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Review

The roles of microphthalmia-associated transcription factor and pigmentation in melanoma

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ABSTRACT

MITF and pigmentation play important roles in both normal melanocyte and transformed melanoma cell biology. MITF is regulated by many pathways and it also regulates many targets, some of which are still being discovered and functionally validated. MITF is involved in a wide range of processes in melanocytes, including pigment synthesis and lineage survival. Pigmentation itself plays an important role as the interface between genetic and environmental factors that contribute to melanoma.

Introduction

Metastatic melanoma is a frequently fatal disease and its incidence has been on the rise throughout the past 30 years, with over 132,000 individuals worldwide diagnosed with melanoma each year [1,2]. While melanoma is highly curable by surgical excision when detected early, it is historically notorious for its therapeutic resistance and propensity to metastasize. Although the 10-year survival rate for stage IV metastatic melanoma has been only 10–15% [3,4], there is reason for optimism due to discoveries ranging from deeper understanding of melanomagenesis to new therapeutic breakthroughs. In this review, we focus on microphthalmia-associated transcription factor (MITF) and pigmentation and their roles in both normal melanocytes and transformed melanoma cells.

Melanomagenesis

Melanoma is likely to arise from a combination of genetic and environmental risk factors. Epidemiological studies have found that having a high number of nevi—neoplastic but benign melanocytic proliferations—correlates with an increased risk of melanoma [1,2,5]. Beyond family history, other known risk factors for melanoma include fair skin and a history of blistering sunburns during youth.

Exposure to sunlight is a widely accepted environmental carcinogen for melanoma development. However, several features have complicated a detailed understanding of its etiologic role. For example, although signature mutations typical of ultraviolet B (UVB)[1] wavelengths (290–320 nm) are commonly present within the genomes of melanoma cells, such mutations are less commonly implicated as "drivers" among melanoma oncogenes and tumor suppressors [3,4,6,7]. This contrasts strikingly with non-melanoma skin cancers, for which UVB mutations are very highly implicated [8,9]. In addition, whereas non-melanoma skin cancers are most commonly found on sun-exposed skin, melanomas do not follow the same restricted anatomic localization [9]. Additional confusing aspects of sunlight's relationship to melanoma include the measurable but surprisingly modest protective effect for sunscreens against melanoma, in contrast to strong protection against cutaneous squamous cell

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[1] *Abbreviations used:* UVB, ultraviolet B; SPF, Sun Protection Factor; RHC, red hair color; MC1R, melanocortin 1 receptor; αMSH, α-melanocyte stimulating hormone; MITF, microphthalmia-associated transcription factor; MiT, microphthalmia-related transcription; CREB, cAMP-responsive element binding protein; LEF, lymphoid enhancer-binding factor; MAPK, mitogen-activated protein kinase; POMC, proopiomelanocortin; PKA, protein kinase A; CRE, cAMP responsive element; DCT, dopachrome tautomerase; Wnt, Wingless-type; MDAs, melanocyte differentiation antigens; RSK, ribosomal S6 kinase; PGC1α, proliferator-activated receptor γ coactivator 1 α; PIAS3, protein inhibitor of activated STAT3; PKC, protein kinase C; HIF1, hypoxia-inducible factor 1; TYR, tyrosinase; TYRP, tyrosinase-related protein; AIM-1, absent in melanoma-1; MART1, melanoma antigen recognized by T-cells 1; PDE4D3, Phosphodiesterase 4D3; CDK2, cyclin-dependent kinase 2; TBX2, T-Box transcription factor 2; CDKN2A, cyclin-dependent kinase inhibitor 2A; BCL2, B-cell lymphoma 2; ML-IAP, Melanoma inhibitor of apoptosis; HGF, hepatocyte growth factor; EMT, epithelial-mesenchymal transition; APEX1, apurinic/apyrimidinic endonuclease I; REF1, redox factor-1; ROS, reactive oxygen species; HDAC, histone deacetylase.

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carcinoma (although numerous potential explanations exist, including followup duration, and other variables). Finally, the Sun Protection Factor (SPF) of dark pigment (eumelanin) is relatively weak (<10) whereas darkly pigmented people exhibit profoundly diminished cutaneous melanoma risk, suggesting an unknown relationship between sunlight's carcinogenic mechanism(s) and melanoma etiology.

Activating mutations in BRAF, a serine/threonine kinase involved in the RAS/RAF/MEK/ERK signaling pathway, have been found in 60–70% of malignant melanomas [10]. A substitution of valine for glutamic acid at position 600 (V600E) resulting from a T1799A transversion in exon 15 accounts for over 90% of activating oncogenic BRAF mutations [11–13]. However, given that up to 80% of benign nevi harbor $BRAF^{V600E}$, this mutation alone is thought to be insufficient for melanomagenesis [12,14]. In fact, $BRAF^{V600E}$ alone has been shown to cause classical oncogene-induced senescence in human melanocytes *in vitro* and also in mouse models [12,15,16]. Patton et al. have shown that in zebrafish, $BRAF^{V600E}$ is sufficient for nevus development and furthermore, in combination with a p53-deficient background, $BRAF^{V600E}$ contributes to melanomagenesis [17]. This suggests that activated BRAF may be a primary cooperating event for melanoma development [11,13,17,18]. In mouse models, BRAF activation along with loss of p53, p16, or PTEN significantly promotes melanomagenesis [12,16,19]. Treatment of $Braf^{V600E}$ mutant melanomas with BRAF inhibitors such as vemurafenib (PLX4032) leads to tumor regression and improved overall survival of patients although these responses are typically not indefinitely durable [20].

