical streaks (Sensitivity: 4.8-23%; Specificity:32-58%), atypical dots/globules (Sensitivity: 13-39.6%; Specificity: 74.3-92%), blue-white veil (Sensitivity: 11.4-92%; Specificity: 74-99%), atypical blotch (Sensitivity: 18-71.3%; Specificity: 30.5-92.6%), regression structures (Sensitivity: 11.4-79%; Specificity: 63-99%), peripheral tan structureless area (Sensitivity: 19-62.5%; Specificity: 92.6-96.1%), shiny white structures (Sensitivity: 70%; Specificity: 80.6%), and finally, atypical vascular structures (Sensitivity: 9.4-62.9%; Specificity: 53.8-96.1%) (72).

Most patients with cutaneous melanoma are asymptomatic, and they come to clinical care only in the presence of a suspicious injury. At the same time, patients with UM are asymptomatic (>40%), those who present symptoms may develop blurred or distorted vision, visual field loss of photopsia, or other ocular symptoms, rarely large tumors induce vitreous hemorrhage (34,35) To make easier the diagnosis of distance and nodular metastasis, extension to adjacent structures and recurrence, there are some diagnostic methods, from examination by imaging to molecular biomarkers, and their accuracy is compared to each other, like seen in Table 2.

The visual inspection of a suspicious skin lesion is the first step in melanoma diagnosis, its sensitivity is about 76% (66-85%) and specificity 75% (57-87%) (71–73) (Figure 2).

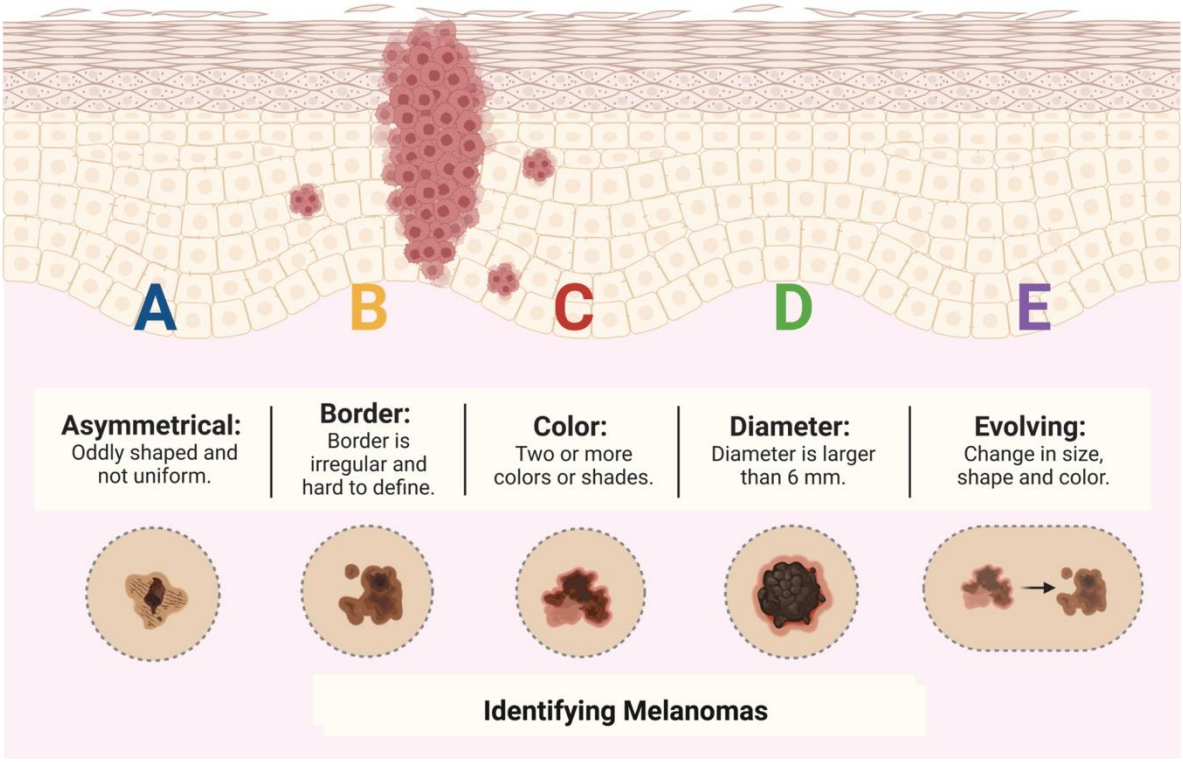

Figure 2. ABCDE for identifying melanoma.

