Microphthalmia-Associated Transcription Factor Controls the DNA Damage Response and a Lineage-Specific Senescence Program in Melanomas

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Abstract

Apoptosis and senescence are cellular failsafe programs that counteract excessive mitogenic signaling observed in cancer cells. Melanoma is known for its notorious resistance to apoptotic processes; therefore, senescence, which remains poorly understood in melanomas, can be viewed as a therapeutic alternative. Microphthalmia-associated transcription factor (MITF), which in its M transcript is specifically expressed in melanocyte cells, plays a critical role in melanoma proliferation, and its specific inhibition is associated with G0-G1 growth arrest. Interestingly, decreased MITF expression has been described in senescent melanocytes, and we have observed an inhibition of MITF expression in melanoma cells exposed to chemotherapeutic drugs that induce their senescence. All these observations thereby question the role of MITF in controlling senescence in melanoma cells. Here, we report that long-term depletion of MITF in melanoma cells triggers a senescence program characterized by typical morphologic and biochemical changes associated with a sustained growth arrest. Further, we show that MITF-silenced cells engage a DNA damage response (DDR) signaling pathway, leading to p53 upregulation, which is critically required for senescence entry. This study uncovers the existence of a lineage-restricted DDR/p53 signaling pathway that is inhibited by MITF to prevent senescence and favor melanoma cell proliferation. Cancer Res; 70(9): 3813–22. ©2010 AACR.

Introduction

The M isoform of microphthalmia-associated transcription factor (MITF), which is specifically expressed in the melanocyte lineage, is critical for melanocyte homeostasis and melanoma development. Several proteins that contribute to cell migration/invasion (DIA1 and c-MET), survival (BCL2, HIF1a, c-MET, and ML-IAP), and proliferation [cyclin-dependent kinase 2 (CDK2)] have been identified as MITF target genes (1). Further, a genetic amplification of MITF has been found in 10% to 20% of melanomas and has been associated with a decreased 5-year survival (2). Additionally, BRAF, whose gene exhibits oncogenic mutation (BRAFV600E) in >60% of advanced melanoma tumors, regulates MITF expression and activity (2). Further, BRAF cooperates with MITF to transform immortalized melanocytes (2). In line with this, MITF expression has been associated with resistance to chemotherapy and correlates with unfavorable prognosis (2, 3). These observations suggest that MITF may function as a lineage-specific "addictive oncogene" in melanoma cells.

Melanoma cells exposed to chemotherapeutic drugs reveal a reduction in MITF expression, suggesting that MITF brings important melanoma proliferative and survival advantages (4). Consistently, inhibition of MITF expression induces a G0-G1 growth arrest of melanoma cells (5–7). MITF has been also involved in the control of melanoma survival through the control of BCL2 or BIRC7 (8, 9), but MITF silencing only marginally causes apoptosis (8, 10). In addition to apoptosis, senescence is another cellular failsafe program that counteracts excessive mitogenic signaling observed in cancer cells. The role of MITF in the control of the senescence program has never been studied thus far. However, a decreased MITF expression has been observed in conditions that promote senescence of melanocytes (11). These observations prompted us to investigate whether MITF might control senescence of melanocytic cells.

Here, we report that long-term suppression of MITF in melanoma cells is associated with typical hallmarks of senescence, including enlarged and flattened cell morphologies, increase in cell granularity, acidic β-galactosidase staining,
change in chromatin structure associated with formation of heterochromatin foci, and cessation of proliferation. We show that the senescence program triggered by MITF silencing operates via a DNA damage response (DDR), leading to ataxia-telangiectasia mutated (ATM), H2AX, p53-binding protein 1 (53BP1), and CHK2 activation and ultimately resulting in p53 phosphorylation and stabilization. Finally, our findings show that the DDR machinery is critically required, as pharmacologic inhibition and genetic ablation of ATM, CHK2, or p53 efficiently bypass the senescence program mediated by MITF depletion.

