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Genetics of Melanocytic Nevi

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Abstract

Melanocytic nevi are a benign clonal proliferation of cells expressing the melanocytic phenotype, with heterogeneous clinical and molecular characteristics. In this review, we discuss the genetics of nevi by salient nevi subtypes: congenital melanocytic nevi, acquired melanocytic nevi, blue nevi, and Spitz nevi. While the molecular etiology of nevi has been less thoroughly studied than melanoma, it is clear that nevi and melanoma share common driver mutations. Acquired melanocytic nevi harbor oncogenic mutations in *BRAF*, which is the predominant oncogene associated with melanoma. Congenital melanocytic nevi and blue nevi frequently harbor *NRAS* mutations and *GNAQ* mutations, respectively, while Spitz and atypical Spitz tumors often exhibit *HRAS* and kinase rearrangements. These initial "driver" mutations are thought to trigger the establishment of benign nevi. After this initial phase of cell proliferation, a senescence program is executed, causing termination of nevi growth. Only upon the emergence of additional tumorigenic alterations, which may provide an escape from oncogene-induced senescence, can malignant progression occur. Here, we review the current literature on the pathobiology and genetics of nevi in the hope that additional studies of nevi promise to inform our understanding of the transition from benign neoplasm to malignancy.

Keywords

congenital melanocytic nevi; acquired melanocytic nevi; Spitz nevi; blue nevi; genetics

Introduction

The term "melanocytic neoplasm" is used to describe the presence of melanocytic cells in epidermal nests, which are defined as three or more melanocytic cells in direct contact, within the dermis or other tissues. Melanocytic neoplasms represent a diverse group of tumors that can be either benign (nevi) or malignant (melanoma). Nevi are believed to be benign clonal proliferations of cells expressing the melanocytic phenotype (Magana-Garcia

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and Ackerman, 1990). The development of melanocytic nevi is a multifactorial and heterogeneous biologic process, and the molecular events resulting in melanocytic neoplasms are multifold and only beginning to be unraveled. In contrast to the extensive and genome-wide studies of melanomas, studies of benign melanocytic lesions have been restricted to mutational analyses targeting genes classically involved in melanomagenesis (Papp et al., 2003; Pollock et al., 2003). This lack of comprehensive molecular profiling is partly an artifact of limited nevi tissue availability after clinically biopsied samples are processed for histologic diagnoses. In melanoma, *BRAF* and *NRAS* gene mutations have been used as genetic markers in studies attempting to integrate genetic and morphologic features to improve classification (Cancer Genome Atlas Network. Electronic address and Cancer Genome Atlas, 2015; Pozzobon et al., 2014; Viros et al., 2008). *BRAF* is a serine-threonine kinase that is activated by the *RAS* family of proteins. Once activated, it triggers the MAPK signaling cascade that ultimately leads to cell cycle progression, upregulation of transcription, and differentiation. *NRAS* is one of the three major isoforms of the *RAS* family of GTPase proteins that are involved in cell growth, differentiation, and survival. A high frequency of oncogenic *BRAF* mutations at a V660E in the kinase domain of exon 15 has been reported in melanomas and melanocytic nevi, suggesting mutational activation of the RAS/RAF/MEK/ERK pathway is a critical step in the development of these melanocytic tumors (Davies et al., 2002; Pollock et al., 2003) (Figure 1).

In contrast to the various mouse models developed to investigate the molecular events underlying melanomagenesis, there are few models to investigate nevus development. The first model system employed targeted overexpression of $BRAF^{V600E}$ in the melanocyte compartment of transgenic zebrafish, which caused proliferation of melanocytes, producing fish nevi (Patton et al., 2005). Similarly, in mice, expression of $BRAF^{V600E}$ in the melanocytic lineage produced nevi with signs of senescence (Goel et al., 2009). Most recently, a mouse model has been developed that closely resembles nevi in humans. In this model, $BRAF^{V600E}$ was conditionally expressed from its endogenous promoter and restricted to melanocytes (Dankort et al., 2009) (Dhomen et al., 2009). Also, pigmented hairless mice with genetic deficiencies in *Ink4a*/*Arf* and *Xpa* genes can produce nevi (~1 to 2 per mouse), but none advance to melanoma (van Schanke et al., 2006). Also, a substantial proportion of these mice remain nevus-free, thus limiting their use as a model for nevus development. Nasti *et al*. developed a mouse model of nevus initiation and progression with C3H/HeN mice using a modified chemical carcinogenesis protocol (Nasti et al., 2015). In this model, dysplastic pigmented skin lesions appeared in 7–9 weeks with 100% penetrance and nests of melanocytic cells appeared in a subset of skin draining lymph nodes. This model may yield insight into acquired mutations at the early stages of nevus initiation and promotion of nevus cell transformation. However, additional nevus/melanoma models are needed to identify genetic underpinnings of nevus formation, novel oncogenic pathways, targets for immune-prevention, and mechanisms of nevus development.

