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MITF: master regulator of melanocyte development and melanoma oncogene

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Microphthalmia-associated transcription factor (MITF) acts as a master regulator of melanocyte development, function and survival by modulating various differentiation and cell-cycle progression genes. It has been demonstrated that MITF is an amplified oncogene in a fraction of human melanomas and that it also has an oncogenic role in human clear cell sarcoma. However, MITF also modulates the state of melanocyte differentiation. Several closely related transcription factors also function as translocated oncogenes in various human malignancies. These data place MITF between instructing melanocytes towards terminal differentiation and/or pigmentation and, alternatively, promoting malignant behavior. In this review, we survey the roles of MITF as a master lineage regulator in melanocyte development and its emerging activities in malignancy. Understanding the molecular function of MITF and its associated pathways will hopefully shed light on strategies for improving therapeutic approaches for these diseases.

Microphthalmia-associated transcription factor

Microphthalmia-associated transcription factor (MITF) is a tissue restricted, basic helix–loop–helix leucine zipper (b-HLH-Zip), dimeric transcription factor. It is encoded by the *Mitf* locus in mice [1] and, when mutated, leads to defects in melanocytes, the retinal pigmented epithelium, mast cells and osteoclasts [1–3].

Functionally, MITF binds to the canonical E-box promoter sequence CACGTG and the non-palindromic sequence CACATG [4–6]. MITF binds to DNA as a dimer, involving a parallel coiled-coil dimerization interface that consists of the helix–loop–helix (four helix bundle) and leucine zipper domains. Several related b-HLH-Zip transcription factors can heterodimerize with MITF and bind to identical DNA sequences. The related factors transcription factor EB (TFEB), TFE3 and TFEC [4], together with MITF, are collectively termed the MiT family [4]. Although MITF expression seems to be mainly restricted to certain specific cell types, other members of the MiT family are thought to be more-ubiquitously expressed. Germline knockouts of TFE3 and TFEC seem to exhibit minimal recognizable phenotypes, whereas germline TFEB homozygous deficiency is associated with embryonic lethality due to placental insufficiency [7]. Elegant genetic studies have demonstrated functional and genetic redundancy between MITF and TFE3 in the development of the osteoclast lineage [8]. From these analyses, it seems that MITF is the only MiT family member that is functionally essential for normal melanocytic development.

MITF is thought to mediate significant differentiation effects of the α-melanocyte-stimulating hormone (α-MSH) [9,10] by transcriptionally regulating enzymes that are essential for melanin production in differentiated melanocytes [11]. Although these data implicate MITF in both the survival and differentiation of melanocytes, little is known about the biochemical regulatory pathways that control MITF in its different roles.

Transcriptional and post-translational MITF regulation

The *MITF* gene has a multi-promoter organization in which at least nine distinct promoter–exon units direct the initiation of specific MITF isoforms that differ in their first one or two exons, which are spliced onto the common downstream exons [12] (Figure 1). The promoter that is located most proximal to the common downstream exons is known as the M promoter and seems to be selectively expressed in melanocytes [13]. The melanocyte-specific exon adds only a small number of amino acids that are not known to alter MITF function dramatically. The *MITF*-M promoter is targeted by several transcription factors that are important in neural-crest development and signaling. Transcription factors implicated in the regulation of the *MITF*-M promoter include paired box gene 3 (PAX3), cAMP-responsive element binding protein (CREB), SRY (sex-determining region Y)-box 10 (SOX10), lymphoid enhancer-binding factor 1 (LEF1, also known as TCF), one cut domain 2 (ONECUT-2) and MITF itself [2,14–16] (Figure 2). α-MSH binds to melanocortin 1 receptor (MC1R), which induces the activation of adenylyl cyclase, followed by cAMP production. cAMP leads to phosphorylation of CREB transcription factors, which in turn stimulate *MITF*-M promoter activation [9]. Interestingly, *MITF*-M promoter expression is cell-type specific despite regulation by the ubiquitous cAMP–CREB pathway. This is explained in part by the obligate cooperativity between CREB and SOX10, the expression of which is restricted mostly to neural-crest derivatives [17]. On the *MITF*-M promoter, Wingless-type (WNT) signaling is crucial for the differentiation of melanocytes from the neural crest. Binding of WNT proteins to the Frizzled receptors results in the interaction of β-catenin with LEF1/TCF transcription factor, which then induces the *MITF*-M promoter [18–20]. PAX3 also activates the

