Review article

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

Ivan Šamija, Josip Lukač, Zvonko Kusić

Department of Oncology and Nuclear Medicine,
University Hospital "Sestre milosrdnice",
Zagreb, Croatia

Corresponding author:
Ivan Šamija
Department of oncology and Nuclear Medicine
University Hospital "Sestre milosrdnice"
Vinogradska cesta 29
10000 Zagreb
Croatia
isamija@kbsm.hr
Tel.: + 385 1 3787647

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.

Received: 28 September 2010
Accepted: 31 October 2010

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 Waardenburd 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).

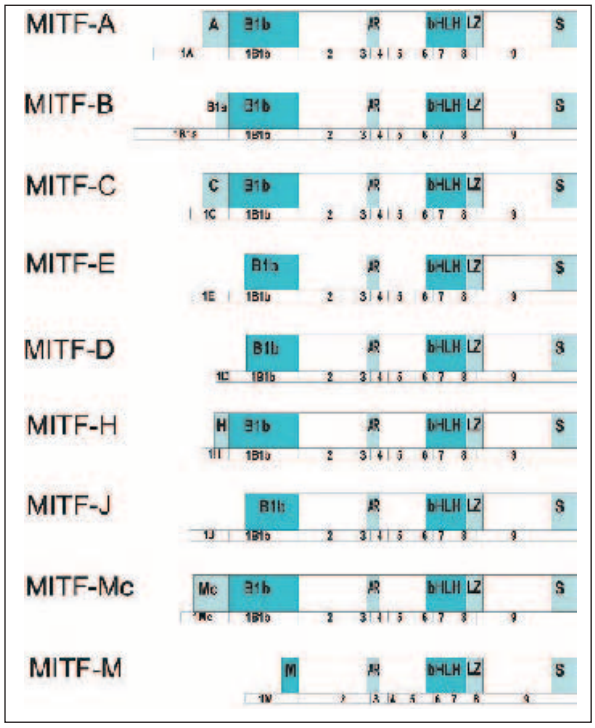

Figure 1 Structures of MITF isoforms. Schematic representations of all described isoforms of MITF protein (MITF-M, MITF-A, MITF-H, MITF-B, MITF-C, MITF-D; MITF-Mc, MITF-E and MITF-J) are shown. An activation region (AR), basic helix-loop-helix leucine-zipper region (bHLH-LZ), serine-rich region (S) and 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, 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 some other 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

(*RAB27A*), 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 (*Orysias 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 1 a (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 tartarate-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, form 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-

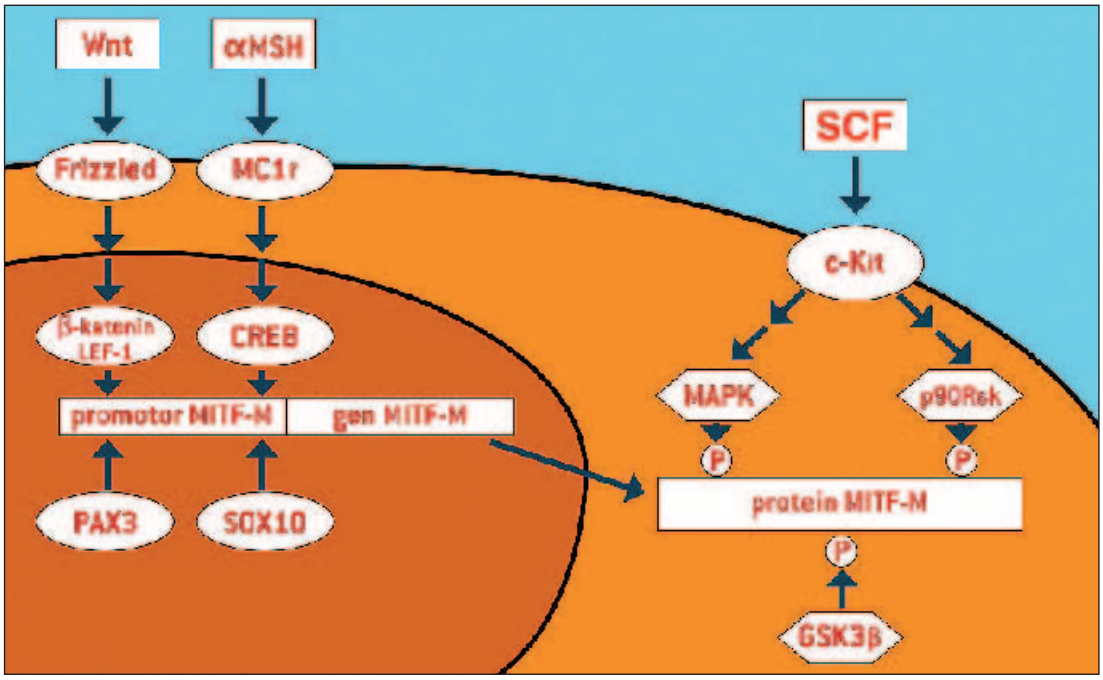

Figure 2 Schematic representation of regulation of MITF gene expression and MITF protein activity. MAPK (mitogen activated protein kinases), p90Rsk, and GSK3b are protein kinases that phosphorylate MITF protein on Ser 73, Ser 409, and Ser 298, respectively and thus regulate its activity. CREB (cAMP response element binding protein), PAX10, SOX10 i 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.

tin 1 receptor (MC1R) on cell surface of melanocytes activating a signalling cascade that involves activation of adenylate cyclase that synthesizes cyclic adenosine monophosphate (cAMP). One of the effects of increased level of intracellular cAMP is activation of cAMP response element-binding (CREB) transcription factor by phosphorylation. Activated CREB can bind to a target sequence in the promoter region of MITF-M and activate its transcription (36, 79).

