Pro-Survival Role of MITF in Melanoma

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Melanoma is a therapy-resistant skin cancer due to numerous mechanisms supporting cell survival. Although components of melanoma cytoprotective mechanisms are overexpressed in many types of tumors, some of their regulators are characteristic for melanoma. Several genes mediating pro-survival functions have been identified as direct targets of microphthalmia-associated transcription factor (MITF), a melanocyte-specific modulator also recognized as a lineage addiction oncogene in melanoma. $BRAF^{V600E}$ and other proteins deregulated in melanoma influence MITF expression and activity, or they are the partners of MITF in melanoma response to radiotherapy and chemotherapeutics. In this review, the pro-survival activity of MITF is discussed.

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INTRODUCTION

Several biological capabilities are acquired during tumor development, and many of them are integral components of most tumors (Hanahan and Weinberg, 2011). DNA damage caused by hyperproliferation and regulatory imbalances resulting from elevated levels of oncogene signaling is compensated by the increased survival capacity of cancer cells. Mechanisms responsible for the enhanced DNA repair (Curtin, 2012) and resistance to multiple apoptotic signals (Hellwig *et al.*, 2011) are common for diverse tumors. The repertoire of other strategies to limit or circumvent cell death largely depends on the type of cells from which the tumor arises.

Melanoma derives from pigment-producing melanocytes. These cells are characterized by pro-survival mechanisms that counteract damage-causing factors, including UV radiation and highly reactive intermediates of melanogenesis (Chen *et al.*, 2014; Denat *et al.*, 2014). These complex mechanisms of resisting cell death, already active in melanocytes, are further extended in melanoma cells. Upstream of anti-apoptotic effectors, a wide range of transcription factors contributes to the melanoma pro-survival phenotype. Several of these transcription factors are constitutively active in a number of tumors. Accordingly, the functional liaison between signal transducer and activator of transcription 3 and NF-κB has been observed in many types of tumors including melanoma, and this association results in the increased expression of anti-apoptotic proteins (Lee *et al.*, 2009; Hartman and Czyz, 2013).

In contrast to signal transducer and activator of transcription 3 or NF-κB, microphthalmia-associated transcription factor (MITF) is a transcription factor unique for melanocyte and melanoma development. It is termed a lineage-addiction oncogene (Garraway *et al.*, 2005); however, it is also recognized as a suppressor of melanoma invasion and metastasis (Pinner *et al.*, 2009; Levy *et al.*, 2010; Shah *et al.*, 2010; Thurber *et al.*, 2011; Cheli *et al.*, 2012; Bell *et al.*, 2014). MITF expression varies between melanoma specimens (Flaherty *et al.*, 2012), and diverse mechanisms contribute to this phenomenon. Genomic amplification of *MITF* was initially found in 15–20% of melanomas (Garraway *et al.*, 2005); however, targeted-capture deep sequencing has shown no alteration in *MITF* copy number in 49 patient-derived melanoma metastases (Harbst *et al.*, 2014). Diverse somatic mutations have been found in *MITF* (Cronin *et al.*, 2009). A SUMOylation-deficient E318K-mutated MITF has been identified as a variant present in patients with familial melanoma (Bertolotto *et al.*, 2011; Yokoyama *et al.*, 2011). Patients carrying this germline substitution, known as a medium-penetrance melanoma gene, suffer from multiple primary melanomas (Sturm *et al.*, 2014). Melanoma-specific $BRAF^{V600E}$ substantially participates in control of MITF expression and activity by maintaining a fine balance of opposing mechanisms (Levy *et al.*, 2006; Goodall *et al.*, 2008; Pinner *et al.*, 2009; Johanessen *et al.*, 2013) (Figure 1). As recently reviewed by Hartsough *et al.* (2014), the response to RAF inhibitors, e.g., vemurafenib and dabrafenib, is heterogeneous in melanoma, and the resistance mechanisms developed during treatment might affect MITF activity as well. *MITF* expression might be also affected by mutations in its transcriptional regulators, e.g., *SOX10* (Cronin *et al.*, 2009) and *ETV1* (Jane-Valbuena *et al.*, 2010), or by alterations in the composition of microRNAs negatively regulating MITF transcript (Bemis *et al.*, 2008; Segura *et al.*, 2009; Haflidadóttir *et al.*, 2010; Luo *et al.*, 2013).