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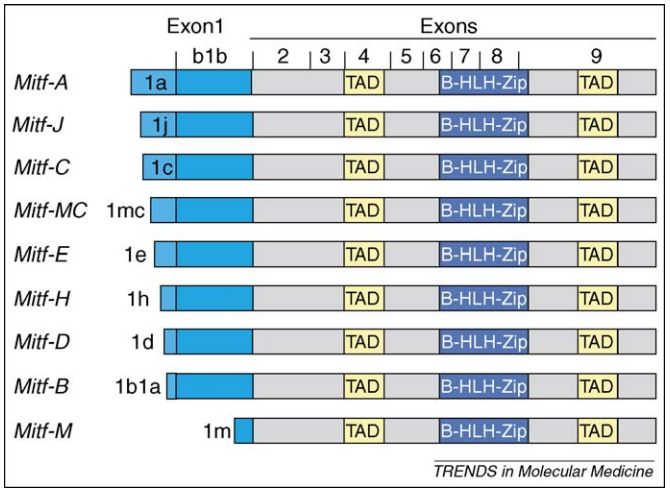

Figure 1. In humans, nine different promoters have been described for *MITF*. These promoters give 5′ specificity for each isoform. However, all the different isoforms have in common exons 2 to exon 9, which encode the functional domains of the transcription factors. These domains are the transactivation domain (TAD) and the b-HLH-Zip. Some of the isoforms are expressed in specific cell types, such as the isoform M, which is expressed specifically in melanocytes, and the isoform MC, which is expressed selectively in mast cells. Exons are marked by a black line above.

MITF-M promoter [21] and might respond to interleukin (IL)-6 signaling via a poorly understood pathway [22].

In addition to transcriptional regulation, MITF is also subject to various post-translational modifications, particularly phosphorylation by mitogen-activated protein kinase (MAPK), ribosomal S6 kinase (RSK), glycogen synthase kinase-3β (GSK3β) and p38 [23–26]. These kinases reside within various important homeostatic signaling pathways and might therefore modulate MITF transcriptional activity in response to specific environmental cues. It remains to be determined exactly how each fits into the specific pathways in which MITF participates.

Activation of c-KIT in melanocytes results in phosphorylation of MITF at Ser73 by extracellular-signal regulated kinase 2 (ERK2) and at Ser409 by p90 ribosomal S6 kinase (p90RSK). Phosphorylation at Ser73 increases the recruitment of the transcriptional coactivator p300 (also known as CREB binding protein, CBP) [27], while simultaneously targeting MITF for ubiquitin-dependent proteolysis [24]. Sumoylation, a post-translational modification mediated by protein inhibitor of the activated STAT3 (PIAS3), affects MITF transcriptional activity in a target-gene-specific fashion. In promoters containing multiple MITF binding sites, sumoylated MITF seems to be less active than unsumoylatable MITF mutants. These observations suggest that MITF activity might vary in a post-translationally regulated way that might alter target-gene specificity based on cellular contexts or signals [28,29]. MITF was also found to be a substrate for proteolytic degradation at its C-terminus by caspase 3, whereas TFE3 and TFEB are resistant to this process [30].

PIAS3 and protein kinase C interacting protein 1 (PKCI, also known as Hint) have been shown to be involved in the repression of MITF transcriptional activity. Both of these proteins directly interact with MITF and inhibit its DNA-binding activity. The interaction of PIAS3 with MITF is affected by the phosphorylation pattern of MITF. When Ser73 is phosphorylated, an increase in PIAS3–MITF interaction is observed; in contrast, when Ser409 is phosphorylated, this interaction is decreased [28,31–33].

Although much is known about regulation of MITF, there is little knowledge about ways to manipulate these mechanisms for therapeutic use. Moreover, more-detailed biological understanding of the impact of these post-translational modifications on MITF activities (e.g. transcription-factor target choices) remains to be learned.