In addition to LEF1/TCF and CREB, SOX10 and PAX3 transcription factors also bind to promoter region of MITF-M and activate its expression (80-84). Mutations in *PAX3* gene are associated with Waardenburg syndrome type 1 and 3 while mutations in *SOX10* gene are associated with Waardenburg syndrome type 4 (85, 86). Role of PAX3 and SOX10 in regulation of MITF expression can explain some of the symptoms associated with Waardenburg syndrome type 1, 3, and 4. It was shown that 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 (87). Waardenburg syndrome type 4 can also be caused by mutations in gene for endothelin (EDN) or gene for its receptor EDNRB. Binding of EDN to EDNRB on melanocytes starts a signal cascade that leads to increased expression of MITF and phosphorylation of MITF at Ser 73 (64). MITF transcription can be activated also by transcription factors Onecut-2 and peroxisome proliferator-activated receptor γ (PPARγ) (88, 89). Recently it was shown that interleukin-1 can significantly down-regu-

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 $BRAF^{V600E}$ activation of extracellular-signal regulated kinase 2 (ERK2) that then phosphorylates MITF thus inducing its degradation (92). The other mechanism involves $BRAF^{V600E}$ 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/CREB-binding protein (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 substitution 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 $BRAF^{V600E}$-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 $BRAF^{V600E}$) 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, $BRAF^{V600E}$ 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 $BRAF^{V600E}$ 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 UISO-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 immunohistochemicaly 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 H_2O_2-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 overexpressed 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 semiquantitatively 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.

Acknowledgements: The research was supported by Research Grant 0134011 from the Ministry of Science Education and Sports, Republic of Croatia.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

References

1. Hodgkinson CA, Moore KJ, Nakayama A, Steingrímsson E, Copeland NG, Jenkins NA, et al. Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. Cell. 1993;74(2):395-404.
2. Tachibana M, Perez-Jurado LA, Nakayama A, Hodgkinson CA, Li X, Schneider M, et al. Cloning of MITF, the human homolog of the mouse microphthalmia gene and assignment to chromosome 3p14.1-p12.3. Hum Mol Genet. 1994;3(4):553-7.
3. Liu XZ, Newton VE, Read AP. Waardenburg syndrome type II: phenotypic findings and diagnostic criteria. Am J Med Genet. 1995;55(1):95-100.
4. Tassabehji M, Read AP, Newton VE, Harris R, Balling R, Gruss P, et al. Waardenburg's syndrome patients have mutations in the human homologue of the Pax-3 paired box gene. Nature. 1992;355(6361):635-6.
5. Widlund HR, Fisher DE. Microphthalamia-associated transcription factor: a critical regulator of pigment cell development and survival. Oncogene. 2003;22(20):3035-41.
6. Tassabehji M, Newton VE, Read AP. Waardenburg syndrome type 2 caused by mutations in the human microphthalmia (MITF) gene. Nat Genet. 1994;8(3):251-5.
7. Tachibana M. Evidence to suggest that expression of MITF induces melanocyte differentiation and haploinsufficiency of MITF causes Waardenburg syndrome type 2A. Pigment Cell Res. 1997;10(1-2):25-33.
8. Amiel J, Watkin PM, Tassabehji M, Read AP, Winter RM. Mutation of the MITF gene in albinism-deafness syndrome (Tietz syndrome). Clin Dysmorphol. 1998;7(1):17-20.
9. Moore KJ. Insight into the microphthalmia gene. Trends Genet. 1995;11(11):442-8.
10. Amae S, Fuse N, Yasumoto K, Sato S, Yajima I, Yamamoto H, et al. Identification of a novel isoform of microphthalmia-associated transcription factor that is enriched in retinal pigment epithelium. Biochem Biophys Res Commun. 1998;247(3):710-5.
11. Fuse N, Yasumoto KI, Takeda K, Amae S, Yoshizawa M, Udono T, et al. Molecular cloning of cDNA encoding a novel microphthalmia-associated

transcription factor isoform with a distinct amino-terminus. J Biochem. 1999;126(6):1043-51.