Therefore, MITF is critically required to prevent senescence and favor melanoma cell proliferation. Our results, which uncover the existence of a melanocyte-specific senescence program, make MITF and p53 potential therapeutic targets and open new perspectives in the treatment of melanoma.

Materials and Methods

**Cell cultures.** Human 501 mel, WM9, and Skmel28 melanoma cells and mouse B16 melanoma cells were grown in DMEM supplemented with 7% FCS and 100 units/mL penicillin/50 μg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO₂.

**Transient transfection of small interfering RNA.** Briefly, a single pulse of 50 nmol/L of small interfering RNA (siRNA) was administrated to the cells at 50% confluency by transfection with 5 μL Lipofectamine RNAiMAX in Opti-MEM for the time indicated in the figure legends. Control and MITF siRNAs were previously described (12). A second MITF siRNA sequence (siMi2) described elsewhere was also used to rule out the risk of off-target effects (5). p53 siRNA was from Santa Cruz Biotechnology.

**Growth curves.** Cells (20 × 10⁴) were seeded in six-well dishes and transiently transfected the following day with control or MITF siRNA. Cells were next detached from days 2 to 6 and manually counted in triplicate with a hemocytometer to assess cell proliferation. The experiment was performed three times.

**Luciferase reporter assays.** 501 mel melanoma cells were transiently transfected as previously described using the Lipofectamine reagent (Invitrogen; ref. 13). Briefly, cells were transiently transfected with 0.3 μg of small interfering RNA (siRNA) using the Lipofectamine reagent. Two hours later, cells were transfected with 0.5 μg of pCMVβ-Gal and 2 μg of pGL3-Basic reporter plasmid. After 24 hours, cells were harvested and lysed in 1× passive lysis buffer (Promega). Luciferase activity of cell lysates was measured using a luminometer (Skanstar; Labsystems). Results are representative of at least three independent experiments.

**Western blot assays.** Western blots were carried out as previously described (14). Briefly, cell lysates (30 μg) were separated by SDS-PAGE, transferred onto a polyvinylidene difluoride membrane, and then exposed to the appropriate antibodies. Proteins were visualized with the enhanced chemiluminescence system. The Western blots shown are representative of at least three independent experiments.

**Statistical analysis.** Data are presented as the average ± SD and were analyzed by Student’s t test using Microsoft Excel software. P ≤ 0.05 was considered significant.

**Results**

**MITF suppression triggers senescence of melanoma cells.** We observed that hydroxyurea, a chemotherapeutic drug, blocked human 501 mel melanoma cell growth and led to morphologic changes that were associated with a stimulation of the SA-β-Gal activity, a widely used biomarker of cellular senescence (Fig. 1A). Noteworthy, hydroxyurea caused a dramatic reduction in the level of MITF without apoptosis induction as observed by the absence of sub-G₁ cells and caspase-3 activation (data not shown). Although hydroxyurea is able to induce senescence independently of MITF by interfering with DNA synthesis, the correlation in melanoma cells between the reduction in MITF level and the senescence phenotype prompted us to hypothesize that MITF may play an active role in controlling senescence in melanocytic cells.

Indeed, we observed that sustained MITF silencing did not promote apoptosis, as shown by the absence of sub-G₁ cells by flow cytometry and the lack of caspase-3 activation by Western blot contrary to tumor necrosis factor–related apoptosis-inducing ligand, which caused apoptosis of melanoma cells.
Figure 1. MITF depletion triggers a senescence program in melanoma cells. A, 501 mel cells were exposed to DMSO (Ct) or 150 μmol/L hydroxyurea (HU) for 96 h and then harvested for flow cytometric analysis (left) or stained for SA-β-Gal activity. Cells were visualized under phase-contrast microscopy. Middle, percentage of means and SDs of SA-β-Gal-positive cells; right, cell lysates from 501 mel cells exposed to DMSO or hydroxyurea (50 or 150 μmol/L) for 96 h were assessed by Western blot for the antibodies indicated. B, left, 501 mel cells were transfected with control (siC) or MITF (siMi) siRNA, stained for SA-β-Gal activity, and observed by phase-contrast microscopy. Percentage of means and SDs of SA-β-Gal-positive cells are indicated. Right, immunofluorescence experiments to HP1β of MITF-depleted cells versus control cells were analyzed by confocal microscopy. Cell nuclei were counterstained with DAPI. C, cells were counted in triplicates from days 2 to 6. Lysates were analyzed by Western blotting with MITF and extracellular signal-regulated kinase 2 (ERK2) antibodies. D, human WM9 and mouse B16 melanoma cells were transfected with control or MITF siRNA and stained for SA-β-Gal activity. Numbers on the panels indicate percent of means and SDs of SA-β-Gal-positive cells.