Mutation of the *Mitf* locus

In humans, mutation of *MITF* causes Waardenburg syndrome (WS) type IIA [34]. This autosomal dominant inherited condition [35] arises from melanocytic deficiencies in the eye, forelock and inner ear. The most serious consequence of *MITF* mutation in affected individuals is sensorineural hearing impairment (ranging from mild to severe). Although their precise actions are uncertain, melanocytes reside within the stria vascularis of the cochlea (inner ear) where their presence is thought to contribute to maintenance of extracellular K^+ in the endolymphatic fluid. WS exists in four variants, all of which share the features of melanocytic-deficiency of type IIA patients. WS type I and type III are caused by mutations in PAX3, a regulator of gene expression that is present in the neural crest, which has been implicated in the direct modulation of the *MITF* promoter [2,21,36]. Because PAX3 affects certain lineages in addition to melanocytes, WS type I and type III patients exhibit additional non-melanocytic phenotypic abnormalities (largely involving musculoskeletal abnormalities of the face). WS type IV is characterized by abnormalities of melanocytes and deficient enteric enervation with Hirschsprung syndrome (megacolon) [37]. Mutations in several genes, including *SOX10*, endothelin 1 and its receptor endothelin receptor B, have been identified in WS type IV patients. Although mutations in *MITF* account for only a minority of WS type II cases, the SNAIL transcription factor has been recently suggested to account for additional cases of WS type II [38]. Remarkably, almost all genes associated with WS can be linked to homeostatic (or signal-related) regulation of

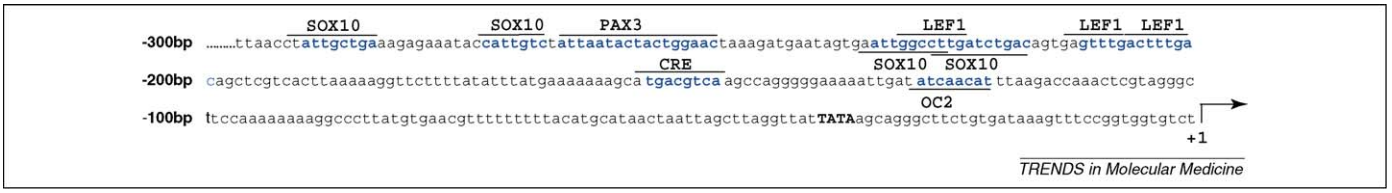

Figure 2. Regulators and transcription-factor binding sites on the *MITF-M* promoter. Transcriptional regulation of the *MITF-M* isoform involves multiple transcription factors. The sequence listed was originally identified by Shibahara and colleagues [100]. Abbreviation: OC2, ONECUT-2.

MITF expression or activity. In the case of endothelin signaling, a direct connection has not yet been established; however, endothelin is known to activate the MAPK pathway, which modulates MITF by phosphorylation [39,40].

When *Mitf* is mutated in mice, the phenotype is characterized by deafness, bone hyperdensity, small eyes and absence of pigment in eyes and skin. Non-functional mutation of *Mitf* leads to a coat that lacks color due to lack of melanocytes, whereas heterozygotes exhibit either no pigment phenotype or have a white-belly spot [1–3], depending on the specific mutation. Because mammalian coat-color mutants were historically easy to identify and study, numerous spontaneous mutations in *Mitf* have been discovered across many species [3]. The subsets of alleles that result in heterozygotes with white-belly spots [4,41] are associated with mutations within the basic domain of MITF, which is responsible for DNA binding, sparing the adjacent HLH-Zip dimerization region. The resulting dominant negative mutants produce ventral spotting, which is the result of defective neural-crest migration of melanocytic precursors. MITF is also necessary in the adult, based on the existence of hypomorphic alleles in mice that result in postnatal melanocyte death and premature graying [42,43]. In addition, although most mutations in *Mitf* produce loss of melanocyte viability, one mutant ($Mitf^{b}$) produces altered coat color with a brownish appearance, providing genetic support to the concept that MITF participates in the pigmentation pathway (independent of melanocyte survival) [44]. The rarity of pigment-specific mutants of *Mitf* is likely to be a reflection of its separate requirement(s) for lineage survival, making it uncommon for a mutant to spare the survival role while affecting pigmentation.

Melanocyte precursors arise from neural-crest progenitor cells, which also seem to depend upon the activity of MITF. Hornyak *et al*. [45] have investigated, using $Mitf^{mi/mi}$ mutant mice, the role that MITF has in progenitors. These studies revealed the importance of MITF in the initial survival of melanocyte progenitors and in the subsequent population increases and migration from the neural tube.

The relevance of MITF has also been shown in melanocyte stem cells (MSCs). MSCs represent a self-renewing population of melanocytes located within the bulge region of the hair follicle [46]. Mutation of *Mitf* in mouse (the $Mitf^{vit}$ allele) results in accelerated hair graying due to premature loss of the MSC population [43]. Maintenance of MSCs prevents physiological hair graying, a process developed through progressive loss of the differentiated progeny during aging. The anti-apoptotic gene B-cell leukemia/lymphoma 2 (*BCL2*) also has a key role in the selective maintenance of murine MSCs shortly after birth [43,47].

