Deregulated MITF sumoylation: A route to melanoma

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Metastatic melanoma is a deadly form of skin cancer. Extraordinarily breakthroughs have been recently achieved in the treatment of the disease [1], leading to objective increase in patient survival. However, early detection of potential dangerous melanocytic lesions remains the best strategy to avoid metastatic dissemination, which is still the main cause of death in melanoma patients. In 2011, our team identified a germline mutation in microphthalmia-associated transcription factor (*MITF*^{E318K}) that predisposes carriers to melanoma. Recently, we demonstrated that this mutation interfered with oncogene-induced senescence, one of the first events that should be overcome to allow melanoma development. Therefore, our works provide important clues on the early steps of melanomagenesis and as such might be useful for prevention or early therapeutic interventions in at risk-patients.

Melanomagenesis is a complex process that involves the effect of ultraviolet radiation and of oncogenic driver mutations, the most frequent being the point mutation (V600E) in the serine/threonine kinase BRAF [2]. BRAF^{V600E} alone appears insufficient in itself to result in malignant transformation. BRAF^{V600E}, is found in 80% of acquired benign melanocytic nevi, thought to be formed of senescent melanocytes. Other epi- or genetic alterations such as cyclin-dependent kinase inhibitor 2A (*CDKN2A)* or phosphatase and tensin homolog (*PTEN)* loss or inherited mutations are required to allow senescence bypass and melanoma development.

Microphthalmia-associated transcription factor (MITF) is a key regulator of melanocyte biology and in some circumstances it behaves as a genuine melanoma oncogene to which melanoma cells are addict. Inactivation of MITF results in senescence entry of melanoma cells [3], indicating that MITF is critically required for proliferation. Previous work identified a germline variant of *MITF* that changes the glutamic acid at codon 318 into a lysine and increases the risk of certain cancers, including melanoma and kidney cancer [4].

MITF can be sumoylated. Sumoylation is a post-translational modification that involves the covalent attachment of a small peptide (SUMO) to a target protein, thereby modifying its fate and/or function. The codon 318 in MITF lies within a sumoylation consensus site (YKXE) and MITF^{E318K} severely perturbs sumoylation of MITF both *in vitro* [4, 5] and *in situ* [6]. How does then hyposumoylated MITF^{E318K} contribute to melanoma progression?

Experiments using new mouse models and normal human melanocytes isolated from healthy and MITF^{E318K} patients allowed to disclose the link between MITF^{E318K} and melanoma pathogenesis.

Mouse harboring Mitf^{E318K} displays no gross phenotype. However, in the BRaf^{V600E} background, Mitf^{E318K} appears nevogenic consistent to what has been reported in humans [5, 7]. Increased nevus number by Mitf^{E318K} was however only observed in the oncogenic BRaf^{V600E} setting [6]. The fact that Mitf^{E318K} alone did not lead to nevus formation might be explained by the lack of ultraviolet exposure that in humans might favor the genetic alterations required for nevus and melanoma development.

Nevus is thought to be a benign lesion formed of melanocytes that entered senescence upon oncogenic activation such as BRAF^{V600E}, a process called oncogene-induced senescence (OIS). Senescence represents a potent barrier against tumor suppression. Acquisition of mutations that suppress senescence and promote cell division is mandatory to melanoma development [8]. Given that about 25% of melanomas derive from preexisting nevi, high nevus/dysplastic nevus count represents a risk factor for melanoma.

Further investigation of how MITF^{E318K} operates, using MITF wild-type or MITF^{E318K} human melanocytes led to the conclusion that MITF^{E318K} delayed BRAF^{V600E}-induced senescence, indicating that MITF^{E318K} melanocytes lost a senescence checkpoint. Mechanistically, these melanocytes showed a reduced expression of the cell cycle inhibitor p16^{INK4A} encoded by the *CDKN2A* locus. The importance of p16^{INK4A} in the senescence ability of melanocytes has been previously demonstrated [8]. Further, patients with biallelic inactivation of *CDKN2A* exhibit an increased number of nevi [8]. The overlapping phenotype between MITF^{E318K} and p16^{INK4A} loss suggests that p16^{INK4A} reduction likely mediates MITF^{E318K} anti-senescence effect, yet the causal role of p16^{INK4A} in MITF^{E318K} effects remains to be firmly demonstrated.