To link the level of MITF with the phenotype of melanoma cells, a ''rheostat'' or ''dynamic epigenetic'' model was created (Carreira *et al.*, 2006). This model was profoundly discussed and extended (Quintana *et al.*, 2010; Bell and Levy, 2011; Giuliano *et al.*, 2011; Eccles *et al.*, 2013; Bell *et al.*,

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Abbreviations: BCL2, B-cell leukemia/lymphoma 2; BCL2A1, BCL2-related protein A1; HGF, hepatocyte growth factor; HIF1α, hypoxia-inducible factor 1α; MITF, microphthalmia-associated transcription factor; ML-IAP, melanoma inhibitor of apoptosis

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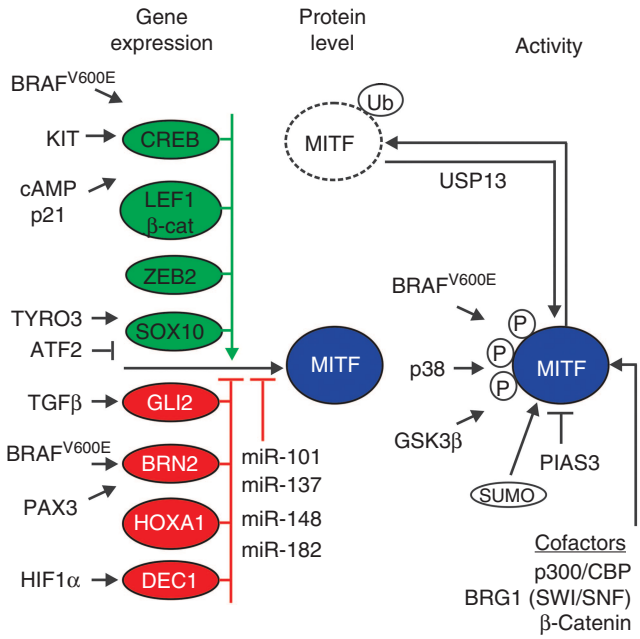

Figure 1. Overview of major mechanisms regulating MITF expression, its protein level, and activity in melanoma. A number of transcription factors promote *MITF* expression (shown in green) or inhibit its transcription (shown in red) via diverse signaling pathways. Recently identified regulators such as HOXA1 (Wardwell-Ozgo *et al.*, 2014) and ZEB2 (Denecker *et al.*, 2014) are included. MITF transcript can be targeted by several microRNAs (Bemis *et al.*, 2008; Segura *et al.*, 2009; Haflidadóttir *et al.*, 2010; Luo *et al.*, 2013). Phosphorylation and SUMOylation of MITF enhance its transcriptional activity (Levy *et al.*, 2006; Yokoyama *et al.*, 2011). BRAF^{V600E} contributes to positive and negative regulation of MITF. MITF can be also subjected to USP13-mediated deubiquitylation preventing its proteasomal degradation (Zhao *et al.*, 2011). HIF1α, hypoxia-inducible factor 1α; LEF1, lymphoid enhancer-binding factor 1; MITF, microphthalmia-associated transcription factor; TGFβ, transforming growth factor-β.