Mutations and genetic models of melanoma

Melanoma, a neoplasm of melanocytic origin, is the most severe human skin cancer and is highly resistant to treatment. During the past ten years, the incidence and annual mortality of melanoma has increased more rapidly than any other cancer. Although progress has been made in deciphering the molecular underpinnings of melanoma, successful treatment for metastatic melanoma remains frustratingly uncommon [48].

The increased incidence of melanoma correlates with discordance between human skin phototypes and environmental sun exposure, a phenomenon typified by fair-skinned individuals residing within sun-belt locations such as Australia [49]. People who are at high risk for melanoma exhibit some of the following characteristics: red hair, fair skin that is resistant to tanning, extended sun exposure (particularly sunburns during childhood), numerous pigmented lesions (nevi) and inherited mutations in various familial melanoma susceptibility loci, which include the cell-cycle regulatory genes cyclin-dependant kinase inhibitor 2A (*CDKN2A*) or cyclin-dependant kinase 4 (*CDK4*) [48,50].

The generation of *in vivo* models of melanoma contributes to the discovery of genomic lesions driving the formation and progression of the disease and will hopefully aid in drug discovery. A wealth of melanoma transgenic models have been generated in recent years, including some tumors that are oncologically transformed by RAS family members and employ inducible oncogene expression in which the requirements of oncogene activity for tumor maintenance could be elegantly assessed [51]. Among the challenges that mouse models of melanoma have to face is that mouse melanocytes reside primarily in hair follicles and dermal locations rather than in the epidermis, which is the site from which most cutaneous melanoma arise in humans. Addressing this problem, Merlino and Noonan [50] have generated a murine model in which transgenic expression of hepatocyte growth factor (HGF) produces epidermal homing of melanocytes. The resulting mice are exquisitely sensitive to perinatal UV-induced melanomagenesis. This represents a striking, genetically tractable mammalian model of UV-induced melanoma, and offers numerous opportunities for understanding UV-mediated carcinogenesis in the melanocyte lineage. In addition, Chin *et al*. [51] have examined genetic interactions between RAS-induced melanomagenesis and defects within p53, ADP-ribosylation factor (ARF) and retinoblastoma (RB) pathways. Yamazaki *et al*. [52] recently generated a melanoma mouse model, using UV-B irradiation to xeroderma pigmentosum group A gene-deficient, stem-cell factor transgenic mice. These mice are defective in the repair of damaged DNA and have epidermal melanocytes. In addition to mouse models, Patton *et al*. [53,54] recently employed a zebrafish model to evaluate genetic interactions implicated in human malignant melanomas. The melanin-producing cells of zebrafish, melanophores, seem to be sufficiently similar to human melanocytes to enable demonstration of interactions between the v-raf murine sarcoma viral oncogene homolog B1 (BRAF) and p53 pathways in melanoma development [53,54].

Recently, molecular and genetic data identified several key molecules involved in melanomagenesis. *BRAF* oncogenic alterations are frequent in melanoma signaling through the RAS–MAPK pathway. *BRAF* is the first gene that has been shown to be mutated in a high fraction of benign and malignant melanocytic lesions [55,56]. In addition, 20% of nevi or melanomas carry mutated

neuroblastoma (N)RAS in a way which seems generally to be mutually exclusive of *BRAF* mutation [50,56,57].

Additionally, the loss of several tumor suppressor genes has been observed in melanoma. The *CDKN2A* tumor suppressor locus encodes $p16^{INK4a}$ and $p19^{ARF}$. Inhibitor of kinase 4a (INK4a) inhibits the cyclin D–CDK4 complex, which is an activator of E2F–RB cell-cycle progression pathway, whereas ARF blocks mouse double minute 2 (MDM2) inhibition of p53. *CDKN2A* mutations appear in 25–40% of melanoma prone families due to an allelic loss of the *CDKN2A* locus [48,56,58]. An activating mutation of *CDK4* that renders the kinase resistant to INK4A suppression, has also been identified in familial melanoma [59]. Mutations or deletions of these genes occur much less commonly in sporadic human melanomas, although epigenetic silencing of INK4a seems to be an alternative mechanism for loss of its tumor-suppression activity [60]. Another tumor suppressor that is lost in a fraction of sporadic melanomas is phosphatase and tensin homolog (PTEN), which is likely to modulate survival via regulation of the canonical phosphoinositide 3-kinase (PI3-K) pathway [56,61].