12. Udono T, Yasumoto K, Takeda K, Amae S, Watanabe K, Saito H, et al. Structural organization of the human microphthalmia-associated transcription factor gene containing four alternative promoters. Biochim Biophys Acta. 2000;1491(1-3):205-19.
13. Takeda K, Yasumoto KI, Kawaguchi N, Udono T, Watanabe KI, Saito H, et al. Mitf-D, a newly identified isoform, expressed in the retinal pigment epithelium and monocyte-lineage cells affected by Mitf mutations. Biochim Biophys Acta. 2002;1574(1):15-23.
14. Oboki K, Morii E, Kataoka TR, Jippo T, Kitamura Y. Isoforms of mi transcription factor preferentially expressed in cultured mast cells of mice. Biochem Biophys Res Commun. 2002;290(4):1250-4.
15. Takemoto CM, Yoon Y, Fisher DE. The identification and functional characterization of a novel mast cell isoform of the microphthalmia-associated transcription factor. J Biol Chem. 2002;277(33):30244-52.
16. Hershey CL, Fisher DE. Genomic analysis of the Microphthalmia locus and identification of the MITF-J/Mitf-J isoform. Gene. 2005;347(1):73-82.
17. Hemesath TJ, Steingrímsson E, McGill G, Hansen MJ, Vaught J, Hodgkinson CA, et al. microphthalmia, a critical factor in melanocyte development, defines a discrete transcription factor family. Genes Dev. 1994;8(22):2770-80.
18. Yasumoto K, Amae S, Udono T, Fuse N, Takeda K, Shibahara S. A big gene linked to small eyes encodes multiple Mitf isoforms: many promoters make light work. Pigment Cell Res. 1998;11(6):329-36.
19. Rehli M, Den Elzen N, Cassady AI, Ostrowski MC, Hume DA. Cloning and characterization of the murine genes for bHLH-ZIP transcription factors TFEC and TFEB reveal a common gene organization for all MiT subfamily members. Genomics. 1999;56(1):111-20.
20. Shibahara S, Yasumoto K, Amae S, Udono T, Watanabe K, Saito H, et al. Regulation of pigment cell-specific gene expression by MITF. Pigment Cell Res. 2000;13 Suppl 8:98-102.
21. Shibahara S, Takeda K, Yasumoto K, Udono T, Watanabe K, Saito H, et al. Microphthalmia-associated transcription factor (MITF): multiplicity in structure, function, and regulation. J Invest Dermatol Symp Proc. 2001;6(1):99-104.
22. Bismuth K, Maric D, Arnheiter H. MITF and cell proliferation: the role of alternative splice forms. Pigment Cell Res. 2005;18(5):349-59.
23. Primot A, Mogha A, Corre S, Roberts K, Debbache J, Adamski H, et al. ERK-regulated differential expression of the Mitf 6a/b splicing isoforms in melanoma. Pigment Cell Melanoma Res. 2010;23(1):93-102.
24. Vachtenheim J, Novotná H. Expression of genes for microphthalmia isoforms, Pax3 and MSG1, in human melanomas. Cell Mol Biol (Noisy-le-grand). 1999;45(7):1075-82.
25. Selzer E, Wacheck V, Lucas T, Heere-Ress E, Wu M, Weilbaecher KN, et al. The melanocyte-specific isoform of the microphthalmia transcription factor affects the phenotype of human melanoma. Cancer Res. 2002;62(7):2098-103.
26. Fuse N, Yasumoto K, Suzuki H, Takahashi K, Shibahara S. Identification of a melanocyte-type promoter of the microphthalmia-associated transcription factor gene. Biochem Biophys Res Commun. 1996;219(3):702-7.
27. Wang Y, Radfar S, Liu S, Riker AI, Khong HT. Mitf-Mdel, a novel melanocyte/melanoma-specific isoform of microphthalmia-associated transcription factor-M, as a candidate biomarker for melanoma. BMC Med. 2010;8(1):14.
28. Opdecamp K, Vanvooren P, Rivière M, Arnheiter H, Motta R, Szpirer J, et al. The rat microphthalmia-associated transcription factor gene (Mitf) maps at 4q34-q41 and is mutated in the mib rats. Mamm Genome. 1998;9(8):617-21.
29. Mochii M, Mazaki Y, Mizuno N, Hayashi H, Eguchi G. Role of Mitf in differentiation and transdifferentiation of chicken pigmented epithelial cell. Dev Biol. 1998;193(1):47-62.
30. Hodgkinson CA, Nakayama A, Li H, Swenson LB, Opdecamp K, Asher JH, et al. Mutation at the anophthalmic white locus in Syrian hamsters: haploinsufficiency in the Mitf gene mimics human Waardenburg syndrome type 2. Hum Mol Genet. 1998;7(4):703-8.
31. Mochii M, Ono T, Matsubara Y, Eguchi G. Spontaneous transdifferentiation of quail pigmented epithelial cell is accompanied by a mutation in the Mitf gene. Dev Biol. 1998;196(2):145-59.
32. Lister JA, Robertson CP, Lepage T, Johnson SL, Raible DW. nacre encodes a zebrafish microphthalmia-related protein that regulates neural-crest-derived pigment cell fate. Development. 1999;126(17):3757-67.
33. Steingrímsson E, Copeland NG, Jenkins NA. Melanocytes and the microphthalmia transcription factor network. Annu Rev Genet. 2004;38:365-411.
34. Bentley NJ, Eisen T, Goding CR. Melanocyte-specific expression of the human tyrosinase promoter: activation by the microphthalmia gene

product and role of the initiator. Mol Cell Biol. 1994;14(12):7996-8006.