2014). Depending on the expression level and posttranslational modifications of MITF, melanoma cells can either differentiate or proliferate. High activity of MITF promotes differentiation preceded by p16- and p21-mediated G1 cell cycle arrest. Low MITF activity is attributed to the stem cell–like and invasive phenotype. Finally, prolonged MITF depletion causes melanoma cell senescence. Thus, MITF exemplifies a regulator operating in the relevant lineage of normal cells during development and differentiation, whose activity is further enhanced by genetic and epigenetic alterations generated within a tumor. Its expression is also dynamically regulated by the microenvironment, and intercellular exchange of microRNAs downregulating MITF transcript via exosomes might be involved (Xiao *et al.*, 2012; Gajos-Michniewicz *et al.*, 2014). Thus, MITF can be expressed at different levels in distinct subpopulations of the heterogeneous tumor mass. This is supported by a study showing variability in the MITF-staining intensity within clinical melanoma samples (Somasundaram *et al.*, 2012). It was suggested that switching back and forth between low and high MITF-expressing phenotypes supported melanoma cells to exhibit different cellular programs (Goding, 2011). Recently, the ''state switching'' model of melanoma progression was described as a double-negative feedback loop, where MITF and MITF-dependent microRNAs can activate one state while suppressing another (Bell *et al.*, 2014), activated by PAX3-BRN2 (Boyle *et al.*, 2011; Eccles *et al.*, 2013). Thus, cellular context and tumor microenvironment are key factors influencing MITF expression and activity, and, as a result, the phenotype of melanoma cells can be dynamically modulated (Hoek and Goding, 2010; Quintana *et al.*, 2010; Bell and Levy, 2011; Bell *et al.*, 2014). In line with this, we have shown recently that even small changes in the composition of the growth medium can significantly alter the MITF level and the phenotype of melanoma cells (Hartman *et al.*, 2014).

Although expression of several genes is regulated by MITF in melanocytes and malignant melanoma cells, this review is focused on MITF-dependent expression of proteins promoting melanoma cell survival.

PRO-SURVIVAL ROLE OF MITF IN MELANOMA

Almost 100 genes encoding proteins with diverse biological functions were identified as direct targets of MITF in melanoma (Hoek *et al.*, 2008; Widmer *et al.*, 2012). MITF is a critical transcription factor for the development of the melanocytic lineage (Opdecamp *et al.*, 1997; Levy *et al.*, 2006) and regulates genes associated with melanogenesis and cell differentiation, proliferation, and survival (Vachtenheim *et al.*, 2010; Haq and Fisher, 2011; Koludrovic and Davidson, 2013). Although pigment synthesis regulated by MITF is an important mechanism protecting the skin against UV radiation–induced damage (Choi *et al.*, 2010; Haq and Fisher, 2011), MITF promotes melanocyte and melanoma cell survival also independently of melanin synthesis.

Several genes encoding anti-apoptotic proteins are direct MITF targets (Figure 2). A microarray-based study identified *BCL2* (B-cell leukemia/lymphoma 2) as an MITF target gene in melanocytes (McGill *et al.*, 2002). A functional E-box sequence at −220 position was found in the *BCL2* promoter, and nearest-neighbor analysis confirmed a tight correlation between the expression of MITF and BCL2 in a panel of primary human melanomas (McGill *et al.*, 2002). BCL2 expression, however, is relatively low in most melanomas compared with other anti-apoptotic regulators (Placzek *et al.*, 2010). Histidine triad nucleotide-binding protein 1, which binds to the chromatin at MITF sites in the *BCL2* promoter and forms a nonfunctional complex with MITF and the transcriptional repressors, HDAC1 and mSIN3a (Genovese *et al.*, 2012), might be responsible for the diminished BCL2 level in melanoma cells compared with melanocytes. Altogether, this might explain why targeting BCL2 demonstrated poor apoptosis-inducing efficacy in melanoma (Senft *et al.*, 2012).

