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Microphthalmic-Associated Transcription Factor Integrates Melanocyte Biology and Melanoma Progression

□□ *Commentary on Koyanagi et al., p. 1137*

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Introduction and Historical Background

In this issue of *Clinical Cancer Research*, Koyanagi et al. (1) present results that indicate that microphthalmic-associated transcription factor (Mitf) levels as measured in circulating tumor cells correlate to stage of disease and thereby further support the notion that this transcription factor is proproliferative. Of particular interest is that the rate of detection of Mitf in circulating tumor cells is considerably higher than that which has been reported for assessment of this marker in pathologic specimens. This suggests that factors, perhaps undescribed, are functioning to up-regulate Mitf *in vivo* that are not present or suppressed *in vitro*.

The clinical outcome of primary melanoma has improved over the last 25 years in large part secondary to earlier diagnosis and appropriate surgical management. Once malignant cells have spread beyond the cutaneous compartment, treatment of the disease and outcome remain poor. An understanding of the role of molecular aberrations that exist in melanoma has been a long time in coming because many aberrations exist and separating the important from the secondary has been difficult. The identification in melanoma of clonal abnormalities in the sea of aneuploidy that characterizes the advanced disease (2) and the recognition that these chromosomal abnormalities had prognostic implications were important early advances (3). The recent identification of distinct sets of genetic alterations by chromosomal genomic hybridization in primary melanomas in different geographic body sites with different UV exposures is an important contribution and identifies subsets of melanoma with distinct genetic pathways (ref. 4; commentary in ref. 5).

Over the last 25 years, a number of investigators have identified the involvement of many growth factors and signaling pathways in melanoma (6, 7). In general, however, these alterations were only loosely linked to the unique features of melanocyte biology. Mitf, the molecule being discussed in this commentary, has its historical origins in the efforts of a German scientist who, over 60 years ago, discovered and described the

first mouse Mitf mutation, white mice with small eyes (8), and in the clinical observations of a Dutch ophthalmologist, Dr. Petrus Waardenburg, who first noticed that people with different colored eyes often had a hearing impairment and described the syndrome now classified as Waardenburg syndrome type 2 (9). In 1971, Arias (10) defined the phenotype of Waardenburg syndrome type 2 as individuals without dystopic canthorum and it is in this group in which autosomal dominant mutations of the Mitf gene are transmitted.

Melanocytes originate in the neural crest and migrate as nonpigmented melanoblasts to their final destinations, including the epidermis and hair follicle, the choroid of the eye, and the inner ear. Differentiation of these cells leads to the manufacture of the pigment melanin that provides skin, hair, and eye color, and protection against solar UV irradiation. Importantly, malignant transformation of the melanocyte characterized by activating mutations in BRAF or NRAS coupled, for example, to loss of cell cycle control, although mutations in the *INK4a/Arf* locus, together with suppression of senescence, gives rise to malignant melanoma, a highly aggressive and increasingly common disease.

Mitf, Survival, and Development

Recently, the attention of many researchers interested in melanocyte biology and development and those occupied with understanding melanoma has converged on a remarkable gene that seems to coordinate almost every aspect of pigment cell behavior. The gene encoding the basic-helix-loop-helix-leucine zipper, *Mitf*, was first isolated as a consequence of transgenic insertions that rendered the gene inactive (11, 12). The resulting Mitf-null mice were completely white and exhibited a microphthalmic phenotype from a failure of the neural crest-derived melanocytes population to survive and the retinal pigment epithelium to differentiate during development. These mice were also deaf because the melanocyte population in the inner ear is required for function of the stria vascularis (13).

Studies from many groups (reviewed in ref. 14) went on to show that Mitf was not only critical to melanoblasts survival, but also played a key role in coordinating the expression of genes, such as *tyrosinase* and *Tyrrp-1*, required for the manufacture of pigment and the genesis of a functional melanosome by binding to a specific subset of E-box motifs in their promoters. These elements, frequently termed M-boxes, characterized by a core CATGTG element flanked by either a 5' T and/or 3' A residue (15), enable Mitf to activate transcription of target genes through recruitment of the cyclic AMP (cAMP)-responsive element binding protein (CREB)-binding protein/p300 transcription cofactors (16).

