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Review Article

Molecular pathogenesis and therapeutic strategies of Malignant melanoma: A brief Review

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ABSTRACT:
Melanoma is one of the most common and fastest growing cutaneous cancers worldwide. It contributes to 71–80 % of skin cancers deaths. UV radiation, melanotic nevi and sunlight are major risk factors leading to development of melanoma. These risk factors lead to various molecular changes allowing melanoma cells to have a growth and survival advantage over others. Depending on the features of the tumor (location, stage, and genetic profile), the therapeutic options may be surgical resection, chemotherapy, radiotherapy, photodynamic therapy (PDT), immunotherapy, or targeted therapy.
In this review, we discuss the various molecular changes associated with cutaneous melanoma and therapies approved or under evaluation for melanoma treatment and relevant research on the molecular mechanisms underlying melanogenesis.
Key words: Metastasis, UV radiation, Sunlight, mutation, BRAF, NRAS.

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INTRODUCTION

Cutaneous melanoma is a malignant neoplasm of the melanocytes which are found in the basal layer of the epidermis, the function of which is production of melanin; responsible for skin colour. It is one of the major types of skin cancer causing vast majority of skin cancer-related deaths even though it accounts for approximately 5% of skin cancer cases. (1, 2) There are four major forms of melanoma including (i) superficial spreading, (ii) nodular, (iii) lentigo maligna and (iv) acral lentiginous melanomas. Of these, the superficial spreading form remains most common; and accounts for about 70% of melanomas followed by nodular form that represents about 15–30% of melanoma cases.(3) Nowadays melanoma is considered as a multi-factorial disease which arises from an interaction between genetic susceptibility and environmental exposure and is known to be associated with various factors like UV radiation, excessive sun exposure, melanotic nevus which lead to its development. (4) Elwood *et al.* studied the correlation between melanoma and sun exposure concluding that intermittent sun exposure appears to be a major determinant of risk for melanoma and is highly mutated tumour, characterised by an abundance of ultraviolet-induced DNA aberrations.(5) Some history of a sunburn may be an indicator of intense intermittent sun exposure. Moreover a history of sunburns in childhood is associated with the highest risk and sun exposure is believed to be involved in the malignant transformation of cutaneous melanocytes. (6,7) Primary melanoma patients demonstrate approximately an 11% mortality rate (2, 6), whereas the mortality rate due to metastatic melanoma is significantly higher and patients typically have a low survival rate due to the poor efficacies of current cancer therapies. (8,9) At the cellular level, cancer cells possess distinguishing molecular properties that allow for apoptosis evasion, limitless growth potential without the need for growth factors, angiogenesis, and metastasis. (10) Identifying specific molecular changes that allow melanoma cells to have a growth and survival advantage over others may aid in the development of more effective targeted therapies to improve the prognosis of melanoma patients.

In this review, we discuss the various molecular changes associated with cutaneous melanoma and therapies approved or under evaluation for melanoma

treatment and relevant research on the molecular mechanisms underlying melanogenesis.

Etiology and Pathogenesis

UV Radiation

Studies have shown that a major risk factor for melanoma development is exposure to Ultraviolet (UV) radiation. Excessive and cumulative exposure to UV radiation results in an increasing level of DNA damage to proliferating melanocytes, which eventually overcomes the capacity for DNA repair mechanisms to compensate usually evoking mechanisms which precipitate apoptotic cell death but occasionally a cell undergoes malignant transformation and nucleates a melanoma. (11,12) One or more blistering sunburn during childhood or adolescence doubles the risk for melanoma in later life and sunburns are known to be a measure of excessive sun exposure. (13) Ultraviolet radiation (UVR) is divided into ultraviolet C (UVC; 200 – 280 nm), ultraviolet B (UVB; 280 – 320 nm) and ultraviolet A (UVA; 320 – 400 nm). (14) The UVC spectrum is highly mutagenic but does not reach the earth's surface because it is absorbed by the stratospheric ozone layer meanwhile UVA and UVB wavelengths represent 95% and 5% of the UV spectrum reaching the earth's surface. (15-20) UVA is generally considered to be less carcinogenic than UVB, is an important factor involved in photocarcinogenesis acting through different mechanisms than UVB and low energy UVA radiation is weakly absorbed by DNA, but can be absorbed by other cellular chromophores, inducing oxidative changes in the cells. (21, 22) Meanwhile high-energy UVB photons are strongly attenuated by stratum corneum and in deeper layers of epidermis UV is absorbed by melanin, DNA, aminoacids, keratin, urocanic acid and other chromophores. Ultraviolet radiation also affects function of immune system causing immunosuppression is not limited only to irradiated area but a systemic suppression of immune system is observed. (24) The main cells affected by UV radiation are Langerhans cells and T lymphocytes which affects the number, function and morphology of Langerhans cells making them less capable of antigen presenting. The three molecular targets proposed to be involved in initiation of suppression of immune system are: DNA damage, plasma membrane damage and trans to cis isomerisation of urocanic acid, the latter affecting the morphology and function of Langerhans cells. (23, 25) Various experiments have shown evidence that UV radiation frequently leads to DNA mutations, such as formation of pyrimidine dimers or deamination of cytosine into thymidine. (26, 27)