Another anti-apoptotic BCL2 family member, BCL2-related protein A1 (BCL2A1), was identified recently as a direct target of MITF (Haq *et al.*, 2013). Putative MITF-binding sites (E-boxes) are present in the *BCL2A1* promoter, and high-level expression of BCL2A1 is restricted to melanoma because of direct control by MITF. BCL2A1 is essential for survival in those melanomas in which *BCL2A1* or *MITF* is genomically amplified. At high levels it promotes melanomagenesis in

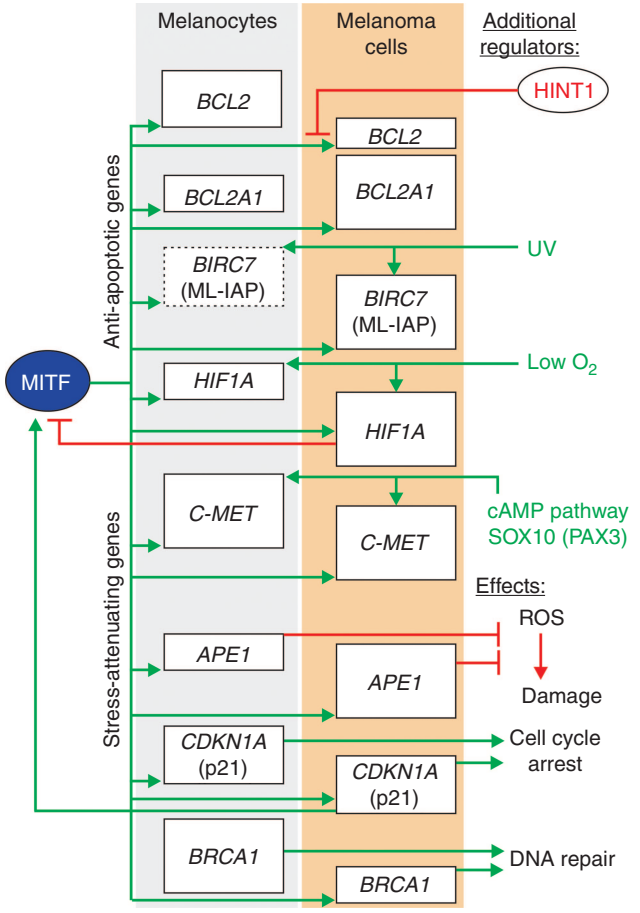

Figure 2. MITF supports survival by regulating expression of anti-apoptotic and stress-attenuating genes in melanocytes and melanoma cells. MITF target genes are expressed in melanocytes and melanoma cells at different levels (as indicated by box sizes). ML-IAP/livin is not detected in melanocytes (dashed line), but it can be expressed in response to UV radiation in an MITF-dependent manner. Additional regulators can interfere with or support the expression of MITF target genes, and p21 and HIF1α might provide a positive and a negative feedback for regulation of MITF expression, respectively. APE1, apurinic/apyrimidinic endonuclease 1; BCL2, B-cell leukemia/lymphoma 2; HIF1α, hypoxia-inducible factor 1α; HINT1, histidine triad nucleotide-binding protein 1; MITF, microphthalmia-associated transcription factor; ML-IAP, melanoma inhibitor of apoptosis; ROS, reactive oxygen species.

cooperation with $BRAF^{V600E}$ (Haq *et al.*, 2013). Interestingly, high expression of BCL2A1 is associated with worse clinical responses to BRAF inhibitors. Suppression of BCL2A1 expression in melanomas harboring amplified *BCL2A1* or *MITF* significantly enhances apoptosis induced by the BRAF inhibitor vemurafenib *in vitro* and decreases tumor volume *in vivo* (Haq *et al.*, 2013). Thus, the MITF-BCL2A1 pathway might be an intrinsic mechanism protecting melanoma cells from drug-induced death, and BCL2A1 might constitute a melanoma-specific therapeutic target. In this respect, an appropriate *in vitro* model for drug testing has to be carefully chosen. We have recently evidenced that multicellular anchorage-independent melanospheres better mirror the original tumors regarding BCL2A1 and MITF expression than do monolayer cultures (Hartman *et al.*, 2014).

