Microphthalmia-associated transcription factor (MITF) – from Waardenburg syndrome genetics to melanoma therapy

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Microphthalmia-associated transcription factor (MITF) was first discovered as protein coded by gene whose mutations are associated with Waardenburg syndrome. Later, MITF was shown to be key transcription factor regulating melanogenesis. Further studies have shown that in addition to regulating melanogenesis MITF also plays central role in regulation of melanocyte development and survival. MITF gene is amplified in a proportion of melanomas and ectopic MITF expression can transform melanocytes so MITF can function as melanoma "lineage survival" oncogene. Different studies have further revealed MITF’s important but complex role in tumorigenesis and progression of melanoma. As expected from its important role in melanocytes and melanoma MITF is intricately regulated on all the levels from transcription to post-translational modifications. Although complex mechanisms of MITF functioning are still being revealed, MITF already has a valuable role in managing melanoma patients. Immunohistochemical analysis of MITF has shown both diagnostic and prognostic value in patients with melanoma. MITF is also a valuable specific marker for detection of circulating melanoma cells by reverse-transcription – polymerase chain reaction. MITF has recently been investigated as a potential target for melanoma therapy.

**Key words:** Microphthalmia-associated transcription factor, Melanoma, Melanocytes, Biological tumor markers, Waardenburg’s syndrome.

Introduction

Microphthalmia-associated transcription factor (MITF) was first discovered as a protein associated with Waardenburg syndrome. Further studies have revealed MITF as a master regulator of melanocyte function, development and survival. As expected from its role in normal melanocytes, MITF has also an important role in melanoma tumorigenesis and progression. The role of MITF in both normal melanocytes and melanoma is complex and depends on intricate system of regulation on different levels, from MITF transcription to post-translational modifications. That system, all components of which are still being discovered, enables MITF to adjust behaviour of melanocytes...
and melanoma cells to various signals coming from within the cell and from the environment of the cell. Although all the components of MITF's functioning are still unknown, MITF already has a valuable role in managing patients with melanoma as diagnostic and prognostic marker. Also, several therapeutic approaches to melanoma targeting MITF are being explored. In addition to its role in melanocytes and melanoma, MITF also plays important role in several other cell types, like osteoclasts and mast cells, but the focus of this review will be on a complex role of MITF in melanocytes and melanoma, and on application of MITF in managing melanoma patients.

MITF and Waardenburg syndrome

MITF was discovered as a protein coded on a gene locus associated with Waardenburg syndrome in humans (1, 2). Waardenburg syndrome is a hereditary autosomal dominant disorder characterized by heterochromia iridis, patchy abnormal pigmentation of the hair and skin and sensorineural deafness (3). Clinically Waardenburg syndrome is classified in four types. Waardenburg syndrome type 1 and Waardenburg syndrome type 3 have been associated with mutations in PAX3 gene (4). Waardenburg syndrome type 4 also known as Waardenburg-Shah syndrome has been associated with mutations in three genes, SOX10, gene for endothelin 3, and gene for endothelin receptor B (EDNRB) (5).

Waardenburg syndrome type 2 has been associated with mutations in one allele of MITF gene (6, 7). It has been shown that mutations in one allele of MITF gene do not influence the activity of protein coded on the other (non-mutated) copy of MITF gene (7). Therefore the dominant inheritance of Waardenburg syndrome type 2 has been explained as a result of haploinsufficiency, mechanism by which a MITF protein coded on a non-mutated allele of MITF gene can't reach the intracellular concentration necessary for its normal function (7). The symptoms of Waardenburg syndrome type 2 (white patches of the skin, altered iris pigmentation and loss of hearing associated with absence of melanocytes in stria vascularis of the cochlea) can be explained as a consequence of the melanocyte depletion (3). Mutations in MITF gene have also been associated with Tietz syndrome, which is a rare hereditary auditory-pigmentary disorder (8). The symptoms characteristic for Tietz syndrome are similar to the ones associated with Waardenburg syndrome type 2 but are present in a more severe form (8).

Mice with mutations in microphthalmia (mi) locus which is responsible for the synthesis of Mitf, a mouse homologue of MITF, have following disorders: loss of pigmentation, reduced eye size (microphthalmia), reduced number of mast cells, osteoporosis as a consequence of disturbed osteoclast function, and hearing impairment (9). These disorders implicate a role of Mitf in the development and function of melanocytes, mast cells, retinal pigment epithelial cells and osteoclasts.