Benign melanocytic neoplasms can be sub-categorized based on clinical and histological characteristics. Common subgroups include congenital melanocytic nevi (CMN), acquired melanocytic nevi, blue nevi, and Spitz nevi (Figure 2). We will discuss the genetics of nevi by type.

CONGENITAL MELANOCYTIC NEVI

Congenital melanocytic nevi (CMN) overwhelmingly occur in a sporadic pattern, with rare reports of familial cases, and as such are believed to result from somatic mutations *in utero* (Charbel et al., 2014). There have been several studies seeking to identify genetic hits underlying CMN pathogenesis, and the aggregate results of these studies suggest *NRAS* mutations and, to a lesser extent, *BRAF* mutations, contribute to the development of CMN (Table 1). The literature on the molecular characterization of CMN often cites discrepancies between studies demonstrating a high prevalence of *BRAF* mutations (Ichii-Nakato et al., 2006; Pollock et al., 2003; Qi et al., 2011; J. Wu et al., 2007; Yazdi et al., 2003), and others that report *NRAS* mutations as more common (Bauer et al., 2007; Carr and Mackie, 1994; Charbel et al., 2014; Dessars et al., 2009; Kinsler et al., 2013; D. Wu et al., 2011). These inconsistencies may be related to differences in study methodology. For example, some studies only sequenced CMN for either *BRAF* or *NRAS* mutations, but not both; others examined one size of CMN, or failed to describe the size of studied lesions altogether. Many of these studies do not report how diagnoses were made, or if lesions diagnosed as CMN were documented as being present at birth (Table 1).

More recent studies, which have included different CMN sizes, have refined the picture of this genetic landscape, revealing a consistent relationship between the size of lesion and mutation status (Table 1). *BRAF or NRAS* mutations are typically found in small CMN, but the prevalence of *NRAS* mutations is greater among medium to giant CMN. The increase in frequency of *NRAS* mutations is concomitant with a decrease in frequency of *BRAF* mutations. The most recent genetic study, conducted by Charbel *et al*. using multiple powerful sequencing techniques and comparable cohorts of small/medium and large/giant CMN documented at birth, affirms this trend. Of the 19 large/giant CMN examined, 18 (94.7%) were positive for an *NRAS* mutation and the remaining one harbored a *BRAF* mutation; 6 out of 20 (30%) small/medium CMN carried a *BRAF* mutation, and the remaining 70% were *NRAS*-mutants (Charbel et al., 2014) (Table 1). The inverse relationship between prevalence of *BRAF* mutations and size of CMN lesion is supported further by reports of zero *BRAF* mutations found in two series of giant CMN (De Raeve et al., 2006; D. Wu et al., 2011) and one series of medium/large CMN (Bauer et al., 2007) (Table 1).

It was aforementioned that many of these studies do not include statements regarding documentation confirming the presence of CMN lesions at birth, or arising shortly thereafter. This is an often overlooked weakness of these studies because, as Bauer *et al*. remarks, the diagnosis of CMN is not always made based on a documented history at birth. The distinctive histopathologic features of CMN are sometimes used to diagnose nevi with an unknown history as “congenital pattern nevi,” and nevi with this classification may be grouped together with documented congenital nevi. The accuracy of such diagnoses has been evaluated, and it has been repeatedly determined that the classic histopathologic features of CMN have limited specificity in predicting which nevi were truly present at birth (Bauer et al., 2007; Clemmensen and Kroon, 1988; Cribier et al., 1999; Rhodes et al., 1985). Misdiagnosing acquired nevi as congenital would presumably falsely increase the prevalence of *BRAF* mutations since acquired melanocytic nevi have high rates of *BRAF*

mutations (Pollock et al., 2003; Poynter et al., 2006; Uribe et al., 2003). Furthermore, as CMN larger than a few centimeters are unlikely to be missed at birth, misdiagnoses based on histological features are more likely to occur with small lesions and may account, in part, for the high rates of *BRAF* mutations reported in small CMN. Another potential cause for the variation in rates of observed mutations may be due to sampling errors secondary to low proportions of mutated nevus cells relative to keratinocytes and stroma, or a clonal heterogeneity of nevus cells (Charbel et al., 2014; Ichii-Nakato et al., 2006).

