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Summary

Cutaneous melanoma is a deadly skin cancer that originates from melanocytes. The development of cutaneous melanoma involves a complex interaction between environmental factors, mainly ultraviolet radiation from sunlight, and genetic alterations. Melanoma can also occur from a pre-existing nevus, a benign lesion formed from melanocytes harboring oncogenic mutations that trigger proliferative arrest and senescence entry.

Senescence is a potent barrier against tumor progression. As such, the acquisition of mutations that suppress senescence and promote cell division is mandatory for cancer development. This topic appears central to melanoma development because, in humans, several somatic and germline mutations are related to the control of cellular senescence and proliferative activity. Consequently, primary melanoma can be viewed as a paradigm of senescence evasion. In support of this notion, a sumoylation-defective germline mutation in microphthalmia-associated transcription factor (*MITF*), a master regulator of melanocyte homeostasis, is associated with the development of melanoma. Interestingly, this MITF variant has also been recently reported to negatively impact the program of senescence.

This article reviews the genetic alterations that have been shown to be involved in melanoma and that alter the process of senescence to favor melanoma development. Then, the transcription factor MITF and its sumoylation defective mutant are described. How sumoylation misregulation can change MITF activity and impact the process of senescence is discussed. Finally, the contribution of such information to the development of anti-malignant melanoma strategies is evaluated.

Melanoma

Cutaneous melanoma is a deadly form of skin cancer that originates from melanocytes, the melanin-producing cells of the skin. Its incidence has dramatically increased in white populations over the past several decades to reach 230,000 new cases worldwide each year (World Health Organization). Early stage skin localized malignant melanoma can be cured with surgery. However, when it has spread, metastatic melanoma has a very poor prognosis.

Cutaneous melanoma is a complex neoplasm that requires multiple environmental and genetic hits that work in concert to steer the acquisition of malignant properties.

In recent years, large-scale genomic studies of cutaneous melanoma established that the mutational signature of ultraviolet radiation (UVR) of sunlight accounts for 46% of driver mutations [1-5], thereby confirming the role of UVR in melanoma pathogenesis. Furthermore, these studies highlighted the recurrent somatic oncogenic mutations in the serine/threonine kinase BRAF (59-66% of the cases) [6, 7] and in the small GTPase NRAS (15-20% of the cases) as well as mutations in NF1 (14% of the cases, 55-75% of them predicted to be loss-of-function and putative driver mutations), which promotes the upregulation of RAS activity. These genetic alterations demonstrated the importance of the ERK signaling pathway in the disease and led to the classification of melanoma into 4 subtypes, including the triple wild-type [5].

The dissection of the molecular mechanisms involved in cutaneous melanoma also showed a frequent activation of the PI3K/AKT signaling pathway, mainly as a consequence of constitutive *NRAS* activation, AKT activation [8] or the inactivation of the phosphatase and tensin homologue (*PTEN*) (20 to 30% of the cases) [9].

Mutations of *β-catenin* (7% in the TCGA cohort), which operates downstream of AKT, were also found in melanoma cells [10, 11]. The mutations described above are the most common and are considered important drivers for melanoma, affecting the process of cellular senescence and proliferation (Figure 1), which will be discussed later in this review. The PI3K effector RAC1, a member of the small GTPase Rho-family that influences cell migration and cell cycle progression, is also mutated (4-7% of the cases) and is considered a driver in melanomagenesis [2, 4, 12-14]. Mutations in equivalent residues in the related Rho family GTPases *RAC2* and *RHOT1* have also been discovered [2], as well as an activating mutation in CDC42 that was shown to be a driver in melanoma [2]. Of note, oncogenic RAC1 was shown to reduce levels of p53 [15]. Furthermore, mutations in other genes, including the transmembrane receptor tyrosine kinase *KIT* (5-8% of cases), the subunit of the PBAF chromatin-remodeling complex *ARID2* (*7%*), the serine/threonine phosphatase *PPP6C (12%)*, the Ras GTPase activating protein *1 RASA2* (5%) and the mitogen-activated protein kinase kinase *MAP2K1 and 2* (*8%*), the *TERT* promoter (75% of metastases), the guanine nucleotide-binding protein G(q) subunit alpha (*GNAQ/11*, 5%), the phospholipase C beta 4 (*PLCB4*, 21% to 28%), and a large repertoire of other, less frequently mutated genes (not detailed here), have also been identified [1, 2, 4, 16-19].

Nevus and Senescence

Remarkably, the main driver mutations on their own do not necessarily translate into melanoma induction. Indeed, in humans, the $BRAF^{V600E}$ oncogenic mutation is frequently expressed in common acquired nevi (80% of the cases) [20], and $NRAS^{Q61K/R}$ is frequently found (80% of the cases) in congenital melanocytic nevi (present at birth) [21]. Spitz nevi, a variant of common acquired nevi, show amplified or

mutated $HRAS^{G12V}$ (30% of the cases) [22]. Thus, these oncogenic mutations represent an early event in the development of pigmented neoplasias, but appear to be insufficient by themselves to result in malignant transformation.

Nevi are benign melanocytic lesions that originate from an initial localized stimulation of melanocyte proliferation followed by a near-complete arrest of their proliferative activity due to senescence. Human nevi can remain growth arrested for decades, and as such, they have been posited as one of the best examples of *in vivo* senescence. Cellular senescence can be replicative due to telomere shortening every time a cell divides or can be premature due to oncogenic activities or stress stimuli. Both are morphologically indistinguishable and display similar characteristics [23]. Nevi express numerous senescence markers, including β-galactosidase activity, the DNA damage markers 53BP1 and γH2AX, an increase in nuclear size, and a lack of proliferative activity and $p16^{INK4A}$ expression [24-27].