The role of p53 in melanoma is incompletely understood. Although p53 is a major regulator of apoptosis and is, indeed, mutated or deleted in a high fraction of human malignancies, *p53* mutations are remarkably uncommon in melanoma, despite the particularly resistant behavior of melanoma cells to most apoptotic triggers (e.g. ionizing radiation and chemotherapy). It is assumed that within melanoma cells additional molecular lesions downstream of the p53 might render melanomas refractory to p53-induced apoptosis. Several potential explanations include the observation that apoptotic peptidase activating factor 1 (APAF1) is frequently lost or silenced in melanoma [62], or that BCL2 has a uniquely vital role in protecting the melanocyte lineage from apoptosis [43,63]. Chudnovsky *et al*. [64] recently characterized the roles of specific genes (*p53DN*, mutant *N-RAS* and *PI3-K*) in the formation of transformed melanocytic lesions. Using transplanted reconstituted human skin tissue containing melanocytes that have been selectively engineered to express these specific genes, they showed that active RAS or PI3K, together with constitutive human telomerase reverse transcriptase (hTERT) expression and suppression of the RB and p53 pathways, facilitated formation of melanoma-like lesions.

MITF in melanoma progression

Evidence that *MITF* is an oncogene in human melanoma came from studies investigating chromosomal alterations in human cancer cell lines using high-density single nucleotide polymorphism (SNP) arrays [65]. These studies identified copy gains at the *MITF* locus in melanoma lines, and corroborated those findings by fluorescence *in situ* hybridization. Using primary melanoma tissue microarrays, *MITF* was found to be amplified in 10–20% of cases, with a higher incidence among advanced (metastatic) melanomas. In metastatic melanoma, *MITF* amplification was associated with a decrease of five years in survival [65]. In addition, functional experiments demonstrated the role of *MITF* in oncogenesis. Human primary melanocytes, immortalized using *hTERT*, dominant negative *p53* and dominant active *CDK4* were transduced with *BRAF* (*V600E*), which conferred growth-factor independence but not soft-agar growth. However, the expression of MITF conferred soft-agar clonogenic growth, providing experimental evidence supporting *MITF* role as a dominant oncogene. In addition, disruption of MITF sensitizes melanomas to conventional chemotherapeutics using *in vitro* assays [65].

MITF expression in melanoma is variable across specimens. In some cases, MITF levels are high (i.e. amplified cases), whereas in others MITF levels are low or decreased [3,66]. MITF expression in melanoma seems to correlate with levels of transient receptor potential cation channel M1 (*TRPM1*), a MITF target gene. *TRPM1* transcripts are expressed at high levels in benign nevi, dysplastic nevi and melanomas *in situ*, variably in invasive melanoma and are commonly absent in melanoma metastases. These data match direct measurements of MITF during melanoma progression [67], which suggest that MITF (or its targets) are commonly downregulated in advanced melanoma, except for those in which MITF is amplified [67,68]. In certain melanoma cell lines, it has been shown that the expression of the melanocyte isoform *MITF*-M promoter is lost, accompanied by the absence of tyrosinase (*TYR*), tyrosinase-related protein 1 (*TYRP1*) and TYR-related protein-2/dopachrome-tautomerase (*DCT*), three transcriptional target genes of MITF. Interestingly, overexpression of exogenous MITF failed to activate expression of these genes, suggesting that a specific nuclear context is required for the transcriptional activation of (at least certain) melanocyte markers by MITF [69]. Taken together with the evidence that MITF amplification is often a late event in melanoma progression, these data suggest that distinct subsets of melanomas exist, with fundamentally different biological activities of MITF. In addition to potential cell-cycle benefits of reduced MITF levels, the decreased expression of MITF in melanoma cells might provide a growth advantage by diminishing levels of pigmentation, and thereby the energy and oxidative stress associated with pigment production [3,48,68,69]. How can MITF be simultaneously lost during progression of some melanomas and amplified in others? One potential explanation is that there are fundamental differences among classes of melanomas that might account for the seemingly opposite effects of MITF expression on melanoma progression.

These data are not the first indicating an association between MITF and melanoma. Within the past decade, MITF has been described as a highly sensitive immunohistochemical marker for melanoma diagnosis [70], Its sensitivity and specificity seemed to be superior to the S-100 and HMB-45 melanoma markers [70]. Currently, a panel of immunohistochemical markers in clinical use include the MITF-specific antibody D5. However, MITF serves as a melanocyte-lineage marker rather than discriminating benign from malignant melanocytic lesions. Although MITF is a useful marker of epithelioid melanomas, it is less sensitive in the rarer desmoplastic and spindle-cell melanoma variants [71,72], raising some questions about the melanocytic nature of these malignancies.