MITF isoforms

Gene MITF is located on a third human chromosome in a region 3p12.3-3p14.1 and is responsible for the synthesis of nine so far described isoforms: MITF-M, MITF-A, MITF-H, MITF-B, MITF-C, MITF-D, MITF-Mc, MITF-E and MITF-J (2, 10-16) (Figure 1.). Protein MITF contains a basic domain required for DNA binding and helix-loop-helix and leucine zipper domains required for dimer formation (bHLH-LZ structure) (1-2). MITF is similar in amino acid sequence to other transcription factors that share the same bHLH-LZ structure: TFE3, TFEB and TFEC (17-19). MITF can bind DNA as a homodimer or as a heterodimer with transcription factors TFE3, TFEB and TFEC (17).
All the isoforms of MITF protein have a common C-terminal region containing a domain required for transcription activation and bHLH-LZ structure but each isoform has a distinct N-terminal region (20, 21) (Figure 1). The shortest isoform MITF-M consists of 419 amino acids and contains a unique N-terminal domain M (amino acid sequence: MLEMLEYNHY) and a six amino acids insert (ACIFPT) close to the basic region of the protein (18). It has been shown that also the MITF-M protein without this six amino acids insert is synthesized (18). Bismuth et al have shown that MITF-M isoform containing six amino acids insert can inhibit cell proliferation unlike the isoform without six amino acids insert (22). In accordance to this finding substantially increased proportion of isoform without six amino acids insert was found in metastatic melanoma in comparison to normal melanocytes (23).

MITF-M is specifically expressed in melanocytes originating from neural crest and in melanoma cells (10, 11, 24, 25). The melanocyte-restricted promoter region from which MITF-M isoform is transcribed is responsible for such a specific expression of this isoform (26). A splice variant of MITF-M named MITF-Mdel containing two in frame deletions, 56 amino acids deletion in exon 2 (from V32 to E87) and 6 amino acids deletion in exon 6 (from A187 to T192), has been identified (27). Like MITF-M, MITF-Mdel is also specifically expressed in melanocytes and melanoma cells (27).

Isoforms MITF-A, MITF-H, MITF-B, MITF-C, MITF-Mc and MITF-J have a common B1b domain of 83 amino acid residues and each its unique N-terminal domain (A, H, B1a, C, Mc and J) (13-16, 18, 20) (Figure 1).

Figure 1 Structures of MITF isoforms. Schematic representations of all described isoforms of MITF protein (MITF-M, MITF-A, MITF-H, MITF-B, MITF-C, MITF-D, MITF-Mc, MITF-E and MITF-J) are shown. An activation region (AR), basic helix-loop-helix leucine-zipper region (bHLH-LZ), serine-rich region (S) and N-terminal regions encoded by isoform-specific exons (A, B1a, B1b, C, H, Mc and M) are indicated for each isoform. Corresponding exons of MITF gene (1A, 1B1a, 1B1b, 1C, 1D, 1E, 1H, 1J, 1Mc, 1M, 1, 2, 3, 4, 5, 6, 7, 8 and 9) are indicated under the schematic representation of each isoform.
Domain A consists of 35 amino acid residues, domain H of 19, domain B1a of 10, and domain C of 34 amino acid residues (12, 20). Unlike the other isoforms, the translation of MITF-E and MITF-D, and probably also MITF-J isoform, does not start from the first exon, because it does not contain a code for methionine, but it starts from within B1b region (Figure 1.). Therefore the protein products for these three isoforms (MITF-E, MITF-D and MITF-J) are the same (13, 14, 16) (Figure 1).

Isoforms MITF-A and MITF-H are expressed in different cell types, including melanocytes and melanoma cells, with varying levels of expression depending on a cell type (10, 11, 24). Isoform MITF-C is expressed in different cell types, but not in melanocytes and melanoma cells (11). Of all the isoforms isoform MITF-A is the most abundantly expressed in retinal pigment epithelial cells (10). The expression of MITF-D isoform has been found in retinal pigment epithelial cells, macrophages, osteoclasts and mast cells, which are all cells affected by mutations in mouse MITF gene (13). In other cell types analyzed, including melanocytes and melanoma cells, no expression of MITF-D isoform has been found (13). Isoforms MITF-E and MITF-Mc are specifically expressed in mast cells (14, 15).

**MITF gene**

Gene MITF consists of nine first exons (1M, 1A, 1H, 1B, 1C, 1D, 1E, 1Mc and 1J) encoding the synthesis of N-terminal domain specific for each isoform and eight exons shared by all isoforms (12, 16) (Figure 1). 3’ part of exon B (B1b) is also a second exon in isoforms MITF-A, MITF-H, MITF-C, MITF-D, MITF-E, MITF-Mc and MITF-J (12, 16) (Figure 1). So the isoforms MITF-M and MITF-B are encoded by nine exons, while the isoforms MITF-A, MITF-H, MITF-C, MITF-D, MITF-E, MITF-Mc and MITF-J are encoded by ten exons. Each isoform is transcribed from its own unique promoter, suggesting the functional diversity of these isoforms in different tissues (12). The amino acid sequences of homologues of human MITF protein have been determined in mice (1), rats (28), chicken (29), hamsters (30), quails (31) and zebrafish (Danio rerio) (32). All of these sequences are highly homologous with the one of human MITF protein. Analysis of publicly available genomic sequence data indicates the existence of genes homologous to human MITF gene also in other vertebrate as well as invertebrate species (33).