The causal role of oncogenic BRAF or NRAS in senescence induction and nevus formation has been demonstrated in cultured cells and/or genetically modified animal models. In cultured human melanocytes, the forced expression of $BRAF^{V600E}$ [28], $NRAS^{Q61R}$ [29] and $HRAS^{G12V}$ [30] or NF1 loss [31] induces senescence. This process is known as oncogene-induced senescence (OIS). BRAF activation in melanocytes results in an increased expression of the cell cycle inhibitors $p16^{INK4A}$ [28, 32, 33] and $p15^{INK4B}$, another member of the INK4 gene family, which halts proliferation [34]. The pRb pathway appears to be the dominant effector of oncogenic N-RAS-induced senescence in human melanocytes, and the loss of $p16^{INK4a}$ weakens senescence [32]. Likewise, the loss of its homologue $p15^{INK4B}$ promotes the transition from a benign melanocytic nevus that harbors oncogenic BRAF to melanoma [34]. However, other studies demonstrated that the $p16^{INK4A}$ role is redundant in senescence mediated by

oncogenic BRAF or NRAS [29, 30, 33]. A panel of seventeen genes was also identified as being essential for $BRAF^{V600E}$-induced senescence in human fibroblasts and melanocytes [35], including insulin-like growth factor binding protein 7 (IGFBP7) [35]. Still, the role of IGFBP7 in the senescence induction of melanocytes by oncogenic BRAF has been questioned and remains to be formally determined [36]. Other secreted factors, such as the cytokines interleukin-6 (IL6) and IL8, also contribute to the senescence of melanocytes caused by $BRAF^{V600E}$ [37, 38].

Transgenic mouse [39] or zebrafish [40] models that express $BRaf^{V600E}$ demonstrate *in vivo* the role of oncogenic mutation in nevus development. Likewise, mouse models that express $BRaf^{V600E}$ from its own promoter confirmed these observations in a more physiological setting [41, 42]. The $BRaf^{V600E}$ nevus-like lesions found *in vivo* stained positive for SA-β-galactosidase activity, and demonstrated low levels of proliferation [39, 42]. Furthermore, transgenic mice that express $NRas^{Q61K}$ display a phenotype reminiscent of congenital melanocytic nevus [43].

The finding that the forced expression of oncogenic BRAF or NRAS in normal human melanocytes elicited senescence in few weeks and the observation that significant telomere attrition was not observed in nevus cells [28] led to the notion that nevi undergo premature senescence due to oncogenic stimuli and replicational stress as described in other cell systems, rather than replicative senescence due to telomere shortening [44-46]. However, because a single eroded telomere can induce senescence, telomere shortening might have a role in nevus formation [47]. In support of this idea, nevus size and number have been associated with telomere length [48], and telomere dysfunction-induced DNA damage foci have been detected in nevi [49]. Collectively, these observations suggest that both premature and replicative dependent senescence might have occurred in nevi (reviewed in [50, 51]). Interestingly,

both types of senescence are associated with stimulation of the DNA Damage Response (DDR) signaling pathway, which converges towards the activation of retinoblastoma protein. Thus, one might hypothesize that both senescence programs have independent abilities to ensure robustness in tumor suppression and may prevent further nevi growth and transformation when a tumor suppressor is inactivated.

Senescence bypass and melanoma

Melanoma originates from a pre-existing nevus in 25% of cases, inferred from a melanoma contiguous to an adjacent nevus remnant; however, melanoma can obliterate an associated nevus [33, 52]. An increased number of nevi/atypical nevi represents a major melanoma risk factor.

The dogma is that senescence bypass represents a prerequisite for malignant transformation. Accordingly, $p16^{INK4A}$ or p53, which are major determinants of cellular senescence, are inactivated in most human cancers [53] and telomerase or an alternative mechanism called ALT (Alternative Telomere Lengthening) is re-expressed [54].

Once formed, the nevus-like lesions can remain static and do not progress to malignancy unless they are coupled with other alterations, such as a loss of $p16^{Ink4a}$ [39, 41, 43, 55-57], p53 [40, 58] or Pten [33, 41]. The receptor tyrosine kinase TYRO3 has also been shown to mediate $BRAF^{V600E}$-induced senescence bypass in primary melanocytes, inducing the transformation of non-tumorigenic cell lines [59]. Likewise, the proto-oncogene C-MYC, the expression of which is enhanced in melanoma cells compared to melanocytes, suppresses OIS [29]. This process involves the loss of the B56α subunit of the PP2A tumor suppressor complex (PP2A-B56α), an MYC

degradation complex, which leads to an increase in C-MYC expression [60]. Accordingly, the depletion of PP2A-B56α in normal human melanocytes upregulated C-MYC protein levels and suppressed $BRAF^{V600E}$- and, less efficiently, $NRAS^{Q61R}$-induced senescence [60].

Of note, melanoma is thought to originate *de novo* in 75% of cases. In this context, senescence might not be efficiently established due to dysfunction in senescence programs and thus might lead immediately to the malignant conversion of melanocytes. This may be the consequence of pre-existing mutations or epigenetic alterations that impair senescence and proliferation arrest. However, even in this case melanoma can originate from senescent melanocytes that are too few to form a visible nevus.