Epidemiological evidence and genome-wide association studies have indicated that people with the red hair color (RHC) phenotype have an increased risk of melanoma [21]. The RHC phenotype is characterized by fair skin, red hair, tendency to burn, inability to tan, and freckles. The RHC phenotype is most often caused by polymorphisms in the melanocortin 1 receptor (MC1R), a G-protein coupled receptor that regulates pigment production in normal melanocytes. Normally, MC1R is essential for tanning. Binding of the ligand α-melanocyte stimulating hormone (αMSH) to MC1R causes cAMP levels to increase in the cell, ultimately resulting in an increase of the melanocyte master regulator MITF and induction of a switch in pigment production from red–yellow pheomelanin towards brown–black eumelanin. RHC individuals contain nonfunctional variants of MC1R, which affect multiple cAMP-mediated signaling events, including pigment production.

Although individuals with the RHC phenotype are unable to tan, D'Orazio et al. showed that in RHC ($Mc1r^{e/e}$) mice, the tanning pathway (and thus pigmentation) could be rescued by topically applying the adenylate cyclase agonist forskolin, thus indicating that even in the absence of functional MC1R, the pigmentation machinery downstream is still available [22]. In these mice, forskolin-induced eumelanin pigmentation was protective against ultraviolet light-induced DNA damage and tumorigenesis [22].

Microphthalmia-associated transcription factor

MITF is the master regulator of melanocytes, playing a key role in their development, differentiation, function, and survival. MITF belongs to the family of basic helix-loop-helix leucine-zipper microphthalmia-related transcription (MiT) factors, which also includes transcription factor E3 (TFE3), transcription factor EB (TFEB), and transcription factor EC (TFEC). These transcription factors bind to DNA recognition sequences called E-boxes, which have the consensus sequence CA[T/C]GTG.

MITF is expressed in pigmented cells including melanocytes and retinal pigment epithelium cells, as well as in certain non-pigmented cell lineages including osteoclasts and mast cells [23]. There are at least nine different isoforms of MITF that exhibit tissue-specific expression patterns. The M isoform of MITF (MITF-M) is selectively expressed in melanocytes [24].

Heterozygous mutation of MITF has been shown to cause Waardenburg syndrome Type IIA, an autosomal dominant condition in humans [25]. Individuals with this condition exhibit a congenital white forelock and sensorineural deafness. Complete absence of MITF in mice results in characteristics such as white fur, deafness, and small eyes (microphthalmia). Loss of pigmentation in these mice is due to an absence of melanocytes, rather than a defect in melanin synthesis within viable melanocytes.

Besides its important roles within normal melanocytes, MITF also appears to play critical roles in melanoma, where it has been shown to be a lineage-specific survival oncogene that is amplified in 5–20% of human melanomas [26]. In melanoma patients, MITF amplification was associated with a decreased five-year survival [26]. MITF has also been shown to control proliferative, invasive, and metastatic properties of melanoma cells [26–28].

MITF has been suggested to have paradoxical roles, which may confer either pro-survival or anti-survival effects. A rheostat model of MITF has been proposed, wherein lower levels of MITF are associated with increased motility and invasive capacity in melanoma cells, and higher levels correspond with increased proliferative capacity [29]. Melanocytes expressing low levels of MITF have high levels of POU3F2 (BRN2) transcription factor, a direct repressor of MITF transcription that has been implicated in melanoma invasiveness [27,30]. MITF has also been shown to control both invasiveness and proliferation by regulating the expression of DIAPH1, the gene that encodes the diaphanous-related formin Dia1, which is involved in actin polymerization and coordination of the actin cytoskeleton [28]. Low MITF levels lead to downregulation of Dia1, a cyclin-dependent kinase inhibitor 1B ($p27^{Kip1}$)-dependent G1 arrest, reorganization of the actin cytoskeleton, and increased invasiveness. Moderate MITF levels lead to actin polymerization and suppressed $p27^{Kip1}$, resulting in proliferation [28]. Hoek et al. have suggested that melanoma cells switch between the proliferative and invasive states during tumor progression [31]. Prolonged MITF suppression has been suggested to trigger senescence in melanoma cells [32].

Regulation of MITF

MITF is regulated both transcriptionally and post-translationally (Fig. 1). At the transcriptional level, MITF-M is regulated by transcription factors including MITF itself, paired box gene 3 (PAX3), cAMP-responsive element binding protein (CREB), sex-determining region Y-box 10 (SOX10), lymphoid enhancer-binding factor 1 (LEF1/TCF), one cut domain 2 (ONECUT-2), and the mitogen-activated protein kinase (MAPK) pathway.

Exposure of skin keratinocytes to UV light causes activation of cellular damage responses, including activation of p53, which leads to proopiomelanocortin (POMC) transcription and ultimately tanning (physiologic pigmentation). POMC can be enzymatically cleaved to produce several physiologically important derivatives including αMSH. During tanning, αMSH is induced and binds to MC1R on melanocytes. This induces activation of adenylyl cyclase, resulting in cAMP production and activation of protein kinase A (PKA). This in turn leads to phosphorylation of CREB, followed by activation of the MITF-M promoter, which contains a cAMP responsive element (CRE) site.

The transcription factors SOX10 and PAX3 are both transcriptional activators of MITF that are important for development. Tissue-specific expression of MITF-M in melanocytes is mediated by cooperation between CREB and SOX10, the latter of which is largely restricted to the neural crest lineage [33]. PAX3 is expressed