Attempts at molecularly characterizing CMN have primarily focused on genetic aberrations in the *BRAF* and *NRAS* loci, in part because of the increased risk of melanoma inherent to larger CMN (Krengel et al., 2006; Watt et al., 2004) and the prominent role of *BRAF* and *NRAS* mutations in melanoma biology. It is becoming clearer that different classes of CMN likely have different genetic signatures, but our knowledge of these genetic signatures has only scratched the surface, with few studies interested in potential targets of mutation other than *BRAF* and *NRAS*. Papp *et al*. screened 18 small/medium CMN for mutations and deletions in *TP53*, *CDKN2A*, and *CDK4* genes, and only found two silent mutations in *TP53* (Papp et al., 1999). Kinsler *et al*. also performed mutational analyses for several genes, reporting one incident each of *GNAQ* and *HRAS* mutations and 3 *KRAS* mutations from a panel of over two-dozen CMN (Kinsler et al., 2013). Interested in identifying other somatic mutations that might be cooperating with *NRAS* mutations to foster CMN pathogenesis, Charbel *et al*. performed whole exome sequencing on 5 large/giant CMN. After eliminating several possible hits as false positives using confirmatory sequencing, the only recurrent somatic mutation identified was, unsurprisingly, in the *NRAS* gene (Charbel et al., 2014).Shakhova *et al*. described a mouse model for giant congenital nevi and show that nevi and melanoma prominently express Sox10. Sox10 haploinsufficiency counteracts $NRAS^{Q61K}$-driven congenital nevus and melanoma formation without affecting the physiological functions of neural crest derivatives in the skin. Furthermore, they showed that SOX10 silencing in human melanoma cells suppresses neural crest stem cell properties, counteracting proliferation and cell survival, and completely abolishes *in vivo* tumor formation (Shakhova et al., 2012). Although much remains to be learned about CMN genetics, as sequencing technology continues to advance in accuracy, ease, and cost, it is plausible that CMN will one day be classified by genotype rather than phenotype.

ACQUIRED MELANOCYTIC NEVI

The term nevus is derived from a Latin root meaning “birthmark,” implying that nevi are present at birth. However, despite the root meaning, the majority of nevi are acquired after birth. Interestingly, these acquired nevi share genetic and environmental risk factors with malignant melanoma. Individuals with fair skin, a tendency to sunburn, and poor tanning ability are at an increased risk for malignant melanoma (Bataille, 2003) and have increased number of melanocytic nevi (Bauer and Garbe, 2003). Additionally, several studies implicate a propensity to develop melanocytic nevi as an independent risk factor for cutaneous melanoma (Bauer and Garbe, 2003). As 20–30% of melanomas arise from preexisting melanocytic nevi (Rivers, 2004), it is not surprising that many of the genetic underpinnings of melanoma have also been found in nevi.

The role of the *BRAF* mutation in the current genetic basis of melanoma development and progression is based on the Clark model for melanoma progression (Bennett, 2003), corresponding to the proposed morphologic changes from benign nevus to dysplastic nevus to melanoma (W. E. Damsky et al., 2014). *BRAF* mutations were first described in 2003 (Pollock et al., 2003), with 81.5% (53 of 65) of acquired nevi studied harboring a *BRAF* mutation. Since this report, many benign acquired melanocytic nevi have been analyzed, and 78% (373/478) were found to have a *BRAF* mutation; an *NRAS* mutation was found in only 6.0% (8/134) of acquired melanocytic nevi (Table 2).

Like melanoma, melanocytic nevi frequently harbor activating *BRAF* mutations, which are thought to be an initial step in melanocytic neoplasia; this idea is supported by the finding that inducible expression of $BRAF^{V600E}$ in melanocytes of mice yields melanocytic nevi and melanoma (Dhomen et al., 2009). However, the initial growth of a melanocytic nevus is followed by loss of proliferative activity and stabilization of size. Mirroring this clinical observation, sustained $BRAF^{V600E}$ expression in normal human melanocytes induces cell cycle arrest accompanied by the induction of both $p16^{INK4a}$ and acidic β-galactosidase activity, which are also demonstrated in lesions of melanoma *in situ* (Gray-Schopfer et al., 2006; Michaloglou et al., 2008). Thus, some melanocytic nevi are likely benign clonal tumors that temporarily undergo proliferation via oncogenic *BRAF* signaling followed by growth arrest due to oncogene-induced senescence (OIS), a concept discussed later (Michaloglou et al., 2008). It is clear that *BRAF* gene mutations are not sufficient to confer malignant change to melanocytes and other factors must play a role. Furthermore, the high concordance rate (80.4%) of *BRAF* mutation between melanoma and its nevus counterpart (Tschandl et al., 2013), as well as the occurrence of *BRAF* wild-type melanoma arising in *BRAF-mutant* nevi (Tan et al., 2015) suggest that other molecular signatures are involved in melanoma development.

