Published in final edited form as:

Dermatol Clin. 2017 January ; 35(1): 85–93. doi:10.1016/j.det.2016.08.001.

Melanocytic Nevi and the Genetic and Epigenetic Control of Oncogene-Induced Senescence

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Synopsis

Melanocytic nevi represent benign melanocytic tumors of the skin which usually remain stable in size and behavior or disappear during life. Infrequently, melanocytic nevi undergo malignant transformation to melanoma. Nevertheless, a considerable percentage of cutaneous melanomas arise from melanocytic nevi, and the presence of large numbers of melanocytic nevi represents an independent risk factor for melanoma development. Identification of oncogenic somatic mutations in melanomas and melanocytic nevi highlights the molecular connection between cutaneous melanomas and their benign precursor lesions. The properties of melanocytic nevi can be explained by the concept of oncogene-induced senescence, a phenomenon which restricts the continued growth of cells expressing oncogenes. Understanding molecular and cellular mechanisms underlying oncogene-induced senescence should help identify pathways underlying melanoma development, leading to the development of new strategies for melanoma prevention and early detection.

Keywords

melanocyte; melanoma; nevi; oncogene; senescence

CONTENT

Melanocytic nevi

A melanocytic nevus is a benign clonal proliferation of melanocytes, the pigment-producing cells of the epidermis, hair follicle, and uveal tract of the eye (Argenziano et al., 2007). Melanocytes are normally interspersed as single cells among keratinocytes in human skin, resting atop the basement membrane. In melanocytic nevi, they are present in greater

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concentrations, either singly or in adherent “nests” or clusters of three or more melanocytic cells. The clinical appearances of melanocytic nevi are heterogeneous, associated in part with when during life the nevus is acquired and likely due as well to the specific differentiation state of the cell of origin and, as we will see, their acquired genetic mutations. They can be considered in two major groups, congenital and acquired melanocytic nevi.

A congenital nevus is present in 1 to 3 % of neonates at birth and shortly thereafter (Price et al., 2010). They are categorized according to their size (small <1.5 cm; medium 1.5–20 cm; large >20 cm–40 cm, giant>40cm) (Schaffer, 2015). Congenital nevi tend to have a globular pattern on dermoscopy and terminal hair follicles. Histologically, congenital nevi consist of big melanocytes which are fusiform, epithelioid, ballooned, or neuroid in shape tracking down from large nests between collagen bundles along cutaneous appendages, vessels and nerves (Argenziano et al., 2007). They extend deep into the reticular dermis and the subcutis. It is postulated that these nevi are a result of clonal proliferation of a melanoblast during embryogenesis.

Acquired nevi appear early in childhood, after the first year of life, and increase in number with age, peaking during the 3rd or 4th decade of life (Bataille et al., 1998, Grulich et al., 1996). Heredity and environment (UV radiation) are predisposing factors. Clinically, they are usually smaller (<6 mm in diameter), flat, superficial and horizontally oriented lesions than congenital nevi. Dermatoscopically, they can have a reticular, globular, or homogeneous appearance, alone in combination (Figure 1). The distinct patterns probably correspond to different arrangements of the nested and adherent lesional melanocytes. Histologically, melanocytes in acquired nevi usually do not involve the reticular dermis or the subcutis and the melanocytes are monomorphous and small with an oval shape.

Most melanocytic nevi either disappear or remain stable during life with fewer than 5% undergoing detectable change when closely monitored (Haenssle et al., 2006, Kittler et al., 2000, Robinson and Nickoloff, 2004). However, it is estimated that 25–50% of cutaneous melanomas arise from melanocytic nevi as precursor lesions (Bevona et al., 2003, Sagebiel, 1993). A recent meta-analysis by Lin et al of 13 studies involving over 4000 cases revealed that 32% of melanomas are in fact associated with a nevus (Lin et al., 2014). Risk of malignant transformation is associated with increased size of congenital nevi (Egan et al., 1998), and risk of cutaneous melanoma correlates with number of total and clinically dysplastic nevi (Tucker et al., 1997) (Newton-Bishop et al., 2010).

