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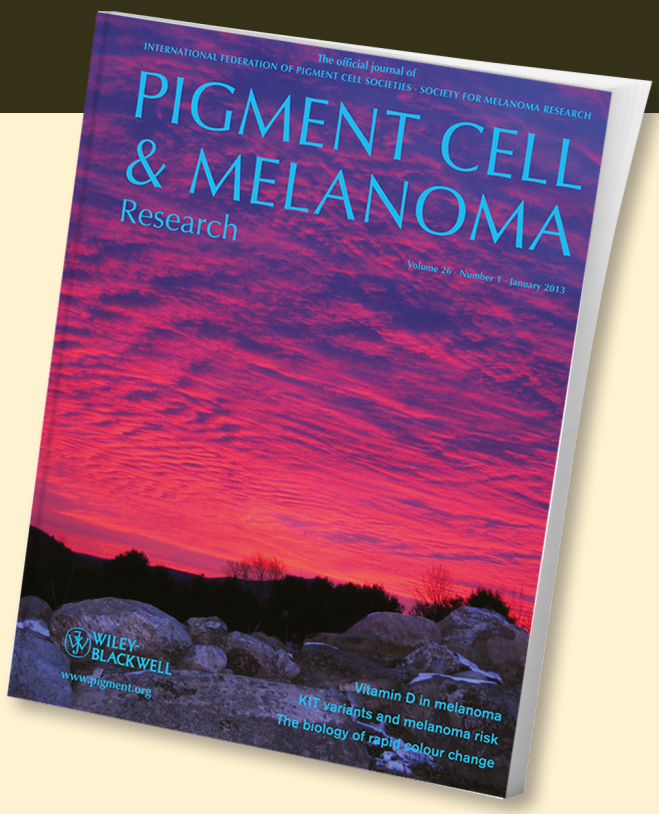

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REVIEW

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Review: Are moles senescent?

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Abstract

Melanocytic nevi (skin moles) have been regarded as a valuable example of cell senescence occurring in vivo. However, a study of induced nevi in a mouse model reported that the nevi were arrested by cell interactions rather than a cell-autonomous process like senescence, and that size distributions of cell nests within nevi could not be accounted for by a stochastic model of oncogene-induced senescence. Moreover, others reported that some molecular markers used to identify cell senescence in human nevi are also found in melanoma cells—not senescent. It has thus been questioned whether nevi really are senescent, with potential implications for melanoma diagnosis and therapy. Here I review these areas, along with the genetic, biological, and molecular evidence supporting senescence in nevi. In conclusion, there is strong evidence that cells of acquired human benign (banal) nevi are very largely senescent, though some must contain a minor non-senescent cell subpopulation. There is also persuasive evidence that this senescence is primarily induced by dysfunctional telomeres rather than directly oncogene-induced.

KEYWORDS
benign nevus, CDKN2A, cell senescence, human, melanoma, mouse, oncogene, telomere dysfunction, TERT

1 | INTRODUCTION: CELL SENESCENCE AND BENIGN TUMORS

Cell senescence is a programmed, permanent arrest of cell division, functioning predominantly in protection against genotoxic stresses and cancer (Gorgoulis et al., 2019). Permanent here means in the absence of genetic manipulation, since senescence can be reversed by experimental abrogation of the core intracellular arrest pathways, or their loss in cancer. Senescence has been categorized into several types according to what triggers it. Not all types are relevant here, but the longest- and best-known is replicative senescence, where chromosomal telomeres shorten with cell division and very short telomeres set up DNA-damage signaling, activating cell-cycle arrest (Gorgoulis et al., 2019). Another type is oncogene-induced senescence (OIS), seen in cell-culture experiments where overexpression of an active oncogene from a strong promoter induces rapid senescence in normal cells (Serrano et al., 1997). OIS is also mediated through DNA-damage signaling, through DNA hyper-replication, and replication stress (Bartkova et al., 2006; Di Micco et al., 2006). OIS occurs in normal diploid cells, as distinct from the outcome of oncogene overexpression in immortalized cells, namely "transformation": reduced growth-factor dependence for proliferation and acquired ability to proliferate in suspension and grow as tumors in mice (e.g., Newbold & Overell, 1983).

In 2005–06, a flurry of papers reported that various types of benign, static lesions in humans and mice were composed of senescent cells, supporting the idea that cell senescence functions to arrest proliferative lesions safely (Braig et al., 2005; Chen et al., 2005; Collado et al., 2005; Gray-Schopfer et al., 2006; Michaloglou et al., 2005).

This provided a mechanism for the established findings that cell senescence and its key effectors function in tumor suppression (e.g., Kamijo et al., 1997; Sharpless et al., 2001; reviewed by Collado & Serrano, 2010; He & Sharpless, 2017; Mooi & Peeper, 2006; Pérez-Mancera et al., 2014). Human pigmented moles (benign or banal nevi) were among the lesions reported as senescent (Gray-Schopfer et al., 2006; Michaloglou et al., 2005), and nevi have been regarded as one of the clearest examples of cell senescence in vivo. Such senescence in nevi, among other benign lesions, has often been described as a kind of OIS (Centeno et al., 2023; Kuilman et al., 2008; Michaloglou et al., 2005). This is because human nevi typically carry an oncogene – a monoclonal oncogenic mutation, including activated *BRAF*, usually $BRAF^{V600E}$, in around 80% of common acquired nevi, and *NRAS* activations in 6%–15% (Bennett, 2016; Roh et al., 2015; Tate et al., 2019 [COSMIC database]). Both normal and activated RAF- and RAS-family proteins mediate cell proliferation via the MAP kinase pathway (pathway details in Bennett, 2016). These are widespread driver mutations in cancer, including melanoma (Cancer Genome Atlas Network, 2015).

What OIS might look like after a natural oncogenic mutation in vivo is not well defined however, as Damsky and Bosenberg (2017) noted. Overexpression of $BRAF^{V600E}$ in cultured human diploid melanocytes does lead to rapid arrest, within days (Gray-Schopfer et al., 2006; Michaloglou et al., 2005). However, a natural $BRAF^{V600E}$ point mutation in an epidermal melanocyte would not give overexpression, only normal abundance of the oncoprotein, or at least 50% normal, being initially heterozygous. Induction of such a point mutation in *BRAF* leads to proliferation of cultured normal human melanocytes (Zeng et al., 2018), rather than replication stress and OIS. McNeal et al. (2021) also report that if $BRAF^{V600E}$ was overexpressed at a level sufficient to arrest melanocytes grown with the mitogenic phorbol ester TPA, but now in melanocytes cultured without TPA, it could stimulate proliferation rather than arrest. Moreover, resumed proliferation was observed in BRAF-arrested cultures on removal of either TPA or the $BRAF^{V600E}$ expression, so at least some cells were reversibly arrested rather than senescent.

A recent paper proposed further that nevus cells are not arrested through OIS, nor any kind of senescence, but are quiescent: arrested reversibly through cell interactions (Ruiz-Vega et al., 2020). This conclusion was based on data from a mouse model of $Braf^{V600E}$-induced nevi, compared to a stochastic mathematical model for OIS timing. They also proposed that this concept could extend to human benign nevi. This conclusion, apparently in conflict with previous ideas and data, has attracted attention and perhaps generated some confusion. Accordingly, this review aims to re-examine the available evidence on nevi and senescence, including the data of Ruiz-Vega et al. in comparison to human nevi.

2 | THE MOUSE MODEL USED BY RUIZ-VEGA ET AL.

The murine melanoma model used by Ruiz-Vega et al. (2020) was that of Dankort et al. (2009). Mice were genetically engineered such that cells of the melanocytic lineage inducibly acquire the $Braf^{V600E}$ mutation, and express the oncogene from its natural (*Braf*) promoter. Thus, the oncogene should not be overexpressed. The mutation was activated postnatally through a *Tyr::CreER* transgene, by 4-hydroxytamoxifen (4-OHT).

Painting of 4-OHT on the back skin of 2- to 4-day-old C57BL6/J mice resulted in growth of numerous, microscopic melanocytic colonies in the dermis, at least 5–10 colonies/mm^2 from images shown, and with a median diameter of 154 μm. These stopped growing by 21 days, and were described as nevi (Ruiz-Vega et al., 2020). They were reported to be composed of smaller cell clusters or nests, as often seen in human nevi (though no clear histological images were provided). These nests had a reported median diameter of around 40 μm.

This biology immediately seems very different from that of human acquired nevi, which are usually defined as at least 2 mm in diameter (Bataille et al., 2007; Shain & Bastian, 2016), range up to 5 mm diameter or more, and are typically spaced centimeters apart. Congenital nevi can be much larger. Acquired nevi grow most commonly along the dermoepidermal border (simple lentigo, junctional nevus), or in both epidermis and dermis (compound nevus) (Mooi & Krausz, 2007). A recent preprint reports that a subclass of dysplastic nevi also shows a lentiginous pattern, associated with driver mutations other than *BRAF* and with older patient age (Lorbeer et al., 2023).

Ruiz-Vega et al. presented convincing evidence that their melanocytic colonies were not arrested via OIS. The evidence included single-cell gene-expression profiles in two cell sets (from whole skin of these mice), both taken to be nevus cells because they included melanocytic markers, and were absent from skin of normal mice. Neither of these sets showed any match to any of several gene expression signatures taken from different types of cell senescence. Secondly, the authors compared the size distribution of their nevi (log-normal), to a mathematical model of OIS in which they assumed stochastic (random) arrest of individual cells with a certain probability per cell division or per time, following oncogene activation. This model predicted highly skewed size distributions on a log axis, rather than log-normal distributions as observed. This was very good evidence that their nevi were not arrested by a stochastic process. Additional considerations, for example sizes versus locations of cell nests within nevi, and the crowding of the nevi (median spacing 79 μm), supported the conclusion that the arrest involved cell interactions, rather than being an autonomous and permanent type of arrest, like senescence (or at least like the initiation of senescence).