Distinct dermoscopic structures have been identified that correspond to histopathological features of nevi (Yadav et al., 1993). For example, a pigment network (or reticulation) corresponds to a junctional, predominantly lentiginous melanocytic proliferation whereas globules correspond to large dermal or epidermal melanocytic nests (Argenziano et al., 2007). Recently, Marchetti *et al*. showed that the frequency of $BRAF^{V600E}$ mutations differs in nevi distinguished by unique dermoscopic structures and microanatomical growth patterns (Marchetti et al., 2014). Globular nevi correspond histopathologically to a predominantly dermal growth pattern or the presence of large junctional nests, and were significantly more likely to express $BRAF^{V600E}$ than reticular nevi(Marchetti et al., 2014).These finding were overall consistent with previous studies showing that intradermal nevi are significantly more likely to have *BRAF* mutations than juctional nevi(Hafner et al., 2009; Karram et al., 2013), and that $BRAF^{V600E}$ positive nevi are less likely to have a junctional component or show lentiginous growth characteristics compared with *BRAF* wild-type nevi (Tschandl et al., 2013).

DYSPLASTIC NEVI

Patients with multiple dysplastic nevi are known to be at higher risk of developing cutaneous melanoma. An individual with a single clinically dysplastic nevus has a 2-fold

increased risk for melanoma, whereas having greater than 10 clinically dysplastic nevi is associated with a 12-fold increased risk (Bataille et al., 1996; Marghoob et al., 1994). Moreover, dysplastic nevi are more common in patients with melanoma; 30% of melanomas seem to arise in association with nevi, most commonly a dysplastic nevus, and a personal and family history of melanoma can significantly increase the incidence of finding dysplastic nevi in a patient (Bataille et al., 1996; Seykora and Elder, 1996; Sober and Burstein, 1995). When dysplastic nevi were separately analyzed, 57.9 % (84/145) of the nevi were positive for a *BRAF* mutation (Table 3). In the late 1970s, Clark *et al*. (Clark et al., 1978) proposed that dysplastic nevi are an evolutionary precursor to melanoma in patients with a family history of melanoma (Clark et al., 1984; Rhodes et al., 1983; Rhodes et al., 1982). However, the biologic and clinical implications of this observation remain unclear as benign, normal-appearing acquired melanocytic nevi can also be found in association with melanomas (Hastrup et al., 1991; Rhodes et al., 1982).

BLUE NEVI

Blue nevi are a group of acquired, pigmented, dermal dendritic melanocytic proliferations comprised of many described variants, the two major ones being common blue nevi and cellular blue nevi (Held et al., 2013; Zembowicz and Mihm, 2004; Zembowicz and Phadke, 2011). Common blue nevi may arise at any age, or be congenital in nature, but have a tendency to appear in adolescence, and most often present as a small (<1 cm) solitary, dark blue-to-black papule (Argenziano et al., 2007; Gonzalez-Campora et al., 1994; Murali et al., 2009; Zembowicz and Phadke, 2011). Like common blue nevi, cellular blue nevi occur at all ages, but are most common in adults younger than 40 years of age (Murali et al., 2009). Cellular blue nevi also have a deep bluish-black color, but present as larger plaques or nodules greater than 1 cm, and sometimes several centimeters, in diameter (Zembowicz and Phadke, 2011). The distinct color of these lesions is due to the dermal location of the melanin, which causes preferential reflection of short-wavelength blue light due to a scattering effect known as Tyndall scattering (Zembowicz and Mihm, 2004). Blue nevi have a predilection for certain body sites, including the scalp, dorsal surfaces of distal extremities, and sacral region; common blue nevi are most often located on extremities, and cellular blue nevi most often in the sacral region (Gonzalez-Campora et al., 1994; Temple-Camp et al., 1988; Zembowicz and Phadke, 2011). It is theorized that blue nevi consistently occur in these body regions because these are the sites with active dermal melanocytes at birth, whereas melanocytes migrate through and eventually disappear from the majority of the body’s dermis during fetal life (Leopold and Richards, 1968; Zembowicz and Phadke, 2011; Zimmermann and Cornbleet, 1948). Histologically, blue nevi are dermal lesions that rarely have a junctional component (may be present in the case of combined nevi), with the diagnostic cell type of common and cellular blue nevi being spindled dendritic and ovoid melanocytes, respectively (Held et al., 2013; Zembowicz and Phadke, 2011).

Clinically and histopathologically, blue nevi are distinguishable as an independent class of melanocytic proliferations, and genetic studies of blue nevi have provided further support that they are a distinct entity separate from acquired melanocytic nevi. Most acquired melanocytic nevi harbor somatic mutations in genes involved in the MAPK signaling pathway, particularly *BRAF* and *NRAS* (Davies et al., 2002; Pollock et al., 2003; Van

Raamsdonk et al., 2009). Mutations in these genes are uncommonly identified in blue nevi; Saldanha *et al*. reported *BRAF* exon 15 mutations in 3 (12%) of 25 blue nevi and did not identify any *NRAS* mutations, while Emley *et al*. reported zero *BRAF* and 1 (5.3%) *NRAS* mutations in 19 common and cellular blue nevi, but found *KRAS* mutations among 16.7% (3/18) of these blue nevi (Emley et al., 2011; Saldanha et al., 2004).