In contrast to their malignant counterparts, most melanocytic nevi are benign tumors that initially proliferate but remain stable or disappear during life. However, despite these differences in behavior, nevi and melanomas share somatic mutations in common. A high proportion of large and giant congenital nevi have activating mutations at codon 61 of *NRAS*, one of the 3 major isoforms of the RAS family of GTPase proteins involved in cell growth, survival, and differentiation (Bauer et al., 2006) (Roh et al., 2015). Small and medium sized congenital melanocytic nevi, as well as many acquired melanocytic nevi, contain a key mutation in the *BRAF* gene, resulting in substitution of glutamic acid for valine at position V600 of the protein within the kinase domain in exon 15 (Pollock et al., 2003). This mutation results in constitutively active *BRAF*^{V600E} (Wellbrock et al., 2004).

BRAF is a serine-threonine kinase that is activated by the RAS family of proteins which, when activated, triggers the MAPK signaling cascade. Up to 80% of benign nevi carry the *BRAF*^{V600E} mutation (Pollock et al., 2003). Both mutations are found at high frequency in cutaneous melanomas, with the *BRAF*^{V600E} mutation being detected in about 60–70 % of malignant melanomas (Curtin et al., 2005). Initial correlation of somatic mutations in nevi with dermatoscopic pattern suggests that *BRAF* mutations may be most closely associated with globular, rather than reticular, melanocytic nevi (Marchetti et al., 2014). Despite activation of the MAPK pathway, which mediates a potent proliferative signal, benign nevi lose all proliferative activity. The paradox of stable melanocytic proliferations exhibiting oncogenic mutations at high frequency led to the suggestion that melanocytic nevi represent the outcome of oncogene-induced senescence in the skin (Bennett, 2003).

Cellular senescence

Most normal mammalian cells are unable to keep growing indefinitely. After 40–70 cell divisions in culture, the cell enters a state of senescence. In this state, basic metabolic processes occur, but the cell neither dies or divides, a phenomenon initially characterized by Hayflick with human fibroblasts (Shay and Wright, 2000, Hayflick and Moorhead, 1961).

Senescent cells display many phenotypic changes which, individually, cannot definitively indicate senescence, but can be used in combination to determine whether a cell or population of cells has undergone senescence. A major indicator of senescence is the cessation of cell division, combined with a resistance to undergo apoptosis. Another commonly accepted marker of senescent cells is the expression of β -galactosidase that is detectable at pH 6, referred to as senescence-associated β -galactosidase (SA β -gal) (Dimri et al., 1995). Several other phenotypic changes are associated with senescence. Senescent cells often show a striking morphological change—the cell flattens and may adopt a more dendritic shape (Campisi and d'Adda di Fagagna, 2007). Finally, a senescence-activated secretory phenotype, which is marked by an increased expression of secreted proteins, including cytokines, is activated (Coppe et al., 2008).

In addition to these cell-wide changes, several changes are also apparent on the molecular level in senescent cells. These cells show punctate condensation of their chromatin known as senescence-associated heterochromatic foci (SAHF). In addition to these chromatin changes, and perhaps in some cases due to these changes, there are a large number of genes that show expression changes after a cell enters senescence. Some of these genes are directly involved in the senescence process, such as the cell cycle regulators p16^{INK4A} and p21^{WAF1}, which are induced in senescence and maintain the tumor suppressor retinoblastoma protein (Rb) in its hypophosphorylated, and active, state (Campisi and d'Adda di Fagagna, 2007).

Another variant of senescence, premature senescence, has been characterized. Premature senescence refers to senescence that occurs without any detectable telomere dysfunction and can be induced by various stimuli. These stimuli include environmental stress in vitro, such as suboptimal nutrient levels or oxygen tension, and oncogene activation. The phenomenon of oncogene-induced senescence was first described as a proliferative cell cycle arrest in primary human and mouse fibroblasts following the retroviral introduction of oncogenic *HRAS*^{G12V}. Forced expression of *HRAS*^{G12V} in these cells results in cellular changes

indistinguishable from the phenotype observed upon replicative senescence, and is associated with increases in the expression of p16, p53, and p21. Oncogene-induced senescence is absolutely dependent upon p16 and p53 in rodent cells, but their loss is not sufficient to abrogate this effect in human cells (Serrano et al., 1997). Several mechanisms of oncogene-induced senescence induction and escape are listed in Table 1.