Melanotic nevi

Nevi are benign lesions composed of a concentrated amount of melanocytes, leading to a dark spot on the skin due to large amount of melanin that is produced, in one study about 81% of melanoma patients observed a changing nevus in the location of malignant lesion.(3, 28) The Clark model for melanoma progression emphasizes a series of histopathological changes beginning from benign melanocytic nevus to melanoma via dysplastic nevus. Various models of the genetic basis of melanoma development and progression are based on this Clark's multi-step model, predicting the acquisition of a BRAF mutation to be a founder event in melanocytic neoplasia. (29) Clark et al.(1984) also proposed a multi step progression model of melanoma. The first phenotypic change in normal melanocytes is the development of benign melanocytic nevus. Michaloglou et al. in 2008 suggested that melanocytic nevus is a benign clonal tumor, which temporarily undergoes proliferation via oncogenic BRAF signaling followed by growth arrest due to oncogene-induced senescence. (29) Dysplastic nevus, which histopathologically shows structural and cytological atypia is the next step towards the melanoma evolution. A dyspeptic nevus may arise from a preexisting melanocyte nevus or as a new lesion. The third step in progression is the radial growth phase (RGP) melanoma, which spreads progressively within or just beneath the epidermis followed by the final stage in melanoma progression which is the vertical growth phase (VGP), growing deep in the dermis and is a metastasis competent. (29) Mutations repressing apoptosis would be required for progression to VGP which allow cells to survive in the absence of keratinocytes. Miller and Mihm (2006) in a review stated that progression from RGP to VGP is also marked by the loss of E-cadherin, as well as the aberrant expression of N-cadherin and aVb3 integrin. (29,30)

Molecular Changes Associated with Malignant Melanoma

BRAF

BRAF is the most frequently targeted gene in melanoma. BRAF gene is otherwise known as V-raf murine sarcoma virus oncogene homolog B1, is a member of the RAF family and is situated on chromosome 7q34. (31,32) It is a proto-oncogene that encodes a serine/threonine protein kinase as part of the RAS-RAF-MEK-ERK kinase pathway, which promotes cell growth and proliferation. (33,34) Studies have shown that around 40–60% of all melanoma cases exhibit an activated BRAF mutation occurring predominantly in melanomas arising on intermittently sun-exposed skin rather than in melanomas arising on chronic sun-exposed or completely unexposed sites. (35,36) Majority of BRAF mutations found in melanocytic nevi and melanomas substitute valine for glutamine at codon 600 which leads to a missense mutation at the kinase activation domain of exon 15. (37) More than 97% of *BRAF* mutations are located in codon 600 of the *BRAF* gene. (38) *BRAF*-mutated melanoma tends to

exhibit distinctive clinical features and is characterized by more aggressive biological behaviour and are more commonly found in younger patients and have tumors with superficial spreading or nodular histology and/or in anatomical regions without chronic sun damage. (39) Molecular testing for *BRAF* mutations in patients with advanced melanoma has become a standard for determining the course of therapy, and testing is recommended by the current National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology guidelines for melanoma. (40,41) Research has revealed that the early stage of melanoma formation, known as the radial growth phase, exhibits a low 10% BRAF mutation rate, which supports the hypothesis that BRAF does not play a part in melanoma initiation. (42) At the same time, 60–70% of vertical growth lesions and metastasized melanomas possess BRAF mutations, which suggests that this oncogenic mutation may be involved in cancer progression .(43-46) Thus identification of somatic mutations in the gene encoding serine-threonine protein kinase B-RAF (BRAF) in majority of melanomas gives an opportunity to test oncogene- targeted therapy for this disease.