Melanoma susceptibility genes and their role in senescence bypass (Figure 1)

Melanoma occurs in a familial context in approximately 10% of cases. Gene mutations that contribute to an inherited susceptibility for melanoma, which increase the risk to the carrier, have been highlighted. These include mutations in rare but clearly highly penetrant melanoma predisposition genes and more common lower penetrant genes.

Even though there is still no disease process, the identification of a melanoma susceptibility allele would help to better predict which patients might benefit from increased surveillance and earlier detection of potentially dangerous lesions. However, these susceptibility alleles, such as $p16^{INK4A}$, which was discussed earlier, can act as modifiers of somatic mutations to dramatically influence the onset of the disease [61, 62]. Importantly, the early detection of potentially dangerous lesions

remains the best strategy to reduce the tumor burden of advance disease and for the optimal clinical outcome of patients suffering from melanoma.

Rare highly penetrant melanoma-causing variants include cyclin-dependent kinase Inhibitor *2A* (*CDKN2A*), cyclin-dependent kinase 4 (CDK4) and retinoblastoma protein 1 (*RB1*) (for review [63]). *CDKN2A* encodes for $p16^{INK4A}$ and $p14^{ARF}$ from an alternate reading frame. Mutations in this gene mainly affect both $p16^{INK4A}$ and $p14^{ARF}$ or $p16^{INK4A}$ only, but in few cases, they can also specifically inactivate $p14^{ARF}$ [64, 65]. $p16^{INK4A}$ inhibits the cyclin-dependent kinases 4/6 and cyclin D complexes [57, 66]. $p14^{ARF}$ inhibits human double minute 2 (HDM2), which triggers p53 stabilization, leading to the increased expression of the cyclin-dependent kinase inhibitor $p21^{Cip1}$ (CDKN1A) [67]. Activating *CDK4* mutations prevent the binding and inhibition of CDK4 by $p16^{INK4A}$ [68]. The relevance of $p16^{INK4A}$ or $p14^{ARF}$ varies among species and cell types. Human melanocyte senescence showed a higher dependency on the $p16^{INK4A}$ pathway compared to the $p14^{ARF}$ pathway [69, 70]. Moreover, the role of p53 with regard to senescence is different in human melanocytes compared to fibroblasts [69, 71]. Both the $p16^{INK4A}$ and the $p14^{ARF}$/p53/$p21^{CIP1}$ axis operate to maintain the retinoblastoma protein (RB) in an inactive state.

Because RB has a critical role in the induction and maintenance of senescence [53], all the above-described alterations in the RB pathway favor senescence bypass and proliferation, which is a mandatory step toward melanoma progression [72].

Accordingly, human melanocytes isolated from individuals with a biallelic inactivation of $p16^{INK4A}$ [69] or with $p16^{INK4A}$-knockdown [73], both of which have functional $p14^{ARF}$, have greater lifespans in culture. Furthermore, mouse melanocytes with a heterozygous inactivation of $p16^{INK4A}$ show defective senescence

[70]. Likewise, individuals that carry a monoallelic inactivation of *CDKN2A* show multiple large nevi suggestive of extended proliferation before senescence [64]. These observations strengthen the critical role of $p16^{INK4A}$ in human melanocyte senescence *in vitro* and *in vivo*. However, $p16^{INK4A}$-deficient human melanocytes still stop proliferating, which indicates that additional factors, such as p53, may impose a $p16^{INK4A}$-independent checkpoint to affect growth arrest [69, 74]. Although $p14^{ARF}$, which controls the p53 level, had no clear reported role in human melanocyte senescence [70, 71], it has been recently reported to control radical oxygen species (ROS) production [75] through a short acidic motif often targeted by familial melanoma mutations in *CDKN2A* [76]. ROS are involved in the maintenance of melanocyte senescence [77, 78].

Other high-risk melanoma susceptibility genes include the *BRCA-1 associated protein (BAP1)* and the telomere maintenance genes *POT1*, *ACD, TERF2IP* and *TERT* (reviewed in [63]). *BAP1* regulates genome stability during cell replication by controlling cellular recovery from DNA damage [79]. The telomere controlling genes protect chromosome extremities from cellular DDR and genomic instability [80]. DDR transiently stops cell-cycle progression until damage is removed, but persistent DDR triggers cellular senescence.

Mutations in the *CDKN2A*, *RB1*, *BAP1* and *TERT* also occur at a somatic level [5].

Collectively, the high-risk melanoma susceptibility genes converge to overcome the RB-dependent growth arrest, which mediates senescence bypass, and to render cells genomically instable, both of which favor melanoma development.

Low- to moderate-risk genes have a weaker impact on melanoma

susceptibility. Low melanoma risk genes are primarily associated with pigmentation that plays a key role in the prevention of sunburns, a risk factor for melanoma. Nevertheless, some are linked to cell survival or metabolism [63].