In humans, the concept of oncogene induced senescence (OIS) is well illustrated in the pathogenesis of melanocytic nevi. The forced expression of *BRAF*^{V600E} in primary human melanocytes leads to a transient cellular proliferation followed by a growth arrest and induction of p16 and SA β -gal expression as senescence markers, similar to the changes reported upon expression of *HRAS*^{G12V} in fibroblasts (Serrano et al., 1997) (Michaloglou et al., 2005). A model for the transient proliferation, followed by growth arrest and senescence, of melanocytes upon oncogene activation to form a nevus is presented in Figure 2.

Melanocytic nevi in vivo express senescence markers as well, leading to the suggestion that oncogene-induced senescence in human melanocytes represents a barrier to tumor progression in melanoma development (Gray-Schopfer et al., 2006) (Michaloglou et al., 2005). Oncogene induced senescence has also been shown to occur in model systems. Patton et al. (Patton et al., 2005) showed that overexpression of *BRAF*^{V600E} in transgenic zebrafish led to the development of fish nevi and in mice, expression of *BRAF*^{V600E} in melanocytes produced nevi with biochemical evidence of senescence (Goel et al., 2009). A concept of how acquisition of an activating *NRAS* mutation in a melanocyte precursor, or melanoblast, during embryonic development might lead to the formation of a congenital melanocytic nevus is depicted in Figure 3.

Maintenance and loss of senescence in nevi and melanoma - role of genetics and epigenetics

Understanding the mechanisms underlying the breakdown of the senescence barrier, leading to further tumor progression, may lead to advances in maintaining or reimposing it as a therapeutic strategy. The mechanisms that these cells develop to escape oncogene-induced senescence are largely unknown but have been explored in vitro as well as in vivo. Table 1 summarizes the effects of many of the proteins and mechanisms on oncogene-induced senescence to be discussed. Studies in zebrafish have shown that oncogene-induced senescence can be reversed in *BRAF*^{V600E} expressing melanocytic nevus cells through experimental suppression of p53 (Patton et al., 2005). Suppression of RB function as well as loss of the phosphatase and tensin homolog (PTEN) and overexpression of SETDB1 have similarly been shown to abrogate oncogene induced senescence experimentally (Sage et al., 2000) (Dankort et al., 2009) (Ceol et al., 2011). Vredeveld et al. recently demonstrated that PI3K pathway activation acts as a crucial step in melanomagenesis in vitro by terminating *BRAF*^{V600E} induced senescence (Vredeveld et al., 2012).

Essential autophagy gene autophagy-related-7, *Atg7*, has also been shown to promote the development of melanoma in *BRAF*^{V600E} mutant *Pten-null* mice by overcoming senescence. In the same study, its loss increased oxidative stress, induced senescence, and improved the response to treatment with BRAF inhibitor dabrafenib (Xie et al, 2015).

Melanoma therapies targeting the OIS pathway are currently under investigation. In addition to the focus on BRAF inhibitors, vemurafenib and trametenib, which are clinically efficacious (Chapman et al., 2011) (Flaherty et al., 2012), there have also been initiatives to test these highly-selective BRAF inhibitors in combination with inhibitors of related pathways in melanomagenesis also relevant to senescence such as PIK3CA and *Atg7* (Liu et al., 2013) (Xie et al., 2015).