35. Yasumoto K, Mahalingam H, Suzuki H, Yoshizawa M, Yokoyama K. Transcriptional activation of the melanocyte-specific genes by the human homolog of the mouse Microphthalmia protein. J Biochem. 1995;118(5):874-81.
36. Bertolotto C, Abbe P, Hemesath TJ, Bille K, Fisher DE, Ortonne JP, et al. Microphthalmia gene product as a signal transducer in cAMP-induced differentiation of melanocytes. J Cell Biol. 1998;142(3):827-35.
37. Aksan I, Goding CR. Targeting the microphthalmia basic helix-loop-helix-leucine zipper transcription factor to a subset of E-box elements in vitro and in vivo. Mol Cell Biol. 1998;18(12):6930-8.
38. Cheli Y, Ohanna M, Ballotti R, Bertolotto C. Fifteen-year quest for microphthalmia-associated transcription factor target genes. Pigment Cell Melanoma Res. 2010;23(1):27-40.
39. Bertolotto C, Buscà R, Abbe P, Bille K, Aberdam E, Ortonne JP, et al. Different cis-acting elements are involved in the regulation of TRP1 and TRP2 promoter activities by cyclic AMP: pivotal role of M boxes (GTCATGTGCT) and of microphthalmia. Mol Cell Biol. 1998;18(2):694-702.
40. Ludwig A, Rehberg S, Wegner M. Melanocyte-specific expression of dopachrome tautomerase is dependent on synergistic gene activation by the Sox10 and Mitf transcription factors. FEBS Lett. 2004;556(1-3):236-44.
41. Lang D, Lu MM, Huang L, Engleka KA, Zhang M, Chu EY, et al. Pax3 functions at a nodal point in melanocyte stem cell differentiation. Nature. 2005;433(7028):884-7.
42. Du J, Miller AJ, Widlund HR, Horstmann MA, Ramaswamy S, Fisher DE. MLANA/MART1 and SILV/PMEL17/GP100 are transcriptionally regulated by MITF in melanocytes and melanoma. Am J Pathol. 2003;163(1):333-43.
43. Vetrini F, Auricchio A, Du J, Angeletti B, Fisher DE, Ballabio A, et al. The microphthalmia transcription factor (Mitf) controls expression of the ocular albinism type 1 gene: link between melanin synthesis and melanosome biogenesis. Mol Cell Biol. 2004;24(15):6550-59.
44. Chiaverini C, Beuret L, Flori E, Busca R, Abbe P, Bille K, et al. Microphthalmia-associated transcription factor regulates RAB27A gene expression and controls melanosome transport. J Biol Chem. 2008;283(18):12635-42.
45. Vachtenheim J, Borovanský J. "Transcription physiology" of pigment formation in melanocytes: central role of MITF. Exp Dermatol. 2010;19(7):617-27.
46. Aoki H, Moro O. Involvement of microphthalmia-associated transcription factor (MITF) in expression of human melanocortin-1 receptor (MC1R). Life Sci. 2002;71(18):2171-9.
47. Tachibana M, Takeda K, Nobukuni Y, Urabe K, Long JE, Meyers KA, et al. Ectopic expression of MITF, a gene for Waardenburg syndrome type 2, converts fibroblasts to cells with melanocyte characteristics. Nat Genet. 1996;14(1):50-4.
48. Béjar J, Hong Y, Schartl M. Mitf expression is sufficient to direct differentiation of medaka blastula derived stem cells to melanocytes. Development. 2003;130(26):6545-53.
49. Bharti K, Liu W, Csermely T, Bertuzzi S, Arnheiter H. Alternative promoter use in eye development: the complex role and regulation of the transcription factor MITF. Development. 2008;135(6):1169-78.
50. McGill GG, Horstmann M, Widlund HR, Du J, Motyckova G, Nishimura EK, et al. Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. Cell. 2002;109(6):707-18.
51. Dynek JN, Chan SM, Liu J, Zha J, Fairbrother WJ, Vucic D. Microphthalmia-associated transcription factor is a critical transcriptional regulator of melanoma inhibitor of apoptosis in melanomas. Cancer Res. 2008;68(9):3124-32.
52. McGill GG, Haq R, Nishimura EK, Fisher DE. c-Met expression is regulated by Mitf in the melanocyte lineage. J Biol Chem. 2006;281(15):10365-73.
53. Beuret L, Flori E, Denoyelle C, Bille K, Busca R, Picardo M, et al. Up-regulation of MET expression by alpha-melanocyte-stimulating hormone and MITF allows hepatocyte growth factor to protect melanocytes and melanoma cells from apoptosis. J Biol Chem. 2007;282(19):14140-7.
54. Du J, Widlund HR, Horstmann MA, Ramaswamy S, Ross K, Huber WE, et al. Critical role of CDK2 for melanoma growth linked to its melanocyte-specific transcriptional regulation by MITF. Cancer Cell. 2004;6(6):565-76.
55. Carreira S, Liu B, Goding CR. The gene encoding the T-box factor Tbx2 is a target for the microphthalmia-associated transcription factor in melanocytes. J Biol Chem. 2000;275(29):21920-7.
56. Prince S, Carreira S, Vance KW, Abrahams A, Goding CR. Tbx2 directly represses the expression of the p21(WAF1) cyclin-dependent kinase inhibitor. Cancer Res. 2004;64(5):1669-74.
57. Vance KW, Carreira S, Brosch G, Goding CR. Tbx2 is overexpressed and plays an important role in maintaining proliferation and suppression of senescence in melanomas. Cancer Res. 2005;65(6):2260-8.