In comparison, the similar $Braf^{V600E}$/*Tyr::CreERT2* mouse strain of Dhomen et al. (2009) gave somewhat different biological results when the oncogene was induced 2–3 months after birth. A smaller number of nevus-like lesions was obtained, somewhat larger than those of Ruiz-Vega et al. (images suggest diameters around 0.5–2 mm), and spaced further apart. This difference likely reflects the changing numbers of epidermal melanocytes in mice, which peak around 2–4 days of age, at ~1000/mm^2 in the closely related C57BL/10J mouse strain, falling to zero by around 30 days when

all melanocytes are normally in hair-follicles (Hirobe, 1982). These lesions were positive for 2/2 tested cell senescence markers, β-galactosidase, and $p16^{CDKN2A}$ (markers detailed later) (Dhomen et al., 2009). The apparently fewer but larger lesions of Dhomen et al. contrast with those seen by Ruiz-Vega et al., consistently with interactions between Ruiz-Vega et al.'s crowded micro-nevi mutually halting lesional growth, but with clones being able to grow larger to the point of senescence if they have more space. Likewise, $Braf^{V600E}$-driven nevus-like clones in mice, initiated at 9 weeks, ceased growth while quite well-spaced, and resembled senescent melanocytes in expressing p16 and BCL-W (BCL2L2) (Kohli et al., 2022). These lesions interestingly showed specific sensitivity to combined "senolytic" inhibitors of the BCL-family and MCL1 anti-apoptotic proteins (Kohli et al., 2022).

In short, Ruiz-Vega et al. (2020) appear correct concerning their small, crowded melanocytic colonies induced in a $Braf^{V600E}$ mouse model just after birth, that the arrest was due to cell interactions rather than senescence. It is questionable however whether these should be called nevi, since they are so different from human nevi. Other groups who induced $Braf^{V600E}$ in similar but older mice found that the sparser clones grew larger and then did show evidence of senescence, more like typical human nevi (Dhomen et al., 2009; Kohli et al., 2022; Wang et al., 2023).

3 | NEST-SIZE MODELING AND HUMAN NEVI

Ruiz-Vega et al. (2020) proposed that their conclusion on non-senescence could also be applied to human nevi, on two specific grounds. One was the clinical evidence that some human nevi can regenerate after excision. The rate of this recurrence varies between reports, from 0.3% to 27% (Vilain et al., 2016). Irrespective of the reasons for such variation, this clearly does imply that some human nevi contain some nevus melanocytes capable of dividing. Indeed, rare mitoses can be observed within excised nevi (Mooi & Krausz, 2007). However, this does not imply that all or most nevus cells can divide.

The other ground was the authors' measured size distribution for nests within a sample of five human nevi. They observed a log-normal distribution of radii of nests, implying that these nests did not fit a stochastic model for OIS, assuming nests represent subclones of the nevus. As a side-issue, not all types of human nevi even have distinct nests. The common simple lentigo consists of a patch of more-closely-spaced than normal melanocytes in the basal epidermis (Mooi & Krausz, 2007). These are rarely excised and studied, however. Excised nevi tend to be larger and thicker, those suspected of malignancy.

Even restricting modeling to nevi containing nests, there are problems with testing predictions of a simple model against a collection of nest radii pooled from several people, since humans are not inbred and have nevus size distributions that differ widely between individuals, so their nests could also differ in size, depending on many possible factors. The core assumption of the model, that only the stochastic process would be generating the variation in size, is not applicable here. Genetic factors are known to affect human nevus size (next section). The person's age at the time of nevus initiation is another factor. Congenital nevi (initiated before birth), for instance, can be far larger than any acquired nevus. This led to the speculation that telomere length is a determinant of human nevus size (Bastian, 2003), a speculation later confirmed (next section). If a stochastic process were the only determinant, then age and telomeres should not matter. Conversely, if nevus cells were capable of dividing but reversibly arrestable by paracrine interactions such as an inhibitory factor(s) made by all colony cells, we would expect cell division to stop in the lesion's center, but to continue around the edges where cells have fewer neighbors, as seen with cell colonies in culture (Stoker & Rubin, 1967). To complicate matters, it has been reported that senescent melanocytes in epidermis can induce senescence in neighboring cells in paracrine fashion (Victorelli et al., 2019). However, by definition this would not be reversible.

Stochastic molecular events are believed to regulate other important cellular processes including reversible telomere uncapping (Rodriguez-Brenes & Peskin, 2010), and initiation of DNA synthesis (Lee et al., 2010). However, a stochastic model for OIS lacks an obvious molecular basis. Our understanding is that "standard" OIS works through the largely deterministic processes of replication stress and DNA damage (Bartkova et al., 2006; Di Micco et al., 2006), rather than through some unknown event(s) that occurs at random and until then allows cells to carry on proliferating.

For all these reasons, the nevus nest size distribution does not weigh strongly against cell senescence in human nevi, especially given the variety of evidence in support—genetic, molecular and biological. These findings, apparently not considered by Ruiz-Vega et al., will now be reviewed.

4 | GENETIC EVIDENCE ON SENESCENCE IN HUMAN NEVI

Genetics provides some of the strongest and broadest evidence connecting cell senescence with human nevi, as well as melanoma suppression, from studies of both population genetics and somatic genetic changes in sporadic melanoma. These will be considered in turn.

4.1 | Human population genetics of nevi

The population genetics of melanoma overlaps with that of nevi. Around 10% of human melanomas worldwide occur in people with a family history of melanoma (with some geographical variation), and over 50 genetic loci associated with melanoma risk have now been identified in genome-wide association studies (GWAS) (Bishop et al., 2002; Landi et al., 2020; Law et al., 2015). Some of these families (not all) also transmit a tendency to develop large numbers

of nevi including unusually large nevi. This is known as dysplastic nevus syndrome (DNS) or familial atypical multiple mole-melanoma (FAMMM) syndrome (Rashid et al., 2022). Of note, nevus number is not entirely separable from nevus size in clinical practice, since counts normally include lesions over 2 mm in diameter, whereas lentigos of diameter <2 mm are also common on adult human skin (as many of us can see on our own skin). Thus, a general tendency for larger lentigo sizes would lead to higher nevus counts. The phenotype of large nevi is found from twin studies to have high heritability of ~65% (Newton-Bishop et al., 2020). A meta-analysis of GWAS studies generated 12 significant loci associated with nevus density (counts), all of which (with one possible exception, *KITLG*) were also associated with melanoma risk (Duffy et al., 2018). The target genes reflected multiple biological pathways, not surprisingly including pigmentation (protects against mutagenic UV light), but also many were linked to telomere maintenance and cell senescence.

The two best-established genes for FAMMM syndrome mutations are *CDKN2A* and *CDK4*, also the best-established high-penetrance genes for familial melanoma (Newton-Bishop et al., 2020; Rashid et al., 2022). *CDKN2A* (chromosome 9p21) encodes two unrelated protein splice variants that both function as cell-senescence mediators: $p16^{INK4A}$ (cyclin-dependent kinase inhibitor 2A, hereafter called p16) and $p14^{ARF}$ ("Alternative reading frame", hereafter ARF) (Bishop et al., 2002; Landi et al., 2020; Law et al., 2015). ARF and p16 have both shared and unique coding segments within *CDKN2A*, so germline or somatic mutations may potentially affect both products or either one selectively. CDK4 (cyclin-dependent kinase 4) is the main CDK inhibited by p16, and next mediator in the p16-RB1 (retinoblastoma) cell senescence pathway (Figure 1).

The majority of melanoma/FAMMM-associated mutations in *CDKN2A* affect the p16-specific segment (exon 1α) or the shared portion with ARF, and cause defects in the binding and inhibition of CDK4 by p16 (Bishop et al., 2002; Rashid et al., 2022). Melanoma/FAMMM-associated mutations in *CDK4* likewise lead to defective p16-binding. Thus, genetic defects in the p16/CDK4/RB1 cell-senescence pathway can lead to large and numerous moles as well as melanoma. ARF functions to upregulate the other chief mediator of cell senescence, p53 (Figure 1). ARF inhibits the degradation of p53, otherwise mediated by the E3 ubiquitin-protein ligase MDM2 (mouse double minute 2 homolog) (Ozenne et al., 2010). Germline mutations specifically affecting ARF have also been reported in a few melanoma families, though indirect transcriptional effects of these mutations on p16 have not been ruled out. These families, and those with combined p16/ARF defects, also appear to have an increased risk of nerve-sheath and other tumors (Sargen et al., 2016).