Polycomb group (PcG) proteins have been implicated as mediators of cellular senescence. PcG proteins are members of distinct macromolecular complexes, the major ones being Polycomb Repressive Complex (PRC) 1 and PRC2, which modify and interact with histones to maintain a cell's epigenetic state. The polycomb protein Bmi-1 was implicated in cellular senescence by virtue of its activity as an epigenetic repressor of the *Cdkn2a* locus, encoding the p16^{Ink4a} tumor suppressor, in murine cells. In human cells, overexpression of BMI-1 extended the time to replicative senescence which was associated with repression of p16 expression (Dimri et al., 1995). EZH2 is the catalytic component of the Polycomb Repressive Complex 2 (PRC2), also consisting of other key members such as SUZ12 and EED, which have not been shown to be catalytic, but play structural roles. EZH2 functions as a histone methyltransferase, adding a third methyl group to the ϵ -amino group of lysine 27 on histone 3, generating the H3K27me3 modification which is associated with gene repression in genome-wide studies. In both replicatively and prematurely senescent cells, a reduction in the expression of EZH2 is associated with reduction in the H3K27me3 histone mark and upregulation of p16 expression at the *Cdkn2a/CDKN2A* locus (Bracken et al., 2007). A partial explanation for the reduction of the H3K27me3 mark in senescent cells was provided when it was established that during RAS-RAF activation, the H3K27me3 demethylase JMJD3 was induced and recruited to the p16 promoter to counter PcG protein-mediated repression of *Cdkn2a* and cause the p16-dependent growth arrest characteristic of oncogene-induced senescence (Agger et al., 2009).

Increased expression of EZH2 is commonly observed in cancer (Kleer et al., 2003). The observation that EZH2 expression might progressively increase during melanoma progression, together with the suppression of the senescent state occurring during that process, suggested that there might be a relationship between EZH2 overexpression in melanoma cells and the suppression of the senescent state, thereby promoting melanoma development and tumorigenicity.

To confirm that EZH2 expression is associated with melanoma progression, we obtained specimens of benign human melanocytic nevi and metastatic melanoma tumors, and observed, on a cell-by-cell basis, a marked increase in the percentage of melanocytic cells expressing EZH2. To test the hypothesis that the induction of EZH2 expression between nevus and melanoma cells was responsible for the loss of senescence, and tumor progression, we utilized RNA interference to suppress the expression of *EZH2* in melanoma cells with *NRAS* activating mutations. Suppression of EZH2 in these cells led to the re-appearance of senescence markers such as SA β -gal, SAHF, and H3K9me3 foci, and was associated as well with other typical senescence changes such a G1 cell cycle arrest and an increase in cell and nuclear size. Stable suppression of *EZH2* expression was also found to

inhibit tumor cell colony formation in soft agar and in vivo tumor xenograft growth in immunodeficient mice (Fan et al., 2011).

Mechanistically, the induction of senescence in melanoma cells upon loss of EZH2 could not be attributed to an induction in p16 expression or activity, since all of the cells tested either did not express p16 or expressed a mutated form (Figure 4). Instead, we detected a p53-independent induction of p21 expression in most of the EZH2-suppressed melanoma cells, an induction that was found to be responsible for a significant proportion of the senescence effect. Nevertheless, the *CDKN1A* gene encoding p21 was not found to be a direct target of EZH2, exhibiting neither significant occupancy by EZH2 nor a high concentration of H3K27me3 modifications, as is typical for direct PRC2 interaction sites, following chromatin immunoprecipitation studies. Hence the direct targets of EZH2 in melanoma genomes responsible for the repression of p21/*CDKN1A* in these cells, and the suppression of the senescent state that promotes tumor progression, remain to be described. However, EZH2 expression has recently been found to be regulated by non-canonical NF- κ B signaling through stabilization of Rb via p21 and p53 (Iannetti et al., 2014) (De Donatis et al., 2015). This may be an explanation for the p53-independent induction of p21 that is seen in EZH2-suppressed melanoma cells. In addition, non-canonical NF- κ B signaling was shown to prevent oncogene-induced senescence. Continued activation of the non-canonical NF- κ B pathway in the context of EZH2 suppression would lead to an increase of p21 in an unsuccessful attempt by the cell to regulate EZH2 expression and bypass senescence. The roles of Polycomb group proteins in melanocyte and melanoma function have been summarized (Tiffen et al., 2015) (Huang and Hornyak, 2015).