Table 2. Accuracy of several methods used in melanoma diagnosis and staging.

Diagnostic method.	Accuracy.		Characteristics.
	Sensitivity.	Specificity.	
Visual inspection	76% (66-85%) (73)	75% (57-87%) (73).	Clinical inspection of pigmented skin lesions using the mnemonic ABCDE (73)
Dermoscopy	Without Artificial Intelligence Support (53.3-65.5%) With Artificial Intelligence Support (81.9-87.6%) (74)	Without Artificial Intelligence Support (62.3-78.9%) With Artificial Intelligence Support (74.8-83.4%) (74)	It's the examination of pigmented and non-pigmented skin lesions with the naked eye (75) With artificial intelligence support like reflectance confocal microscopy increases accuracy (74)
Histopathology	91% (84-95%) (73)	94% (86-98%) (73).	The histological examination of a pigmented skin lesion. It's considered the gold standard for melanoma diagnosis (73)
Immunohistochemistry (IHC)	Adjuvant to histopathology, it consists in the examination of melanoma antigens using anti-H4K20me and anti-H3K27me3 monoclonal antibodies, which interact with their respective antigens (76)		
Comparative Genomic Hybridization (CGH)	92-96% (77)	87-100% (77)	Adjuvant to histopathology detects genome-wide changes in DNA copy number, but it doesn't detect actual mutations. It can detect genetic anomalies in chromosomes 6p, 1q, 7p, 7q, 8q, 17q and 20q and/or losses of 9p, 9q, 10q, 10p, 6q and 11q (77)
Fluorescent In Situ Hybridization (FISH)	43-100% (77)	29-80% (77)	Adjuvant to histopathology detects cytogenetic abnormalities by direct visualization (77)
Ultrasound. (US)	Nodal metastasis 35.4% (17-59. 4%) (78)	Nodal metastasis 93.9% (86.1-97.5%) (78)	Ultrasound uses high-frequency sound waves to create images in the body, it can be used to assist in detection of lymph node metastasis (78)

Ultrasound with Fine Needle Aspiration Cytology (US FNAC)	Nodal metastasis 18% (3.58-56.5%) (78)	Nodal metastasis 99.8% (99.1-99.9%) (78)	The cytologic examination of skin lesions using a fine needle aspiration guided by ultrasound (78)
Computed Tomography (CT)	Nodal metastasis 87.2 (76.5-93.4%). Distant metastasis 73.4% (63.6-81.3%) (78)	Nodal metastasis 69.2% (34.6-90.5%). Distant metastasis 72% (64.3-78.5%) (78)	Uses ionizing radiation in the form of X-rays to take cross sectional images of the body, is used to evaluate metastasis (78)
Magnetic Resonance Imaging. (MRI)	Nodal metastasis 83.7% (68.8-92.3%). Distant metastasis 74.5% (62.1-83.9%) (78)	Nodal metastasis 77.7% (72.4-82.1%). Distant metastasis 85.8% (70.4-93.9%) (78)	Uses large magnets and non-ionizing radiation in the form of radio waves to generate images of the body, is used to evaluate metastasis (78)
Positron Emission Tomography (PET/CT).	Nodal metastasis 86.5% (80.2-91.1%). Distant metastasis 92.5% (85.3-.96.4%) Detection of bone metastasis 90.2% (78.5-95.9%) (78)	Nodal metastasis 92.5% (85-96.4%). Distant metastasis 89.7% (78.8-.95.3%) Detection of bone metastasis 88.2% (72.5-95.5%) (78)	A nuclear medicine imaging technique, it uses a radioactive component (^{18}FDG intravenous) which is taken up by cancer cells (78)

New treatment strategies

Most patients are diagnosed in early-stage disease, in which surgical excision is the treatment of choice, because it's curative in most of the cases (79). About 10% of new-diagnosed patients, presents an advanced-stage disease (unresectable or metastatic disease). They can be treated with kinase inhibitors (BRAF and MEK inhibitors) alone or in combination with immune checkpoint inhibitors (anti-CTLA4 and anti-PD1 monoclonal antibodies) (80).