Another potential connection between nevi and cell senescence concerns telomeres, the protective structures at the ends of linear chromosomes, consisting of highly repetitive DNA coated in the protein complex shelterin (Gorgoulis et al., 2019). The ribonucleoprotein enzyme telomerase is required for maintenance or extension of telomere length, and includes subunits TERT (telomerase reverse transcriptase) and TERC (telomerase

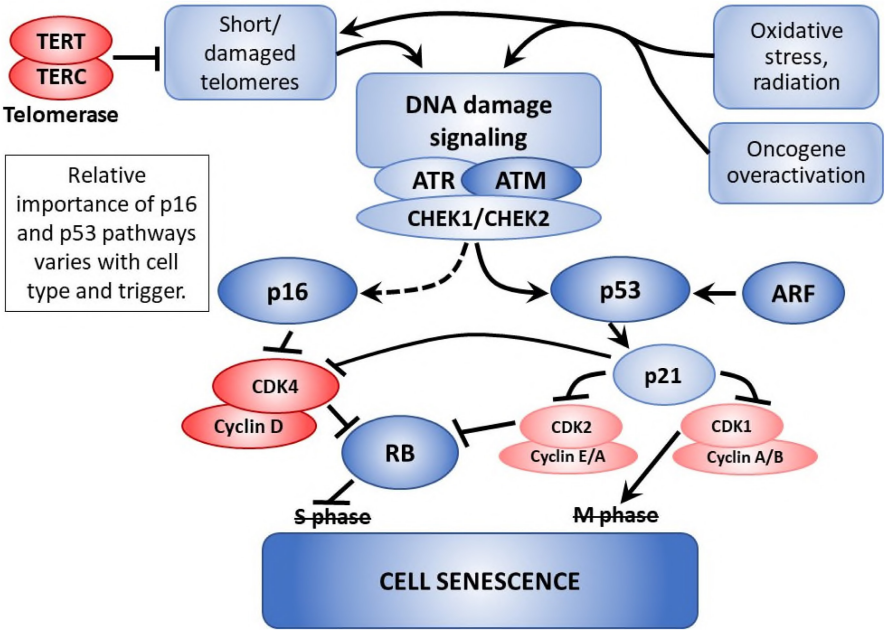

FIGURE 1 Senescence signaling in humans: core pathways. Simplified to show the roles of key intermediates relevant to nevi. See Bennett (2016) for more detail and interacting pathways. Blue: pro-senescence signaling; red: anti-senescence signaling. T-bars: inhibition. Darker shades indicate components subject to significant mutation in familial and/or sporadic melanoma (Cancer Genome Atlas Network, 2015; Landi et al., 2020; Law et al., 2015; Tate et al., 2019). RB represents the RB protein family (RB1, RBL1, RBL2). p21 is also called CDKN1A. Dashed arrow: upregulation of p16 by senescence/DNA-damage signaling is functionally established but the mechanism is unknown. S and M phases refer to the cell cycle. The p16 pathway appears more important than the p53 pathway in human nevi and generally in primary senescence of human melanocytes (see text for details).

RNA component). TERT protein is absent or nearly so in most normal human somatic cells, resulting in telomere shortening in dividing cells with age, and replicative senescence through DNA-damage signaling from short telomeres following extensive division (Figure 1) (Gorgoulis et al., 2019). Most immortal cancers (cells capable of indefinite proliferation) reactivate TERT expression, usually through promoter mutations (Gorgoulis et al., 2019). Telomere length distribution at birth varies between individuals. Concerning nevi, Bataille et al. (2007) observed that an individual's age-normalized telomere length was correlated with their numbers of melanocytic nevi, also with numbers of large nevi. The *TERC* and *TERT* loci are linked to high nevus counts (Duffy et al., 2018). These and other telomere-related genes including several for shelterin components are also high-penetrance loci for melanoma risk (Newton-Bishop et al., 2020). A genetic score predicting telomere length was specifically found to be a strong predictor of melanoma risk (Newton-Bishop et al., 2020). Rare germline mutations in the *TERT* promoter are also associated with familial melanoma, and large, atypical and multiple nevi have also been reported in members of these families (Horn et al., 2013; Zaremba et al., 2022). An interesting biological finding was that when normal epidermal melanocytes were explanted from multiple-melanoma patients carrying no known melanoma susceptibility gene, they consistently showed significantly longer culture lifespans than those from single-melanoma patients, implying a general, systematic connection between individual melanoma susceptibility and delayed melanocyte senescence (Kohli, Tolomio, et al., 2017).

Taken together, these genetic findings imply that telomeres must have a role in nevus size, just as Bastian (2003) surmised, and so must the p16/CDK4 pathway. This suggests contributions of short telomeres as well as (or upstream of) the p16 pathway in control of human nevus size. Yet Michaloglou et al. (2005) assessed telomere length within human nevi and found no significant difference from that of neighboring normal cells. This apparent conflict is revisited below in the light of more-recent data.

4.2 | Evidence from genetics of nevus-to-melanoma progression

If nevi are arrested by cell senescence, then to resume proliferation and progress to dysplasia or melanoma, genetic (or epigenetic) alterations disrupting cell senescence will be expected. To become immortal, human cancers need both to inactivate the signaling pathways that effect senescence arrest, and to gain telomerase function, allowing telomere maintenance (Hayashi et al., 2015). We know the requirements for immortalization of human melanocytes in culture: telomere maintenance and full immortality can be conferred in cultured human melanocytes by the combination of p16 pathway loss/inactivation and exogenous expression of TERT (Gray-Schopfer et al., 2006; Sviderskaya et al., 2003). Both these changes (p16 loss and TERT upregulation) are seen in the great majority of advanced human melanomas and indeed are now the two commonest known alterations in melanomas. Activating *TERT* promoter mutations are found in around 70% of melanomas, or 85% of metastatic melanomas (Horn et al., 2013; Huang et al., 2013; Newton-Bishop et al., 2020). For p16, if we assume that loss by homozygous deletion, deleterious mutation or silencing by DNA methylation will usually be mutually exclusive (only one needed), then we can add their incidences together, in which case p16 is defective in ~88% of melanomas (Bennett, 2016).

The incisive studies of Shain, Bastian et al. (Shain et al., 2015; Shain & Bastian, 2016) have related such changes to specific stages of progression. They sequenced 293 cancer-related genes from sporadic human melanomas and their spatially associated precursor lesions (benign nevi and/or "intermediate" lesions between nevus and melanoma) (Shain et al., 2015). They observed that nevi typically showed only a single mitogenic driver mutation (*BRAF*, *NRAS*), while associated intermediate lesions (and corresponding melanomas) displayed the same driver plus most commonly (77%) an activating *TERT* promoter mutation; or a heterozygous (usually) deletion or mutation in *CDKN2A*, or both. Homozygous *CDKN2A* deletions were observed only in invasive melanomas, where they were frequent. This work showed that the commonest first step in escape from the growth arrest in benign nevi is upregulation of *TERT* via a promoter mutation, or alternatively a defect in *CDKN2A*. A recent preprint from the same center reported that *TERT* promoter mutations in dysplastic nevi were seen only in those nevi without *BRAF* mutations, but with other MAPK (proliferative signaling) pathway mutations such as in *NRAS*, instead. Dysplastic nevi with *TERT* promoter mutations had shorter average telomere lengths than others, and were found in older patients (Lorbeer et al., 2023). Chiba et al. (2017) further reported that a single *TERT* promoter mutation within a nevus is insufficient to prevent overall telomere shortening, but repairs the shortest telomeres, allowing resumed proliferation. Telomere shortening continues into early melanomas, leading to telomeric crisis and chromosomal instability at this stage, which can be overcome by further *TERT* upregulation (Chiba et al., 2017). This accords with the common presence of crisis markers in primary melanomas and their common lack of immortality when explanted (Soo, MacKenzie Ross, Kallenberg, et al., 2011).

4.3 | Human-mouse molecular differences in cell senescence and nevus arrest

Cell-senescence mediators appear to have undergone relatively recent evolution, consistent with the widely varying lifespans and cancer rates of different vertebrates. For example, ARF in mice ($p19^{ARF}$) is different from and larger than $p14^{ARF}$ in humans (Ozenne et al., 2010). p16 is not even present in the chicken, only an even-smaller ARF and p16's paralog p15 (INK4B; *CDKN2B*) (Kim et al., 2003). Mice also have much longer telomeres than humans and yet mouse replicative cell senescence occurs quite rapidly, within weeks, in normal cell-culture conditions, while human cells

often take many months to senesce. Mouse cell senescence in culture is not triggered by extensive division and telomere shortening (as in human cells), but typically by oxidative damage through the near-atmospheric oxygen level usually used for culture, well above tissue levels (Coppé et al., 2010). Likewise, mouse cells including melanocytes (Sviderskaya et al., 2002) do not require *TERT* activation to become immortal, only *Cdkn2a* loss. Another mammal, the elephant, has 20 copies of the *TP53* (p53) gene, and low cancer incidence (Sulak et al., 2016). Caution is thus required in extrapolating senescence-related findings between different mammals and vertebrates; core pathways (Figure 1) are largely shared, but can differ markedly in detail and triggering.

Of the two *CDKN2A* protein products, p16 broadly appears the more important in humans (see sections 4.1 and 4.2), while ARF appears the more important in mice. Mice with ARF deletion and normal p16 developed a variety of spontaneous tumors, not including melanoma (Kamijo et al., 1997), while p16-null, ARF-competent mice also developed tumors, but at later ages and of only a few types, including melanoma (Sharpless et al., 2001). The difference is also seen in melanocyte senescence and its role in nevus arrest and tumor suppression. Dhomen et al. (2009) reported that p16 was not required for the arrest of nevi initiated in older $Braf^{V600E}$/*Tyr::CreERT2* mice. They did not study ARF, but Ferguson et al. (2010) found that intact ARF was required for arrest of nevi induced by neonatal UV irradiation in other model mice, including $Tyr::Nras^{Q61K}$, where specific abrogation of ARF led to "skipping" of a nevus stage and early onset of melanoma.

Regarding requirements for senescence, mouse melanocyte strains explanted from skin of ARF-null, p16-competent mice completely failed to senesce, and grew immediately as immortal cells (Ha et al., 2007). In comparison, p16-null, ARF-competent mouse melanocytes were also immortal from the outset, but were dependent for survival on keratinocytes or keratinocyte-derived growth factors (Ha et al., 2007), recalling the similar dependence seen in human p16-null, ARF-competent melanocytes (Sviderskaya et al., 2003).