**Function of MITF**

MITF is a transcription factor that activates the transcription of genes for tyrosinase, tyrosinase-related protein 1 (TYRP1), and dopachrome tautomerase (DCT), enzymes specifically expressed in melanocytes that have a key role in synthesis of pigment melanin (34-36). MITF activates the transcription of these and other target genes by binding in their promoter regions to a restricted subset of E-box motives containing canonical CATGTG sequence flanked by a 5’ thymidine (37). More recent analysis of more than 40 MITF target genes came out with T-C-A-T/C-G-T-G-A as a MITF-binding consensus sequence (38). The regulation of DCT promoter is more complex than for tyrosinase and TYRP1, as other some proteins like CREB and SOX10 cooperate with MITF in activating DCT expression, while PAX3 has an antagonistic effect on activation of DCT expression by MITF (39-41). In addition to activating the transcription of genes involved in melanin synthesis, MITF also activates the transcription of genes involved in melanosome structure (PMEL17/SILV/ GP100, MLANA/MELAN-A/MART-1), melanosome biogenesis (ocular albinism type 1 gene (OA1)), and melanosome transport.
which makes MITF a central regulator of melanogenesis on a transcription level (38, 42-45). MITF also activates the transcription of gene for melanocortin 1 receptor (MC1R), a receptor on plasma membrane of melanocytes for α-melanocyte stimulating hormone (α-MSH) (38, 45, 46). Binding of α-MSH to MC1R is first step in the mechanism of the hormonal regulation of pigmentation, the mechanism that also involves activation of MITF as an important downstream step (45). Therefore, activation of MC1R transcription by MITF represents a positive feedback mechanism in the hormonal regulation of pigmentation.

Association of mutations in human MITF gene with Waardenburg syndrome and study of mice with mutations in microphthalmia locus coding for mouse homologue of MITF imply important role for MITF in melanocyte development and survival. Several experiments have confirmed that MITF-M has an important role in differentiation and normal function of melanocytes. Induced expression of gene for MITF-M in NIH/3T3 fibroblasts in which it is normally not expressed converted these cells into cells expressing melanocyte-specific genes for tyrosinase and TRP-1 (47). In one experiment zebrafish (Danio rerio) embryos lacking melanophores due to mutations in nacre, a functional homologue of MITF gene, were transfected with wild-type nacre gene, which was sufficient to restore the development of melanophores (32). In another experiment embryonic stem-like cells form medaka (Orysis latipes) were transfected with the melanocyte-specific isoform of Xiphophorus mitf gene and cells with all the characteristics of differentiated, functional pigment cells were observed (48). Unlike for melanocytes, differentiation of retinal pigment epithelium cells and melanogenesis in these cells is not regulated by melanocyte specific MITF-M isoform but by other MITF isoforms like MITF-D, MITF-H and MITF-A (10, 49).