Transcriptional target genes of MITF

Because MITF seems to have a pivotal role in melanocyte development and melanoma, it is crucial to examine the transcriptional target genes of MITF. Although it is probably an oversimplification, the growing number of MITF target genes can be classified into two groups: differentiation or growth and/or survival genes (Figure 3).

MITF regulates the transcription of three major pigmentation enzymes: TYR, TYRP1 and DCT. The promoters of these genes contain the *MITF* consensus E-box sequence and they are expressed in melanocytes [4,6,10,73–78]. MITF regulates several other genes with less-well-understood function. Among these genes, absent in melanoma-1 (*AIM-1*) is mutated in human oculocutaneous albinism type IV [79,80] and melanoma antigen recognized by T-cells 1 (*MART1*) [81] is expressed in melanosomes. MART1 and silver homolog *SILV*, also known as *PMEL17*, a melanosomal structural protein also regulated by MITF, are commonly employed for melanoma immunohistochemical diagnosis [81]. PMEL17 (gp100) and tyrosinase peptides are being studied as antigens in melanoma vaccines. The regulation of multiple pigmentation and differentiation related genes by MITF has solidified the hypothesis that MITF functions as a central regulator of melanogenesis.

MITF is an amplified oncogene in a subset of human melanomas. It might transcriptionally regulate a distinct set of target genes that control melanocytic proliferation or survival (or other neoplasia-related phenotypes that are distinct from pigmentation). T-Box transcription factor 2 (*TBX2*) mediates repression of the $p19^{ARF}$ promoter [82], and has been described as one of the MITF target genes that is not involved in pigmentation [83]. TBX2 is overexpressed in melanoma cell lines and it has been recently suggested to be required to maintain proliferation and suppress senescence in melanomas via downregulation of p21 [84]. This indicates that MITF, as a transcriptional activator of TBX2, is required for proliferation and antisenescence processes.

The ability of MITF to regulate cell-cycle progression might also be mediated by modulation of CDK2, the expression of which is essential for melanoma clonogenic growth. MITF and CDK2 expression levels were tightly correlated in several melanoma cell lines and clinical specimens [85]. The anti-apoptotic factor *BCL2* has also been found to be a direct MITF target gene. Apoptosis induced by MITF depletion in primary melanocytes and melanoma was partially rescued by constitutive BCL2 overexpression [63]. Recent evidence has also suggested that MITF might regulate the expression of hypoxia inducible factor 1 α (HIF1α), which is the key mediator of transcriptional responses to hypoxia [86]. It will be interesting to determine in which biological contexts this mode of regulation is important, because much of HIF1α regulation is known to occur via post-translational regulation of stability via 'oxygen sensing' [87]. MITF has also been linked to two different CDK inhibitors (p21 and p16/INK4a) [88,89]; high levels of MITF might hinder cell-cycle progression, which is necessary for melanocyte differentiation. Transcriptional regulation of RB pathway inhibitors provides an attractive model for understanding how MITF might induce cellular differentiation and cell-cycle arrest versus dysregulated growth and/or survival. The latter potentially occurs within the context of cells in which the RB pathway has been disabled by independent mutational events.

As described earlier, MITF can interact with the histone acetyl transferases CBP/p300 to activate target genes [27,90]. In a recent study, MITF has been suggested to use the chromatin-remodeling ATPase-complex SWI/SNF to activate expression of certain pigmentation-related genes, based upon a fibroblast-cell system in which MITF was introduced together with a dominant negative suppressor of the SWI/SNF complex [91]. This interesting study has suggested the possibility that chromatin-remodeling events might participate in choosing the biological pathways that MITF regulates.

Another transcriptional target of MITF is the *c-MET* proto-oncogene [92]. It has been shown that MITF directly regulates *c-MET* promoter and is essential for the homeostatic upregulation of c-MET expression following activation of the receptor by its ligand HGF [92]. c-MET is highly expressed in human melanomas and has been linked to the metastatic potential of melanomas. It was also observed that MITF regulates c-MET expression in primary melanocytes and that the ability of HGF to stimulate invasive growth of melanocytes and melanoma cells in culture was abolished upon suppression of endogenous MITF [92].

