Melanoma Development: Current Knowledge on Melanoma Pathogenesis

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ABSTRACT The pathogenic features of melanomas include growth and amplification of atypical melanocytes associated with several features (self-sufficiency of growth factors, insensitivity to growth inhibitors, evasion of cellular apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion, and metastasis). These melanoma pathogenic events can be triggered by activating oncogenes or inactivating tumor-suppressor genes by means of molecular mechanisms such as dotted mutations, deletions, and translocations or epigenetic mechanisms such as microRNA expression and promoter methylation. In melanomas, an analysis of the gene aberrations in the genome has led to the discovery of the complex interaction of signaling pathways. Progression of melanomas also involves genetic instability and selective growth of cells with favorable mutations. Additional factors include genetic predisposition, mutagenesis, and suppressed host immune response. Some of the most important signaling pathways involved in the pathogenesis of melanoma are the MAPK, PI3K/PTEN/AKT, and MITF signaling pathways. Obtaining insight into the biology of melanocytes and pathogenesis of melanomas is important for the development of a targeted therapy (such as vemurafenib, dabrafenib, trametinib) as well as the immunotherapy (e.g. pembrolizumab, nivolumab, ipilimumab), which has enabled a substantial breakthrough in the treatment of patients with melanoma.

KEY WORDS: melanoma, pathogenesis, signaling pathways, mutations, targeted therapy, immunotherapy

INTRODUCTION

A melanoma develops through malignant transformation of melanocytes, the cells producing the pigment melanin (1,2). The origin of melanocytes is associated with the fetal period and the melanocyte precursors which are produced in the neural ridge and which migrate to various localizations in the body during fetal development (including the skin, meningeal coverings, mucous membrane, the upper part of esophagus, and the eyes). This is why a melanoma can develop by malignant transformation of melanocytes in any of these places, but it most frequently develops in the skin – specifically, the hair follicles in the skin – where it develops from melanocytes on the dermal-epidermal junction (3,4). The

pathogenic features of melanomas are associated with their growth and with amplification of atypical melanocytes. Several features have been observed: self-sufficiency of growth factors, insensitivity to growth inhibitors, evasion of cellular apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion, and metastasis (5-7). These pathogenic events can be triggered by activating oncogenes or inactivating tumor-suppressor genes by means of molecular mechanisms such as dotted mutations, deletions, and translocations or epigenetic mechanisms such as microRNA expression and promoter methylation. Analysis of the aberrations of genes in the genome has led to the discovery of the complex interaction of signaling pathways in melanomas (2). Progression of melanomas also involves genetic instability and selective growth of cells with favorable mutations. Additional factors include genetic predisposition, mutagenesis, and suppressed host immune response (2,7). Obtaining insight into the biology of melanocytes and the pathogenesis of melanoma is important for the design and development of anti-cancer therapy (8,9).

RISK FACTORS IN MELANOMA DEVELOPMENT

Environmental and genetic factors are involved in the pathogenesis of melanomas. The environmental factors include UV radiation, burns suffered early in life, physical features such as blonde or red hair, green or blue eyes, presence of multiple (>100) melanocytic nevi, five or more atypical nevi, and fair skin, although melanomas can also develop in persons with dark-pigmented skin, as acral or mucosal melanomas, etc. (10,11).

Melanomas of the trunk and limb skin are connected with intermittent exposure to UV radiation and frequent BRAF (50%) and NRAS (20%) mutations. On the other hand, lower rates of BRAF mutations (5-20%) and higher rates of KIT mutations (5-10%) have been demonstrated in mucosal and acral lentiginous melanomas (11).