Several targets of MITF, including genes that play important role in the control of apoptosis and cell cycle, have been identified elucidating the important role of MITF in melanocyte development and survival. MITF controls the transcription of BCL2, gene for Bcl-2, an important inhibitor of apoptosis (50). The importance of this interaction for melanocyte survival was shown in experiment in which overexpression of BCL2 rescued melanocytes from apoptosis induced by dominant-negative MITF mutation (50). Regulation of BCL2 expression by MITF could explain reduced number of melanocytes in persons with Waardenburg syndrome type 2. Mutated MITF in persons with Waardenburg syndrome type 2 is less efficient in inducing the expression of BCL2, and reduced expression of BCL2 has a consequence of more melanocytes dying by apoptosis. MITF induces the transcription of another inhibitor of apoptosis, BIRC7 (also known as melanoma inhibitor of apoptosis (ML-IAP) or LIVIN) (51). MITF also regulates the expression of MET, receptor for hepatocyte growth factor (HGF), the activation of which protects melanocytes from apoptosis (52-53). MITF promotes proliferation of melanocytes by regulating the transcription of several genes involved in the cell-cycle regulation. In melanocytes and melanoma cells MITF binds to a sequence upstream of the transcription start of cyclin-dependent kinase 2 (CDK2) gene and induces transcription of this important cell-cycle regulator that induces cell-cycle progression (54). MITF induces the expression of the gene for transcription factor TBX2 that prevents senescence and cell-cycle arrest through repression of cyclin-dependent kinase inhibitor 1A (p21) expression (55-57). Another mechanism by which MITF promotes proliferation of melanocytes and melanoma cells is through activation of the
expression of **DIAPH1**, a gene for Dia1 protein that controls actin polymerization (58). Activity of Dia1 results in increased degradation of cyclin-dependent kinase inhibitor 1B (p27), which leads to an increased cellular proliferation (58). MITF trough activation of **DIAPH1** expression also reduces invasiveness of melanoma cells (58). In contrast to described pro-proliferative effects, MITF also has anti-proliferative effects. MITF activates transcription of genes for two proteins that induce cell cycle arrest, cyclin-dependent kinase inhibitor 1A (CDKN1A/p21) and cyclin-dependent kinase inhibitor 2A (CDKN2A/p16) (59, 60). The effect of MITF on cell cycle arrest indicates the important role of MITF in melanocyte differentiation. It is possible that level of expression of MITF determines whether it will have pro-proliferative or anti-proliferative effect. It was shown that both MITF depletion and MITF forced expression inhibit proliferation of melanoma cells while normal level of MITF expression favours cell proliferation (61). Some other genes that play a role in promoting melanocyte survival and preventing apoptosis have been identified as MITF targets, like gene for a DNA repair enzyme apurinic / apyrimidinic endonuclease1 (APEX1), gene for a transcription factor hypoxia inducible factor 1a (HIF1A), and gene for endothelin receptor B (ENDRB) (38, 62-64). Also some other MITF target genes that are not directly related to melanocyte survival and development have been identified, like TRPMN1/melastatin, glycoprotein-nmb (GNMB), and SNAI2/SLUG, a gene that plays important role in epithelial-mesenchymal transition (38, 65-67).

In addition to its’ central role in melanocyte development and biology, MITF is also important for osteoclasts and mast cells development and function (68). In osteoclasts MITF has been shown to activate the transcription of several genes for proteins important for osteoclast function like tartar-ate-resistant alkaline phosphatase (TRAP), cathepsin K, OSCAR, E-cadherin, OSTM1 and Clcn7 (69, 70). In mast cells MITF activates the expression of several genes important for mast cell differentiation and function like genes for mast cell proteases 2, 4, 5, 6, and 9, granzyme B, tryptophan hydroxylase, Kit, and some others (71, 72).

**Regulation of MITF**

MITF is regulated on different levels, from transcription to post-translational modifications. It was shown that Wnt signalling pathway induces the transcription of MITF (73). Wnt are secreted cysteine rich glycoproteins that play an important role in embryonic development and differentiation. Wnt proteins are especially important for differentiation of melanocytes and other neural crest derived cells (74, 75). Binding of Wnt molecules to specific cell-surface receptors of the Frizzled family activates these receptors and initiates the sequence of signals that leads to increased stability and accumulation of cytoplasmic β-catenin which then enters the nucleus and interacts there with lymphoid enhancer factor 1/T cell factor (LEF1/TCF) transcription factor inducing the transcription of LEF1/TCF target genes (75) (Figure 2.). In a promoter region of MITF-M a functional binding site for LEF1/LCF was discovered which explains the mechanism of activation of MITF expression by Wnt signaling pathway (73). MITF also interacts directly with LEF1 to activate expression of some MITF target genes, as well as expression of MITF itself (76, 77). It was shown that dickkopf 1 (DKK1), an inhibitor of Wnt pathway, has a suppressing effect on the expression of MITF (78).

MITF expression can also be activated by α melanocyte-stimulating hormone (αMSH) signalling pathway (36, 79). αMSH is synthesized and secreted in epidermal keratinocytes and binds to the melanocor-
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The microphthalmia-associated transcription factor (MITF) plays a crucial role in the development of melanocytes. This transcription factor is activated by various pathways, including the mitogen-activated protein kinase (MAPK) pathway, which involves the phosphorylation of MITF at Ser 73, Ser 409, and Ser 298 by protein kinases such as p90Rsk and GSK3β. Additionally, the CREB (cAMP response element-binding protein) transcription factor can bind to a target sequence in the promoter region of MITF to activate its transcription.

In addition to CREB, the transcription factors SOX10 and PAX3 also bind to the promoter region of MITF and activate its expression. Mutations in the PAX3 gene are associated with Waardenburg syndrome type 1, 3, and 4, while mutations in the SOX10 gene are associated with Waardenburg syndrome type 4. The cooperation between SOX10 and CREB is required to activate MITF expression, which can explain how ubiquitous CREB can regulate cell-type specific expression of MITF.