MiT family cancers

In addition to the identification of *MITF* as an amplified oncogene in melanoma, the human *TFE3* and *TFEB* genes have also been implicated in human cancer. Translocations involving fusions of pediatric renal-cell carcinoma (*PRCC*), non-POU domain-containing octamer-binding protein (*NONO*, also known as $p54^{nrb}$), PSF, regulator of chromosome condensation 1 (*RCC1*) and alveolar soft-part sarcoma (*ASPL*) to *TFE3* have been identified in renal-cell carcinomas and in alveolar soft-part sarcomas [93–98]. Interestingly, these translocations follow a prototypical pattern in which the DNA-binding domain of the MiT family member (encoded by the b-HLH-Zip domain) is always preserved. Moreover, the *TFEB* translocations present in renal-cell carcinoma [97,98] fuse an abundantly expressed gene to the 5′ end of *TFEB* at a location upstream of the translation initiation codon. This results in a 'promoter swap' event that does not alter the coding region of *TFEB* – a mechanism that, similar to gene amplification, seems to induce oncogenic transformation via dysregulated expression rather than altered or mutated function of the transcription factor.

Another correlation of the MiT family to tumorgenesis has been recently made for clear cell sarcoma (CCS). CCS, a soft-tissue malignancy that expresses melanocytic markers, harbors a chromosomal translocation event that results in cAMP-independent transcriptional activity. It was shown that the chimera EWS–activating transcription factor 1 (*ATF1*) occupies the *MITF* promoter and induces its expression, which then mediates CCS survival [99]. CCS cells could not survive in the absence of MITF, although their survival could be rescued by either MITF or the closely related family members TFE3 or TFEB. Reciprocally, papillary renal carcinoma cells that harbor