Additional recent studies have extended findings about the role of PcG proteins in senescence. A major role of Cbx4, a PRC1 component, is to suppress senescence of epidermal stem cells through its chromodomain and interactions with the H3 tail domain. Cbx4 expression seems to be important in these cells to fine tune the transition of epidermal stem cells between the quiescent and active states (Luis et al., 2011). Cbx7, another PRC1 component, mediates senescence by binding to and repressing the classic PcG protein target *Ink4b/ARF/Ink4a*. Interestingly, its ability to bind to the locus and inhibit senescence is dependent not only upon its ability to recognize and bind H3K27me3, but also to bind *ANRIL*, a non-coding RNA transcribed from the locus which bridges the interactions between H3K27me3 and PRC1 (Yap et al., 2010). Ras-induced senescence in mouse embryonic fibroblasts is associated with dramatic changes in both H3K4me3 and H3K27me3 trimethylation that alter expression of critical components of the Bmp2-Smad1 signaling pathway, enforcing senescence through the induction of a senescence-associated secretory phenotype (Kaneda et al., 2011) Though each of these studies were performed using primary cells, it is likely that genome-wide chromatin immunoprecipitation studies with cancer cells will also reveal important and unrecognized Polycomb-regulated genes responsible for the suppression of senescence in these cells.

SUMMARY/DISCUSSION

Melanocytic nevi most likely represent the outcome of clonal proliferation of single melanocytes that acquire highly-specific, somatic mutations which escape DNA repair and

cause them to proliferate to a variable extent in the skin. These particular mutations, such as mutations at *BRAF*^{V600E} and at codon 61 in *NRAS*, likely function in a highly cell type-specific context to drive proliferation, since benign tumors arising from other cell types do not feature identical mutations at high frequency. Concomitantly, expression of the oncogene induces a senescence response in the proliferating melanocytes which stops cell growth and presents a barrier to further malignant transformation. Infrequently, nevi undergo transformation to malignant melanoma. This transition is associated not only with further genetic changes, such as loss or mutation of the *CDKN2A* locus encoding the p16 tumor suppressor, but also with epigenetic changes, such as chromatin-level silencing of tumor suppressor gene expression that is also a factor in tumor progression. Polycomb proteins, including proteins comprising Polycomb Repressive Complex 2 (PRC2), have important roles in cancer development. The members of this complex have been found to be overexpressed in a variety of cancers including melanoma. EZH2 expression increases progressively from being undetectable in normal melanocytes to low in nevi and high in melanoma, with an increase seen between primary and metastatic lesion. EZH2 depletion from melanoma cell lines has been shown to decrease proliferation, increase the percentage of cells with a senescent phenotype, and decrease the volume of tumors generated with a xenograft experiment. In addition, several mutations of EZH2 have been identified that increase the H3K27Me3 activity of the PRC2 complex in lymphoma. Growing evidence suggests that the epigenetic regulation catalyzed by PRC2 may play a role in regulating senescence. Senescence is a barrier to uncontrolled proliferation, but can be bypassed. Reversing senescence bypass and rendering proliferating tumor cells dormant as a result may be a useful adjunct to targeted therapies and immunotherapies that are currently utilized for advanced melanoma and remain under investigation for other cutaneous malignancies.

Acknowledgments

This work was supported in part by Merit Review Award 1I01BX002582 from the United States (U.S.) Department of Veterans Affairs, Biomedical Laboratory Research and Development Service; and by NIH Award R01 AR064810, National Institutes of Health, U.S. Department of Health and Human Services. The contents do not represent the views of the U.S. Department of Veterans Affairs, the U.S. Department of Health and Human Services, or the United States Government.

Abbreviations and Acronyms

BRAF

A member of the RAF family of serine/threonine-specific kinases which is frequently mutated in human melanoma and is a molecular target for therapy

OIS

oncogene-induced senescence

NRAS

member of the RAS family of GTPases (small GTPase proteins) which mediate growth factor receptor signaling and are critical for cell proliferation, survival, and differentiation. Activating mutations in NRAS proto-oncogene, particularly at codon 61, are also common in human melanoma

BRAF^{V600E}

The V600E mutation results in an amino acid substitution of valine (V) to glutamic acid (E) at position 600 of BRAF.