58. Carreira S, Goodall J, Denat L, Rodriguez M, Nuciforo P, Hoek KS, et al. Mitf regulation of Dia1 controls melanoma proliferation and invasiveness. Genes Dev. 2006;20(24):3426-39.
59. Carreira S, Goodall J, Aksan I, La Rocca SA, Galibert M, Denat L, et al. Mitf cooperates with Rb1 and activates p21Cip1 expression to regulate cell cycle progression. Nature. 2005;433(7027):764-9.
60. Loercher AE, Tank EMH, Delston RB, Harbour JW. MITF links differentiation with cell cycle arrest in melanocytes by transcriptional activation of INK4A. J Cell Biol. 2005;168(1):35-40.
61. Kido K, Sumimoto H, Asada S, Okada SM, Yaguchi T, Kawamura N, et al. Simultaneous suppression of MITF and BRAF V600E enhanced inhibition of melanoma cell proliferation. Cancer Sci. 2009;100(10):1863-9.
62. Liu F, Fu Y, Meyskens FL. MiTF regulates cellular response to reactive oxygen species through transcriptional regulation of APE-1/Ref-1. J Invest Dermatol. 2009;129(2):422-31.
63. Buscà R, Berra E, Gaggioli C, Khaled M, Bille K, Marchetti B, et al. Hypoxia-inducible factor 1{alpha} is a new target of microphthalmia-associated transcription factor (MITF) in melanoma cells. J Cell Biol. 2005;170(1):49-59.
64. Sato-Jin K, Nishimura EK, Akasaka E, Huber W, Nakano H, Miller A, et al. Epistatic connections between microphthalmia-associated transcription factor and endothelin signaling in Waardenburg syndrome and other pigmentary disorders. FASEB J. 2008;22(4):1155-68.
65. Miller AJ, Du J, Rowan S, Hershey CL, Widlund HR, Fisher DE. Transcriptional regulation of the melanoma prognostic marker melastatin (TRPM1) by MITF in melanocytes and melanoma. Cancer Res. 2004;64(2):509-16.
66. Loftus SK, Antonellis A, Matera I, Renaud G, Baxter LL, Reid D, et al. Gpnmb is a melanoblast-expressed, MITF-dependent gene. Pigment Cell Melanoma Res. 2009;22(1):99-110.
67. Sánchez-Martín M, Rodríguez-García A, Pérez-Losada J, Sagrera A, Read AP, Sánchez-García I. SLUG (SNAI2) deletions in patients with Waardenburg disease. Hum Mol Genet. 2002;11(25):3231-36.
68. Kawaguchi N, Noda M. Mitf is expressed in osteoclast progenitors in vitro. Exp Cell Res. 2000;260(2):284-91.
69. Mansky KC, Marfatia K, Purdom GH, Luchin A, Hume DA, Ostrowski MC. The microphthalmia transcription factor (MITF) contains two N-terminal domains required for transactivation of osteoclast target promoters and rescue of mi mutant osteoclasts. J Leukoc Biol. 2002;71(2):295-303.
70. Meadows NA, Sharma SM, Faulkner GJ, Ostrowski MC, Hume DA, Cassady AI. The expression of Clcn7 and Ostm1 in osteoclasts is coregulated by microphthalmia ranscription factor. J Biol Chem. 2007;282(3):1891-904.
71. Kitamura Y, Morii E, Jippo T, Ito A. Effect of MITF on mast cell differentiation. Mol Immunol. 2002;38(16-18):1173-6.
72. Kitamura Y, Oboki K, Ito A. Molecular mechanisms of mast cell development. Immunol Allergy Clin North Am. 2006;26(3):387-405.
73. Takeda K, Yasumoto K, Takada R, Takada S, Watanabe K, Udono T, et al. Induction of melanocyte-specific microphthalmia-associated transcription factor by Wnt-3a. J Biol Chem. 2000;275(19): 14013-6.
74. Dorsky RI, Moon RT, Raible DW. Control of neural crest cell fate by the Wnt signalling pathway. Nature. 1998;396(6709):370-3.
75. Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer. 2008;8(5):387-98.
76. Saito H, Yasumoto K, Takeda K, Takahashi K, Fukuzaki A, Orikasa S, et al. Melanocyte-specific microphthalmia-associated transcription factor isoform activates its own gene promoter through physical interaction with lymphoid-enhancing factor 1. J Biol Chem. 2002;277(32):28787-94.
77. Yasumoto K, Takeda K, Saito H, Watanabe K, Takahashi K, Shibahara S. Microphthalmia-associated transcription factor interacts with LEF-1, a mediator of Wnt signaling. EMBO J. 2002;21(11):2703-14.
78. Yamaguchi Y, Passeron T, Watabe H, Yasumoto K, Rouzaud F, Hoashi T, et al. The effects of Dickkopf 1 on gene expression and Wnt signaling by melanocytes: mechanisms underlying its suppression of melanocyte function and proliferation. J Invest Dermatol. 2007;127(5):1217-25.
79. Price ER, Horstmann MA, Wells AG, Weilbaecher KN, Takemoto CM, Landis MW, et al. alpha-Melanocyte-stimulating hormone signaling regulates expression of microphthalmia, a gene deficient in Waardenburg syndrome. J Biol Chem. 1998;273(49):33042-7.
80. Watanabe A, Takeda K, Ploplis B, Tachibana M. Epistatic relationship between Waardenburg syndrome genes MITF and PAX3. Nat Genet. 1998;18(3):283-6.
81. Bondurand N, Pingault V, Goerich DE, Lemort N, Sock E, Le Caignec C, et al. Interaction among SOX10, PAX3 and MITF, three genes altered

in Waardenburg syndrome. Hum Mol Genet. 2000;9(13):1907-17.