The observation that mice with activating mutations in the G protein α-subunits, *GNAQ* and *GNA11*, acquire a phenotype of dermal hyperpigmentation similar in appearance to blue nevi in humans prompted the discovery that the majority of blue nevi harbor a somatic mutation of either *GNAQ* or *GNA11* (Van Raamsdonk et al., 2004). In their first study, Van Raamsdonk *et al*. sequenced the entire coding regions of these genes in 29 blue nevi and reported *GNAQ* mutations in 24 (82.8%); no *GNA11* mutations were reported (Van Raamsdonk et al., 2009). In a follow up study, out of a group of 96 common and cellular blue nevi, 65 (67.7%) and 8 (8.3%) harbored *GNAQ* and *GNA11* mutations respectively, with all but one affecting glutamine at codon 209 (Q209) (Van Raamsdonk et al., 2010). More recently, a genetic analysis of 30 common and cellular blue nevi revealed that 86.7% (26/30) and 3.6% (1/28) had *GNAQ* and *GNA11* mutations, respectively, all in codon 209 (Bender et al., 2013). The previously mentioned study by Emley *et al*. reported a lower prevalence of *GNAQ* mutations in common and cellular blue nevi, as only 42.1% (8/19) lesions carried mutations, but once again, all were in codon 209 (Emley et al., 2011). These mutations cause a loss of GTPase activity, leading to constitutive activation and subsequent upregulation of MAPK pathway signaling, a consequence also seen with constitutively active mutated BRAF and NRAS. Additionally, like mutated BRAF and NRAS, in vivo tumorigenicity studies have shown that, although mutated GNAQ acts as an oncogenic protein, it is not sufficient for melanomagenesis when acting alone (Van Raamsdonk et al., 2009). This is corroborated by the generally benign and stable nature of blue nevi, which rarely progress to malignancy.

Genetic analyses of rare blue nevi variants and blue nevi-related dermal melanocytoses (i.e. Nevus of Ito and Nevus of Ota) are sparse, with small sample sizes, but the findings tend to agree that *GNAQ* and *GNA11* mutations are much less prevalent in these lesions than in common and cellular blue nevi (Bender et al., 2013; Emley et al., 2011; Held et al., 2013; Van Raamsdonk et al., 2009; Van Raamsdonk et al., 2010). Further genetic studies and functional validation investigations are needed to better elucidate how *GNAQ* and *GNA11* mutations influence the biology and pathophysiology of blue nevi and related dermal melanocytic lesions.

SPITZ NEVI

Spitz tumors are a subgroup of melanocytic neoplasms, ranging from benign to malignant, with a predominance of large epithelioid or spindled melanocytes. Cases on the benign end of the spectrum, with no overlapping morphologic features of melanoma are designated as Spitz nevi, whereas cases with unequivocal features of melanoma are designated as spitzoid melanoma. Consistent with Spitz nevi's lack of morphologic features of melanoma, the genetics of Spitz nevi also do not align well with melanoma. *BRAF and NRAS* mutations are prevalent in melanomas, but the majority of studies investigating the genetic status of Spitz

nevi (Table 4) have demonstrated an absence of these mutations. In total, *BRAF* and *NRAS* mutations were detected in 6.4% (21/330) and 2.2% (4/178) of Spitz nevi respectively (Table 4). The most frequently observed genetic alterations in Spitz nevi involve the *HRAS gene*; an aggregate of data from multiple studies revealed 48 of 293 (16.4%) Spitz nevi harbored *HRAS* alterations (copy number gain or mutation) (Table 4). *HRAS* belongs to the family of *RAS* genes, which contains two additional members, *KRAS* and *NRAS* (Barbacid, 1987). In contrast to *NRAS*, *HRAS* is rarely mutated in melanoma (Jiveskog et al., 1998; van Elsas et al., 1996). *HRAS* mutation seems to occur almost exclusively in Spitz nevi. Bastian *et al*. first described *HRAS* copy number amplification in 23.5% (4/17) of Spitz nevi in 1999, and subsequently confirmed the presence of increased *HRAS* copy number in 11.8% (12/102) of Spitz nevi, while also showing *HRAS* mutations in 66.7% (8/12) of the Spitz nevi with increased copy number (Bastian et al., 2000; Bastian et al., 1999). HRAS is known to have higher affinity for the PI3K/ATK pathway when compared to other *RAS* isoforms (Yan et al., 1998), however, the significance of *HRAS* mutations in the nevogenesis of Spitz nevi remains unclear. It has been suggested that HRAS drives the symmetrical overgrowth of cells with epitheloid morphology via preferential PI3K/AKT activation, without marked increase of the melanin activation pathway (Ross et al., 2011). Clinically, the presence of an *HRAS* mutation seems to be associated with a favorable prognosis. Thus far, studies have found no metastases in patients with *HRAS*-mutated Spitz tumors, and no *HRAS* mutations have been reported in Spitzoid melanomas with distant metastasis or fatal outcome (Da Forno et al., 2009; Takata et al., 2007; van Dijk et al., 2005; van Engen-van Grunsven et al., 2010). Based on the frequency of *HRAS* mutations in Spitz nevi and the lack thereof in spitzoid melanomas, it seems unlikely that *HRAS*-mutated Spitz nevi progress to spitzoid melanomas.