MAP kinase (MAPK) signaling pathway:

Responsible for relaying extracellular signals from cell membrane to nucleus. Dysregulation of this pathway due to activating mutations in BRAF, RAS and other genes leads to increased signaling activity leading to cell proliferation, invasion, metastasis, migration, survival and angiogenesis.

Senescence-associated β -galactosidase- (SA β -gal)

β -galactosidase activity that is detectable at pH 6.0 in cells undergoing replicative or induced senescence that is absent in proliferating cells. This is the most commonly used biomarker for senescence.

Senescence-associated heterochromatic foci (SAHF)

Domains of condensed chromatin, or heterochromatin, that often form in senescent human cells. They play a role in repressing proliferation-promoting genes and their detection can help identify senescent cells.

p16 (also p16^{INK4A})

tumor suppressor protein that functions as a cyclin-dependent kinase inhibitor and is encoded by the CDKN2A gene. Plays a significant role in cell cycle regulation; tumor suppressor implicated in the prevention of melanoma and many other cancers. It is one of the genes associated with hereditary melanoma and plays a role in cell senescence.

p21^{WAF1}

cyclin-dependent kinase inhibitor that mediates p53-dependent cell cycle arrest and likely plays a role as a tumor suppressor. This protein also inhibits apoptosis and may promote cell proliferation in some tumors.

p53

mutation of this tumor suppressor gene is common in melanoma, more so in many other cancers. In normal cells, p53 plays a role in cell cycle arrest and DNA repair or apoptosis, and can mediate cellular senescence.

HRAS^{G12V}

oncogenic Ras protein that is frequently mutated in cancers. When the amino acid glycine G replaced with amino acid valine V at codon 12, it becomes permanently activated within the cell (proto-oncogene), leading to uncontrolled cell division and tumor formation.

RB

tumor suppressor gene which normally arrests cells in the G1 or G1/S phase of the cell cycle by acting as a transcriptional repressor. Loss or inactivation can lead to uncontrolled cell proliferation

Phosphatase and tensin homolog (PTEN)

tumor suppressor gene which is frequently lost/inactivated in melanoma. The PTEN protein is a phosphatase which negatively regulates the PI3K/Akt pathway and influences cell adhesion, migration, and invasion.

SETDB1

Histone methyltransferase which is overexpressed in melanoma and accelerates its onset in zebrafish melanoma models harboring the BRAF V600E mutation

PI3K-AKT pathway

activation of this pathway is one of the most significant signaling pathways in melanoma. It plays a role in melanoma initiation and resistance to therapeutics.

Atg7

Essential autophagy gene, autophagy-related-7, promotes melanoma by limiting oxidative stress and overcoming senescence; inhibition may be of therapeutic value.

PRC

polycomb repressive complexes 1 and 2 (PRC1 and PRC2) which are protein complexes associated with chromatin condensation and transcriptional repression (epigenetic modifications) PRC1 catalyzes the ubiquitylation of histone H2A and PRC2 methylates H3K27.

Bmi-1

polycomb complex protein, member of PRC1, which regulates cell cycle inhibitor genes (p16). Its overexpression may promote tumor invasion and metastasis.

EZH2 (Enhancer of zeste homolog 2)

epigenetic modifier and catalytic component of the polycomb repressive complex 2 (PRC2), which is thought to promote growth and metastasis of melanoma. Increased expression is associated with uncontrolled proliferation in melanoma.

SUZ12

component of the PRC2 complex

EED (embryonic ectoderm development)

component of PRC2

JMJD3

Histone demethylase which promotes melanoma progression and metastasis through regulation of NF-kappa B and BMP signaling

NF-kappaB

inducible transcription factor which regulates the expression of genes involved in the immune response and is involved in the regulation of apoptosis, angiogenesis, and tumor cell invasion. Its activity is upregulated in human melanoma and many other cancers

Cbx4-PRC1

associated protein which protects epidermal stem cells from senescence by maintaining them in their undifferentiated state.

ANRIL

antisense non-coding RNA which is involved in chromatin remodeling, transcription, posttranscriptional processing. It is often abnormally expressed in cancer.