Genetic factors are particularly important for the pathogenesis of melanomas (sporadic and family melanomas). Genetic factor analysis has shown that it is the sporadic melanomas that account for most of the melanomas (90% of them). These are the melanomas which are not familial, their most marked mutation being BRAF (V600E) (1). On the other hand, familial melanomas account for only 10% of melanomas. Familial melanomas are mostly associated with the dysregulation of cell cycle regulators. Tumor suppressor gene cyclin-dependent kinase inhibitor 2A (CDKN2A) which encodes p16INK4A and p14 ARF proteins has been identified as a main high-risk gene for melanoma (10,12,13). It has been established that p16INK4α
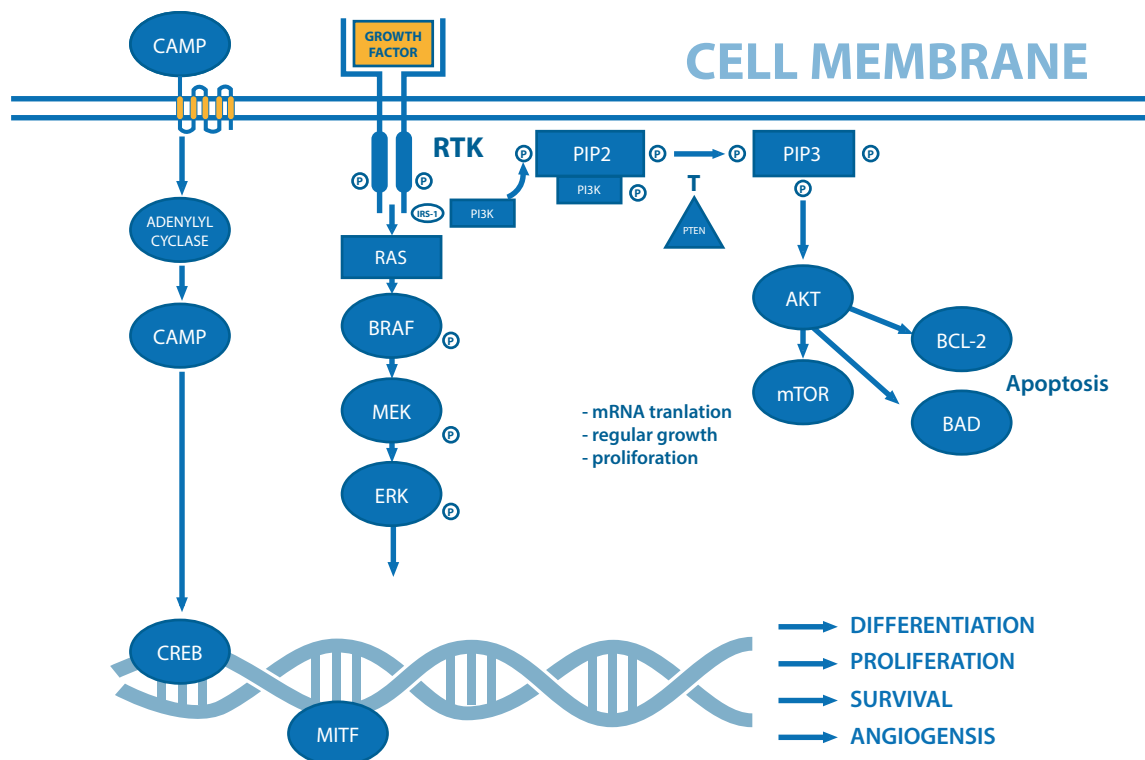
Figure 1. Crucial pathways in melanoma pathogenesis (based on current knowledge).

regulates the transition from G1 to S phase of the cell cycle, functioning as a CDK inhibitor and blocking the phosphorylation and inactivation of the Rb protein at the same time, which is the key control point in the cell cycle. On the other hand, p14ARF also acts anti-proliferatively and inhibits the disintegration of the p53 tumor suppressor. The normal expression of p53 puts a stop to the cell cycle in G2/M phase or induces the programmed cell death (apoptosis) (e.g. as a response to DNA damage caused by UV radiation) (1).