MITF Controls a DDR/p53-Dependent Senescence Program

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In agreement with our hypothesis, MITF silencing induced dramatic morphologic changes associated with a G0-G1 growth arrest and the accumulation of cells positive for SA-β-Gal staining (∼50% after 3 days and >70% after 4 days; Fig. 1B). Noteworthy, SA-β-Gal staining was observed very occasionally in 501 mel cells transfected with control siRNA (2% of cells). Quantification of the morphologic changes by flow cytometry showed a 30% to 50% enlargement of the cell size and up to a 60% increase in cell granularity, two parameters of cellular senescence (Supplementary Fig. S2). Senescence is also associated with changes in chromatin structure, and we indeed found senescence-associated heterochromatin foci (SAHF) using heterochromatin protein 1β (HP1β) labeling of melanoma cells depleted for MITF (Fig. 1B). Consistently, MITF silencing triggered long-term growth arrest of melanoma cells (Fig. 1C). To rule out the possibility that the effect of MITF silencing resulted from nonspecific cellular responses, we used a second siRNA duplex containing a different MITF targeting sequence, which showed a comparable efficiency in inhibiting MITF and in triggering senescence of melanoma cells (Supplementary Fig. S3).

The senescence phenotype caused by MITF depletion was also observed in other human melanoma cells such as WM9 and in mouse B16 cells as well as in melanoma cells freshly isolated from tumor specimens (Fig. 1D; Supplementary Fig. S4). On the other hand, MITF siRNA did not promote SA-β-Gal staining in nonmelanoma cells (data not shown).

Our findings therefore show that MITF silencing triggers a lineage-restricted growth arrest and senescence program of melanoma cells.

**MITF depletion promotes mitotic defects and causes DNA damages.** Data from the literature indicate that senescence operates in a signal- and context-dependent manner, and therefore, it remained to elucidate which pathways were engaged during the process of MITF-induced senescence in melanoma cells. We found that MITF silencing promoted an increase in p16INK4A and p27KIP1 and a decrease in CDK2, but loss-of-function experiments revealed that none of them were key mediators of the senescence program mediated by MITF invalidation (Supplementary Fig. S5).

On the other hand, careful examination of DNA using 4′,6-diamidino-2-phenylindole (DAPI) staining of nuclei showed that cells transfected with the MITF siRNA encountered...
several chromosome segregation defects with a high prevalence of lagging chromosome (anaphase chromatin forming continuous bridges connecting the two sets of separating chromosomes) compared with control cells (Fig. 2A). Noteworthy, for better contrast, DAPI staining has been depicted in green. Anaphase bridges have been associated with DNA double-strand breaks (DSB; ref. 17). Further, in mammals, histone H2AX phosphorylation (γH2AX) and 53BP1 are rapidly recruited to sites of DSB and act as key mediators in the transduction of DNA damage signals (18, 19). In line with this, immunofluorescence studies showed a weak and diffuse γH2AX staining all through the nucleus in control cells, whereas 70% of cells transfected with MITF siRNA exhibited γH2AX foci at the site of DNA damages. Western blot analysis confirmed the phosphorylation of H2AX in MITF-depleted cells (Fig. 2B). Additionally, a large number of MITF-depleted cells were positive for 53BP1 foci compared with control cells, and the number of 53BP1 foci per nucleus was more important than those found occasionally in control cells (Fig. 2C).