Moderate-risk genes include microphthalmia-associated transcription factor *(MITF)* [81-84] and its upstream regulator melanocortin 1 receptor (*MC1R*). In addition to controlling pigmentation [85, 86], both MC1R and MITF could also exert pro-melanoma effects and regulate cellular senescence. Indeed, MC1R signaling induces the phosphorylation of DNA repair proteins to mediate the repair of UVR-induced DNA damage [87, 88]. Melanocytes that express loss of function MC1R variants display compromised DDR activation and genomic stability [88], which predisposes the cell to malignant transformation. Furthermore, MC1R has been shown to interact with PTEN and to stabilize its expression [89]. Some MC1R variants, in contrast to the wild-type form, do not interact with PTEN [89] and therefore might favor the sustained activation of the PI3K pathway that facilitates senescence.

Microphthalmia-associated transcription factor (MITF) is the conductor of melanocyte lineage development [90] and function [85, 91] (reviewed in [92]). Furthermore, MITF may act, *per se*, as a bona fide melanoma oncogene [93, 94]. It has also been recently associated with intrinsic and acquired resistance to targeted therapies [94-97] and it has a negative impact on immunotherapy efficiency [98, 99]. To explain the different functions of MITF in the melanocyte lineage, a rheostat model has been established that stipulates that the expression level, post-translational modification and co-factors create a bar code-like situation, which channels MITF towards a specific subset of target genes and determines either pro- or anti-oncogenic MITF activity [100].

The recurrent germline mutation in MITF that changes glutamate 318 into a lysine

(MITF^{E318K}) predisposes carriers to certain cancers, including melanoma [81-84, 101]. Furthermore, the MITF^{E318K} variant has been associated with multiple primary melanoma and nodular melanoma and thus could play a role in the fast-growing and aggressive form of the disease [82, 84, 101]. Mechanistically, MITF^{E318K} has recently been shown to weaken the process of cellular senescence in melanocytes [102]. The way in which the MITF E318K mutation impacts MITF activity and the process of senescence is the focus of the following section.

MITF and sumoylation

MITF can be sumoylated [81, 84, 103, 104]. Sumoylation is a highly dynamic and reversible ubiquitination-like post-translational modification that triggers the covalent attachment of a small peptide (SUMO) to a target protein [105]. Sumoylation events usually occur at a consensus motif site (YKXE) and are critically dependent on the acidic residue at +2 (E) of the acceptor lysine (K). Sumoylation is a multistep process that involves the consecutive actions of E1 (SAE1/SAE2), E2 (UBC9) and E3 (PIAS) enzymes that catalyze the attachment of SUMO to target proteins, while deconjugation is promoted by SUMO-specific proteases [106]. The ligase involved in the SUMO modification of MITF has not been pinpointed. However, MITF has been previously demonstrated to bind PIAS3 [107, 108], which functions as a SUMO ligase for MITF. Thus, PIAS3 might act as an E3 sumo ligase for MITF.

Sumoylation dysregulation has been reported at the global level by affecting the activity of the enzymes of the conjugation/deconjugation machinery or at the level of individual proteins by modifying the accessibility of the targeted lysines.

Although a number of individual proteins exhibit changes in sumoylation, only a few examples of mutations exist, such as in lamin-A, that directly impact a SUMO-

consensus site [109]. The clear establishment of the relevance of sumoylation alterations in these diseases is difficult. The hyposumoylated MITF^{E318K} variant is another example. Indeed, the codon 318 in MITF is located in a sumoylation consensus site (YKXE), and accordingly, MITF^{E318K} severely impaired the sumoylation of MITF both *in vitro* [81, 84] and *in situ* [102].

To better understand how MITF^{E318K} mediates pro-tumoral effects, a combination of immortalized human melanocytes, human melanocytes isolated from healthy or MITF^{E318K} patients and mouse models was used [102].

MITF^{E318K} appears nevogenic in mice, in agreement with what has been reported in humans [83, 84, 101]. However, an increased number of nevi due to the presence of MITF^{E318K} was only observed in the oncogenic BRaf^{V600E} setting [102]. Compared to humans, mice grow in a relatively homogenous environment with less variation in diet and environmental exposures and have much longer telomeres that restrain chromosomal abnormalities, thereby preventing the acquisition of other potential alterations required for nevus development [110]. Moreover, non-follicular melanocytes are scarce in normal mouse skin and are mainly located in the hair follicle, which may have implications for the incidence of nevi.

These additional (epi)genetic alterations mediated by environmental factors likely co-exist in human MITF^{E318K} nevi. Of note, MITF^{E318K} melanocytes have a shorter doubling time compared to wild-type MITF. Thus, melanocytes that constitutively express MITF^{E318K} could have an altered ability to enter senescence upon the induction of oncogenic BRAF^{V600E}, leading to an increased nevus number *in vivo*. Accordingly, cultured MITF^{E318K} melanocytes displayed delayed BRAF^{V600E}-induced senescence and reduced expression of p16^{INK4A}; how MITF^{E318K} inhibits p16^{INK4A}

expression remains to be elucidated. In agreement with this finding, patients with a biallelic inactivation of the cell cycle inhibitor *CDKN2A* exhibit an increased number of nevi [69]. Furthermore, *Mitf*^{E318K} facilitates melanomagenesis on the oncogenic BRaf^{V600E} and Pten-deficient background; these two mutations are frequently identified in human melanomas, thereby recapitulating the genetic events found in a subset of human melanomas [111].