Another subset of spitzoid neoplasms are characterized by *BRAF* mutations combined with bi-allelic *BAP1* loss (Wiesner et al., 2012; Wiesner et al., 2011). BAP1 is a deubiquitinating enzyme whose gene is located on chromosome region 3p12 (Jensen et al., 1998). *BAP1* has been suggested to be a tumor suppressor gene with a role in cell proliferation and growth inhibition (Jensen and Rauscher, 1999). This subset of melanocytic neoplasm described by Wiesner et al. histologically resembled so-called “atypical Spitz tumors” (ASTs), which are an ill-defined and heterogeneous group of melanocytic tumors that display histologic features seen in both Spitz nevi and melanoma. These *BRAF* mutant tumors had similar cytologic features to Spitzoid tumors characterized by *HRAS* mutations, however, the *HRAS* mutant neoplasms were associated with marked desmoplasia and did not show dense cellular aggregates compared to $BRAF^{V600E}$/$BAP1^{-/-}$ tumors (Wiesner et al., 2012).

Recently, gene rearrangements of *ROS1*, *NTRK1*, *ALK*, *BRAF*, and *RET*, resulting in in-frame kinase fusions, were reported through genomic analysis of 140 Spitzoid neoplasms (Wiesner et al., 2014). These kinase fusions occurred across the entire biologic spectrum of spitzoid neoplasms, including Spitz nevi, ASTs, and spitzoid melanoma. All fusions occurred in a mutually exclusive pattern, and no fusions were detected in tumors with *HRAS* mutations (Wiesner et al., 2014). The results of the study suggest that these fusions occur early in the pathogenesis of spitzoid neoplasms and are therefore necessary, but not sufficient, for malignant transformation. In this respect, the oncogenic role of these gene

arrangements seems to be similar to that of mutations in oncogenes (such as *BRAF*, *NRAS*, *GNAQ*, and *GNA11*) commonly found in melanocytic neoplasms (Wiesner et al., 2014).

NEVI AND MELANOMAGENESIS

To understand the molecular basis of melanomagenesis, it is imperative to identify the genetic changes that facilitate the different steps in progression from normal melanocyte to nevus and melanoma. Commonly, an initial "driver" mutation, either activation of an oncogene or the loss of a tumor suppressor gene, triggers the establishment of a benign growth. After this initial phase of proliferation, a senescence program is executed, causing termination of the tumor expansion. Cellular senescence is a physiologic process by which cells gradually lose their growth potential and eventually exit cell cycle progression. In normal human cells, senescence can occur due to either telomere shortening that takes place after a certain number of cell divisions (replicative senescence) (Dimri et al., 1996) or, paradoxically, because of the aberrant activation of oncogenes (oncogene-induced senescence) (Serrano et al., 1997). In humans, naturally occurring oncogene-induced senescence (OIS) is best exemplified by acquired melanocytic nevi and CMN, which represent benign aggregations of non-proliferative melanocytes that often harbor activating mutations in *BRAF* and *NRAS* genes, respectively. Michaloglou *et al*. showed that overexpression of $BRAF^{V600}$ in cultured human melanocytes caused growth arrest and also observed hallmarks of OIS in human congenital melanocytic nevi: expression of an activated oncoprotein ($BRAF^{V600}$), stable and total, or near-total, proliferative arrest, upregulation of a tumor suppressor ($p16^{INK4A}$), and emergence of senescence-associated acidic β-galactosidase (Michaloglou et al., 2008). Additionally, there was no significant difference in telomere fluorescence between congenital nevi and surrounding tissues, which supports results from common acquired nevi and Spitz nevi (Miracco et al., 2002), and further suggests that nevi are growth arrested due to OIS rather than replicative senescence. In spite of the activation of the MAPK pathway (RAS/RAF/MEK/ERK) that mediates a potent proliferative signal, benign nevi eventually lose all proliferative activity, and their growth remains arrested for decades, until they gradually disappear; however, with additional genetic alterations, a few nevus cells can develop into malignant melanoma (Kuwata et al., 1993; Maldonado et al., 2004). In large CMN, Charbel *et al*. demonstrated that after birth, certain large CMN cell subtypes still display clonogenic potential and can expand into nevus-like structures when interacting with adjacent keratinocytes(Charbel et al., 2015). This explains nevus secondary resurgences, which have been described following nevus excisions, as well as the natural expansion of nevi during childhood.