These observations therefore show that MITF depletion causes mitotic defects that culminate in DNA damages, most likely DNA DSBs.

Activation of the DDR signaling cascade is required for the senescence phenotypes mediated by MITF silencing. DNA damages, through activation of the DDR signaling pathway, trigger cellular senescence (20). Thus, we next wished to delineate the role of the DDR signaling cascade in the senescence program triggered by MITF suppression.

CHK2 has a fundamental role in the network of genome surveillance pathways by regulating cell cycle progression. We observed that MITF silencing promoted a time-dependent phosphorylation of CHK2 on Thr68 that is localized in discrete

![Figure 3. Inhibition of the ATM-dependent DDR cascade prevents senescence engagement.](image)
foci within the nucleus (Fig. 3A). In cells transfected with control siRNA, low level of CHK2 phosphorylation and almost no foci were observed. Noteworthy, in Western blot assay, CHK2 phosphorylation was clearly visible at 24 hours after transfection and therefore was observed before SA-β-Gal detection that appeared at 48 hours, thereby indicating that CHK2 phosphorylation was not a consequence of senescence induction by MITF silencing. Autophosphorylation at Thr387, which is required for full CHK2 activity, was also stimulated by MITF suppression and was abrogated by a cell-permeable indoloazepine that specifically inhibits CHK2 (InhChk2; Fig. 3B). The CHK2 inhibitor did not affect MITF expression. Interestingly, CHK2 inhibition dramatically impaired induction of the senescence phenotypes mediated by MITF siRNA, as shown by the blockade of SA-β-Gal staining and by the decrease of cell size and granularity (Fig. 3B; Supplementary Fig. S6A).

Figure 4. MITF silencing increases the level of transcriptionally active p53. A, fluorescence images show MITF and p53 labeling in control siRNA–transfected or MITF siRNA–transfected cells. Nuclei were counterstained with DAPI. B, left, Western blot revealed increased phosphorylation at Ser15 (pS15-p53) and increased protein levels of p53 after 36 h of MITF depletion; middle, cells were transfected with PG13-Luc, a p53-dependent firefly luciferase reporter gene, and then with control or MITF siRNA; right, supernatants from these cells were examined by Western blot for PAI-1. Inset, efficiency of siMi. C, right, luciferase assay using 501 mel cells transfected with PAI-1 promoter, pCMV-β-galactosidase, and various siRNA constructs or empty or pS3-encoding plasmid. D, ChIP was performed using 501 mel cells and specific anti-p53 antibody and mouse IgG as control. Primers spanning the PAI-1 promoter region were used for PCR amplification. A control of PCR amplification was performed on nonimmunoprecipitated extracts (Input). Another control was performed using a primer pair to the human GAPDH promoter.

Noteworthy, debromohymenialdisine, another pharmacologic CHK2 inhibitor, gave comparable results in inhibiting senescence phenotypes (Supplementary Fig. S6B).

The DDR pathway activates members of the phosphoinositide 3-kinase–related protein kinase (PIKK) family (i.e., DNA-PK, ATM, and ATR). We therefore assessed their relative involvement in the senescence program mediated by MITF depletion by using specific inhibitors, namely, KU-55993, an inhibitor of ATM (21) and NU-7026, an inhibitor of DNA-PK (22). Phosphorylation of CHK2 at Thr387 by PIKK was compromised in the presence of the pharmacologic inhibitors showing their efficiency (Fig. 3C). Noteworthy, the inhibition mediated by KU-55993 was stronger than that caused by NU-7026. Consistently, compared with the DNA-PK inhibitor, the ATM inhibitor KU-55993 severely compromised the number of senescent cells induced by MITF silencing.
Consistently, cell size and granularity returned to control levels in the presence of KU-55993, whereas NU-7026 barely affected these two parameters (Supplementary Fig. S7). Furthermore, aggregation of phosphorylated ATM (Ser<sup>1981</sup>) in discrete nuclear foci was more important in MITF-depleted cells compared with control cells (Fig. 3D). We therefore identify ATM and CHK2, two components of the DDR pathway, as key molecules in the senescence program triggered by MITF depletion.