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Properties of Mitf and Postnatal Survival of Melanocytes

In addition to the ability of Mitf to promote melanoblast survival during development, perhaps in part through its capacity to regulate expression of the hypoxia-induced transcription factor HIF1 α (17), Mitf is also required for postnatal melanocyte survival. One allele, *Mitf^{vit}*, characterized by a single amino acid substitution in helix 1 of the bHLH-LZ domain, leads to premature hair graying in mice and depletion of the melanocyte stem cell population normally found in the niche of the hair follicle (18). Given its critical role in prenatal and postnatal melanocyte biology, it is not surprising that Mitf expression and activity is tightly regulated at the transcriptional and posttranslational levels. In the neural crest-derived melanocyte population, a proximal promoter directs expression of the Mitf-M isoform (Fig. 1). In other cell types where Mitf is present, the retinal pigment epithelium, for example, a range of other promoters drive expression leading to versions of Mitf that share a coding sequence encoded by exons 2 to 9, but that have different first exons (reviewed in ref. 14). The Mitf-M promoter is by far the best characterized and is regulated by a variety of transcription factors, including Sox10, Pax3, CREB, and LEF1/Tcf, that enable Mitf expression to be regulated during development and in response to different signal transduction pathways. Particularly important is regulation by cAMP and CREB that seems to increase Mitf expression and promote activation of the pigmentation genes (19), and the Wnt/ β -catenin pathway that normally activates Mitf expression in the neural crest in response to Wnt signaling (20), but which is constitutively activated in a significant proportion of melanomas (21).

At the protein level, Mitf is the target of a range of modifications that control its expression or activity. Thus, phosphorylation on Ser²⁹⁸ or Ser³⁰¹ by GSK3 β (22) or the p38 stress-activated kinase (23), respectively, seems to promote the ability of Mitf to activate transcription, whereas phosphorylation of Mitf on Ser⁷³ by the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase 2, possibly in concert with RSK-mediated modification of Ser⁴⁰⁹, promotes ubiquitination on Lys²⁰¹ and degradation (24, 25). In addition, sumoylation of Mitf may regulate its ability to differentiate between different target promoters (26, 27).

Mitf and Proliferation: Pro or Anti

Precise transcriptional regulation and combinatorial post-translational modification enables Mitf to respond appropriately to specific environmental cues (reviewed in ref. 14). Significantly, deregulation of signaling pathways known to regulate Mitf expression and activity is implicated in melanoma