82. Potterf SB, Furumura M, Dunn KJ, Arnheiter H, Pavan WJ. Transcription factor hierarchy in Waardenburg syndrome: regulation of MITF expression by SOX10 and PAX3. Hum Genet. 2000;107(1):1-6.
83. Lee M, Goodall J, Verastegui C, Ballotti R, Goding CR. Direct regulation of the Microphthalmia promoter by Sox10 links Waardenburg-Shah syndrome (WS4)-associated hypopigmentation and deafness to WS2. J Biol Chem. 2000;275(48):37978-83.
84. Verastegui C, Bille K, Ortonne JP, Ballotti R. Regulation of the microphthalmia-associated transcription factor gene by the Waardenburg syndrome type 4 gene, SOX10. J Biol Chem. 2000;275(40):30757-60.
85. Tassabehji M, Newton VE, Liu XZ, Brady A, Donnai D, Krajewska-Walasek M, et al. The mutational spectrum in Waardenburg syndrome. Hum Mol Genet. 1995;4(11):2131-7.
86. Pingault V, Bondurand N, Kuhlbrodt K, Goerich DE, Préhu MO, Puliti A, et al. SOX10 mutations in patients with Waardenburg-Hirschsprung disease. Nat Genet. 1998;18(2):171-3.
87. Huber WE, Price ER, Widlund HR, Du J, Davis IJ, Wegner M, et al. A tissue-restricted cAMP transcriptional response: SOX10 modulates alpha-melanocyte-stimulating hormone-triggered expression of microphthalmia-associated transcription factor in melanocytes. J Biol Chem. 2003;278(46):45224-30.
88. Grabacka M, Placha W, Urbanska K, Laidler P, Płonka PM, Reiss K. PPAR γ regulates MITF and β-catenin expression and promotes a differentiated phenotype in mouse melanoma S91. Pigment Cell Melanoma Res. 2008;21(3):388-96.
89. Jacquemin P, Lannoy VJ, O'Sullivan J, Read A, Lemaigre FP, Rousseau GG. The transcription factor onecut-2 controls the microphthalmia-associated transcription factor gene. Biochem Biophys Res Commun. 2001;285(5):1200-590.
90. Kholmanskikh O, van Baren N, Brasseur F, Ottaviani S, Vanacker J, Arts N, et al. Interleukins 1alpha and 1beta secreted by some melanoma cell lines strongly reduce expression of MITF-M and melanocyte differentiation antigens. Int J Cancer. 2010;127(7):1625-36.
91. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417(6892):949-54.
92. Wellbrock C, Marais R. Elevated expression of MITF counteracts B-RAF-stimulated melanocyte and melanoma cell proliferation. J Cell Biol. 2005;170(5):703-8.
93. Wellbrock C, Rana S, Paterson H, Pickersgill H, Brummelkamp T, Marais R. Oncogenic BRAF regulates melanoma proliferation through the lineage specific factor MITF. PLoS ONE. 2008;3(7):e2734.
94. Hemesath TJ, Price ER, Takemoto C, Badalian T, Fisher DE. MAP kinase links the transcription factor Microphthalmia to c-Kit signalling in melanocytes. Nature. 1998;391(6664):298-301.
95. Wu M, Hemesath TJ, Takemoto CM, Horstmann MA, Wells AG, Price ER, et al. c-Kit triggers dual phosphorylations, which couple activation and degradation of the essential melanocyte factor Mi. Genes Dev. 2000;14(3):301-12.
96. Price ER, Ding HF, Badalian T, Bhattacharya S, Takemoto C, Yao TP, et al. Lineage-specific signaling in melanocytes. C-kit stimulation recruits p300/CBP to microphthalmia. J. Biol. Chem. 1998;273(29):17983-6.
97. Takeda K, Takemoto C, Kobayashi I, Watanabe A, Nobukuni Y, Fisher DE, et al. Ser298 of MITF, a mutation site in Waardenburg syndrome type 2, is a phosphorylation site with functional significance. Hum Mol Genet. 2000;9(1):125-32.
98. Mansky KC, Sankar U, Han J, Ostrowski MC. Microphthalmia transcription factor is a target of the p38 MAPK pathway in response to receptor activator of NF-kappa B ligand signaling. J Biol Chem. 2002;277(13):11077-83.
99. Miller AJ, Levy C, Davis IJ, Razin E, Fisher DE. Sumoylation of MITF and its related family members TFE3 and TFEB. J Biol Chem. 2005;280(1):146-55.
100. Larribere L, Hilmi C, Khaled M, Gaggioli C, Bille K, Auberger P, et al. The cleavage of microphthalmia-associated transcription factor, MITF, by caspases plays an essential role in melanocyte and melanoma cell apoptosis. Genes Dev. 2005;19(17):1980-5.
101. Bemis LT, Chen R, Amato CM, Classen EH, Robinson SE, Coffey DG, et al. MicroRNA-137 targets microphthalmia-associated transcription factor in melanoma cell lines. Cancer Res. 2008;68(5):1362-8.
102. Haflidadóttir BS, Bergsteinsdóttir K, Praetorius C, Steingrímsson E. miR-148 regulates Mitf in melanoma cells. PLoS ONE. 2010;5(7):e11574.
103. Segura MF, Hanniford D, Menendez S, Reavie L, Zou X, Alvarez-Diaz S, et al. Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. Proc Natl Acad Sci U S A. 2009;106(6):1814-9.