**MITF suppression promotes p53 accumulation and activation.** Upregulation of p53 protein is also a key feature of DNA damages and a pivotal player in cellular senescence. Interestingly, MITF silencing clearly induced a nuclear accumulation of p53 (Fig. 4A). Western blot analysis confirmed the accumulation of p53. Noteworthy, activation of CHK2 and increased expression of p53 were also observed in other melanoma cells (Supplementary Fig. S8).

Consistently, an increased phosphorylation of p53 on Ser<sup>15</sup>, a site dominantly phosphorylated by ATM, was observed. Phosphorylation of p53 on Ser<sup>15</sup> is crucial for its stabilization but also stimulates p53 transcriptional activity (23, 24). We indeed observed in MITF-depleted cells an enhanced activity of a p53-responsive reporter plasmid, showing that MITF depletion induced the expression of a transcriptionally active p53 (Fig. 4B). Consistently, MITF silencing stimulated the secretion of PAI-1, a p53 target gene, which has been reported to be a key mediator of cellular senescence (25). Ponceau staining revealed equal loading of the two lanes (data not shown). Furthermore, MITF depletion increased the mRNA level of PAI-1 (data not shown) and stimulated the transcriptional activity of the PAI-1 promoter to a level comparable with that of p53 (Fig. 4C). p53 siRNA, which abolished both basal and MITF-induced p53, prevented PAI-1 transcription (Figs. 4C and 5A). ChIP experiments revealed that, in melanoma cells, p53 bound to the promoter of PAI-1, thereby indicating that MITF silencing increased, through upregulation of p53, the transcription of PAI-1 (Fig. 4D). In conclusion, stimulation of H2AX, 53BP1, CHK2, and p53, three ATM

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**Figure 5.** p53 plays a critical role in the senescence program mediated by MITF depletion. A, co-knockdown of p53 and MITF prevented p53 expression and severely compromised SA-β-Gal staining. Percentage of means and SDs of SA-β-Gal-positive cells are indicated. B, p53-deficient SKMel28 cells were transfected with two different MITF siRNAs. Cells were next analyzed by Western blot for MITF and ERK2 and for SA-β-Gal activity. C, SKMel28 cells transfected with MITF siRNAs were also counted in triplicates from days 2 to 6. D, model for MITF silencing-induced senescence.
targets, together with p53 accumulation and increased activity, reveals mobilization of the DNA damage detection machinery on MITF silencing.

**p53 is a key mediator of the senescence program mediated by MITF depletion.** We next investigated the importance of p53 and found that p53 suppression caused cells to efficiently bypass the senescence program mediated by MITF depletion (Fig. 5A). Interestingly, in p53-mutant SKMel28 melanoma cells, although the two MITF siRNAs promoted a strong reduction in the level of MITF, only a small proportion of cells became positive for SA-β-Gal staining (Fig. 5B). In these cells, MITF suppression slowed down proliferation, but these cells kept growing (Fig. 5C).

These findings thereby show that MITF silencing requires activation of the ATM-CHK2-p53 signaling cascade to reactivate a senescence growth arrest of melanoma cells (Fig. 5D).

**Discussion**

Cutaneous melanoma is a highly aggressive tumor recognized for its notorious resistance to all current therapeutic intervention. Numerous studies have ascribed this resistance to alterations in apoptotic processes (26). Less attention has been paid to senescence, a process that limits cell proliferation and oncogene-induced transformation. Alteration in senescence processes might also play a key role in melanoma development. Therefore, deciphering the mechanism of senescence regulation in melanoma could offer new therapeutic strategies for the treatment of this disease.