In cultured human melanocytes however, p16 (not ARF) is required for the normal timing of replicative senescence. Either germline or engineered loss of *CDKN2A* or of active p16 from human melanocytes, with normal ARF, permits many more cell doublings than in normal adult melanocytes with wild-type p16 (Fung et al., 2013; Gray-Schopfer et al., 2006; Sviderskaya et al., 2003; Zeng et al., 2018). Delayed senescence then occurs in culture, with activation of the p53-p21 pathway (Gray-Schopfer et al., 2006). p53 signaling also appears in melanoma progression in vivo, but appears incomplete and ineffectual in arresting the tumor (MacKenzie Ross et al., 2013), consistent with the finding that p53 remains wild-type in around 80% of human melanomas (review: Bennett, 2016). Normal repression of TERT is also of course important in melanocyte senescence and normal nevus size. Reexpression of TERT plus abrogation of the p16/RB pathway were found to be necessary and sufficient to immortalize human melanocytes (Gray-Schopfer et al., 2006). Chudnovsky et al. also blocked human melanocyte senescence by viral transduction of three dominant mutations blocking the p16/RB and p53 pathways and expressing TERT, and they added activated NRAS as driver. They showed that diploid human melanocytes were thereby converted to invasive melanoma-like lesions upon xenografting to mice. Without TERT, noninvasive nevus-like lesions were formed (Chudnovsky et al., 2005).

5 | PROLIFERATION-RELATED MARKERS AND PERMANENT ARREST

As mentioned, some human nevi can regenerate after incomplete excision, proving that these contained some non-senescent nevus cells, but this does not tell us how many cells. Senescence markers label up to 90% of acquired nevus cells where quantitated (section 6), leaving the possibility of a minority of non-senescent cells. Such cells are not easy to detect, however. Nevi show no or very few mitotic figures, varying with stage of evolution (e.g., Mooi & Krausz, 2007). Proliferation markers are likewise reported absent or almost absent from excised human nevi, including Ki67 (Johmura et al., 2014; Michaloglou et al., 2005); cyclins A, D1 and D3 and survivin/BIRC5 (Alonso et al., 2004), and cyclin B (Johmura et al., 2014). Lack of division markers may reflect either senescence, quiescence (reversible arrest), or a mixture. Melanocytes in human nevi are by definition more crowded than normal epidermal melanocytes, whether forming nests or not (Mooi & Krausz, 2007), so density-dependent arrest of some residual non-senescent cells is possible.

A strong test for permanent versus reversible arrest is explantation into culture, whereupon any local inhibitory tissue factors are diluted out and quiescent cells such as normal melanocytes can resume division. Soo, MacKenzie Ross, Kallenberg et al. (2011) prepared such explants from samples of 22 nevi and reported that the great majority of human benign nevus cells failed to proliferate, under culture conditions where normal human melanocytes grew well. The newly-explanted nevus cells often showed a flat and/or multidendritic morphology, typical of senescent human melanocytes (Soo, MacKenzie Ross & Bennett, 2011). Some nevus cultures (15/22) did yield a small number of colonies of proliferating melanocytes among the arrested cells; these dividing cells could be subcultured but senesced after one to several passages, showing several markers of senescence (Soo, MacKenzie Ross, Kallenberg, et al., 2011). They were not able to exclude that the rare proliferating cells were normal melanocytes potentially trapped in the nevus. McNeal et al. (2015) also explanted human nevi ($n=27$). They did not observe flat and multidendritic cells, possibly because of a different explantation protocol. Still, they observed no proliferation, again under conditions where normal melanocytes grew well, and they used sequencing to demonstrate the heterozygous presence of the $BRAF^{V600E}$ oncogene in all the arrested cultures tested. These explantation studies provide persuasive evidence that few to no cells within most human benign nevi can proliferate.

6 | MOLECULAR AND MORPHOLOGICAL MARKERS OF SENESCENCE IN HUMAN NEVI

It has been noted that some molecular senescence markers found in nevi, such as DNA damage markers, can also be found in malignant melanomas (MacKenzie Ross et al., 2013; Tran et al., 2012). This has led to questioning of whether these can really be considered as senescence markers or as evidence that nevus cells are senescent (Tran et al., 2012). However, cancer cells are a special case. Senescence gene-expression programs are complex (Gorgoulis et al., 2019), and can be upstream of or parallel to the actual effectors of arrest (Figure 1; Bennett, 2016). Accordingly, when cancer cells escape from senescence by mutations and deletions in the arrest pathways, they do not necessarily lose other elements of the program such as the SASP (senescence-associated secretory program—secretion of cytokines, proteases etc; Gorgoulis et al., 2019). DNA-damage signaling from telomeres can likewise continue after abrogation of its downstream arrest pathways (as seen in early melanoma: MacKenzie Ross et al., 2013), unless or until TERT is sufficiently reactivated to suppress telomere dysfunction (Suram et al., 2012). Accordingly, we should separate three different aims of studying senescence markers in nevi:

1. To ascertain whether nevi are generally senescent, to understand their arrest. Suitable for this would be previously established markers, functionally associated with cell senescence, and absent from normal, non-senescent melanocytes.
2. To investigate to what extent nevi can also contain non-senescent cells. This could involve multi-senescence-marker studies on tissue sections (seeking cells that lack all such markers), or positive markers/functional studies of ability to proliferate, as discussed above (Section 5).
3. To seek senescence-related markers that may help with differential diagnosis: present in nevi and reliably lost in early melanoma. These may be only a small subset of senescence markers in nevi. Among markers considered below, to the author's knowledge, only p16 is currently in clinical use as a diagnostic aid (Joselow et al., 2017).

No one molecular marker has been found that is present in all kinds of senescent cells and only in senescent cells. Thus, the presence of several established markers is required to demonstrate cell senescence clearly (Gorgoulis et al., 2019). An impressive range of molecular markers of senescence has indeed been detected by immunohistochemistry and immunofluorescence in human nevi (Table 1). One is the popular β-galactosidase (Gray-Schopfer et al., 2006; Michaloglou et al., 2005), a lysosomal enzyme that is "senescence-associated" if detected at pH 6 or higher (Dimri et al., 1995), simply representing abundant lysosomes. Another is protein p16, in nuclear (or nuclear plus cytoplasmic) location (references in Table 1). Nuclear location is important because mutant, inactive p16 can be highly expressed but cytoplasmic only (Gray-Schopfer et al., 2006). All investigators detect a mosaic expression pattern for p16, that is, not all nevus cells are labeled, and sometimes fewer than 50%. At least eight other established molecular senescence markers have been reported in human nevi (Table 1). Most of them are detected in most but not all nevus cells (Table 1), the highest quantitated rate being for DNA-damage marker 53BP1 (around 90% of cells). An unlabeled subpopulation does not in itself rule out all cells of a nevus being senescent, since completely senescent melanocytic cultures, showing no increase in cell number, typically show only about 60%–80% cells positive for any one senescence marker (Soo, MacKenzie Ross, Kallenberg, et al., 2011), much as with senescent human fibroblasts and β-galactosidase (Dimri et al., 1995). The report of McNeal et al. (2015) on p15(CDKN2B) (paralog of p16) was interesting, as they reported higher detection levels than for p16, in nevi and $BRAF^{V600E}$-transduced melanocytes. They also reported common loss of p15 in melanoma, although genetic *CDKN2B* defects were not found to arise commonly at the stage between melanoma and adjacent nevus (Shain et al., 2015).

An important marker common in nevi and other benign tumors is telomere-associated foci of DNA damage (TAFs, also known as TIFs: telomere-induced foci): colocated labeling for a DNA-damage marker and a telomere probe, indicating dysfunctional telomeres (Lai et al., 2018; Suram et al., 2012; Victorelli et al., 2019). The majority of cells in human nevi were found to show TAFs (Suram et al., 2012). TAFs can represent either critically short telomeres, with shelterin loss, or stable DNA damage within longer telomeres. Damage within telomeres is poorly repaired, exactly because the shelterin complex inhibits DNA repair. It can thus persist stably and trigger senescence. Such persistent telomeric DNA damage may be the cause of TAFs and senescence in nevi, since their TAF telomeres are not unusually short (Suram et al., 2012). Such DNA damage may arise through ultraviolet irradiation; through reactive oxygen species that are generated by melanin exposed to ultraviolet, especially pheomelanin (Brenner & Hearing, 2008); other unknown pathways, or a combination of these. Interestingly though, it has been noted that there is a minimum telomere length able to bind the PNA probe used in fluorescence (Q-FISH) measurements of telomere length, so the shortest telomeres will give no signal by this method (Lai et al., 2018). Accordingly, it is possible after all that nevi contain critically short telomeres, not visible by Q-FISH in the TAF method, as a cause of senescence. This would help to explain the finding that a single TERT promoter mutation, found to extend only the shortest telomeres (Chiba et al., 2017), is the commonest genetic change linked to progression of benign nevi (Shain et al., 2015). It also aligns with data in the preprint of Lorbeer et al. (2023), that dysplastic nevi with *TERT* promoter mutations contain shorter telomeres than other dysplastic nevi (so clonal TERT expression would give a selective advantage).

Certain morphological senescence features are also seen in some types of human nevi. These include large cells with large, pale nuclei and prominent nucleoli; and a multinucleate cell subpopulation (Gray-Schopfer et al., 2006; Mooi & Krausz, 2007), also partial tetraploid DNA content (Johmura et al., 2014). There is evidence that diploid human cells can arrest for senescence in both G1 and

TABLE 1 Senescence markers found in human banal/benign nevi.