Waardenburg syndrome type 4 can also be caused by mutations in the gene for endothelin (EDN) or its receptor EDNRB. Binding of EDN to EDNRB on melanocytes initiates a signal cascade that leads to increased expression of MITF and phosphorylation of MITF at Ser 73. MITF transcription can also be activated by transcription factors Onecut-2 and peroxisome proliferator-activated receptor γ (PPARγ). Interleukin-1 can significantly down-regulate MITF expression, further affecting melanocyte development.

Figure 2 Schematic representation of regulation of MITF gene expression and MITF protein activity. MAPK (mitogen activated protein kinases), p90Rsk, and GSK3b are protein kinases that phosphorylate MITF protein on Ser 73, Ser 409, and Ser 298, respectively, and thus regulate its activity. CREB (cAMP response element binding protein), PAX10, SOX10, and LEF1 are transcription factors that induce transcription of MITF gene. Wnt, SCF (stem cell factor), and αMSH (a melanocyte stimulating hormone) are growth factors that activate cellular signaling pathways leading to activation of named protein kinases and transcription factors. c-Kit is receptor for SCF (stem cell factor), Frizzled is receptor for Wnt, and MC1r is receptor for αMSH.
late the expression of MITF-M in melanoma cells expressing interleukin 1 receptor (90).

It was shown that oncogenic BRAF with V600E mutation found in ~60% melanomas also regulates MITF by two different mechanisms (91-93). First mechanism involves BRAFV600E activation of extracellular-signal regulated kinase 2 (ERK2) that then phosphorylates MITF thus inducing its degradation (92). The other mechanism involves BRAFV600E mediated induction of the expression of transcription factor BRN2 that is not normally expressed in melanocytes and that can bind to MITF promoter and induce the transcription of MITF (93). These apparently contradictory effects of BRAF V600E on MITF can be reconciled in a previously described model according to which normal level of MITF activity promotes cell proliferation while both its downregulation and upregulation suppress this pro-proliferative action of MITF.

MITF is also regulated by post-translational modifications. Binding of stem cell factor (SCF) to its cell surface receptor c-Kit starts a signalling cascade that leads to phosphorylation of MITF on Ser 73 by a mitogen activated protein kinase (MAPK) extracellular-signal regulated kinase 2 (ERK2) and on Ser 409 by serine-threonine kinase p90 ribosomal s6 kinase (RSK) (94, 95). It was shown that Ser 73 phosphorylation of MITF enables binding of transcription coactivator p300/CBP to MITF increasing the activity of MITF as transcription factor (96). Both Ser 73 and Ser 409 phosphorylations also downregulate MITF by enhancing its ubiquitination followed by proteosome degradation (95). MITF can also be phosphorylated at Ser 298 by glycogen synthase kinase 3β (GSK3β) which activates MITF by enhancing its binding to its target DNA sequences (97). The importance of this phosphorylation is confirmed by finding of point mutation in a subset of Waardenburg syndrome 2 patients that results in substitu-

tion of Ser 298 with a proline in MITF (97). It was shown in osteoclasts that MITF is phosphorylated on Ser 307 by p38 MAPK as a part of NF-κB signalling pathway, which increases MITF activity in inducing the transcription of its target genes (98). The activity of MITF can also be modulated by sumoylation at Lys 182 and Lys 316 mediated by protein inhibitor of activated STAT3 (PIAS3) (99). It was shown that sumoylated MITF has decreased transcriptional activity on target genes with more than one MITF binding site (99). It was shown that in melanocytes and in melanoma cells MITF can be cleaved by caspases after Asp 345 producing C-terminal fragment that has pro-apoptotic activity (100).

The expression of MITF can also be regulated by microRNAs (miRNA). It was shown that expression of miRNA-137 suppresses MITF expression (101, 102). Also miRNA-182, that is frequently upregulated in melanoma, suppresses the expression of MITF (103). MITF expression is also regulated by miRNA-148 (102).

It was shown that activity of MITF as transcription factor depends on its interaction with several other proteins. MITF binds a known transcriptional co-activators p300/CBP, which enhances MITF’s activity as a transcription factor (96, 104). It was shown that interaction with p300/CBP turns MITF from repressor to activator of DCT gene (105). MITF was shown to interact with Rb to activate CDKN1A gene expression (59). Direct interaction of MITF with β-catenin increases activation of expression of MITF target genes (106). It was shown that activity of chromatin-remodelling enzymes SWI/SNF is required for MITF activation of some MITF target genes (TRP1 and tyrosinase gene) but not for other MITF target genes (MC1R) (107).