Melanoma inhibitor of apoptosis (ML-IAP; livin) can inhibit both extrinsic and intrinsic apoptotic pathways by the interaction with caspases (Vucic *et al.*, 2000). It is encoded by *BIRC7*, which contains two functional consensus MITF-binding sites (−49 and −290). The transcript levels of MITF and ML-IAP correlate in melanoma samples. The small interfering RNA–mediated knockdown of MITF in melanoma cells led to a substantial decrease in ML-IAP expression (Dynek *et al.*, 2008). ML-IAP is not detected in melanocytes (Saladi *et al.*, 2013), whereas in melanoma its expression correlates with diminished patient survival (Lazar *et al.*, 2012). Recently, the melanoma-specific mechanism underlying MITF-mediated ML-IAP/livin expression in response to UV radiation was demonstrated (Saladi *et al.*, 2013). BRG1, a catalytic subunit of the chromatin-remodeling complex SWI/SNF, cooperates with MITF to promote a transcriptionally permissive chromatin structure on the *BIRC7* promoter. As an earlier report demonstrated that BRG1 acted as a cofactor of MITF in promoting cell differentiation (Keenen *et al.*, 2010), a study by Saladi *et al.* (2013) extended its role for pro-survival activity. However, an MITF-independent pro-survival role of the BRG1-containing SWI/SNF complex in melanoma was also delineated (Ondrusova *et al.*, 2013).

Other MITF targets, *HIF1A* and *MET*, involved in melanoma cell survival are regulated by the cAMP pathway. MITF overexpression is sufficient to increase hypoxia-inducible factor 1α (HIF1α). Two motifs for MITF binding are present in the *HIF1A* promoter. Increased levels of MITF and HIF1α are observed in melanoma cells exposed to the cAMP-elevating agent forskolin (Busca *et al.*, 2005). Forskolin-dependent HIF1α expression protects melanoma cells from staurosporine-induced apoptosis; thus, HIF1α exerts a pro-survival function (Busca *et al.*, 2005). Although HIF1α is rapidly degraded under normoxic conditions, the analysis of *HIF1A* expression in melanoma cell lines representing different tumor stages confirmed its expression also in normoxia. A splice variant lacking a moiety promoting oxygen-dependent HIF1α degradation was detected in some metastatic cell lines (Mills *et al.*, 2009). Interestingly, MITF downregulation is mediated by HIF1, through recruitment of the HIF1-inducible differentially expressed in chondrocytes protein 1 to the *MITF* promoter (Feige *et al.*, 2011). This conclusion is supported by diminished MITF-dependent expression of differentiation marker Melan-A/MART-1 after HIF1α induction (Widmer *et al.*, 2013).

The cAMP/MITF pathway also contributes to the regulation of c-MET expression. The *MET* promoter contains three E-box sequences; however, only one mediates the transactivating effect of MITF (Beuret *et al.*, 2007). In response to forskolin or α-melanocyte-stimulating hormone, which is substantially increased by UV radiation, MITF-dependent c-MET expression is observed, both in melanoma cells and melanocytes. *MET* transcription can also be regulated by other factors. One of them, SOX10, mediates transcription of *MET* but only synergistically with either MITF or PAX3 (Mascarenhas *et al.*, 2010). c-MET is also regulated by its ligand, hepatocyte growth factor (HGF; McGill *et al.*, 2006). Upregulation of c-MET allows HGF to protect melanocytes and melanoma

cells from apoptosis. HGF derived from stromal cells in the tumor microenvironment might be of special importance for the pro-survival effects (Kankuri *et al.*, 2005) and c-MET-mediated resistance to RAF inhibitors (Straussman *et al.*, 2012). In melanoma patients, high serum HGF levels before vemurafenib treatment is predictive of reduced overall survival (Wilson *et al.*, 2012). The anti-apoptotic signals maintained by HGF/c-MET signaling involve the activation of the extracellular signal–regulated kinase and phosphatidylinositol 3 kinase/AKT pathways (Xiao *et al.*, 2001; Chattopadhyay *et al.*, 2014). In melanoma cells pretreated with forskolin, the HGF/c-MET axis protects cells from staurosporine-induced apoptosis (Beuret *et al.*, 2007). c-MET can also inhibit cell death independently of HGF by sequestering the Fas receptor, thus preventing the binding of Fas ligand and initiation of the extrinsic pathway of apoptosis (Wang *et al.*, 2002). Accordingly, some melanoma cell lines are particularly insensitive to Fas ligand–induced apoptosis (Raisova *et al.*, 2000; Chetoui *et al.*, 2008).