RELEVANT SIGNALING PATHWAYS INVOLVED IN MELANOMA PATHOGENESIS

The MAPK pathway

The MAPK signal pathway (mitogen-activated protein kinase cascade) regulates the cellular proliferation, growth, and migration and is activated in almost all melanomas. The MAPK pathway activates normally as well, but in case of melanoma the pathway becomes increasingly activated so the activation becomes excessive (2). The activation of the MAPK pathway takes place when a growth factor binds with a tyrosine kinase receptor (Figure 1). The stimulation of the receptor triggers activation of the members of RAS family – the monomer G proteins causing cascade activation of serine/threonine kinases which in turn lead to activation of ERK (also known as MAPK). This ERK is a serine/threonine kinase that activates the transcription factors, thus intensifying the transcription of the genes involved in cellular growth, proliferation, and migration. The crucial role of this pathway is emphasized by the fact that either NRAS or BRAF are mutated in approx. 80% of all skin melanoma or melanocytic nevi (2). The aberrations leading to activation of the MAPK pathway in melanomas mostly include the activating mutations of the BRAF serine/threonine kinase (40-50%) and G protein NRAS (15-20%) (14).

Understanding of the MAPK signaling pathway has led to development of targeted therapy for a subset of patients with BRAF-activating mutations. Although BRAF inhibitors showed clinical activity, combination therapy with BRAF + MEK inhibitos (vemurafenib + cobimetinib, dabrafenib + trametinib) further improved outcomes of patients with metastatic and resected BRAF mutated melanoma and has therefore become the standard of care in this subset of patients (15,16).

BRAF

BRAF is a serine/threonine kinase which is activated directly via RAS and is markedly expressed in me-

lanocytes, neural tissue, testicles, and hematopoietic cells. It has been established that BRAF phosphorylates and activates MEK (a component of the signaling pathway; also the kinase participating in that cascade) which in turn activates the ERK by phosphorylation and stimulates the growth and transformation crucial for the pathogenesis of melanomas (10,16.)

The most frequent BRAF mutation (~70% of all BRAF mutations) is the transversion of thymidine into adenine (T → A), resulting in the substitution of valine into glutamate (V600E) and leading to constitutive activation of the kinase domain. These mutations indirectly activate BRAF by disrupting the normal intramolecular interactions that keep BRAF inactive (14). BRAF (V600E) mutations are more often observed in melanomas in the persons intermittently exposed to the sun than in those with acral and mucosal melanomas. Although BRAF mutations can be associated with exposure to the sun, the said transversion (T → A) is not classically associated with UV damage (10). Other substitutions are also possible, particularly of V600K mutations (accounting for ~20% of the BRAF mutations in a melanoma) which are more frequent in the melanomas in the persons who were chronically exposed to the sun. Furthermore, BRAF V600 mutations constitute an early event in melanoma development and are observed in most of the benign and dysplastic nevi (approx. 80%) (14). BRAF (V600E) mutation induces forming of a nevus, including the initial cellular proliferation followed by oncogene-induced aging, most likely as part of the accumulation of p16 ink4A. Thus, a mutated BRAF was observed in only 10% of the melanomas in the radial growth phase and in 6% of *in situ* melanomas. Additionally, many nevi and primary melanomas are polyclonal (containing both wild-type BRAF and BRAF mutated cells). The metastatic melanomas, on the other hand, are not polyclonal (10). It was also observed that the BRAF V600E mutations were more frequent in women and that they correlated in inverse proportion with age (9). BRAF V600K, on the other hand, was the most common sub-type and its incidence increased with age (14).

RAS

The mutations leading to increased RAS activity in melanomas also promote cellular proliferation, but significantly more rarely than in other solid tumors (10). The somatic mutation of NRAS genes can trigger the constitutive activity of the NRAS protein (which then cannot be "excluded"), resulting in serial activation of the serine/threonine kinases that stimulate the course of the cellular cycle, cellular transformation, and survival of the cells. This cascade of events

can be mediated by an excessive expression and/or hyperactivation of various growth factor receptors (like c-Met, epidermal growth factor receptors – EGFR and KIT), as well as by the loss of function of the neurofibromatosis type 1 (NF1) tumor suppressor gene that suppresses the NRAS signaling (9,17). These activating RAS mutations were observed in only 10-20% of melanomas (mostly in amelanotic nodular sub-types), usually the NRAS. While BRAF mutations activate the MAPK signaling pathway only, the NRAS-activating mutations simultaneously activate both MAPK and PI3K pathways (10,17).