**MITF and melanoma**

Cronin et al analyzed somatic mutations in MITF and SOX10, upstream regulator
of MITF, and found them in more than 14% primary melanomas and 22% metastatic melanoma (108). Garraway et al. have shown that MITF gene was amplified in 10.5% of primary melanomas and 15.2% of metastatic melanomas, but no amplification was detected in melanocytic nevi, which are considered a pre-malignant lesions associated with melanoma (109). Also, the amplification of MITF gene was associated with decreased 5-year survival in the same study (109). In another study strong MITF gene amplification in metastatic melanoma patients was also associated with reduced disease-specific survival (110). Furthermore, it was shown that ectopic expression of MITF together with V600E mutated BRAF can transform immortalized melanocytes genetically engineered to have inactivated CDKN2A/CDK4/RB and p53 pathways and to express human telomerase reverse transcriptase (hTERT) (109). Also it was shown that MITF is at least partly responsible for melanoma chemoresistance (109). Based on these results it was proposed that MITF might play a role as “lineage specific” oncogene in melanoma. “Lineage specific” (also called “lineage survival” and “lineage addiction”) oncogenes are genes that play important role in normal proliferation and survival of particular cell lineage during development and deregulated expression of which in a subset of cancers of the same cell lineage is important for cancer survival and progression (111). It is possible that MITF amplification is one way to compensate for MITF downregulation trough BRAFV600E-ERK, supporting a role for MITF as “lineage specific” oncogene in melanoma. The role of MITF as a “lineage specific” melanoma oncogene is in apparent contradiction with its role in melanocyte differentiation and cell cycle arrest exhibited trough activation of CDKN2A and CDKN1A gene transcription (59, 60). These contradicting roles of MITF could be reconciled if we hypothesize that MITF plays a role as “lineage specific” melanoma oncogene only in subset of melanoma cells in which CDKN2A/CDK4/RB pathway is inactivated, for example by mutations in CDKN2A gene which are well documented in melanoma (112). This explanation is supported by finding that all cell lines with MITF gene amplification in previously described study by Garraway et al also had CDKN2A pathway inactivation (109). In the same study MITF (in cooperation with BRAFV600E) could transform melanocytes that had inactivated CDKN2A pathway (109). Also, it was shown that inactivation of CDKN2A can enable melanocytes to escape MITF induced growth inhibition while maintaining MITF expression (60). Studies that have shown that MITF is important activator of expression of several genes that play important role in melanoma cell survival, growth and proliferation, like BCL2, CDK2, HIF1A, TBX2, BIRC7/ML-IAP support the role of MITF as “lineage specific” oncogene in melanoma (50, 51, 54, 55, 57, 63).

In a different genetic context when CDKN2A/CDK4/RB pathway is not inactivated it is most probable that MITF activity should be kept at a certain level to promote melanoma cell survival and proliferation because too high expression of MITF would lead to cell cycle arrest and too low or no expression would lead to apoptosis. In line with this model is two-fold regulation of MITF by V600E mutated BRAF in melanoma. As previously described, BRAFV600E found in ~60% melanomas, downregulates MITF trough ERK2 mediated phosphorylation and upregulates MITF transcription trough BRN2 transcription factor (92, 93). It is probable that in that way oncogenic BRAFV600E keeps MITF at a level needed to maintain melanoma cells proliferation and survival. Several studies have shown results corroborating that model. It was shown that upregulation of MITF expression in mela-
noma cells inhibits their proliferation (92). Also, MITF reexpression in melanoma cells that do not express MITF reduced their tumorigenecity in vivo (25). Transfection of aggressive UI5O-Mel-6 melanoma cells with MITF-M decreased their proliferation and metastatic potential leading to a less aggressive phenotype (113). Expression of MITF was studied by immunohistochemistry and was shown to decrease with melanoma progression (114). Another study has shown that MITF expression analyzed immunohistochemically is associated with longer overall survival and disease-free survival and fewer lymph node metastases (115). On the other hand, abolished MITF expression in melanoma cells resulted in profound apoptosis that could be rescued by BCL2 or BIRC7/ML-IAP overexpression (50, 51). In other study downregulation of MITF suppressed colony formation by melanoma cells that could be rescued by overexpression of CDK2, a cell cycle regulator which was shown to be controlled by MITF and indispensable for growth and cell cycle progression only in melanoma cells (54).

Kido et al have shown that both depletion and forced expression of MITF significantly inhibited melanoma cell proliferation (61). Although many of the previously mentioned studies indicate important role for MITF in melanoma, MITF was not expressed in a proportion of melanoma samples analyzed, indicating that there are different subsets of melanomas which differ regarding the role and importance of MITF for their progression and survival (115-117). Furthermore, it is possible that importance and role for MITF in melanoma can change during melanoma progression (118).