A comparison of Mitf wild-type or Mitf^{E318K} tumors revealed that *Mitf*^{E318K} reduces the levels of the cell cycle inhibitors *CDKN2B* and *CDKN2A,* which, as discussed earlier, are critically required to prevent the melanocyte to melanoma progression. Remarkably, *CDKN2B* is a kidney cancer predisposition gene [112], the incidence of which is also increased in MITF^{E318K} carriers [81, 113]. Consistently, β-catenin, which regulates MITF expression [114], has also been shown *in vivo* to promote senescence evasion via the inhibition of p16^{INK4A} expression [56] and to facilitate melanomagenesis in the oncogenic BRaf^{V600E} and Pten-deficient background [115]. Collectively, these observations strengthen the idea that MITF^{E318K} impairs the implementation of the senescence program and favors melanoma progression.

Sumoylation impacts protein localization, stability or activity [116]. It has been shown to modulate the activity of a large number of proteins, mostly transcription factors, and to be involved in many biologically important functions, including DNA damage repair, the response to stress, and cellular senescence [116, 117]. Perturbations of such modifications are therefore likely to contribute to human diseases such as cancer [118]. Accordingly, in humans, environmental stresses that influence melanoma progression, such as UVR [119] and hypoxia [120], trigger the production of reactive oxygen species (ROS). These stimuli alter the process of sumoylation [121] and senescence [122].

One evident answer to why MITF^{E318K} favors melanomagenesis is that the mutation reduces the sumoylation of MITF. The inhibition of MITF sumoylation does not appear to affect MITF cellular localization or stability [81, 103, 104]; sumoylation changes the transcriptional activity [81, 84] and target sequences of MITF, which could explain the pro-tumoral effects. Indeed, compared to wild-type MITF, MITF^{E318K} is redistributed over the genome on a larger repertoire of low affinity genomic sequence [81]. Actually, the E318K mutation modulates MITF DNA binding due to its preference for an extended palindromic E box 5'-TCACGTGA (versus 5'-CACGTGAC/T for wild-type MITF), thereby altering MITF's target gene specificity (Figure 2). Briefly, strong MITF^{E318K} binding requires an extended 8-base pair palindrome or an M-box [123]. Sites with a 6-base pair E-box are bound with lower affinity, and the diminished specificity allows MITF^{E318K} to bind with lower affinity to more degenerate E-boxes, thus accounting for the larger number of weakly bound sites that were found with this mutant. In contrast, few sites with the extended 8-base pair palindrome are among the top 500 bound by wild-type MITF, which indicates that the full palindrome is not required for the strong binding of wild-type MITF. Nevertheless, an understanding of the precise mechanisms of changes in site-selection will require further studies.

Additionally, we cannot rule out the possibility that the E318K mutation exerts a sumoylation independent effect by altering other post-translation modifications such as acetylation or ubiquitination of the new lysine, or more generally, the MITF conformation.

Therapeutic options that target sumoylation and senescence

Recent genomic studies permitted a better understanding of melanoma pathogenesis and have been instrumental in the development of newer therapies to target driver

mutations. An understanding of the molecular mechanisms also permitted the development of efficient immunotherapy strategies. Despite the success of recent treatments that utilized targeted therapies (BRAF, MEK) and immunotherapies (anti-CTLA-4, anti-PD1) that substantially extended the median overall survival of patients suffering from metastatic melanoma [124, 125], the therapeutic options are far from perfect, as clinical responses are either transient or limited to restricted subsets of melanoma patients.

Therefore, approaches that could represent therapeutic strategies to improve malignant melanoma treatment and/or to impair its progression are discussed below.

The SUMO pathway has been considered a potential target in cancer treatment. The loss of SAE1/2 enzymatic activity in human mammary epithelial cells drives synthetic lethality with cMYC [126]. The RNAi depletion of UBC9 impairs the *in vitro* and *in vivo* growth of KRAS mutant colorectal cancer cells [127]. Furthermore, the global inhibition of sumoylation with chemicals such as the E1 inhibitor anacardic acid has been shown to promote the death of acute myeloid leukemia cell lines *in vitro* and to reduce tumor growth *in vivo* [121]. However, sumoylation inhibition might not be appropriate for melanoma as it is expected to reduce MITF sumoylation and to favor melanoma progression. In contrast, the global upregulation of sumoylation via the use of inhibitors of SENP activity that are currently under development [128] could represent a valid anti-melanoma strategy.

As discussed in this review, cancers develop via senescence suppression and the stimulation of cell proliferation. Given that $p16^{INK4A}$ is a barrier to senescence evasion and plays a key role in melanomagenesis, the restoration of genetic alterations and the epigenetic reactivation of *CDKN2A* may represent strategies for the prevention and therapy of cancer [129]. Melanoma cells already have a powerful

pro-senescence signal that exists in dormancy via the activation of BRAF or NRAS that discerns the cancer cells from their normal counterparts. In support of this notion, the depletion of MITF [130-132] or cMYC [29] reactivated senescence in $BRAF^{V600E}$ or $NRAS^{Q61R}$ expressing melanoma cells. As such, pro-senescence therapy already has a built-in specificity for melanoma cells that might be exploited to improve current melanoma treatment.

Accordingly, senescence induction in human breast cancer and lung carcinoma following chemotherapy is correlated to a favorable outcome [133, 134]. However, *in vivo*, effective tumor regression has been associated with the clearance of senescent cells by the immune system [135, 136]. Therapy-induced senescence can enhance long-term outcomes, but tumors eventually reappear [137].