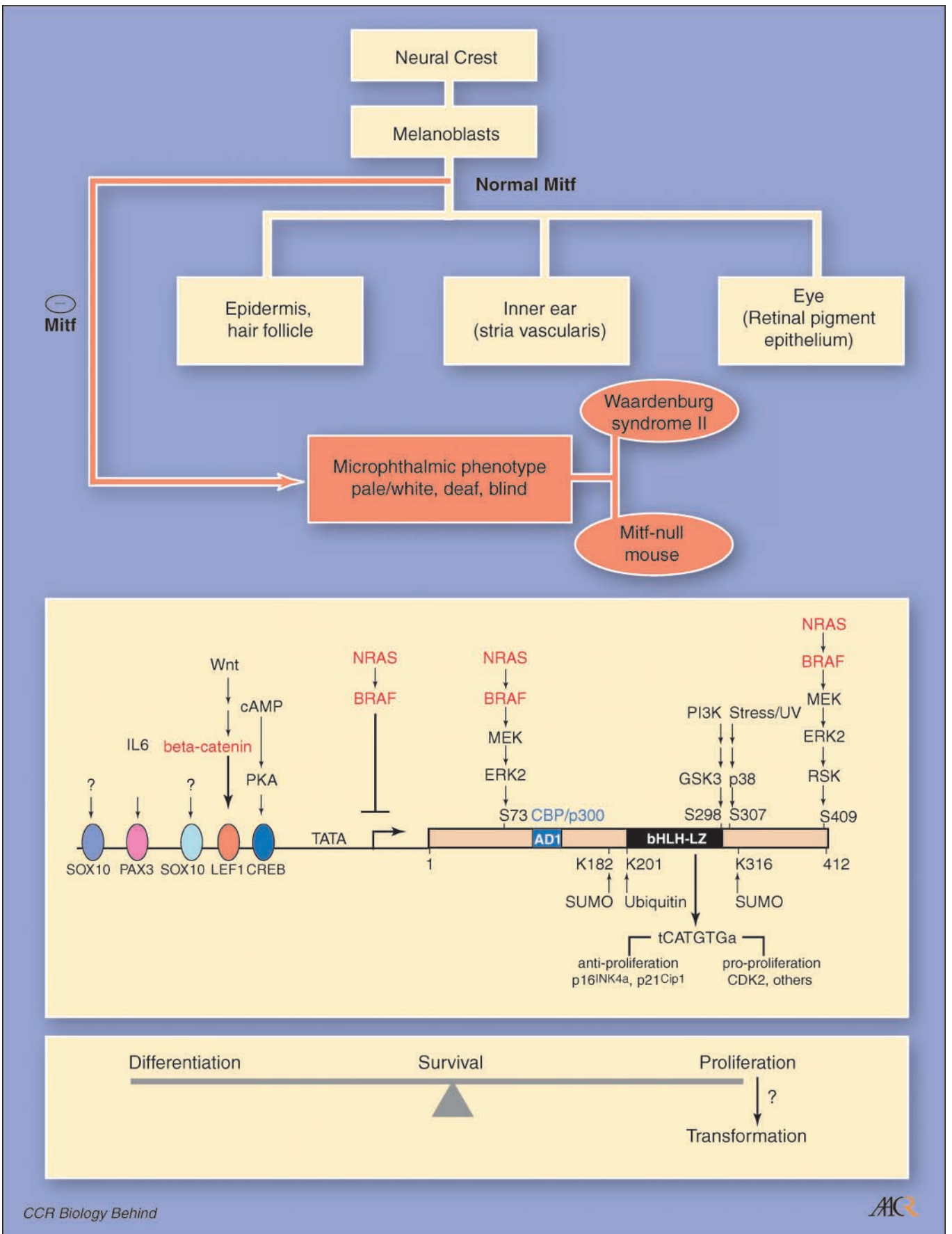
(28). Consequently, understanding how Mitf functions has become a key to understanding this disease. Moreover, the importance of Mitf in melanoma has been highlighted by a series of recent articles that have identified Mitf as an important regulator of melanocyte and melanoma proliferation. A proproliferative role for Mitf was initially signaled by the observation that Mitf expression is generally conserved in melanoma (29) and was recently reinforced by the study of Garraway et al. (30) in which up to 100-fold amplification of the *Mitf* gene was detected in some melanoma samples, particularly those from metastases, and Mitf was found to cooperate with BRAF in melanocyte transformation assays. Moreover, Mitf can up-regulate expression of the cyclin-dependent kinase CDK2 (31) and is required for the proproliferative effects of β -catenin in melanoma cells in culture (32). These data together seem to mark Mitf as a proproliferative factor in melanoma, although precisely how Mitf operates to promote proliferation is yet to be understood.

Koyangi et al.'s results indicate that Mitf levels as measured in circulating tumor cells correlate to stage of disease, which supports the idea that Mitf is proproliferative. Of particular interest in their results is that the rate of detection of Mitf in circulating tumor cells is considerably higher than that which has been reported for assessment of this marker in pathologic specimens, suggesting that factors, perhaps undescribed, are functioning to up-regulate Mitf *in vivo* that are not present or suppressed *in vitro*.

Paradoxically, however, as highlighted previously (28), equally strong evidence implicates Mitf as an antiproliferative factor. Thus, elevated Mitf expression leads to a G₁ cell cycle arrest mediated by up-regulation of the p21^{CIP1} and p16^{INK4a} cyclin-dependent kinase inhibitors (33, 34); melanoblasts *in vivo* in an Mitf+/- background exhibit increased proliferation (35); in the eye, the absence of Mitf induces hyperproliferation and a failure of the RPE to differentiate (36), whereas the presence of Mitf inhibits proliferation in CHX10-/- mice (37); and significantly, activated BRAF leads to strong suppression of Mitf expression, increasing Mitf expression in melanoma cells counteracts the proproliferative signal arising from BRAF-mediated activation of the MAPK pathway (38).