Marker	Comments	% Nevus cells labeled	References
β-galactosidase	Lysosomal enzyme detected at suboptimal pH, reflecting abundant lysosomes[a]	~100% (continuous stain)	Michaloglou et al. (2005); Gray-Schopfer et al. (2006)
p16 protein[b,c]	Reliable marker only if present in nuclei. Cytoplasmic-only p16 can be mutant and is common in melanoma.	Variable, ~20–70%	Michaloglou et al. (2005); Gray-Schopfer et al. (2006); Suram et al. (2012); MacKenzie Ross et al. (2013); Fox et al. (2016); Kohli, Mir, et al. (2017)
p15 protein (CDKN2B)	p16 paralog. Proposed also important in growth-arrest.	NS. Most cells in image shown; most nevi.	McNeal et al. (2015)
PML bodies		~70% median	Tran et al. (2012)
γH2AX	Nuclear foci, component of DNA-damage signaling (DDS).	~50% median	Tran et al. (2012)
Phospho-CHEK2 (pThr68)	Nuclear foci, component of DDS.	Variable (below or above 50%)	MacKenzie Ross et al. (2013)
53BP1[b]	Nuclear foci, component of DDS.	~90%	Suram et al. (2012)
H2AFY (macroH2A)	Component of senescence-associated heterochromatin. Proposed as good senescence marker in vivo.	Almost all	Suram et al. (2012)
Dysfunctional telomeres	Telomere-associated foci of DDS (Colocated telomere probe and 53BP1).	~65%	Suram et al. (2012)
H4K40me3	Trimethylated lysine 40 of histone H4. Colocates with senescence-associated heterochromatin.	Most (from "typical" image)	Nelson et al. (2016)

Abbreviation: NS, not stated.

[a]Only congenital nevi tested, in both studies, by histochemistry (frozen sections). All other markers were assessed in formalin-fixed sections of acquired nevi.

[b]Note, a few normal melanocytes in adult human skin, in numbers increasing with age, label with p16 (Ressler et al., 2006) and 53BP1 (Suram et al., 2012). Presumed to be senescent.

[c]Antigen retrieval at pH 9.0 recommended (Kohli, Mir, et al., 2017).

G2 phases of the cell cycle, or in G1 with tetraploidy after skipping mitosis (Johmura et al., 2014; Mao et al., 2012). This may sometimes be because the M-phase CDK, CDK1, can be inhibited by p21, the universal CDK inhibitor (Figure 1; Sherr & Roberts, 1995), although unlikely in benign nevi, where little or no p21 is detectable (Gray-Schopfer et al., 2006; MacKenzie Ross et al., 2013). It may also happen through inhibition of CDK4 by p16, since CDK4 activates the G2/M master regulator FOXM1 (Anders et al., 2011), required for transcription of various mitotic effectors.

7 | CONCLUSION: WHAT KIND OF SENESCENCE?

To summarize concerning the mouse lesions called nevi by Ruiz-Vega et al. (2020), they were unlike human nevi. There was good evidence that these crowded, microscopic colonies were arrested by cell interactions rather than cell senescence. However, extrapolation of this conclusion to human nevi seems unwarranted, in the face of wide-ranging evidence for senescence in banal, acquired human nevi, as reviewed above. Regeneration by some human nevi shows that some nevus cells can divide, especially in recently initiated nevi that are still growing, and also in some clinically suspicious nevi that are excised. However, marker and explantation studies indicate that any such non-senescent subpopulation is typically small.

Should we call the senescence in nevi "oncogene-induced"? I agree with Suram et al. (2012), Damsky and Bosenberg (2017) and Ruiz-Vega et al. (2020) that it does not match the concept of OIS. As with fibroblasts, overexpression of $BRAF^{V600E}$ from a strong viral or housekeeping promoter in human melanocytes induced immediate OIS, after zero to a few cell divisions, not nearly sufficient to generate a visible nevus (Gray-Schopfer et al., 2006; Michaloglou et al., 2005; Zeng et al., 2018). This was not prevented by prior abrogation of p16, showing that OIS following oncogene overexpression does not require p16. However, when CRISPR-Cas9 was used to introduce the activating $BRAF^{V600E}$ mutation into genomic *BRAF* with its own promoter, in human melanocytes, the cells proliferated rather than undergoing immediate OIS (Zeng et al., 2018). This mimics nevi, which have obviously proliferated initially following the mutation. Zeng et al. also confirmed that specific impairment of p16 delayed melanocyte replicative senescence (allowed additional population doublings), as previously reported (Fung et al., 2013; Gray-Schopfer et al., 2006;

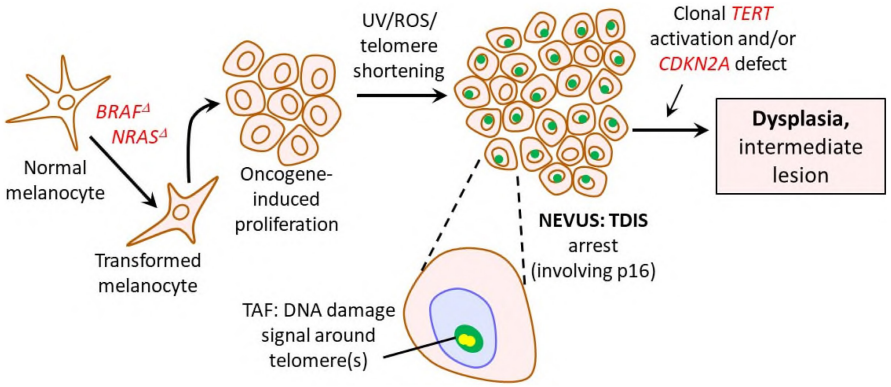

FIGURE 2 Updated model for human nevus senescence and escape. Activation of *BRAF* or *NRAS* by natural mutation (Δ) leads to transformation – proliferation through reduced requirement for external mitogens and endogenous upregulation of the MAPK mitogenic pathway. A combination of DNA damage within telomeres and telomere shortening then leads to TDIS in most cells, arresting growth as a banal nevus. A few cells are shown without TAFs, representing the possible presence of some non-senescent cells. Escape from this senescence is rare, and its most common first step is either a *TERT* promoter mutation or a *CDKN2A* defect.

Sviderskaya et al., 2003). Accordingly, the eventual senescence in nevi does not resemble OIS as seen in culture. Instead, nevi undergo oncogene-induced proliferation, followed after many divisions by cell senescence.

Reports of limited telomere shortening in nevi (Michaloglou et al., 2005) implied that nevus cell senescence did not resemble replicative senescence closely either. However, this now needs to be revisited following evidence that the shortest telomeres are invisible to a PNA probe (Lai et al., 2018), and preprint data that *TERT* promoter mutations in dysplastic nevi are associated with shorter telomeres (Lorbeer et al., 2023). At any rate there are abundant TAFs in cells of nevi, indicating either critically short telomeres or telomeric DNA damage, and *TERT* promoter mutations are associated with nevus progression (Chiba et al., 2018; Shain et al., 2015, 2016; Suram et al., 2012). The broad implication is that senescence in human nevi may sometimes after all be replicative senescence, and at present can broadly be described as TDIS: telomere-dysfunction-induced senescence (Suram et al., 2012). These conclusions are summarized in Figure 2.

Some unanswered questions remain. One is that of how to integrate the genetic and biological evidence for a role of p16 in nevus arrest with the histopathological finding that p16 is detectable in only a proportion of nevus cells by immunostaining. Another is the issue that telomerase is thought to be active only in proliferating cells, not in arrested cells (Holt et al., 1997), so how does it begin to repair telomeres after arrest? Perhaps rare nevus cells acquire such *TERT* promoter mutations already in the growth phase of the nevus, especially given that senescence can be a gradual process rather than an abrupt switch: some human cells can continue some divisions with one or a few DNA-damage foci (Nassrally et al., 2019). Colebatch et al. (2019) did report subclonal *TERT* promoter mutations within clinically benign nevi.

To conclude, concerning whether moles are senescent: the literature provides strong evidence that cell senescence is the primary cause of arrest in human banal nevi and that this arrest is a key tumor-suppression mechanism for melanoma, although some such nevi must include a minority of reversibly arrested cells. The senescence in nevi has the properties of TDIS rather than canonical OIS.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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REFERENCES

Alonso, S. R., Ortiz, P., Pollan, M., Perez-Gomez, B., Sanchez, L., Acuna, M. J., Pajares, R., Martínez-Tello, F. J., Hortelano, C. M., Piris, M. A., & Rodriguez-Peralto, J. L. (2004). Progression in cutaneous malignant melanoma is associated with distinct expression profiles - a tissue microarray-based study. *American Journal of Pathology*, *164*(1), 193–203. https://doi.org/10.1016/s0002-9440(10)63110-0

Anders, L., Ke, N., Hydbring, P., Choi, Y. J., Widlund, H. R., Chick, J. M., Zhai, H., Vidal, M., Gygi, S. P., Braun, P., & Sicinski, P. (2011). A systematic screen for CDK4/6 substrates links FOXM1 phosphorylation to senescence suppression in cancer cells. *Cancer Cell*, *20*(5), 620–634. https://doi.org/10.1016/j.ccr.2011.10.001

Bartkova, J., Rezaei, N., Liontos, M., Karakaidos, P., Kletsas, D., Issaeva, N., Vassiliou, L. V., Kolettas, E., Niforou, K., Zoumpourlis, V. C., Takaoka, M., Nakagawa, H., Tort, F., Fugger, K., Johansson, F., Sehested, M., Andersen, C. L., Dyrskjot, L., Ørntoft, T., … Gorgoulis, V. G. (2006). Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature*, *444*(7119), 633–637. https://doi.org/10.1038/nature05268

Bastian, B. C. (2003). The longer your telomeres, the larger your nevus? *American Journal of Dermatopathology*, *25*(1), 83–84.