It was established that NRAS and BRAF mutations almost never take place concomitantly, indicating that mutation of any one of them was sufficient for constitutive activation of the MAPK pathway (10). The activating BRAF mutations are more frequent (in 70-80% of dysplastic nevi) and NRAS mutations are rare (in most of the congenital nevi). HRAS mutations, on the other hand, are associated with Spitz nevi. Alternatively, the dysregulation of the AMPK signaling pathway in melanomas can be caused by an excessive expression or hyperactivation of the growth factor receptors (such as c-Met, KIT and epidermal growth fator receptor – EGFR) (10).

c-KIT

c-KIT (tyrosine kinase receptor) and its ligand (stem cell factor) have an important role in the development of melanocytes (16). c-KIT mutations thus cause insufficient pigmentation. The results of numerous immunohistochemical studies indicate that the transition from a benign condition into a primary or metastatic melanoma is associated with the loss of c-KIT expression. Additionally, the activating mutations and amplifications of KIT genes were observed in cutaneous melanomas in persons chronically exposed to sun and in acral melanomas (hands, feet, nail bed). The KIT mutations can activate numerous signaling pathways, particularly the PI3K-AKT pathway. The dotted mutations of the KIT gene correspond with those in gastrointestinal stromal tumors (GIST). The functional characteristic of these mutations turned out to be clinically significant because KIT inhibitors are clinically efficient in melanomas, but with a much lower clinical response rate (10-30%) when compared with GIST (> 70%) (14).

c-MET and HGF

Excessive expression of c-MET (another tyrosine kinase receptor) and its ligand HGF (hepatocyte growth factor) correlates with the progression of melanomas. It is known that c-MET controls numer-ous biological functions (proliferation, survival, motility, and invasion); thus, tumors can develop and malignant cells can metastasize in the case of their dysregulation by means of aberrant c-MET activation. Importantly, c-MET (tyrosine kinase receptor) can be excessively active in the case of excessive secretion of its ligand HGF, produced by the cells of the tumorous melanoma microenvironment. This paracrine effect causes the activation of the PI3K-AKT pathway in tumor cells and stimulates resistance to the MAPK inhibitors (14)

Other factors

Other factors include: neurofibromatosis type 1 (NF1) tumor suppressor and negative RAS regulator, which are also important in this context. NF1 mutations were identified in 5 out of 21 tumors without BRAF and NRAS mutations. In the context of BRAF (V600E), it was observed that NF1 dysregulates the MAPK and PI3K pathways, suppressing the development and proliferation of melanomas. In some melanomas, inactivating mutations of neurofibromatosis type 2 (NF2) tumor suppressor was observed; it was also found that germline mutations NF1 and NF2 were associated with the hereditary neurofibromatosis (10). Additionally, somatic mutations were identified in the downstream MAPK effectors such as MAP3K5, MAP3K9, MEK1, and MEK2, in melanomas (10).

PI3K/PTEN/AKT pathway (phosphatidylinositol 3-kinase pathway)

PI3K-AKT is another important signaling pathway involved in the regulation of cellular survival, growth, and apoptosis (2). In carcinomas, this pathway can be activated genetically, by means of either activating mutations (e.g. PIK3CA, AKT1) or loss of functions of certain components of this pathway (e.g. PTEN). The PI3 kinases (phosphatidylinositol 3-kinases) (lipid kinases) are activated directly (by activating the tyrosine kinase receptor) or indirectly (by means of RAS), causing phosphorylation of PIP2 into PIP3 and, subsequently, phosphorylation of AKT (14). PTEN lipid phosphatase has an important role, as it antagonizes this pathway by converting PIP3 back to PIP2 (1). Phosphorylation of AKT (serine/threonine protein kinase) leads to phosphorylation of the multiple effector proteins that regulate the cellular processes (proliferation, survival, motility, angiogenesis, and metabolism). It was observed that a large number of melanomas showed increased activation of the PI3K signaling, usually by inactivation (by mutation, deletion, methylation of promoters) of the genes encoding PTEN inhibitor (2).