Actually, senescent cells are growth arrested but they remain viable and metabolically active, and this leaves a door open for growth reset. The inactivation of p53 was reported to reverse the replicative senescence of some cells, but it failed to overcome oncogene-induced senescence [138]. Senescence reversibility might depend on the expression of cellular $p16^{INK4A}$, which provides a dominant barrier against proliferation [138]. Furthermore, senescence is associated with the chronic secretion of multiple bioactive factors, which is called senescence-associated secretory phenotype (SASP). SASP comprises pro-inflammatory cytokines or chemokines, extracellular matrix proteases and growth factors [139, 140] that influence the cellular microenvironment. Some of these secreted molecules may have autocrine effects that enforce cellular senescence [37, 141], whereas others may exert non-cell-autonomous effects that favor tumorigenesis in nearby non-senescent cells [131, 142, 143].

Thus, cellular senescence can exert both beneficial and detrimental effects,

depending on the senescence signal, tissue context and secreted molecules. The challenge lies in the suppression of the activity of the anti-senescence factors and the enhancement of the activity of pro-senescence factors.

Of note, SASP was not detected when cells were induced to senesce with the forced expression of $p16^{INK4A}$, and it was suggested that p53 signaling was necessary [144]. In melanocytes, compelling evidence shows the critical role of $p16^{INK4A}$ in the control of proliferative activity and the ability to senesce *in vitro* and *in vivo* [64, 69, 70]. Thus, one might hypothesize that nevi do not produce a secretome. However, in atypical nevi, which are associated with an increased risk of developing melanoma, $p16^{INK4A}$ can be mutated and p53 can be reactivated. This finding suggests that some nevi can produce SASP factors and create a microenvironment that causes regrowth in the nevus.

In the context of aging, senescent cells accumulate in tissues. They might disrupt tissue structure and be causally implicated in the generation of age-related diseases, including cancers. As such, the clearance of senescent cells can prevent or postpone tissue dysfunction and extend the healthspan. Indeed, the selective removal of senescent cells by the BH3 mimetic ABT263 in sublethally irradiated or normally aged mice has health benefits in part through the rejuvenation of aged tissue stem cells [145]. Furthermore, the elimination of $p16^{Ink4a}$-positive senescent cells in progeroid mice, via the stimulation of apoptosis, delays the acquisition of age-related pathologies [146]. Likewise, a FOXO4 peptide that perturbs the FOXO4 interaction with p53 has been shown to induce apoptosis in senescent cells [147]. *In vivo*, FOXO4 peptides neutralize doxorubicin-induced chemotoxicity and restore tissue function [147]. Anti-senescence therapies that utilize senolytic drugs, which selectively kill senescent cells, are under development [148-150]. These drugs may improve the healthy

lifespan, but they might also be useful in cancer treatment, as a prophylactic approach to eliminate benign pro-tumoral cells. This prophylactic approach could be exploited in patients at a high risk of developing melanoma. In addition, the BRAF inhibitor vemurafenib, which is approved for clinical use, or chemotherapeutic drugs have been shown to induce a senescence-like phenotype in melanoma cells [131, 142, 151]. Thus, pro-senescence therapies associated with senolytic drugs might also be used to kill malignant melanoma cells (Figure 3).

Acknowledgements

The authors are very grateful to Dr. I. Davidson (IGBMC, Strasbourg) for the analysis of the ChIP-sequencing dataset by the MEME program and his helpful discussion.

This work was funded by INCa grants INCa_10573 to CB and INCa_2013-070 to RB and ANR-13-BSV1-0025-01 to RB.

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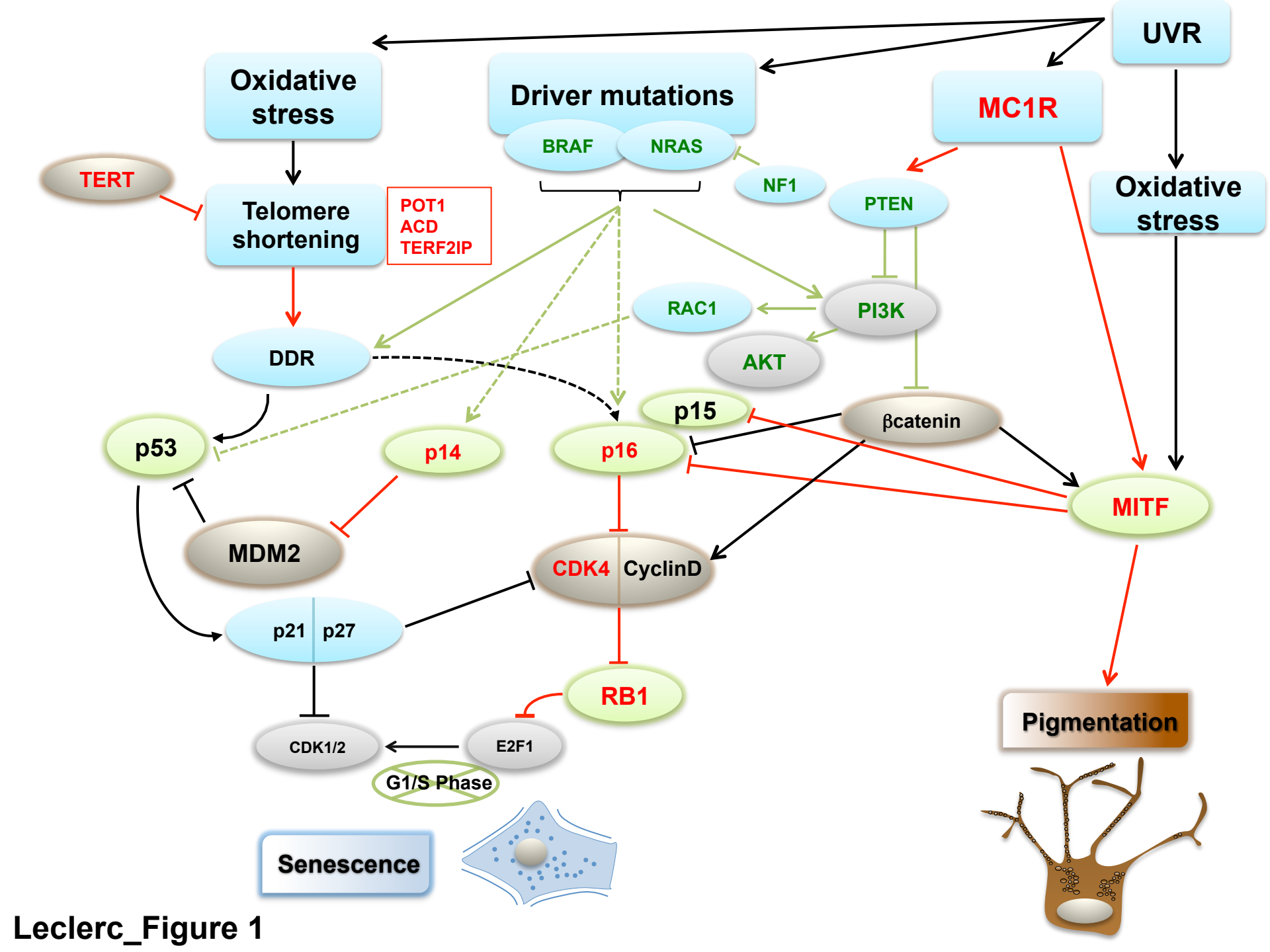
Figure legends