Bataille, V., Kato, B. S., Falchi, M., Gardner, J., Kimura, M., Lens, M., Perks, U., Valdes, A. M., Bennett, D. C., Aviv, A., & Spector, T. D. (2007). Nevus size and number are associated with telomere length and represent potential markers of a decreased senescence in vivo. *Cancer Epidemiology, Biomarkers & Prevention*, *16*(7), 1499–1502. https://doi.org/10.1158/1055-9965.EPI-07-0152

Bennett, D. C. (2016). Genetics of melanoma progression: The rise and fall of cell senescence. *Pigment Cell and Melanoma Research*, *29*(2), 122–140. https://doi.org/10.1111/pcmr.12422

Bishop, D. T., Demenais, F., Goldstein, A. M., Bergman, W., Bishop, J. N., Bressac-de Paillerets, B., Chompret, A., Ghiorzo, P., Gruis, N., Hansson, J., Harland, M., Hayward, N., Holland, E. A., Mann, G. J., Mantelli, M., Nancarrow, D., Platz, A., & Tucker, M. A. (2002). Geographical variation in the penetrance of CDKN2A mutations for melanoma. *Journal of the National Cancer Institute*, *94*(12), 894–903. https://doi.org/10.1093/jnci/94.12.894

Braig, M., Lee, S., Loddenkemper, C., Rudolph, C., Peters, A. H., Schlegelberger, B., Stein, H., Dörken, B., Jenuwein, T., & Schmitt, C. A. (2005). Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature*, *436*(7051), 660–665. https://doi.org/10.1038/nature03841

Brenner, M., & Hearing, V. J. (2008). The protective role of melanin against UV damage in human skin. *Photochemistry and Photobiology*, *84*(3), 539–549. https://doi.org/10.1111/j.1751-1097.2007.00226.x

Cancer Genome Atlas Network. (2015). Genomic classification of cutaneous melanoma. *Cell*, *161*(7), 1681–1696. https://doi.org/10.1016/j.cell.2015.05.044

Centeno, P. P., Pavet, V., & Marais, R. (2023). The journey from melanocytes to melanoma. *Nature Reviews Cancer*, *23*(6), 372–390. https://doi.org/10.1038/s41568-023-00565-7

Chen, Z., Trotman, L. C., Shaffer, D., Lin, H. K., Dotan, Z. A., Niki, M., Koutcher, J. A., Scher, H. I., Ludwig, T., Gerald, W., Cordon-Cardo, C., & Pandolfi, P. P. (2005). Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature*, *436*(7051), 725–730. https://doi.org/10.1038/nature03918

Chiba, K., Lorbeer, F. K., Shain, A. H., McSwiggen, D. T., Schruf, E., Oh, A., Ryu, J., Darzacq, X., Bastian, B. C., & Hockemeyer, D. (2017). Mutations in the promoter of the telomerase gene. *Science*, *357*(6358), 1416–1420. https://doi.org/10.1126/science.aao0535

Chudnovsky, Y., Adams, A. E., Robbins, P. B., Lin, Q., & Khavari, P. A. (2005). Use of human tissue to assess the oncogenic activity of melanoma-associated mutations. *Nature Genetics*, *37*(7), 745–749. https://doi.org/10.1038/ng1586

Colebatch, A. J., Ferguson, P., Newell, F., Kazakoff, S. H., Witkowski, T., Dobrovic, A., Johansson, P. A., Saw, R. P. M., Stretch, J. R., Mcarthur, G. A., Long, G. V., Thompson, J. F., Pearson, J. V., Mann, G. J., Hayward, N. K., Waddell, N., Scolyer, R. A., & Wilmott, J. S. (2019). Molecular genomic profiling of melanocytic nevi. *Journal of Investigative Dermatology*, *139*(8), 1762–1768. https://doi.org/10.1016/j.jid.2018.12.033

Collado, M., Gil, J., Efeyan, A., Guerra, C., Schuhmacher, A. J., Barradas, M., Benguría, A., Zaballos, A., Flores, J. M., Barbacid, M., Beach, D., & Serrano, M. (2005). Tumour biology: Senescence in premalignant tumours. *Nature*, *436*(7051), 642. https://doi.org/10.1038/436642a

Collado, M., & Serrano, M. (2010). Senescence in tumours: Evidence from mice and humans. *Nature Reviews Cancer*, *10*(1), 51–57. https://doi.org/10.1038/nrc2772

Coppé, J. P., Patil, C. K., Rodier, F., Krtolica, A., Beauséjour, C. M., Parrinello, S., Hodgson, J. G., Chin, K., Desprez, P. Y., & Campisi, J. (2010). A human-like senescence-associated secretory phenotype is conserved in mouse cells dependent on physiological oxygen. *PLoS ONE*, *5*(2), e9188. https://doi.org/10.1371/journal.pone.0009188

Damsky, W. E., & Bosenberg, M. (2017). Melanocytic nevi and melanoma: Unraveling a complex relationship. *Oncogene*, *36*(42), 5771–5792. https://doi.org/10.1038/onc.2017.189

Dankort, D., Curley, D. P., Cartlidge, R. A., Nelson, B., Karnezis, A. N., Damsky, W. E., Jr., You, M. J., Ra, D. P., Mcmahon, M., & Bosenberg, M. (2009). *Braf*(V600E) cooperates with *Pten* loss to induce metastatic melanoma. *Nature Genetics*, *41*(5), 544–552. https://doi.org/10.1038/ng.356

Dhomen, N., Reis-Filho, J. S., Da Rocha, D. S., Hayward, R., Savage, K., Delmas, V., Larue, L., Pritchard, C., & Marais, R. (2009). Oncogenic Braf induces melanocyte senescence and melanoma in mice. *Cancer Cell*, *15*(4), 294–303. https://doi.org/10.1016/j.ccr.2009.02.022

Di Micco, R., Fumagalli, M., Cicalese, A., Piccinin, S., Gasparini, P., Luise, C., Schurra, C., Garre, M., Nuciforo, P. G., Bensimon, A., Maestro, R., Pelicci, P. G., & d'Adda di Fagagna, F. (2006). Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature*, *444*(7119), 638–642. https://doi.org/10.1038/nature05327

Dimri, G. P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E. E., Linskens, M., Rubelj, I., Pereira-Smith, O., & Campisi, J. (1995). A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proceedings of the National Academy of Sciences, USA*, *92*, 9363–9367. https://doi.org/10.1073/pnas.92.20.9363

Duffy, D. L., Zhu, G., Li, X., Sanna, M., Iles, M. M., Jacobs, L. C., Evans, D. M., Yazar, S., Beesley, J., Law, M. H., Kraft, P., Visconti, A., Taylor, J. C., Liu, F., Wright, M. J., Henders, A. K., Bowdler, L., Glass, D., Ikram, M. A., … Martin, N. G. (2018). Novel pleiotropic risk loci for melanoma and nevus density implicate multiple biological pathways. *Nature Communications*, *9*(1), 4774. https://doi.org/10.1038/s41467-018-06649-5

Ferguson, B., Konrad Muller, H., Handoko, H. Y., Khosrotehrani, K., Beermann, F., Hacker, E., Soyer, H. P., Bosenberg, M., & Walker, G. J. (2010). Differential roles of the pRb and Arf/p53 pathways in murine naevus and melanoma genesis. *Pigment Cell and Melanoma Research*, *23*(6), 771–780. https://doi.org/10.1111/j.1755-148X.2010.00752.x

Fox, C., Lambie, D., Wilmott, J. S., Pinder, A., Pavey, S., Lê Cao, K. A., Akalin, T., Karaarslan, I. K., Ozdemir, F., Scolyer, R. A., Yamada, M., Soyer, H. P., Schaider, H., & Gabrielli, B. (2016). Multiparameter analysis of naevi and primary melanomas identifies a subset of naevi with elevated markers of transformation. *Pigment Cell and Melanoma Research*, *29*(4), 444–452. https://doi.org/10.1111/pcmr.12489

Fung, C., Pupo, G. M., Scolyer, R. A., Kefford, R. F., & Rizos, H. (2013). p16INK4a deficiency promotes DNA hyper-replication and genetic instability in melanocytes. *Pigment Cell and Melanoma Research*, *26*(2), 236–246. https://doi.org/10.1111/pcmr.12062

Gorgoulis, V., Adams, P. D., Alimonti, A., Bennett, D. C., Bischof, O., Bishop, C., Campisi, J., Collado, M., Evangelou, K., Ferbeyre, G., Gil, J., Hara, E., Krizhanovsky, V., Jurk, D., Maier, A. B., Narita, M., Niedernhofer, L., Passos, J. F., Robbins, P. D., & Demaria, M. (2019). Cellular senescence: Defining a path forward. *Cell*, *179*(4), 813–827. https://doi.org/10.1016/j.cell.2019.10.005

Gray-Schopfer, V. C., Cheong, S. C., Chow, J., Moss, A., Abdel-Malek, Z. A., Marais, R., Wynford-Thomas, D., & Bennett, D. C. (2006). Cellular senescence in naevi and immortalisation in melanoma: A role for p16? *British Journal of Cancer*, *95*, 496–505. https://doi.org/10.1038/sj.bjc.6603283

Ha, L., Ichikawa, T., Anver, M., Dickins, R., Lowe, S., Sharpless, N. E., Krimpenfort, P., Depinho, R. A., Bennett, D. C., Sviderskaya, E. V., & Merlino, G. (2007). ARF functions as a melanoma tumor suppressor by inducing p53-independent senescence. *Proceedings of the National Academy of Sciences, USA*, *104*(26), 10968–10973. https://doi.org/10.1073/pnas.0611638104

Hayashi, M. T., Cesare, A. J., Rivera, T., & Karlseder, J. (2015). Cell death during crisis is mediated by mitotic telomere deprotection. *Nature*, *522*(7557), 492–496. https://doi.org/10.1038/nature14513

He, S., & Sharpless, N. E. (2017). Senescence in health and disease. *Cell*, *169*(6), 1000–1011. https://doi.org/10.1016/j.cell.2017.05.015

Hirobe, T. (1982). Genes involved in regulating the melanocyte and melanoblast populations in the epidermis of newborn mouse skin. *Journal of Experimental Zoology*, *223*, 257–264. https://doi.org/10.1002/jez.1402230307