Several studies have shown that MITF is involved in other aspects of melanoma behaviour in addition to regulating melanoma cell survival and proliferation. MITF can regulate melanoma angiogenesis by activating the expression of HIF1A, which in turn activates the expression of vascular endothelial growth factor (VEGF) (63). Through regulating the expression of DIAPH1, gene for Dia1 protein, MITF in addition to increasing proliferation also reduces invasiveness of melanoma cells (58). MITF induced by HGF signalling upregulates the expression of MET receptor and so plays a central role in HGF-MET regulated invasion of melanocytes and melanoma cells (52). MITF also activates the expression of TRPM1/melastatin, a prognostic factor in melanoma patients the expression of which is inversely correlated with melanoma metastatic potential and prognosis so it may play a role as melanoma metastasis suppressor (65). MITF can also be implicated in regulation of melanoma invasion and metastases trough regulating the expression of SNAI2/SLUG, a gene that plays important role in epithelial-mesenchymal transition (67).

Hoek et al analyzed expression profiles for 86 melanomas and separated them based on expression profile in two cohorts, one with high proliferative and low metastatic potential characterized by high MITF expression and other with low proliferative and high metastatic potential characterized by low MITF expression (119). In a further study it was shown that during tumour progression melanoma cells could switch from one to other of these two expression profiles (118). MITF was shown also to play important role in regulating cellular response to reactive oxygen species through regulating the expression of apurinic/apyrimidinic endonuclease 1 (APE-1/Ref-1) (62). The expression of MITF makes melanoma cells more resistant to H2O2-induced cell death implicating another role for MITF in melanoma carcinogenesis (62).

Studies showing that signalling pathways deregulation of which is implicated in melanoma tumorigenesis and progression regulate MITF expression and activity further imply an important role for MITF in mela-
noma. MITF is an important downstream target of Wnt/β-catenin signalling pathway that is deregulated with aberrant nuclear accumulation of β-catenin in significant proportion of melanomas (73, 120). Widlund et al. have shown that β-catenin is important for growth and survival of melanoma in a manner dependent on downstream activation of MITF (121). As previously elaborated, MITF is downstream target of c-Kit signalling pathway (95). Somatic oncogenic mutations in KIT, gene coding for c-Kit, have been implicated in melanomas arising on acral, mucosal and chronically sun-damaged cutaneous surfaces and therapy targeting c-Kit has shown promising results in these melanoma patients (122, 123). Also, MITF expression is induced by TYRO3, which is upregulated in melanoma and plays important role in melanoma tumorigenesis and progression (124). Furthermore, miRNA-182, which is upregulated in melanoma and plays a role in melanoma progression regulates MITF transcription further emphasizing role of MITF in melanoma (103).

In addition to melanoma, MITF has also been implicated in the development of clear cell sarcoma and TFE3 and TFEB, transcription factors closely related to MITF, have been implicated in the development of some other cancers (125).

**MITF as immunohistochemical marker for melanoma**

The most common routinely used markers for immunohistochemical diagnosis of melanoma, S100 and gp100 (detected with HMB45 antibody) show either relatively low specificity (S100) or relatively low sensitivity (gp100/HMB45) (126). Therefore, a particular interest was shown for the results of the study in which all the tissue samples of primary melanoma and melanoma metastases, including the ones that were negative for S100 and gp100, stained positively with a nuclear staining pattern when D5 antibody specific for human MITF was used (127). In that study samples of healthy skin and benign melanocyte lesions were also positive for MITF. However, none of the samples of tumours of non-melanocyte origin was positive for MITF.

Such highly specific and sensitive immunohistochemical staining for MITF in melanocyte lesions was confirmed in other studies (128, 129). In one study MITF was analyzed as immunocytochemical marker for melanoma, showing specificity of 97% and sensitivity of 100% superior to S100 and gp100 in the same study (130). However, some other studies have shown a lower sensitivity (88% and 81%) of MITF protein as a marker for immunohistochemical diagnosis of melanoma (116, 117). In one study only 64% of S100 positive, HMB45 negative, epitheloid melanomas stained with MITF (131). Also staining for MITF not specific for melanocyte lesions has been shown in some studies. In one study 1 out of 8 breast carcinomas, 2 out of 17 renal carcinomas, and 2 out of 5 leiomyosarcomas were MITF positive (117). In other study immunoreactivity for MITF was seen also in macrophages, lymphocytes, fibroblasts, Schwann cells, and smooth muscle cells at various sites, and in tumours derived from these cells (132).