MITF plays a crucial role in melanocyte homeostasis and melanoma development. Therefore, several attempts have been made to further elucidate how MITF exerts its effects. Here, we show that long-term silencing of MITF in melanoma cells induces a phenotype displaying all the hallmarks of senescence, such as enlarged and flattened cell, vacuolization, SA-β-Gal activity at acidic pH, and SAHF labeled by HP1β. SAHF are subnuclear structures containing heterochromatin proteins, which result in stable repression of promoters of proliferation-associated genes (27) that might explain the robust and sustained cell cycle arrest observed in MITF-depleted melanoma cells.

MITF suppression triggers senescence-like phenotypes in several human and mouse melanoma cells of different genetic background (Supplementary Fig. S9), thereby showing that this process is not restrained to species or to a unique melanoma cell line. In normal human melanocytes, MITF silencing displays weak senescence-promoting activity (data not shown). Importantly, MITF silencing mediates senescence in melanoma cells harboring BRAFV600E, the most frequently mutated oncogene in this disease (28). Additionally, MITF silencing may also engage senescence of freshly isolated melanoma cells, two of them being BRAFV600E. Altogether, these observations highlight the physiopathologic relevance of our observations and provide a rational basis for the use of MITF suppression in the treatment of melanoma. More importantly, the control of senescence by MITF, a cell-specific transcription factor, evidences for the first time the existence of a lineage-specific cell-autonomous senescence program.

Interestingly, knockdown of the c-myc oncogene, a transcription factor of the bHLH-LZ family, such as MITF, also caused senescence of melanoma cells (29). Therefore, MITF, which was proposed to function as a melanocyte-specific oncogene, and c-myc may both function in convergent pathways controlling cellular senescence, recently referred as oncogene inactivation-induced senescence (OIIS; refs. 2, 29, 30). However, unlike MITF, the effect of c-myc depletion was not restrained to melanoma cells because it also triggered senescence in lymphoma and osteosarcoma cells (30). Further, c-myc inactivation in these different cell types did not engage the same cell cycle regulators and checkpoint proteins, thereby showing that OIIS, caused by c-myc suppression, operates in a context-dependent manner. Therefore, it was important to unveil the molecular mechanism by which MITF depletion exerted its prosenescent effects.

We also show that MITF suppression clearly promotes senescence in p16INK4a-proficient (501 mel) and p16INK4a-deficient melanoma cells (WM9 and B16) and that genetic ablation of p16INK4a in 501 mel cells does not abrogate the senescence growth arrest phenotype in agreement with previous reports (31). Additionally, CDK2 and p27Kip1, two other cell cycle regulators deregulated on MITF depletion and involved in cellular senescence (32, 33), do not seem as key mediators of MITF siRNA prosenescent effect. Noteworthy, a 25% increase in the number of senescent cells is observable in CDK2-depleted cells that could be explained by a reduced MITF expression (Supplementary Fig. S5). The decrease in MITF expression on CDK2 suppression may reflect a negative feedback loop that remains to be investigated.

On the other hand, when examining MITF-silenced cells, nuclei reveal severe chromosome segregation defects with a high prevalence of lagging chromosome at anaphase. None of the MITF target genes described thus far have been involved in this process. Therefore, our observations ascribe to MITF novel functions in correct chromosome segregation. Anaphase bridges have been associated with DNA DSBs, suggesting the presence of DNA damages (17). These observations prompted us to investigate, in response to MITF depletion, the involvement and the role of the DDR pathway that has been already implicated in the control of senescence (20, 34). In agreement with our hypothesis, we show here that the senescence program mediated by MITF silencing engages a broad DDR signaling pathway marked by activation of ATM, γH2AX, 53BP1, CHK2, and p53, culminating in p53 accumulation. Noteworthy, although we cannot rule out the existence of DNA single-strand breaks, engagement of the ATM/γH2AX/53BP1/CHK2/p53 cascade in MITF-silenced cells strongly indicates that the type of DNA damages is mainly DSB.