104. Sato S, Roberts K, Gambino G, Cook A, Kouzarides T, Goding CR. CBP/p300 as a co-factor for the Microphthalmia transcription factor. Oncogene. 1997;14(25):3083-92.
105. Schwahn DJ, Timchenko NA, Shibahara S, Medrano EE. Dynamic regulation of the human dopachrome tautomerase promoter by MITF, ER-alpha and chromatin remodelers during proliferation and senescence of human melanocytes. Pigment Cell Res. 2005;18(3):203-13.
106. Schepsky A, Bruser K, Gunnarsson GJ, Goodall J, Hallsson JH, Goding CR, et al. The microphthalmia-associated transcription factor Mitf interacts with beta-catenin to determine target gene expression. Mol Cell Biol. 2006;26(23):8914-27.
107. de la Serna IL, Ohkawa Y, Higashi C, Dutta C, Osias J, Kommajosyula N, et al. The microphthalmia-associated transcription factor requires SWI/SNF enzymes to activate melanocyte-specific genes. J Biol Chem. 2006;281(29):20233-41.
108. Cronin JC, Wunderlich J, Loftus SK, Prickett TD, Wei X, Ridd K, et al. Frequent mutations in the MITF pathway in melanoma. Pigment Cell Melanoma Res. 2009;22(4):435-44.
109. Garraway LA, Widlund HR, Rubin MA, Getz G, Berger AJ, Ramaswamy S, et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. Nature. 2005;436(7047):117-22.
110. Ugurel S, Houben R, Schrama D, Voigt H, Zapatka M, Schadendorf D, et al. Microphthalmia-associated transcription factor gene amplification in metastatic melanoma is a prognostic marker for patient survival, but not a predictive marker for chemosensitivity and chemotherapy response. Clin Cancer Res. 2007;13(21):6344-50.
111. Garraway LA, Sellers WR. Lineage dependency and lineage-survival oncogenes in human cancer. Nat Rev Cancer. 2006;6(8):593-602.
112. Bennett DC. How to make a melanoma: what do we know of the primary clonal events? Pigment Cell Melanoma Res. 2008;21(1):27-38.
113. Lekmine F, Chang C, Sethakorn N, Dasgupta T, Salti G. Role of microphthalmia transcription factor (Mitf) in melanoma differentiation. Biochem Biophys Res Commun. 2007;354(3):830-5.
114. Zhuang L, Lee CS, Scolyer RA, McCarthy SW, Zhang XD, Thompson JF, et al. Mcl-1, Bcl-XL and Stat3 expression are associated with progression of melanoma whereas Bcl-2, AP-2 and MITF levels decrease during progression of melanoma. Mod Pathol. 2007;20(4):416-26.
115. Salti GI, Manougian T, Farolan M, Shilkaitis A, Majumdar D, Das Gupta TK. Micropthalmia transcription factor: a new prognostic marker in intermediate-thickness cutaneous malignant melanoma. Cancer Res. 2000;60(18):5012-6.
116. Miettinen M, Fernandez M, Franssila K, Gatalica Z, Lasota J, Sarlomo-Rikala M. Microphthalmia transcription factor in the immunohistochemical diagnosis of metastatic melanoma: comparison with four other melanoma markers. Am J Surg Pathol. 2001;25(2):205-11.
117. Granter SR, Weilbaecher KN, Quigley C, Fisher DE. Role for microphthalmia transcription factor in the diagnosis of metastatic malignant melanoma. Appl Immunohistochem Mol Morphol. 2002;10(1):47-51.
118. Hoek KS, Eichhoff OM, Schlegel NC, Dobbeling U, Kobert N, Schaerer L, et al. In vivo switching of human melanoma cells between proliferative and invasive states. Cancer Res. 2008;68(3):650-6.
119. Hoek KS, Schlegel NC, Brafford P, Sucker A, Ugurel S, Kumar R, et al. Metastatic potential of melanomas defined by specific gene expression profiles with no BRAF signature. Pigment Cell Res. 2006;19(4):290-302.
120. Rimm DL, Caca K, Hu G, Harrison FB, Fearon ER. Frequent nuclear/cytoplasmic localization of beta-catenin without exon 3 mutations in malignant melanoma. Am J Pathol. 1999;154(2):325-9.
121. Widlund HR, Horstmann MA, Price ER, Cui J, Lessnick SL, Wu M, et al. Beta-catenin-induced melanoma growth requires the downstream target Microphthalmia-associated transcription factor. J Cell Biol. 2002;158(6):1079-87.
122. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol. 2006;24(26):4340-6.
123. Woodman SE, Davies MA. Targeting KIT in melanoma: a paradigm of molecular medicine and targeted therapeutics. Biochem Pharmacol. 2010;80(5):568-574.
124. Zhu S, Wurdak H, Wang Y, Galkin A, Tao H, Li J, et al. A genomic screen identifies TYRO3 as a MITF regulator in melanoma. Proc Natl Acad Sci U S A. 2009;106(40):17025-30.
125. Davis IJ, Fisher DE. MiT transcription factor associated malignancies in man. Cell Cycle. 2007;6(14):1724-9.
126. Ohsie SJ, Sarantopoulos GP, Cochran AJ, Binder SW. Immunohistochemical characteristics of melanoma. J Cutan Pathol. 2008;35(5):433-44.
127. King R, Weilbaecher KN, McGill G, Cooley E, Mihm M, Fisher DE. Microphthalmia transcription factor. A sensitive and specific melanocyte marker for melanoma diagnosis. Am J Pathol. 1999;155(3):731-8.