Holt, S. E., Aisner, D. L., Shay, J. W., & Wright, W. E. (1997). Lack of cell cycle regulation of telomerase activity in human cells. *Proceedings of the National Academy of Sciences*, *USA*, *94*(20), 10687–10692. https://doi.org/10.1073/pnas.94.20.10687

Horn, S., Figl, A., Rachakonda, P. S., Fischer, C., Sucker, A., Gast, A., Kadel, S., Moll, I., Nagore, E., Hemminki, K., Schadendorf, D., & Kumar, R. (2013). TERT promoter mutations in familial and sporadic melanoma. *Science*, *339*(6122), 959–961. https://doi.org/10.1126/science.1230062

Huang, F. W., Hodis, E., Xu, M. J., Kryukov, G. V., Chin, L., & Garraway, L. A. (2013). Highly recurrent TERT promoter mutations in human melanoma. *Science*, *339*(6122), 957–959. https://doi.org/10.1126/science.1229259

Johmura, Y., Shimada, M., Misaki, T., Naiki-Ito, A., Miyoshi, H., Motoyama, N., Ohtani, N., Hara, E., Nakamura, M., Morita, A., Takahashi, S., & Nakanishi, M. (2014). Necessary and sufficient role for a mitosis skip in senescence induction. *Molecular Cell*, *55*(1), 73–84. https://doi.org/10.1016/j.molcel.2014.05.003

Joselow, A., Lynn, D., Terzian, T., & Box, N. F. (2017). Senescence-like phenotypes in human nevi. *Methods in Molecular Biology*, *1534*, 175–184. https://doi.org/10.1007/978-1-4939-6670-7_17

Kamijo, T., Zindy, F., Roussel, M. F., Quelle, D. E., Downing, J. R., Ashmun, R. A., Grosveld, G., & Sherr, C. J. (1997). Tumor suppression at the mouse *INK4a* locus mediated by the alternative reading frame product p19^{ARF}. *Cell*, *91*(5), 649–659. https://doi.org/10.1016/s0092-8674(00)80452-3

Kim, S. H., Mitchell, M., Fujii, H., Llanos, S., & Peters, G. (2003). Absence of p16INK4a and truncation of ARF tumor suppressors in chickens. *Proceedings of the National Academy of Sciences*, *USA*, *100*(1), 211–216. https://doi.org/10.1073/pnas.0135557100

Kohli, J., Ge, C., Fitsiou, E., Doepner, M., Brandenburg, S. M., Faller, W. J., Ridky, T. W., & Demaria, M. (2022). Targeting anti-apoptotic pathways eliminates senescent melanocytes and leads to nevi regression. *Nature Communications*, *13*(1), 7923. https://doi.org/10.1038/s41467-022-35657-9

Kohli, J. S., Mir, H., Wasif, A., Chong, H., Akhras, V., Kumar, R., Nagore, E., & Bennett, D. C. (2017). ETS1, nucleolar and non-nucleolar TERT expression in nevus to melanoma progression. *Oncotarget*, *8*(61), 104408–104417. https://doi.org/10.18632/oncotarget.22254

Kohli, J. S., Tolomio, E., Frigerio, S., Maurichi, A., Rodolfo, M., & Bennett, D. C. (2017). Common delayed senescence of melanocytes from multiple primary melanoma patients. *Journal of Investigative Dermatology*, *137*(3), 766–768. https://doi.org/10.1016/j.jid.2016.10.026

Kuilman, T., Michaloglou, C., Vredeveld, L. C., Douma, S., Van, D. R., Desmet, C. J., Aarden, L. A., Mooi, W. J., & Peeper, D. S. (2008). Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell*, *133*(6), 1019–1031. https://doi.org/10.1016/j.cell.2008.03.039

Lai, T. P., Wright, W. E., & Shay, J. W. (2018). Comparison of telomere length measurement methods. *Philosophical Transactions of the Royal Society B*, *373*(1741), 20160451. https://doi.org/10.1098/rstb.2016.0451

Landi, M. T., Bishop, D. T., MacGregor, S., Machiela, M. J., Stratigos, A. J., Ghiorzo, P., Brossard, M., Calista, D., Choi, J., Fargnoli, M. C., Zhang, T., Rodolfo, M., Trower, A. J., Menin, C., Martinez, J., Hadjisavvas, A., Song, L., Stefanaki, I., Scolyer, R., … Law, M. H. (2020). Genome-wide association meta-analyses combining multiple risk phenotypes provide insights into the genetic architecture of cutaneous melanoma susceptibility. *Nature Genetics*, *52*(5), 494–504. https://doi.org/10.1038/s41588-020-0611-8

Law, M. H., Bishop, D. T., Lee, J. E., Brossard, M., Martin, N. G., Moses, E. K., Song, F., Barrett, J. H., Kumar, R., Easton, D. F., Pharoah, P. D. P., Swerdlow, A. J., Kypreou, K. P., Taylor, J. C., Harland, M., Randerson-Moor, J., Akslen, L. A., Andresen, P. A., Avril, M. F., … Iles, M. M. (2015). Genome-wide meta-analysis identifies five new susceptibility loci for cutaneous malignant melanoma. *Nature Genetics*, *47*(9), 987–995. https://doi.org/10.1038/ng.3373

Lee, T. J., Yao, G., Bennett, D. C., Nevins, J. R., & You, L. (2010). Stochastic E2F activation and reconciliation of phenomenological cell-cycle models. *PLoS Biology*, *8*(9), e1000488. https://doi.org/10.1371/journal.pbio.1000488

Lorbeer, F. K., Rieser, G., Goel, A., Wang, M., Oh, A., Yeh, I., … Hockemeyer, D. (2023). Distinct senescence mechanisms restrain progression of dysplastic nevi. *bioRxiv*, 548818. https://doi.org/10.1101/2023.07.14.548818

MacKenzie Ross, A. D., Cook, M. G., Chong, H., Hossain, M., Pandha, H. S., & Bennett, D. C. (2013). Senescence evasion in melanoma progression: Uncoupling of DNA-damage signaling from p53 activation and p21 expression. *Pigment Cell and Melanoma Research*, *26*(2), 226–235. https://doi.org/10.1111/pcmr.12060

Mao, Z., Ke, Z., Gorbunova, V., & Seluanov, A. (2012). Replicatively senescent cells are arrested in G1 and G2 phases. *Aging (Albany NY)*, *4*(6), 431–435. https://doi.org/10.18632/aging.100467

McNeal, A. S., Belote, R. L., Zeng, H., Urquijo, M., Barker, K., Torres, R., Curtin, M., Shain, A. H., Andtbacka, R. H., Holmen, S., Lum, D. H., Mccalmont, T. H., Vanbrocklin, M. W., Grossman, D., Wei, M. L., Lang, U. E., & Judson-Torres, R. L. (2021). BRAF^{V600E} induces reversible mitotic arrest in human melanocytes via microRNA-mediated suppression of AURKB. *eLife*, *10*, e70385. https://doi.org/10.7554/eLife.70385

McNeal, A. S., Liu, K., Nakhate, V., Natale, C. A., Duperret, E. K., Capell, B. C., Dentchev, T., Berger, S. L., Herlyn, M., Seykora, J. T., & Ridky, T. W. (2015). CDKN2B loss promotes progression from benign melanocytic nevus to melanoma. *Cancer Discovery*, *5*(10), 1072–1085. https://doi.org/10.1158/2159-8290.CD-15-0196

Michaloglou, C., Vredeveld, L. C., Soengas, M. S., Denoyelle, C., Kuilman, T., Van der Horst, C. M., Majoor, D. M., Shay, J. W., Mooi, W. J., & Peeper, D. S. (2005). BRAF^{E600}-associated senescence-like cell cycle arrest of human naevi. *Nature*, *436*(7051), 720–724. https://doi.org/10.1038/nature03890

Mooi, W. J., & Krausz, T. (2007). *Pathology of melanocytic disorders* (2nd ed.). Hodder Arnold.

Mooi, W. J., & Peeper, D. S. (2006). Oncogene-induced cell senescence—halting on the road to cancer. *New England Journal of Medicine*, *355*(10), 1037–1046. https://doi.org/10.1056/NEJMra062285

Nassrally, M. S., Lau, A., Wise, K., John, N., Kotecha, S., Lee, K. L., & Brooks, R. F. (2019). Cell cycle arrest in replicative senescence is not an immediate consequence of telomere dysfunction. *Mechanisms of Ageing and Development*, *179*, 11–22. https://doi.org/10.1016/j.mad.2019.01.009

Nelson, D. M., Jaber-Hijazi, F., Cole, J. J., Robertson, N. A., Pawlikowski, J. S., Norris, K. T., Criscione, S. W., Pchelintsev, N. A., Piscitello, D., Stong, N., Rai, T. S., McBryan, T., Otte, G. L., Nixon, C., Clark, W., Riethman, H., Wu, H., Schotta, G., Garcia, B. A., … Adams, P. D. (2016). Mapping H4K20me3 onto the chromatin landscape of senescent cells indicates a function in control of cell senescence and tumor suppression through preservation of genetic and epigenetic stability. *Genome Biology*, *17*(1), 158. https://doi.org/10.1186/s13059-016-1017-x

Newbold, R. F., & Overell, R. W. (1983). Fibroblast immortality is a prerequisite for transformation by EJ c-Ha-ras oncogene. *Nature*, *304*(5927), 648–651. https://doi.org/10.1038/304648a0

Newton-Bishop, J., Bishop, D. T., & Harland, M. (2020). Melanoma genomics. *Acta Dermato-Venereologica*, *100*(11), adv00138. https://doi.org/10.2340/00015555-3493