MITF can evoke pro-survival effects not only by positive regulation of strictly anti-apoptotic genes but also by neutralizing death-inducing signals through diverse mechanisms (Figure 2). MITF can regulate the expression of *CDKN1A* encoding p21 (Carreira *et al.*, 2005), which is endowed with multiple activities including cell cycle inhibition and pro-survival protection (Abbas and Dutta, 2009). p21 was also identified as a CRE-binding protein cofactor promoting MITF expression in melanoma (Sestakova *et al.*, 2010). This mutual regulation reinforcing MITF expression may enhance MITF-dependent survival of melanoma cell under cellular stress. *BRCA1*, another MITF target, is engaged in the DNA repair process that highlights the cytoprotective role of MITF in response to DNA damage (Beuret *et al.*, 2011). A DNA-protecting role of MITF was evidenced by a direct regulation of apurinic/apyrimidinic endonuclease 1 expression, a crucial redox sensor. Accordingly, melanoma cells with higher levels of MITF are less vulnerable to ROS (reactive oxygen species) (Liu *et al.*, 2009).

The cooperation between MITF and other key regulators of melanoma cell maintenance highlights the complex mechanisms of the MITF-dependent support for melanoma cell survival. β-Catenin, through MITF, promotes melanoma cell growth and survival, and MITF rescues these processes in β-catenin-depleted cells (Widlund *et al.*, 2002). MITF can function as a target, as well as a nuclear effector of Wnt signaling, because of its interaction with lymphoid enhancer-binding factor 1 (Yasumoto *et al.*, 2002; Eichhoff *et al.*, 2011). The direct interaction between β-catenin and MITF can result in the redistribution of β-catenin from LEF1 target genes to MITF target genes; thus, MITF can use β-catenin as its own cofactor in melanoma (Schepsky *et al.*, 2006).

The role of anti-apoptotic MITF targets in apoptosis-unrelated processes makes the contribution of MITF to the melanoma cell phenotype even more complicated. BCL2 promotes angiogenesis through the signal transducer and activator of transcription 3–mediated (Kaneko *et al.*, 2007) or HIF1-mediated upregulation of vascular endothelial growth factor (Trisciuoglio *et al.*, 2011). This cooperation between two MITF targets involves heat-shock protein 90–dependent stabilization of HIF1 by BCL2 under hypoxic conditions (Trisciuoglio *et al.*, 2010). ML-IAP/livin inhibits cell migration by direct repression of CRAF in melanomas bearing mutated *NRAS* (Oberoi-Khanuja *et al.*, 2012). An extremely high capacity to disseminate throughout the body is another way of supporting melanoma cell survival (Braeuer *et al.*, 2014). In this respect, MITF contributes to melanoma progression by increasing the expression of proteins such as endothelin receptor B, HIF1α, and cyclin-dependent kinase 2 (Mobley *et al.*, 2012).

Although MITF is generally associated with pro-survival functions in melanocytes and melanoma cells, its apoptosis-promoting properties were also demonstrated (Larribere *et al.*, 2005). During TRAIL (tumor necrosis factor–related apoptosis-inducing ligand)-induced apoptosis, MITF is a substrate for caspase-mediated cleavage, resulting in the generation of a small C-terminal moiety with the pro-apoptotic activity. Cells transfected with a non-cleavable but transcriptionally active MITF mutant exerted higher resistance to TRAIL-induced apoptosis. Interestingly, such proteolytically generated pro-apoptotic moieties from pro-survival molecules were also identified for MITF targets (Del Bello *et al.*, 2001; Nachmias *et al.*, 2003; Kucharczak *et al.*, 2005; Yan *et al.*, 2006; Lefebvre *et al.*, 2013).