Figure 1: Cell senescence pathways associated with genes commonly altered in melanoma. Partly adapted from a figure in Bennett et al. (2016). UVR from sunlight is the main environmental risk factor for melanoma development. UVR contributes to carcinogenesis through the induction of oxidative stress, DNA damage and mutations and triggers the activation of signaling cascades, including the BRAF/NRAS/ERK and PI3K/AKT(PTEN)/RAC1 pathways that are critically required for melanoma cell survival, proliferation and differentiation. The mutations in effectors of these pathways (green) are considered important drivers for melanoma and affect the process of DNA damage, cellular senescence, proliferation and survival. Melanoma susceptibility alleles can act as modifiers of somatic mutations to influence the onset of the disease. High (CDKN2A, CDK4, RB1, POT1, ACD, TERF2IP) and moderate (MC1R, MITF) melanoma susceptibility genes are indicated (red).
This schema illustrates how the cooperation of germline variants with oncogenic activities can alter key biologic pathways to favor senescence bypass and contribute to melanoma progression.

Figure 2: MITF^{E318K} displays modified DNA binding activity. The MEME program was used to analyze the top 500 wild-type MITF binding sites in the ChIP-seq dataset from Bertolotto et al. [81]. This analysis revealed that highly occupied MITF sites are composed of the CACGTG or CATGTG core motif flanked by a highly represented A and a C/T (List 1, upper panel). In contrast, an analogous analysis of 500 sites only bound by wild-type MITF (List 2, middle panel), mainly sites that exhibit low occupancy, indicates the presence of non-canonical Ebox motifs. A subset of sites in this list are also bound by MITF^{E318K}, although these sites are not necessarily among

the top 500 bound by the mutant. An analysis of the top 500 sites bound selectively by $MITF^{E318K}$ (List 3, lower panel) revealed the presence of an extended palindromic E box, in which the CACGTG or CATGTG core motifs are flanked by A and T. In each panel, the numbers of motifs with each consensus sequence are shown along with the relevant P and E values.

Figure 3: Model of melanocyte to melanoma transformation and therapeutic strategies. Melanoma can develop *de novo* from a melanocyte or from a pre-existing nevus. A nevus is a benign proliferation of melanocytes. The growth arrest of nevus melanocytes results from senescence induced by oncogenes such as $BRAF^{V600E}$. Once formed, a nevus can remain growth arrested for decades. However, a nevus can reverse “oncogene-induced senescence” and undergo malignant conversion. The development of prophylactic treatments to prevent the switch from benign nevi to melanoma by allowing the clearance of senescent cells by senolytic drugs or the development of curative treatments that use pro-senescence therapy associated with senolytic drugs could represent therapeutic strategies (in blue).