BRAF is a serine/threonine protein kinase, encoded on chromosome 7q34, which activates the MAPK/ERK-signaling pathway. The most frequent BRAF mutation (90%) is located at codon 600, in which a single nucleotide mutation results in the substitution of glutamic acid for valine (V600E) (81). Melanomas with BRAF V600E mutation are associated with poor prognosis by promoting angiogenesis, immune evasion, invasion, and metastasis, whose can be used BRAF inhibitors such as dabrafenib and vemurafenib, however, the upregulation of MEK 1/2 is associated with a prominent escape from the mechanism, so it's necessary to use a combination of BRAF inhibitor and a MEK inhibitor (such as trametinib), demonstrating a survival advantage in both resectable and unresectable/metastatic disease (80).

As mentioned above, melanoma cells express PD-L1 in their membrane surfaces, and the interaction of CTLA-4 in T cells membrane surfaces results in T cell anergy. These two immune checkpoints are important for an effective immune response. Immune checkpoint inhibitors play key roles, when a tumor does not have targeted mutations, or it does not respond to BRAF/MEK inhibitors. There are two types of immune checkpoint inhibitors, PD-1 inhibitors (nivolumab and pembrolizumab) and CTLA-4 antibody inhibitors (ipilimumab). The inhibition of these two immune checkpoints helps the immune system to recognize cancer cells by suppressing melanoma's immune evasion system (82). The combination of both types is associated with a high inflammatory cell infiltration compared with ipilimumab alone (80). Nowadays, these new treatment strategies are considered the backbone of systemic therapy, while chemotherapy is considered the second line of treatment (83) since in one systematic review made by Pasquali S, et al. using Cochrane Library Database concluded that the combination of anti-CTLA4 plus anti-PD1 monoclonal antibodies was associated with better progression-free survival (HR 0.40, 95% CI 0.35 to 0.46, 2 studies, 738 participants); and the combination of BRAF plus MEK

inhibitors was associated with better overall survival (HR 0.70, 95% CI 0.59 to 0.82, 4 studies, 1784 participants) (82).

Future directions

Numerous phase I and II clinical trials are currently underway to explore innovative agents and multimodal approaches to enhance the prognosis of patients facing melanoma. Many of these trials are centered on monoclonal antibodies, which represent vital components of targeted strategies in the era of precision medicine. While monoclonal antibodies hold considerable promise, their mechanism of action often entails inhibiting critical pathways associated with melanoma pathogenesis. Consequently, these interactions can lead to adverse effects.

Discussion

Various researchers have conducted exhaustive investigations into the mechanisms discussed earlier, underscoring their significance in driving carcinogenesis in melanocytes and their correlation with various molecular subclassifications. While new treatment strategies have emerged based on these mechanisms, some still lack targeted therapies, necessitating further research into the yet uncharted direct and indirect contributors to tumorigenesis. Genetic, epigenetic alterations and tumor microenvironment have all been associated with this unfavorable prognosis due to their facilitation of uncontrolled proliferation of malignant cells. Therefore, this article seeks to consolidate valuable insights on melanoma, to contribute to the formulation of treatment strategies.

Conclusions

Melanoma is the most aggressive skin cancer, with poor prognosis and high mortality. Its pathogenesis encompasses many molecular mechanisms, incorporating genetic and epigenetic factors. These mechanisms operate within various signaling pathways, often displaying interconnectedness and interplay. They exert their influence on pro- and anti-apoptotic proteins, sculpting the microenvironment by regulating cell proliferation, invasiveness, and immune evasion. Intriguingly, these emerging mechanisms are not confined to melanoma but are also observed in other solid tumors, including breast, colorectal, urogenital, pancreatic, and lung tumors. Nowadays, these new molecular mechanisms open the possibility of investigating new alternatives for possible targeted therapies. The primary objective of this review article is to provide a comprehensive account of the molecular mechanisms involved in melanoma pathogenesis and how the alteration of any of them leads to the genesis of cancer, to foster the development of novel targeted therapy strategies.

Author contribution

This manuscript was written entirely by **JMM**.

Conflict of interest

The authors report no conflict of interest.

Funding

There is no funding.

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