Most likely, Mitf plays both proproliferative and antiproliferative roles depending on its level of expression and its activity. In melanoma, therefore, the repertoire of signaling pathways would be directed toward maintaining the low level of Mitf required to promote proliferation while simultaneously preventing Mitf from attaining a level of activity that would inhibit proliferation. Moreover, within any tumor mass, it is likely that different microenvironments would lead to variations in Mitf activity and, consequently, a range of phenotypes varying from highly pigmented, more differentiated cells to those that exhibit decreased pigmentation but increased proliferation.

Fig. 1. Regulation of Mitf-M expression. The study of patients with microphthalmia associated with congenital deafness led to studies of developmental abnormalities known as the Waardenburg syndromes in humans and mice, and of Mitf and the eventual recognition of its importance in melanocyte biology and melanoma progression. Expression of Mitf from the melanocyte-specific M promoter is controlled by an array of transcription factors. Importantly, Mitf expression is up-regulated by Wnt/ β -catenin signaling, which is constitutively activated in a substantial proportion of melanomas, but strongly down-regulated by activating mutations in BRAF or NRAS. The Mitf protein is subject to a wide range of modifications that regulate its stability or activity. p38 and GSK3 β -mediated phosphorylation up-regulates activity, whereas signaling downstream from NRAS or BRAF decreases protein stability and turnover via phosphorylation on S73 and S409, leading to ubiquitination on K201. Sumoylation on K182 and 316 seems to differentially affect the ability to Mitf to activate transcription depending on the target gene (41). Whether Mitf provides a proproliferative or antiproliferative signal is likely to depend on its activity and expression level.



Markers of Melanoma and Host Survival

The detection of circulating tumor cells as a measure of tumor burden or relapse has been going on for over 40 years. In general, however, the effort to detect whole tumor cells in blood has not been a useful measure of tumor burden or treatment effect, at least not in nonhematopoietic neoplasms. Of more use has been the detection of biochemical and molecular markers in circulating tumor cells in the blood, especially for melanoma. Recently, detection of tyrosinase in circulating tumor cells of peripheral blood showed a strong association with disease-specific survival time in stage II and III disease (39). A similar result was obtained in the current study using *Mitf* as the biomarker. Because tyrosinase is downstream of *Mitf* control, it would be of considerable interest to know the relationship between the two markers in circulating tumor cells, as well as in the nodal metastases and in the original lesions of these patients. A major limitation of all biomarkers is that although any one or group of markers may predict survival reasonably well in a population of characterized patients, their discriminatory accuracy in individuals needs to be improved. In this regard, the data for *Mitf* in the current study seem strong, although it is likely that in the end a panel of markers will be needed to increase accuracy.

Markers of Melanoma and Treatment Response

The results obtained in the current study are striking as they suggest that the therapy has separated two or more separate groups of melanoma patients—those who respond and have a long survival and those who do not and die quickly. The history of oncology has repeatedly shown that successful therapy frequently identifies underlying biological features of a

particular cancer that have been previously ignored or not appreciated and leads to useful subclassification based on defined biological features. Further genetic and molecular characterization of the responder and nonresponder in this trial should lead to further deepening of our knowledge of melanoma. The relationship of the responders and nonresponders to the comparative genome hybridization classification cited earlier (4) might also provide an important link and provide further understanding of melanoma subtypes. The investigators in the current study have recently also reported their experience with this same set of patients using a panel of melanoma-associated markers that probably do not interact directly with *Mitf* (40). Because these markers are antigenic in nature, however, a possible association by down-regulation of immunologic proteins by an inappropriate level of *Mitf* is worth considering. Further characterization of these results with the *Mitf* findings should be informative.

Mitf beyond Melanoma

Several other genes, especially *PAX3*, are mutated in the various Waardenburg syndromes and involve the frontal bone, back muscles, and enteric ganglia. Mutations and aberrant expression of *Mitf* family members have also been reported in papillary renal cell carcinoma and alveolar soft parts sarcoma. Because *PAX3* transactivates the *Mitf* promoter, it is likely that *Mitf* will play a critical role in other malignancies as well.

Given the critical importance of *Mitf* as a coordinator of melanocyte and melanoma survival, proliferation, and differentiation, it is clear that understanding how *Mitf* operates to integrate wide range of signals to regulate gene expression will be key to deciphering melanoma and probably other malignant and nonmalignant diseases.

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