FINAL CONCLUSIONS AND PERSPECTIVES

MITF is a master regulator of melanocytes, but it also constitutes one of the most pivotal contributors to the melanoma cell phenotype. MITF can operate within a wide range of activity levels, resulting in the promotion of strikingly extreme phenotypes. This is supported by the analysis of the biological functions of MITF target genes that operate within mutually exclusive processes. These targets involve, e.g., cyclin-dependent kinase 2-promoting proliferation versus p16 and p21 responsible for cell cycle inhibition (Yajima *et al.*, 2011). MITF and MITF-dependent miR-211 block the melanoma invasion program (Bell *et al.*, 2014). MITF exerts pro-survival functions in melanoma cells exposed to radio- and chemotherapy. MITF at high level protects melanoma cells from MEK inhibitor cytotoxicity (Smith *et al.*, 2013), and MITF activity has been linked to the resistance to MAPK pathway inhibition (Van Allen *et al.*, 2014), in relapsing tumors as well (Johannessen *et al.*, 2013). Thus, the reduction of MITF activity may sensitize melanoma cells to chemotherapeutics, and modalities affecting MITF can be beneficial for melanoma patients. Different strategies against anti-apoptotic MITF targets are tested (Hartman and Czyz, 2012; Mohana-Kumaran *et al.*, 2014), but the only pharmacological approach that suppresses MITF is the use of HDACi, which shows anti-melanoma efficacy both *in vitro* and in xenograft models (Yokoyama *et al.*, 2008). Two histone deacetylase inhibitors, LBH589 (panobinostat) and valproic acid (vorinostat), are currently evaluated in clinical trials (ClinicalTrials.gov). Recently, we have shown that several natural compounds affecting heterogeneous populations of melanoma cells either increased or reduced the MITF transcript level (Sztiller-Sikorska *et al.*, 2014). As MITF can be widely regulated at the transcriptional level and numerous

posttranslational modifications influence the MITF protein level and stability (Figure 1), targeting MITF-related regulatory pathways, although less specific, seems promising as well. This approach was demonstrated for the dietary flavonoid fisetin that efficiently disrupted Wnt/β-catenin signaling and reduced the MITF level, leading to the inhibition of tumor development *in vivo* (Syed *et al.*, 2011). Moreover, particular elements of MITF-dependent pathways might be employed against melanoma cells. For example, a high tyrosinase activity due to methotrexate-mediated MITF upregulation was used for the activation of the antifolate prodrug, 3-O-(3,4,5-trimethoxybenzoyl)-(-)-epicatechin (Saez-Ayala *et al.*, 2013). This combination induced apoptosis in melanoma cells both *in vitro* and *in vivo*. It should be emphasized, however, that agents increasing the MITF level may favor the pro-survival phenotype of melanoma cells. This is supported by experiments showing an upregulation of pro-survival genes in response to induced MITF overexpression (McGill *et al.*, 2002; Busca *et al.*, 2005; Beuret *et al.*, 2007; Dynek *et al.*, 2008). A most recently published study performed in the $BRAF^{V600E}$ animal model with conditionally controlled endogenous MITF activity has revealed that obliterating MITF activity in melanoma is a potent antitumor mechanism that leads to tumor regression, but low level of wild-type MITF activity is oncogenic (Lister *et al.*, 2014). The authors suggest that lowering MITF activity in $BRAF^{V600E}$ melanomas instead of its full abrogation would rather lead to the enhancement of oncogenic potential than tumor regression. Thus, even if MITF is considered as a potential element of targetome in melanoma, a direct inhibition of MITF might be a difficult therapeutic strategy (Haq and Fisher, 2013).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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