Pharmacologic or genetic inhibition of ATM, CHK2, or p53 severely compromises the senescence program mediated by MITF suppression, showing the requirement of the DDR cascade in this process. Notably, like ATM, DNA-PK has also been reported to phosphorylate CHK2 (35) and to promote p53 stabilization (36). However, using pharmacologic inhibition of these kinases, we show that ATM plays a more active role on senescence program.
role in the senescence program engaged by MITF depletion. In SKMel28 melanoma cells, which harbor a mutant form of p53, MITF silencing induces a modest accumulation of cells that stained positive for SA-β-Gal and does not completely block cell growth, suggesting that p53 is a key actor of the senescence program mediated by MITF silencing. This hypothesis was confirmed because siRNA to p53 prevents almost completely the SA-β-Gal staining induced on MITF depletion in p53-proficient melanoma cells.

Previous reports indicated that p53 mediated G1 cell cycle arrest through transcriptional repression of c-myc (37) and c-myc depletion promoted senescence of melanoma cells (29). However, no change in the intracellular level of c-myc is observed, ruling out the possibility that MITF depletion acts through c-myc suppression to trigger senescence (data not shown).

p21^{CIP1} is one of the most studied p53 targets facilitating the cell cycle arrest that characterizes senescence (38). Although MITF silencing causes p53 upregulation and activation, the expression of p21^{CIP1} seems rather inhibited in the different melanoma cell lines. The uncoupling between p21^{CIP1} and p53 is not unprecedented particularly in melanoma cells (39). In support of these observations, in melanoma cells, MITF is a direct transcriptional activator of p21^{CIP1}, and therefore, MITF suppression would cause an inhibition of p21^{CIP1} expression (40). Additionally, p21^{CIP1} expression is repressed at the transcriptional level by TBX2, a transcription factor of the T-box family overexpressed in melanoma cells (41). Therefore, MITF and TBX2 could be responsible for the uncoupling of p53 and p21^{CIP1} in melanoma cells. Further, p21^{CIP1} can limit the DDR (42). Therefore, the decrease in p21^{CIP1} could take part to a genetic program activated by MITF silencing that would favor activation of the DDR, upregulation of p53, and induction of senescence.

Kortlever and colleagues (25) recently showed that PAI-1 was necessary and sufficient to trigger, through p53, cellular senescence. Here, we observe a marked increase in the production of PAI-1 in MITF-silenced cells, showing the functional activation of p53. Noteworthy, our results, in melanoma cells, indicate that p53 binds to the promoter of PAI-1 and controls PAI-1 expression at the transcriptional level. Consistently, p53 has been already negatively associated with melanoma development. Indeed, PAI-1 was described to inhibit the activity of the secreted protease urokinase-type plasminogen activator, which can cause cells to progress through G1 to S phase (43). Further, inhibition of proliferation and invasive properties of melanoma cells was associated with an increased expression of PAI-1 (44).

As previously reported, we find no induction of cell death in response to MITF depletion (5, 12). The fact that increased expression of p53 observed on MITF depletion causes senescence instead of cell death may be linked to the genetic background of melanoma cells. Indeed, alteration of several molecules involved in the regulation and execution of apoptosis, such as BCL2 and APAF1, was reported (26). Further, increased expression of p27^{kip1} has been reported to exert antiapoptotic activity (45). Therefore, it is likely that the high antiapoptotic background of melanoma cells associated with the genetic program mediated by MITF depletion would favor the establishment of a senescence program rather than cell death.

Several lines of evidence indicate that p53 signaling can suppress melanoma development, as inactivation of p53 favors melanoma development induced by oncogenic NRAS or BRAF (46–48). However, unlike in other solid tumors, p53 is rarely mutated in melanomas. Therefore, it is crucial to understand how melanoma cells could escape from the growth-suppressive function of p53. According to our data, we can propose that MITF, in which its expression is conserved in 80% of melanomas, maintains a low level of p53, inactivates the senescence barrier, and allows melanoma development. This hypothesis is in complete agreement with data showing an absence of p53 staining in some melanoma biopsies and a positive correlation between p53 detection and overall survival of patients (49, 50). Therefore, our results might bring important clues to explain the enigmatic absence of p53 mutations in a vast majority of melanoma and foster the re-assessment of drugs targeting the p53 pathway in the treatment of melanoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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