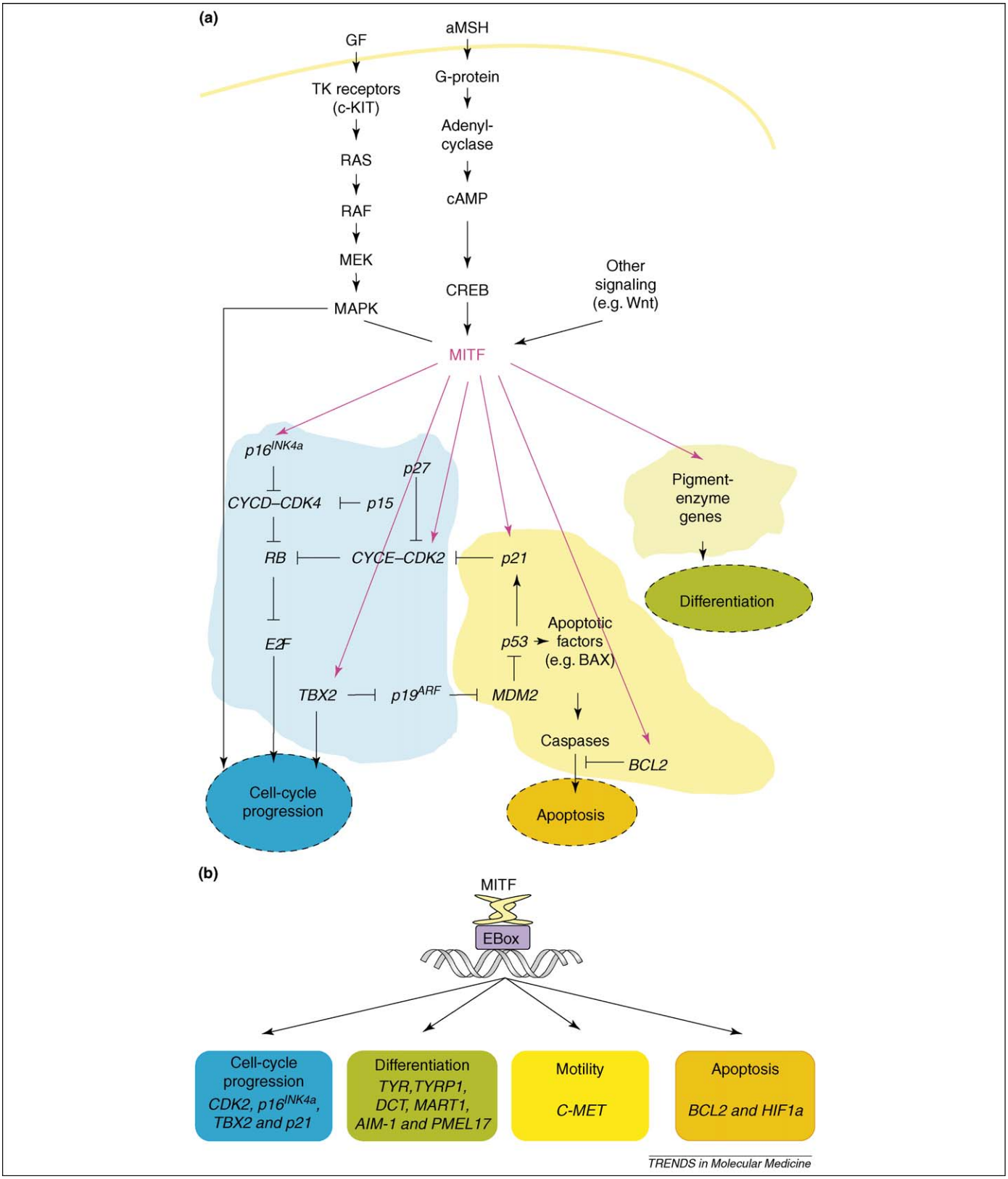

Figure 3. **(a)** MITF is involved in the induction of melanoma, melanocyte differentiation, cell-cycle progression and survival. Pink lines represent MITF target genes, which include genes involved in cell-cycle arrest (*p21* and *INK4a*), cell proliferation (*TBX2* and *CDK2*), cell survival (*BCL2*) or differentiation (*TYR, TYRP1, DCT, MART1, AIM-1* and *PMEL17*). Given the diverse biological activities of MITF and the antagonistic behaviors of certain target genes, it is likely that the repertoire of MITF targets depends upon cellular context. Black lines represent some of the signaling pathways connected to MITF, such as growth factors (GF), which lead to activation of tyrosine kinase (TK) receptors followed by a phosphorylation cascade, and α-MSH [101,102]. **(b)** MITF target genes. MITF-M regulates the transcription of multiple genes by binding specific sequences, as subset of E-boxes, present in promoter or enhancer elements containing the consensus CATGTG, CACATG or CACGTG. MITF regulates multiple targets in melanocytes and melanoma cells involved in various cellular processes such as cell-cycle control, survival, motility, invasion and differentiation and/or pigmentation.

Box 1. Potential clinical implications of MITF in melanoma

- Use of MITF as a diagnostic marker for melanocytic neoplasms.
- Transcriptional regulation of melanocytic proteins as vaccine antigens in melanoma.
- MITF amplification as a prognostic marker and regulator during melanoma progression.
- Identification of 'drugable' MITF target genes that mediate its oncogenic activity in melanoma.
- Drug strategies for suppression of MITF activity in melanoma.
- Application of MITF-related biological pathways to non-melanoma MiT family cancers in humans.

TFE3 translocation could not survive upon TFE3 knockdown by small interference RNA (siRNA) but were rescued by expression of MITF. These studies thus demonstrated functional oncogenic overlap among MiT family factors, and also led to a re-evaluation of tumor classifications based upon common oncogenic factors [99]. The presence of oncogenic gene fusion–translocation events or dysregulation for MITF-related family members, coupled to genetic evidence of functional redundancy among several of these factors [9] collectively implicate the MiT family in a new (and growing) set of human malignancies.

Concluding remarks

Unlike most normal cellular precursors of specific cancers in humans, melanocytes have a specifically important role in protecting humans from UV-induced skin cancers. For this reason, additional understanding of pigment biology and its regulation might impact cancer through the development of novel prevention strategies. Conversely, MITF oncogenic activities are still poorly understood, although directly implicated in melanoma oncogenesis (Box 1). The discovery of the roles of MITF in melanoma formation and progression have just begun to reveal new therapeutic strategies for melanoma that are based, for example, upon suppression of key MITF target genes (e.g. *CDK2*). The concept of discovering direct small molecule inhibitors of MITF itself is conceptually attractive, although its lack of a ligand-binding pocket or of a measurable catalytic activity makes it a challenging drug target. It is striking that MiT family members (TFEB and TFE3) are implicated in non-melanoma human cancers, some of which were discovered by virtue of the unanticipated expression of melanocytic genes that are transcriptionally regulated by MITF in melanocytes. The MiT-related malignancies (melanoma, translocation-associated renal-cell carcinomas and alveolar soft-part sarcoma) differ in various clinical features but share the frustrating feature of chemotherapy refractoriness and poor prognosis if surgically incurable. For these reasons, there is hope that information gleaned from studies of MITF in melanocytes might contribute to therapeutic advances in both melanoma and the MiT family non-melanoma cancers of humans.

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