NRAS

Another source of molecular changes that may allow for melanoma initiation or propagation is found in the NRAS GTPase or neuroblastoma RAS oncogene. (3) NRAS GTPase mutations are found in 15–20% of melanoma patients and activating mutations are usually found in the Q60/61 and G12/13 codons. (46,47) Activation of the MAP kinase pathway has been identified as a key player in melanoma. (48,49) Activating mutations in *NRAS* have been identified in approximately 15% – 20% of melanoma tumors. (50) The most common *NRAS* mutations are in exon 2 at codon 61, specifically Q61L (leucine substitution for glutamine) resulting in a constitutively active form of the protein leading to uncontrolled cellular proliferation. (51,52) The small GTPase, NRAS, was the first oncogene identified in melanoma and other mutational subtypes of melanoma. (52) Patients with mutant NRAS tumors tend to be older and have a history of chronic ultraviolet (UV) exposure. (53,54) Histologically, mutant NRAS tumors are more aggressive than other subtypes and have thicker lesions, elevated mitotic activity, and higher rates of lymph node metastasis.(55,56)

PI3K-ATK/PTEN

PI3K (phosphatidylinositol 3-kinase)–AKT pathway is a separate route that also plays a role in cell proliferation and survival. (57) Constitutive activation of PI3K-AKT leads to a competitive growth advantage that makes way for melanoma proliferation and metastasis. (58) High levels of PI3K-AKT activity can also result from activation via an NRAS mutation or a lack of inhibition due to PTEN inactivation and PTEN containing a phosphatase domain, is inactivated in 12% of melanomas through mutation or methylation. (59, 60) Mutations of PTEN contribute to antiapoptosis, abnormal proliferation, angiogenesis, and invasion for melanoma development and progression. But analysis of melanoma tumor samples has identified about 3% PI3K missense mutation rate and such a low rate makes this pathway appear as an unlikely contributor to melanoma development and progressions. (61,62)

CDK4/CDKN2A

Cyclin-dependent kinase 4 (CDK4)classic cell cycle kinase forming complexes with D-type cyclins and Tumor suppressor gene CDKN2A, which encodes p16INK4a have been implicated in familial melanoma development indicating the genetic susceptibility to develop melanoma. (63- 65) CDK4 and p16INK4a regulate cell cycle progression from the G1 to S phase upon activation by Cyclin D1. CDK4 acts as a proto-oncogene that promotes the progression from G1 to S phase, allowing cell proliferation, whereas, p16INK4a inhibits CDK4 action, arresting cell division. (66) In view of these molecular changes, the logical step would be to inhibit CDK4 to prevent further cell cycle progression and limit the uncontrolled growth in melanoma.

MC1R

Melanocortin 1 receptor (MC1R) acts as a G-protein coupled receptor in melanocytes located on chromosome 16q24.3 and plays a critical role in determining skin pigmentation. (3) *MC1R* is the most important gene found to play a role in predisposition to sporadic cutaneous melanoma and its association with cutaneous melanoma has been replicated and confirmed by meta-analyses and genome-wide association studies. (67) Exposure to UV radiation leads to generation of alpha-melanocyte stimulating hormone, which activates MC1R to generate melanin via the process of melanogenesis. (68) In normal conditions, melanin along with the glutathione pathway acts as antioxidants that neutralize the destructive effects of reactive oxygen species, however, in MC1R variant individuals, the decreased melanin production would lead to an increased state of oxidative stress and thus, DNA damage that may trigger mechanisms of melanoma initiation. (69) Individuals with MC1R variant leading to low melanin production are more vulnerable to UV-induced DNA mutagenesis making them susceptible to acquiring mutations in genetic loci of BRAF, NRAS, or CDKN2A, which have been found with constitutively activated BRAF melanoma patients who typically do not possess a history of chronic-sun damage of the skin. (68,70)

THERAPEUTICS STRATIGIES

Current therapeutic approaches include surgical resection, chemotherapy, photodynamic therapy

(PDT), immunotherapy, biochemotherapy, and targeted therapy. The therapeutic strategy includes single agents or combined therapies, depending on the patient's health, stage, and location of the tumor. (71) Primary melanomas are typically treated with surgical excision, yielding a high survival rate; but following metastasis, surgical excision of the tumor only yields about 10% five-year survival rate. (72) IFN-α is an FDA approved adjuvant therapy, which is administered after surgery in order to help prevent any remaining melanoma cells from proliferating.(73) Various treatment options available:

- *Chemotherapy*: It was the earliest treatment option for advanced melanoma. Chemotherapy combinations have been evaluated to improve the clinical responses, but the overall survival (OS) did not show improvement. (74) Dacarbazine, an alkylating agent approved in 1974 by FDA, is the standard chemotherapy medication for metastatic melanoma. Studies reported that a complete response was achieved in <5% and 5-year survival in 2%–6% of patients.(75) Electrochemotherapy is a technique that combines the use of cytotoxic drugs, bleomycin and cisplatin, with high-intensity electric pulses, which facilitates drug delivery into the cells. (76,77)
- PDT: It is a minimally invasive procedure requiring a photosentisizer and light of a defined wavelength, to activate the photosensitizer.(78) Both create reactive oxygen species (ROS) when combined with oxygen. ROS unleash irreversible damage to tumor cells and tumor-associated blood vessels, also activating antitumor, immune, and inflammatory responses. (79,80)
- IMMUNOTHERAPY: It is known in many types of cancer that complex interactions between the tumor and the immune system play a role in the metastatic spread to distant sites. (81) Tumor infiltrating lymphocytes (TILs) have been considered in many studies as independent markers for the occurrence of lymph node metastasis. TILs can mediate immune responses of the host against cancer cells, being associated with a positive outcome and improved survival in patients with malignant melanomas. (82) The immunogenic tumor microenvironment (TME), with mediators and cellular effectors of inflammation, influences the success of immunotherapies, the molecular pathways involved in this cancer-related inflammation are now being clarified, in order to establish new target molecules that may lead to improvements in the diagnosis and treatment of cancer. (83,84) Improved knowledge of the pathophysiology and a better understanding of the role of immune system in tumor evolution have led to the development and approval of several immunotherapies involving Interferon (IFN) α-2b, Peginterferon α-2b (Peg-IFN), Interleukin-2 (IL-2), Treg inhibition, Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) blockade, Programmed cell death protein 1 (PD-1)/PD-1 ligand (PD-L1) blockade, Oncolytic virus therapy, gp100 peptide vaccine, Adoptive T-cell therapy.
- *Targeted Therapy:* About 70% of patients with cutaneous melanoma harbor mutations in genes of key signaling pathways. These oncogenic mutations may be associated with melanoma cell proliferation and a malignant phenotype. (85) Targeted therapies include:

BRAF inhibitors

Vemurafenib: It is a highly selective inhibitor of mutated BRAF, is extremely active in patients with metastatic melanoma who harbor a mutation in *BRAF*V600 (including patients with non V600E mutations) by inducing response rates in ~50% of patients and prolonging survival when compared to traditional chemotherapeutic agents. (86,87) Despite the clinical success of vemurafenib, most, if not all, patients eventually develop resistance. Multiple resistance mechanisms have been defined and are generally grouped as either those with reactivation of the MAPK pathway (intrinsic) or those outside of the MAPK pathway (extrinsic). (88) Vemurafenib is compared with dacarbazine for unresectable stage IIIC and stage IV metastatic BRAFV600E positive melanomas.

Dabrafenib: It is considered as a next generation agent and has a mechanism of action similar to that of vemurafenib. Dabrafenib is also a selective BRAF-mutant inhibitor approved by the FDA (2013) for the treatment of unresectable or metastatic melanomas harboring *BRAFV600E* mutations. (89,90)

MEK Inhibitors

Trametinib: It is a pharmacological MEK1/2 inhibitor with antitumoral activity, was approved (2013) as a monotherapy by the FDA for the treatment of unresectable or metastatic malignant melanomas with *BRAF* mutations. (91) Trametinib inhibits MEK i.e. the extraceullular signal-regulated kinase that is downstream of BRAF, its recommended dose is 2 mg orally once daily and the duration of treatment remains until disease progression or unacceptable side effects develop. (3) Combined therapy of trametinib and dabrafenib (BRAF-mutant inhibitor) showed durable objective responses in a randomized, multicenter, open-label studyand the combination was approved (2014) by the FDA for the treatment of unresectable and metastatic melanomas harboring *BRAF* mutations. (92,93)

CKIT Inhibitors:

Imatinib is an oral CKIT inhibitor that reveals significant activity in patients with metastatic

melanoma harboring *CKIT* aberrations, with a response rate of 30%. (94,95) Other multikinase inhibitors, such as sunitinib, dasatinib, and nilotinib, may have activity in patients with melanoma harboring *KIT* mutations. (90)

Cyclin-dependent kinase (CDK) inhibitors:
A new generation of selective CDK4/6 inhibitors, including ribociclib, abemaciclib, and palbociclib, has enabled tumors to be targeted with improved effectiveness and fewer adverse effects. (96) Abemaciclib has also been reported to induce growth regression in vemurafenib-resistant melanoma models, in which high levels of cyclin D1 expression and MAPK-pathway reactivation were observed. (97)

CONCLUSION

Melanoma represents a serious public health problem, due to its high case-fatality rate. Identification of individuals at high risk would be of major interest to improve early diagnosis and ultimately survival. The understanding of melanoma pathogenesis is crucial for the development of new therapeutic modalities. Characterization of oncogenic signaling pathways and interactions allow the identification of novel targets for clinically effective treatments, such as pathways inhibitors and immune checkpoint antibodies. Although it represents an advancement for melanomas treatment, these types of approaches face several challenges. The comprehensive features of patients that will benefit from each strategy aim to establish biomarkers (eg, specific mutations) for the best (eg, targeted) therapy in advanced melanomas. The convergence of immunology with other disciplines of biomedical research would lead to further improvement in the development of newer and more efficacious therapeutic approaches to manage this debilitating and deadly disease.

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