It is widely believed that a substantial percentage of melanomas arise from melanocytic nevi (Mooi and Peeper, 2006) and several groups have provided genetic evidence that supports the progression model (Bogdan et al., 2003; Dadzie et al., 2009; Demunter et al., 2001). It seems that OIS, similar to apoptosis, is a fail-safe mechanism to prevent malignant transformation of normal cells. Therefore, overcoming OIS may act as a rate-limiting event for melanomagenesis (Bennett, 2003; Mooi and Peeper, 2006). That is, only upon the emergence of additional tumorigenic alterations can a malignant process occur. The molecular mechanisms underlying this malignant transformation from nevus to melanoma are not yet resolved. Loss of $p16^{INK4A}$ has long been suspected to play a critical role in the

abrogation of OIS and collective evidence support a redundant role for *p16*^{INK4A} in senescence induced by mutant *BRAF* or *NRAS in vitro* (Michaloglou et al., 2008) and *in vivo* (Dhomen et al., 2009). Another common genetic event in melanoma is activation of the PI3K pathway (Dhawan et al., 2002). Vredeveld *et al*. recently demonstrated that PI3K pathway activation serves as a critical event in melanomas arising from nevi, acting in part by abrogating OIS (Vredeveld et al., 2012). They showed that *PTEN* depletion and consequent activation of the PI3K pathway abrogates *BRAF*^{V600E}-induced senescence. Similarly, Damsky *et al*. reported that simultaneous *Cdnk2a* and *Lkb1* inactivation in *BRAF*^{V600E} melanocytes results in activation of both *mTORC1* and *Akt*/*mTORC2*, inducing rapid melanoma formation in mice (W. Damsky et al., 2015). Loss of *Cdkn2a* is insufficient to release melanocytes from OIS; however, this primes a subset of growth-arrested, *BRAF*^{V600E} nevi for later stochastic progression to melanoma, which appears to occur through activation of *Akt*/*mTORC2* and *mTORC1* signaling (W. Damsky et al., 2015).

Melanomas associated with nevi have been studied thoroughly with the hope of better understanding melanoma biology. Tschandl *et al*. demonstrated that no significant difference in the distribution of *BRAF* or *NRAS* mutations is found between melanomas and associated nevi or between melanoma-associated nevi and control nevi (Tschandl et al., 2013). Notably, this study concludes that the presence of a *BRAF*^{V600E} or *NRAS*^{Q61} mutation within a nevus does not alter the risk of malignant transformation. A recent meta-analysis of 13 studies, with 4,346 cumulative patients, revealed that 32% of melanomas are associated with a nevus, however, there was no prognostic implication in sentinel lymph node status or overall survival for these nevus-associated melanomas compared to *de novo* melanomas (Lin et al., 2015).

Since the use of specific *BRAF*^{V600E} inhibitors has become more commonplace in the treatment of melanoma, interesting findings have been reported regarding the effect of these drugs on benign nevi, which frequently harbor *BRAF*^{V600E} mutations (Pollock et al., 2003); (Dalle et al., 2011; Haenssle et al., 2012). Dermoscopic changes to preexisting nevi in color, appearance and disappearance of globules, dermoscopic island pigmentation, and increases in size have been reported following BRAF inhibitor therapy (Perier-Muzet et al., 2014). Interestingly, the evolving nevi were *BRAF*^{V600E} mutated while the stable nevi were *BRAF* wild-type. This phenomenon maybe related to decreased MAPK pathway activity due to BRAF inhibition (McClenahan et al., 2014). Others have reported that BRAF^{V600E} inhibitor-treated patients show increased size and pigmentation in some nevi and development of new BRAF wild-type melanomas driven by paradoxical MAPK activation (Hatzivassiliou et al., 2010). Clearly, *BRAF* and its associated pathway are linked to nevogenesis and stability, but larger studies are required to better understand escape pathways and paradoxical MAPK pathway activation.

SUMMARY

Nevi are a heterogeneous group of benign melanocytic neoplasms, with varying clinical and molecular characteristics. While the molecular etiology of nevi have been less thoroughly studied than melanoma, it is clear that nevi and melanoma share common driver mutations. Acquired melanocytic nevi harbor oncogenic mutations in *BRAF*, which is the predominant

oncogene associated with melanoma. While congenital melanocytic nevi, blue nevi, and acquired nevi predominantly harbor *NRAS* mutations, *GNAQ* mutations, and *BRAF* alterations, respectively, Spitz nevi and spitzoid tumors have now been linked to an assortment of oncogenic drivers including *HRAS* and *BAP1* alterations and kinase fusions (Figure 1). Excepting *HRAS*, all of the "nevus" genes have also been described in melanoma development and progression. Thus, the distinction between melanoma and nevi seems to be a nevus cell's ability to undergo senescence after sustaining pro-proliferative mutations. Melanoma's ability to escape this senescence pathway, tipping the balance from benign neoplasm to malignancy, remains an area of active investigation. The genetics of nevi have not been as thoroughly studied as its malignant counterpart, despite the potential to provide insight into how melanomas undergo malignant transformation. Molecular studies have been limited by a lack of robust nevi mouse models and limited availability of excised tissue for analysis. Nevertheless, further genetic studies of nevi promise to shape our understanding of melanoma and the delicate tipping-point from benign neoplasm to malignancy.