MITF signaling

The microphthalmia-associated transcription factor (MITF) is a transcription factor required for the differentiation of melanocytes, which can promote malignant behavior in some melanomas. The most frequent genetic alteration of MITF is amplification, which takes place in 15-20% of melanomas (more often in metastatic melanomas) (18). It is believed that MITF occurs later in the melanoma progression and that it is associated with a lower 5-year survival rate (19). It was observed that MITF increased the expression of the genes involved in the cell cycle progression, cellular proliferation, and cellular survival. It is a transcription factor for cellular cyclin kinases (CDK2), CDK inhibitors p16INK4a and p21 and antiapoptotic mitochondrial membrane protein BCL-2 (B-cell lymphoma 2) (20,21).

MITF expression is activated by the activation of the melanocortin 1-receptor (MC1R). The latter is activated by binding of melanocortin (ACTH, α-MSH) (2). Its activation triggers the activation of adenylate cyclase and creation of c-AMP, which in turn activates the protein kinase A (PKA). PKA then activates CREB (cAMP response-element binding protein), which works as a transcription factor and intensifies the expression of MITF (2).

MITF signaling is also closely connected with and regulated by MAPK signaling. Importantly, MITF can also act as an anti-proliferative transcription factor that induces the termination of the cell cycle. In this connection, it was observed that a mutated oncogenic BRAF could regulate MITF expression, thus ensuring the levels of the proteins compatible with the proliferation and survival of melanoma cells (2). In melanocytes, MITF expression takes place after MSH (melanocyte-stimulating hormone) has bound with MC1R (melanocortin 1 receptor). In this process MITF targets the genes involved in the regulation of differentiation and pigmentation, proliferation, and survival.

OTHER FACTORS IMPORTANT FOR MELANOMA PATHOGENESIS

Numerous cellular interactions are also important in pathogenesis, with adhesion molecules (cadherins, adherents) taking part in them. The immune system also plays a significant role in melanoma carcinogenesis. Important factors in antitumor immunity are both humoral and cell-mediated immune response. Cancer cells can modify the immunological response in order to survive with several mechanisms such as down-regulated or disabled antigen presentation, immunologic barriers within the tumor microenvironment, negative regulatory pathways targeting T-cells, and T-cell dysfunction (22,23).

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD1) are two immune checkpoints who regulate immune homeostasis by inhibition of T-cell activation. Development of monoclonal antibodies targeting immune checkpoints CTLA-4 (ipilimumab) and PD1 (nivolumab, pembrolizumab) which remove the inhibition of T-cell activation and restore T cell recognition has revolutionized the treatment of this disease showing more durable responses and benefits in long-term survival and had become the standard of care in most patients with advanced and metastatic melanoma (16,24,25).

Combination of immune-checkpoints inhibitors with BRAF/MEK targeted therapies, radiotherapy, other immunotherapies such as Indoleamine 2, 3-dioxygenase 1 (IDO 1) inhibitors, as well with an oncolytic herpes virus Talimogene laherparepvec (TVEC) are currently ongoing with the goal of further improving response and activity of immune-checkpoints inhibitors (26).

A distinguishing feature of malignant neoplasms is their ability to metastasize. Normally, melanocytes are connected to basal keratinocytes with cell-cell adhesion molecules, such as transmembrane glycoprotein E-cadherin. Loss of E-cadherin and upregulation of N-cadherin leads to detachment of the melanoma cells from the epidermis and promotes melanoma invasion. Phosphatase and tensin homolog (PTEN) has been suggested as a potential regulator in this process of cell adhesion (1,27).

CONCLUSION

Pathogenesis of melanoma is a very complex process that includes a series of events and factors, genetic mutations, enzymes, and numerous molecules. It should be noted that obtaining insight into the biology of melanocytes and pathogenesis of melanomas is important primarily for devising and developing targeted therapy and immunotherapy, which has enabled a substantial breakthrough in the treatment of patients with this disease.

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