Mitf^{E318K} accelerates melanomagenesis on the oncogenic BRaf^{V600E} and Pten-deficient background, two mutations frequently identified in human melanomas, thereby recapitulating the genetic events found in a subset of human melanomas. Comparative microarray analysis revealed a reduced *CDKN2A* and *CDKN2B* level in Mitf^{E318K} mouse tumors compared to wild-type Mitf tumors. Likewise, β-catenin, which regulates MITF, has been shown to repress p16^{Ink4A} expression [9] and to facilitate

BRaf^{V600E} and Pten deficient-driven melanomagenesis [10]. Thus, both loss of Pten and p16^{INK4A} reduction may work in concert to weaken senescence and their brakes on melanoma development.

MITF stimulates transcription but also it can mediate repression, among which that of p16^{INK4A} [4]. How MITF^{E318K} regulates p16^{INK4A} transcription is not known. However, amino acid change (E318K) could change MITF interaction with its partner proteins by inducing MITF conformational change or by altering the MITF-SUMO interaction with SIM-containing peptides.

In conclusion, although the mechanisms of MITF^{E318K} effects have not been fully disclosed, our findings indicate that hyposumoylated MITF^{E318K}, through enhanced p16^{INK4A} repression, delays the BRAF^{V600E}-driven senescence process and is associated with more nevi. Additional genetic alterations such as loss of PTEN, overcome the senescence barrier, favoring proliferation and melanoma development (Figure 1).

Understanding the mechanisms that control senescence is not only important to gain critical insights into melanoma biology but may also lead to the identification of much needed biomarkers of malignancy and the development of prophylactic treatments that prevent the switch from benign nevi to malignant melanocytic lesions.

Footnote

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The authors apologize for not citing all relevant references because of space limitations.