128. King R, Googe PB, Weilbaecher KN, Mihm MC, Fisher DE. Microphthalmia transcription factor expression in cutaneous benign, malignant melanocytic, and nonmelanocytic tumors. Am J Surg Pathol. 2001;25(1):51-7.
129. Dorvault CC, Weilbaecher KN, Yee H, Fisher DE, Chiriboga LA, Xu Y, et al. Microphthalmia transcription factor: a sensitive and specific marker for malignant melanoma in cytologic specimens. Cancer. 2001;93(5):337-43.
130. Sheffield MV, Yee H, Dorvault CC, Weilbaecher KN, Eltoum IA, Siegal GP, et al. Comparison of five antibodies as markers in the diagnosis of melanoma in cytologic preparations. Am J Clin Pathol. 2002;118(6):930-6.
131. Xu X, Chu AY, Pasha TL, Elder DE, Zhang PJ. Immunoprofile of MITF, tyrosinase, melan-A, and MAGE-1 in HMB45-negative melanomas. Am J Surg Pathol. 2002;26(1):82-7.
132. Busam KJ, Iversen K, Coplan KC, Jungbluth AA. Analysis of microphthalmia transcription factor expression in normal tissues and tumors, and comparison of its expression with S-100 protein, gp100, and tyrosinase in desmoplastic malignant melanoma. Am J Surg Pathol. 2001;25(2):197-204.
133. Granter SR, Weilbaecher KN, Quigley C, Fletcher CD, Fisher DE. Microphthalmia transcription factor: not a sensitive or specific marker for the diagnosis of desmoplastic melanoma and spindle cell (non-desmoplastic) melanoma. Am J Dermatopathol. 2001;23(3):185-9.
134. O'Reilly FM, Brat DJ, McAlpine BE, Grossniklaus HE, Folpe AL, Arbiser JL. Microphthalmia transcription factor immunohistochemistry: a useful diagnostic marker in the diagnosis and detection of cutaneous melanoma, sentinel lymph node metastases, and extracutaneous melanocytic neoplasms. J Am Acad Dermatol. 2001;45(3):414-9.
135. Iwamoto S, Burrows RC, Kalina RE, George D, Boehm M, Bothwell MA, et al. Immunophenotypic differences between uveal and cutaneous melanomas. Arch Ophthalmol. 2002;120(4):466-70.
136. Li KKC, Goodall J, Goding CR, Liao S, Wang C, Lin Y, et al. The melanocyte inducing factor MITF is stably expressed in cell lines from human clear cell sarcoma. Br J Cancer. 2003;89(6):1072-8.
137. Medic S, Pearce RL, Heenan PJ, Ziman M. Molecular markers of circulating melanoma cells. Pigment Cell Res. 2007;20(2):80-91.
138. Tsao H, Nadiminti U, Sober AJ, Bigby M. A meta-analysis of reverse transcriptase-polymerase chain reaction for tyrosinase mRNA as a marker for circulating tumor cells in cutaneous melanoma. Arch Dermatol. 2001;137(3):325-30.
139. Reynolds SR, Albrecht J, Shapiro RL, Roses DF, Harris MN, Conrad A, et al. Changes in the presence of multiple markers of circulating melanoma cells correlate with clinical outcome in patients with melanoma. Clin Cancer Res. 2003;9(4):1497-1502.
140. Samija I, Lukac J, Marić-Brozić J, Kusić Z. Microphthalmia-associated transcription factor and tyrosinase as markers of melanoma cells in blood of patients with melanoma. Croat Med J. 2004;45(2):142-8.
141. Koyanagi K, O'Day SJ, Gonzalez R, Lewis K, Robinson WA, Amatruda TT, et al. Microphthalmia transcription factor as a molecular marker for circulating tumor cell detection in blood of melanoma patients. Clin Cancer Res. 2006;12(4):1137-43.
142. Samija I, Lukac J, Marić-Brozić J, Buljan M, Alajbeg I, Kovacević D, et al. Prognostic value of microphthalmia-associated transcription factor and tyrosinase as markers for circulating tumor cells detection in patients with melanoma. Melanoma Res. 2010;20(4):293-302.
143. Nakai N, Kishida T, Shin-Ya M, Imanishi J, Ueda Y, Kishimoto S, et al. Therapeutic RNA interference of malignant melanoma by electrotransfer of small interfering RNA targeting Mitf. Gene Ther. 2007;14(4):357-65.
144. Yokoyama S, Feige E, Poling LL, Levy C, Widlund HR, Khaled M, et al. Pharmacologic suppression of MITF expression via HDAC inhibitors in the melanocyte lineage. Pigment Cell Melanoma Res. 2008;21(4):457-63.