BMP (Bone Morphogenic Protein)

member of the transforming growth factor-beta (TGF-beta) superfamily which are involved in proliferation, apoptosis, differentiation, chemotaxis, and angiogenesis. It has been shown to have potent anti-tumor activity in the skin. In melanoma, it is overexpressed and is thought to promote cell invasion and migration.

SMAD1

gene which encodes a protein involved in the downstream signaling pathway of bone morphogenic protein (BMP)

BMP-Smad1

this signal and its regulation by epigenetic alterations are significant in Ras-induced senescence

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Key Points

- Melanocytic nevi share oncogenic molecular mutations with melanomas
- Oncogene-induced senescence explains in part why most nevi are stable and do not undergo progression to malignant melanoma
- Determining mechanisms which reverse melanoma cells to a senescent phenotype could lead to the identification of therapeutic adjuncts in advanced melanoma

Figure 1.

Dermoscopic patterns of acquired melanocytic nevi. (Left) Acquired melanocytic nevus with reticular, or net-like, pattern visible through homogeneous brown pigmentation.

(Center) Acquired nevus with distinct globular dermoscopic pattern. (Right) Acquired nevus with combined reticular and globular dermoscopic pattern.

Oncogene activation and cellular senescence

Figure 2.

Model for the development of a melanocytic nevus following activation of BRAF with acquisition of $BRAF^{V600E}$ somatic mutation. Acquisition of the activating mutation (most likely from an unrepaired oxidative modification to guanine) results in transient melanocyte proliferation driven by MAP kinase signaling (center). Subsequently a senescence response is induced and growth arrest occurs, leaving a clonal population of oncogene-harboring, melanocytic cells forming the nevus.

Figure 3.

Concept for the development of large congenital melanocytic nevus following activation of NRAS following Q61 mutation in melanoblast. Depicted is a mouse *Dct-lacZ* embryo (Horn yak et al., 2001) where individual melanoblasts are stained blue. Melanoblasts migrate from the neural tube, at the “back” of the embryo, across to the ventral surface. Acquisition of a somatic activating mutation at codon 61 of NRAS in a single melanoblast may lead to massive proliferation of clonal progeny before oncogene-induced senescence results in growth arrest.

Adapted from Horn yak TJ, Hayes DH, Chiu LY, et al. Transcription factors in melanocyte development: distinct roles for Pax-3 and Mitf. *Mech Dev* 2001;101:47–59; with permission.

Figure 4.

Model for the bypass of senescence in melanoma cells induced by EZH2 and/or non-canonical NF-κB signaling. Senescence in melanocytes can be mediated by either the tumor suppressors p16 or p53, but p16 function is invariably inactivated in most cutaneous melanomas whereas p53 gene sequence and function usually remains intact. EZH2 induction in melanoma cells results in senescence bypass by suppressing the expression of pro-senescent p21 downstream of p53-p21 activation. EZH2 expression is dependent upon non-canonical NF-κB signaling through the p52 and RelB subunits.

Table 1**Mechanisms of Oncogene-Induced Senescence Induction and Escape**

Mechanism	Epigenetic or Signaling Consequence	Effect on Senescence
BRAF ^{V600E} mutation	Constitutive activation of MAPK signaling	Senescence induction
NRAS activating mutations	Constitutive activation of MAPK signaling	Senescence induction
p53 suppression	Abrogated DNA Damage checkpoints, cell cycle changes	Suppress oncogene-induced senescence
RB suppression	Cell cycle changes	Suppress oncogene-induced senescence
SETDB1 overexpression	Chromatin remodeling	Suppress oncogene-induced senescence
PI3K pathway activation	Cell cycle changes	Suppress oncogene-induced senescence
Atg7 depletion	Increased oxidative stress	Suppress oncogene-induced senescence
BMI-1 activation	Repression of <i>CDKN2A</i>	Suppress oncogene-induced senescence
EZH2 activation	H3K27me3 trimethylation	Suppress oncogene-induced senescence
NF- κ B signaling (non-canonical)	RB stabilization	Suppress oncogene-induced senescence
CBX4	Chromatin remodeling	Suppress oncogene-induced senescence
CBX7	Repression of <i>Ink4b/ARF/Ink4a</i>	Suppress oncogene-induced senescence