Ozenne, P., Eymin, B., Brambilla, E., & Gazzeri, S. (2010). The ARF tumor suppressor: Structure, functions and status in cancer. *International Journal of Cancer*, *127*(10), 2239–2247. https://doi.org/10.1002/ijc.25511

Pérez-Mancera, P. A., Young, A. R., & Narita, M. (2014). Inside and out: The activities of senescence in cancer. *Nature Reviews Cancer*, *14*(8), 547–558. https://doi.org/10.1038/nrc3773

Rashid, S., Gupta, S., McCormick, S. R., & Tsao, H. (2022). New insights into melanoma tumor syndromes. *JID Innovations*, *2*(6), 100152. https://doi.org/10.1016/j.xjidi.2022.100152

Ressler, S., Bartkova, J., Niederegger, H., Bartek, J., Scharffetter-Kochanek, K., Jansen-Durr, P., & Wlaschek, M. (2006). p16INK4A is a robust in vivo biomarker of cellular aging in human skin. *Aging Cell*, *5*(5), 379–389. https://doi.org/10.1111/j.1474-9726.2006.00231.x

Rodriguez-Brenes, I. A., & Peskin, C. S. (2010). Quantitative theory of telomere length regulation and cellular senescence. *Proceedings of the National Academy of Sciences, USA*, *107*(12), 5387–5392. https://doi.org/10.1073/pnas.0914502107

Roh, M. R., Eliades, P., Gupta, S., & Tsao, H. (2015). Genetics of melanocytic nevi. *Pigment Cell and Melanoma Research*, *28*(6), 661–672. https://doi.org/10.1111/pcmr.12412

Ruiz-Vega, R., Chen, C. F., Razzak, E., Vasudeva, P., Krasieva, T. B., Shiu, J., Caldwell, M. G., Yan, H., Lowengrub, J., Ganesan, A. K., & Lander, A. D. (2020). Dynamics of nevus development implicate cell cooperation in the growth arrest of transformed melanocytes. *eLife*, *9*, e61026. https://doi.org/10.7554/eLife.61026

Sargen, M. R., Merrill, S. L., Chu, E. Y., & Nathanson, K. L. (2016). CDKN2A mutations with p14 loss predisposing to multiple nerve sheath tumours, melanoma, dysplastic naevi and internal malignancies: A case series and review of the literature. *British Journal of Dermatology*, *175*(4), 785–789. https://doi.org/10.1111/bjd.14485

Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D., & Lowe, S. W. (1997). Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell*, *88*, 593–602. https://doi.org/10.1016/s0092-8674(00)81902-9

Shain, A. H., & Bastian, B. C. (2016). From melanocytes to melanomas. *Nature Reviews Cancer*, *16*(6), 345–358. https://doi.org/10.1038/nrc.2016.37

Shain, A. H., Yeh, I., Kovalyshyn, I., Sriharan, A., Talevich, E., Gagnon, A., Dummer, R., North, J., Pincus, L., Ruben, B., Rickaby, W., Darrigo, C., Robson, A., & Bastian, B. C. (2015). The genetic evolution of melanoma from precursor lesions. *New England Journal of Medicine*, *373*(20), 1926–1936. https://doi.org/10.1056/NEJMoa1502583

Sharpless, N. E., Bardeesy, N., Lee, K. H., Carrasco, D., Castrillon, D. H., Aguirre, A. J., Wu, E. A., Horner, J. W., & DePinho, R. A. (2001). Loss of $p16^{Ink4a}$ with retention of $p19^{Arf}$ predisposes mice to tumorigenesis. *Nature*, *413*(6851), 86–91. https://doi.org/10.1038/35092592

Sherr, C. J., & Roberts, J. M. (1995). Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes and Development*, *9*(10), 1149–1163. https://doi.org/10.1101/gad.9.10.1149

Soo, J. K., MacKenzie Ross, A. D., Kallenberg, D. M., Milagre, C., Chong, H., Chow, J., Hill, L., Hoare, S., Collinson, R. S., Hossain, M., Keith, W. N., Marais, R., & Bennett, D. C. (2011). Malignancy without immortality? Cellular immortalization as a possible late event in melanoma progression. *Pigment Cell and Melanoma Research*, *24*, 490–503. https://doi.org/10.1111/j.1755-148X.2011.00850.x

Soo, J. K., MacKenzie Ross, A. D., & Bennett, D. C. (2011). Isolation and culture of melanoma and naevus cells and cell lines. *Methods in Molecular Biology*, *731*, 141–150. https://doi.org/10.1007/978-1-61779-080-5_13

Stoker, M., & Rubin, H. (1967). Density dependent inhibition of cell growth in culture. *Nature*, *215*, 171–172. https://doi.org/10.1038/215171a0

Sulak, M., Fong, L., Mika, K., Chigurupati, S., Yon, L., Mongan, N. P., Emes, R. D., & Lynch, V. J. (2016). *TP53* copy number expansion is associated with the evolution of increased body size and an enhanced DNA damage response in elephants. *eLife*, *5*, e11994. https://doi.org/10.7554/eLife.11994

Suram, A., Kaplunov, J., Patel, P. L., Ruan, H., Cerutti, A., Boccardi, V., Fumagalli, M., Di Micco, R., Mirani, N., Gurung, R. L., Hande, M. P., d'Adda di Fagagna, F., & Herbig, U. (2012). Oncogene-induced telomere dysfunction enforces cellular senescence in human cancer precursor lesions. *EMBO Journal*, *31*(13), 2839–2851. https://doi.org/10.1038/emboj.2012.132

Sviderskaya, E. V., Gray-Schopfer, V. C., Hill, S. P., Smit, N. P., Evans-Whipp, T. J., Bond, J., Hill, L., Bataille, V., Peters, G., Kipling, D., Wynford-Thomas, D., & Bennett, D. C. (2003). p16/cyclin-dependent kinase inhibitor 2A deficiency in human melanocyte senescence, apoptosis, and immortalization: Possible implications for melanoma progression. *Journal of the National Cancer Institute*, *95*(10), 723–732. https://doi.org/10.1093/jnci/95.10.723

Sviderskaya, E. V., Hill, S. P., Evans-Whipp, T. J., Chin, L., Orlow, S. J., Easty, D. J., Cheong, S. C., Beach, D., Ra, D. P., & Bennett, D. C. (2002). p16(Ink4a) in melanocyte senescence and differentiation. *Journal of the National Cancer Institute*, *94*, 446–454. https://doi.org/10.1093/jnci/94.6.446

Tate, J. G., Bamford, S., Jubb, H. C., Sondka, Z., Beare, D. M., Bindal, N., Boutselakis, H., Cole, C. G., Creatore, C., Dawson, E., Fish, P., Harsha, B., Hathaway, C., Jupe, S. C., Kok, C. Y., Noble, K., Ponting, L., Ramshaw, C. C., Rye, C. E., … Forbes, S. A. (2019). COSMIC: The catalogue of somatic mutations in cancer. *Nucleic Acids Research*, *47*(D1), D941–D947. https://doi.org/10.1093/nar/gky1015

Tran, S. L., Haferkamp, S., Scurr, L. L., Gowrishankar, K., Becker, T. M., Desilva, C., Thompson, J. F., Scolyer, R. A., Kefford, R. F., & Rizos, H. (2012). Absence of distinguishing senescence traits in human melanocytic nevi. *Journal of Investigative Dermatology*, *132*(9), 2226–2234. https://doi.org/10.1038/jid.2012.126

Victorelli, S., Lagnado, A., Halim, J., Moore, W., Talbot, D., Barrett, K., Chapman, J., Birch, J., Ogrodnik, M., Meves, A., Pawlikowski, J. S., Jurk, D., Adams, P. D., van Heemst, D., Beekman, M., Slagboom, P. E., Gunn, D. A., & Passos, J. F. (2019). Senescent human melanocytes drive skin ageing via paracrine telomere dysfunction. *EMBO Journal*, *38*(23), e101982. https://doi.org/10.15252/embj.2019101982

Vilain, R. E., McCarthy, S. W., & Scolyer, R. A. (2016). The regenerating naevus. *Pathology*, *48*(2), 108–112. https://doi.org/10.1016/j.pathol.2015.12.009

Wang, X., Ramos, R., Phan, A. Q., Yamaga, K., Flesher, J. L., Jiang, S., Oh, J. W., Jin, S., Jahid, S., Kuan, C. H., Nguyen, T. K., Liang, H. Y., Shettigar, N. U., Hou, R., Tran, K. H., Nguyen, A., Vu, K. N., Phung, J. L., Ingal, J. P., … Plikus, M. V. (2023). Signalling by senescent melanocytes hyperactivates hair growth. *Nature*, *618*(7966), 808–817. https://doi.org/10.1038/s41586-023-06172-8

Zaremba, A., Meier, F., Schlein, C., Jansen, P., Lodde, G., Song, M., Kretz, J., Möller, I., Stadtler, N., Livingstone, E., Zimmer, L., Hadaschik, E., Sucker, A., Schadendorf, D., & Griewank, K. (2022). Clinical and pathological characteristics of familial melanoma with germline TERT promoter variants. *Pigment Cell and Melanoma Research*, *35*(6), 573–586. https://doi.org/10.1111/pcmr.13060

Zeng, H., Jorapur, A., Shain, A. H., Lang, U. E., Torres, R., Zhang, Y., As, M. N., Botton, T., Lin, J., Donne, M., Bastian, I. N., Yu, R., North, J. P., Pincus, L., Ruben, B. S., Joseph, N. M., Yeh, I., Bastian, B. C., & Judson, R. L. (2018). Bi-allelic loss of *CDKN2A* initiates melanoma invasion via BRN2 activation. *Cancer Cell*, *34*(1), 56–68.e59. https://doi.org/10.1016/j.ccell.2018.05.014

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