Leclerc_Figure 1

Sites bound by wild-type MITF

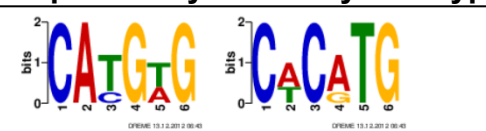
Details

Positives	Negatives	P-value	E-value	Unerased E-value
364/499	14/499	8e-135	2.1e-130	2.1e-130

Enriched Matching Words

Word	Positives	Negatives	P-value	▼E-value
CACGTGAC	260/499	2/499	5.0e-95	1.3e-90
CACGTGAT	107/499	0/499	1.0e-35	2.8e-31
CATGTGAC	53/499	8/499	5.2e-10	1.4e-5
CATGTGAT	24/499	5/499	2.3e-4	6.0e+0

Sites specifically bound by wild-type MITF



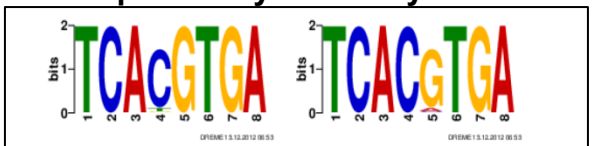
Details

Positives	Negatives	P-value	E-value	Unerased E-value
234/499	82/499	8.7e-26	2.4e-21	2.4e-21

Enriched Matching Words

Word	Positives	Negatives	P-value	▼E-value
CATGTG	132/499	46/499	4.4e-13	1.2e-8
CACGTG	30/499	3/499	4.9e-7	1.3e-2
CACGAG	36/499	9/499	2.3e-5	6.1e-1
CATGAG	75/499	35/499	3.5e-5	9.4e-1

Sites specifically bound by MITF^{E318K}



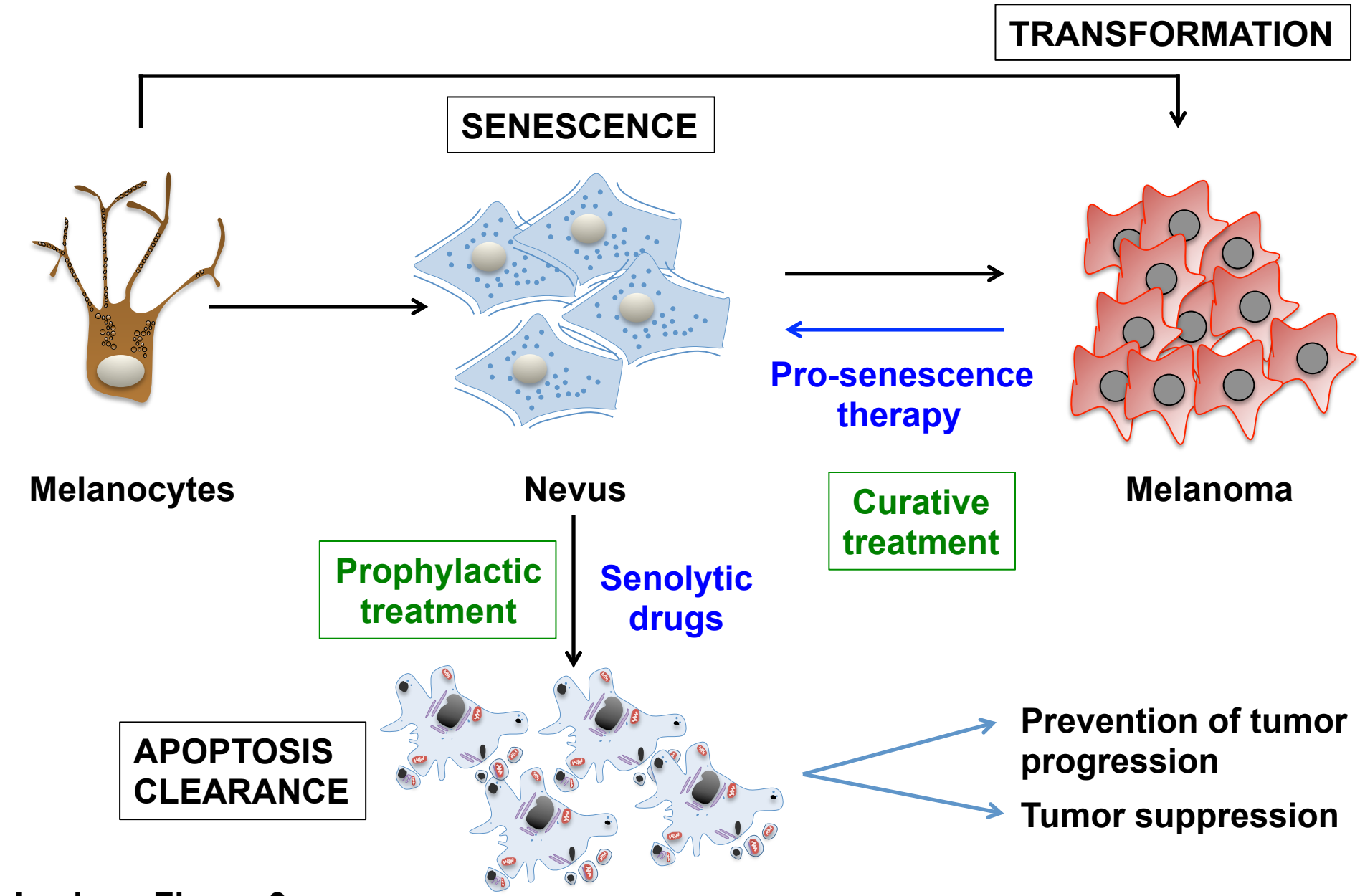
Details

Positives	Negatives	P-value	E-value	Unerased E-value
430/499	3/499	1.2e-202	3.3e-198	3.3e-198

Enriched Matching Words

Word	Positives	Negatives	P-value	▼E-value
TCACGTGA	407/499	0/499	6.5e-190	1.8e-185
TCATGTGA	25/499	3/499	1.1e-5	3.0e-1
TCAGGTGA	12/499	0/499	2.3e-4	6.4e+0

Leclerc_Figure 2



Leclerc_Figure 3