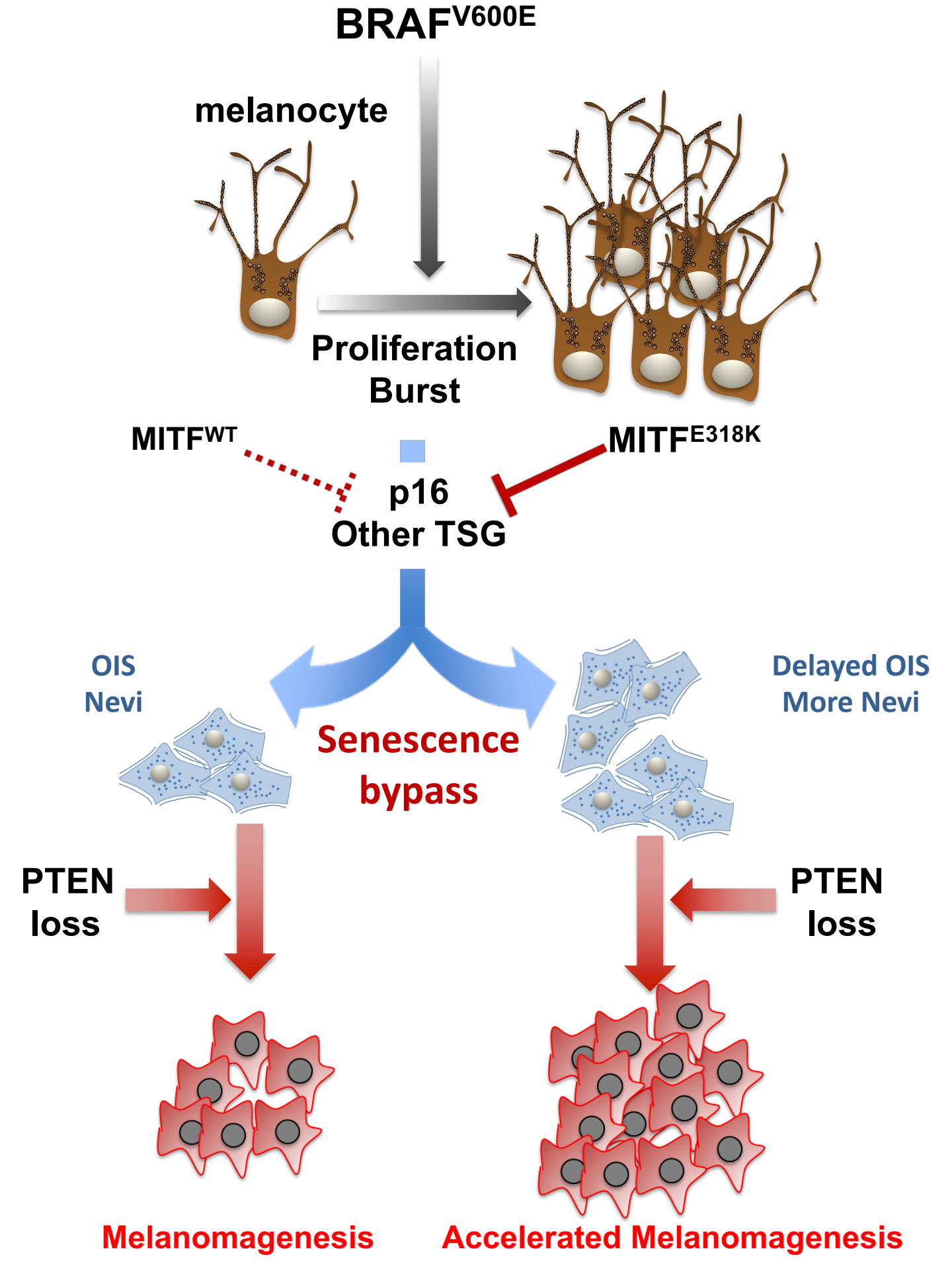
References

1. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, *et al.* Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med* **372**, 2521-2532 (2015).
2. Cancer Genome Atlas Network, Genomic Classification of Cutaneous Melanoma. *Cell* **161**, 1681-1696 (2015).
3. Giuliano S, Cheli Y, Ohanna M, Bonet C, Beuret L, Bille K, Loubat A, Hofman V, Hofman P, Ponzio G, *et al.* Microphthalmia-associated transcription factor

controls the DNA damage response and a lineage-specific senescence program in melanomas. *Cancer Res* **70**, 3813-3822 (2010).
4. Bertolotto C, Lesueur F, Giuliano S, Strub T, De Lichy M, Bille K, Dessen P, de la Fouchardière A, Molinié V, d'Hayer B *et al.* A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. *Nature* **480**, 94-98 (2011).
5. Yokoyama S, Woods SL, Boyle, G. M, Aoude LG, MacGregor S, Zismann V, Gartside M, Cust AE, Haq R, Harland M, Taylor JC. *et al.* A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. *Nature* **480**, 99-103 (2011).
6. Bonet C, Luciani F, Ottavi JF, Leclerc J, Jouenne FM, Boncompagni M, Bille K, Hofman V, Bossis G, de Donatis GM, *et al.* Deciphering the role of oncogenic MITFE318K in senescence delay and melanoma progression. *J Natl Cancer Inst* **109** (2017).
7. Sturm RA, Fox C, McClenahan P, Jagirdar K, Ibarrola-Villava M, Banan P, Abbott NC, Ribas G, Gabrielli B, Duffy DL, *et al.* Phenotypic Characterization of Nevus and Tumor Patterns in MITF E318K Mutation Carrier Melanoma Patients. *J Invest Dermatol*, **134**, 141-149 (2014).
8. Bennett DC. Genetics of melanoma progression: the rise and fall of cell senescence. *Pigment Cell Melanoma Res* **29**, 122-140 (2016).
9. Delmas V, Beermann F, Martinozzi S, Carreira S, Ackermann J, Kumasaka M, Denat L, Goodall J, Luciani F, Viros A, *et al.* Beta-catenin induces immortalization of melanocytes by suppressing p16INK4a expression and cooperates with N-Ras in melanoma development. *Genes Dev* **21**, 2923-2935 (2007).
10. Damsky WE, Curley DP, Santhanakrishnan M, Rosenbaum LE, Platt JT, Gould Rothberg BE, Taketo MM, Dankort D, Rimm DL, McMahon M, Bosenberg M, *et al.* Beta-catenin signaling controls metastasis in Braf-activated Pten-deficient melanomas. *Cancer Cell* **20**, 741-754 (2011).

Figure legend

Figure 1: Proposed model for MITF^{E318K} role in melanocyte to melanoma progression. Oncogenic activation such as BRAF^{V600E} stimulates proliferation of melanocytes, then upregulation of p16^{INK4A} or other tumor suppressor genes (TSG) allows the implementation of oncogene-induced senescence (OIS) and nevus formation. Hyposumoylated microphthalmia-associated transcription factor MITF^{E318K}, which triggers an enhanced inhibition of p16^{INK4A} expression compared to wild-type MITF, delays senescence entry and is associated with more nevi. Nevus can remain static and do not progress to malignancy unless they are coupled with other alterations, such as loss of phosphatase and tensin homolog (PTEN), that allow senescence bypass, proliferation and melanoma development.


BRAF V600E
melanocyte
Proliferation
Burst
MITF WT
MITF E318K
p16
Other TSG
OIS
Nevi
Senescence
bypass
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More Nevi
PTEN
loss
PTEN
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Melanomagenesis
Accelerated Melanomagenesis