MITF was specially investigated as a marker for immunohistochemical diagnostics of desmoplastic melanoma, a rare histological type of melanoma that is often difficult to distinguish from some other tumours or benign lesions, but did not show value for that purpose. In one study only one out of 30 investigated histological samples of desmoplastic melanoma was positive for MITF (116). Another study has shown that MITF is neither specific nor sensitive marker for immunohistochemical diagnostics of desmoplastic and spindle-cell melanomas (133). MITF has also shown a value as immunohistochemical marker for the
detection of melanoma metastases in sentinel lymph nodes (134). Using the specific antibodies the expression of MITF protein was confirmed in cell lines and tissue specimens of other tumours of melanocytic origin: uveal melanomas, central nervous system melanocytomas and clear cell sarcomas (134-136).

In one study the expression of MITF protein was investigated as a prognostic factor in patients with intermediate-thickness (1-4 mm) cutaneous melanoma (115). The expression of MITF evaluated semi-quantitatively by immunohistochemistry in that study was associated with statistically significantly longer overall survival and disease-free survival. In another study MITF gene amplification was analyzed by quantitative real-time PCR in tumour tissue samples from metastatic melanoma patients (110). In that study strong MITF gene amplification was associated with a reduced disease-specific survival but no correlation was found between MITF copy number and chemotherapy response. These results indicate that MITF gene copy number could be a valuable prognostic marker but not a predictive marker for chemotherapy response in patients with metastatic melanoma.

**MITF as a marker for the detection of circulating melanoma cells**

The detection of circulating melanoma cells by reverse-transcription – polymerase chain reaction (RT-PCR) has been investigated as a potential prognostic and predictive marker in melanoma patients. The most widely used melanoma-specific marker for RT-PCR detection of circulating melanoma cells is tyrosinase. Tyrosinase has shown high specificity, low threshold for the detection of circulating melanoma cells and association with overall and progression-free survival in many studies (137). Still, the clinical value of tyrosinase is limited due to significant proportion of patients with confirmed distant metastases being tyrosinase negative (137, 138). Several studies have shown that analysis of additional markers together with tyrosinase can improve the detection of circulating melanoma cells (137, 139).

We were first to confirm that MITF-M can be analyzed as a specific marker with a low threshold for the detection of circulating melanoma cells by RT-PCR (140). In that study, we have shown that analysis of MITF-M as an additional marker to tyrosinase improves the detection of circulating melanoma cells in melanoma patients (140). Koyanagi et al. have subsequently shown that MITF detection in blood by real-time quantitative RT-PCR is a significant independent prognostic factor for relapse-free and overall survival and can indicate subclinical metastatic disease and predict treatment outcome in melanoma patients (141). In a recent study we investigated MITF as a marker for the detection of circulating melanoma cells by RT-PCR on 201 melanoma patients in all stages of the disease (142). In this study positive value of MITF was associated with significantly shorter overall survival and progression-free survival.

A recently identified splice variant of MITF-M, MITF-Mdel is widely expressed in melanocytes, melanoma cell lines and tissues, but almost undetectable in non-melanoma cell lines or peripheral blood mononuclear cells from healthy donors (27). Therefore, MITF-Mdel is also a promising marker for the detection of circulating melanoma cells by RT-PCR.

**MITF as target for melanoma therapy**

The role of MITF in normal melanocyte development and in melanoma progression and survival makes it a potential target for melanoma therapy. Electroporation mediated transfer of short interfering RNA specific for MITF gene was studied in mice with
melanoma. That treatment induced apoptotic death of tumour cells leading to significant growth retardation of the tumour (143). Another approach using histone deacetylase (HDAC) inhibitors has been studied showing that HDAC inhibitors suppress the expression of MITF-M in melanoma cells and systemic HDAC inhibitors treatment significantly suppressed the growth of melanoma in a human melanoma xenograft model (144).

Conclusion

Results of many different studies described in this review have established MITF as a master regulator of melanocyte and melanoma function, development and survival. MITF is already routinely applied as melanoma marker and has shown promising results as a target for melanoma therapy. Still, regulation of MITF in melanocytes and melanoma cells is immensely intricate and consequently its role and importance in melanoma is complex and depending on different factors. As described previously, just one example of complex role of MITF in melanocytes and melanoma is finding that MITF has both proproliferative and antiproliferative effects. Also, as described, MITF gene is amplified in a proportion of melanomas where MITF plays a role of a “lineage specific” oncogene, while in another proportion of melanomas MITF is not expressed at all. Therefore, further elucidation of complex regulation and effects of MITF in different genetic, intracellular and extracellular contexts could enable further and clinically more relevant applications of MITF, primarily in the management of melanoma patients.

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