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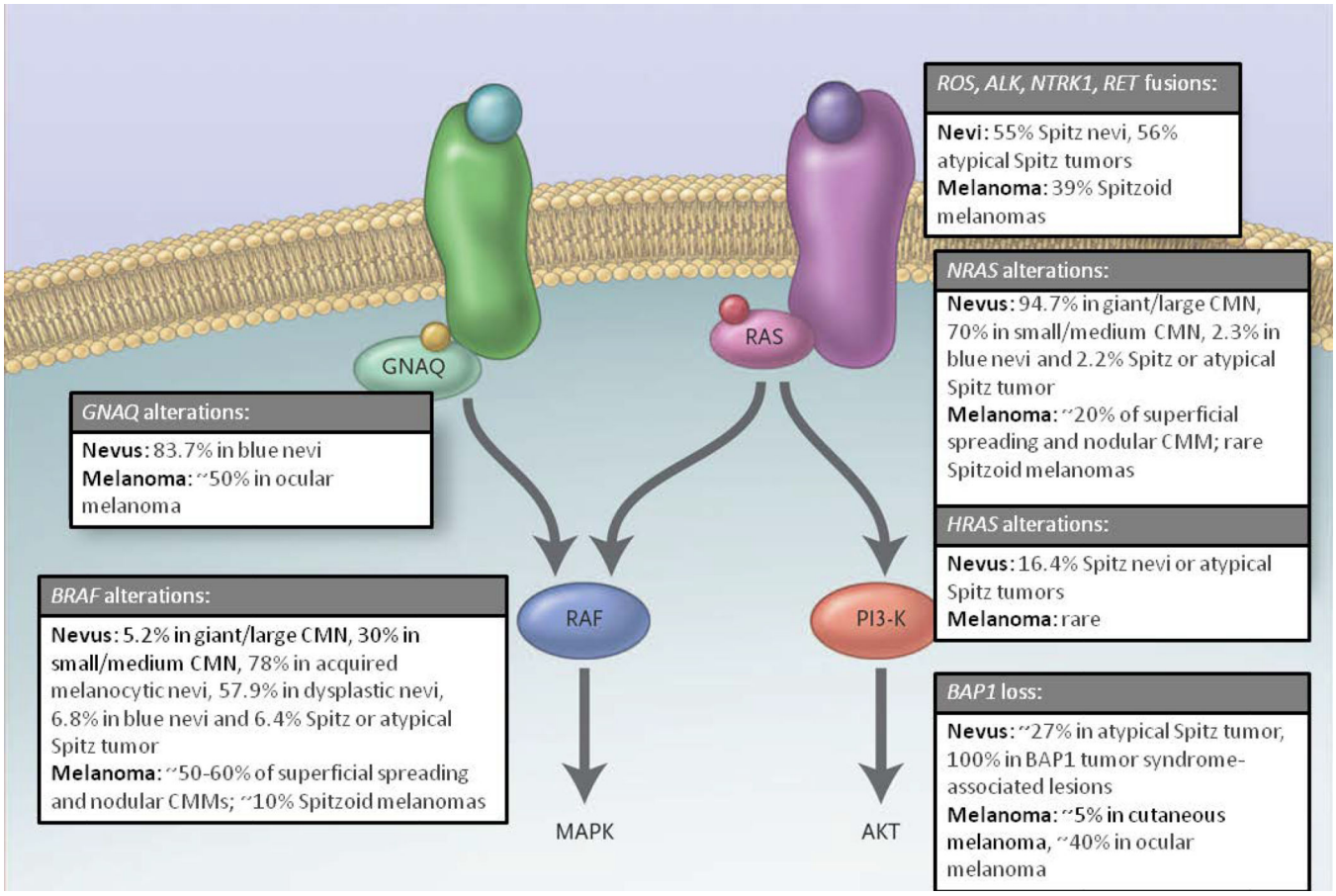

Figure 1. The RAS Signaling pathways and Nevi

MAPK signaling promotes cell growth and survival and is constitutively active in nevi. RAS family members are activated by receptor tyrosine kinases and signal through effector proteins, including RAF kinases and PI3K. Acquired melanocytic nevi harbor oncogenic mutations in *BRAF*, which is the predominant oncogene associated with melanoma. Congenital melanocytic nevi, blue nevi, and Spitz nevi frequently harbor *NRAS* mutations, *GNAQ* mutations, and *HRAS* alterations, respectively (Modified from N Engl J Med. 2010 Sep 30;363(14):1352-60 Copyright © (2010) Massachusetts Medical Society. Reprinted with permission.)

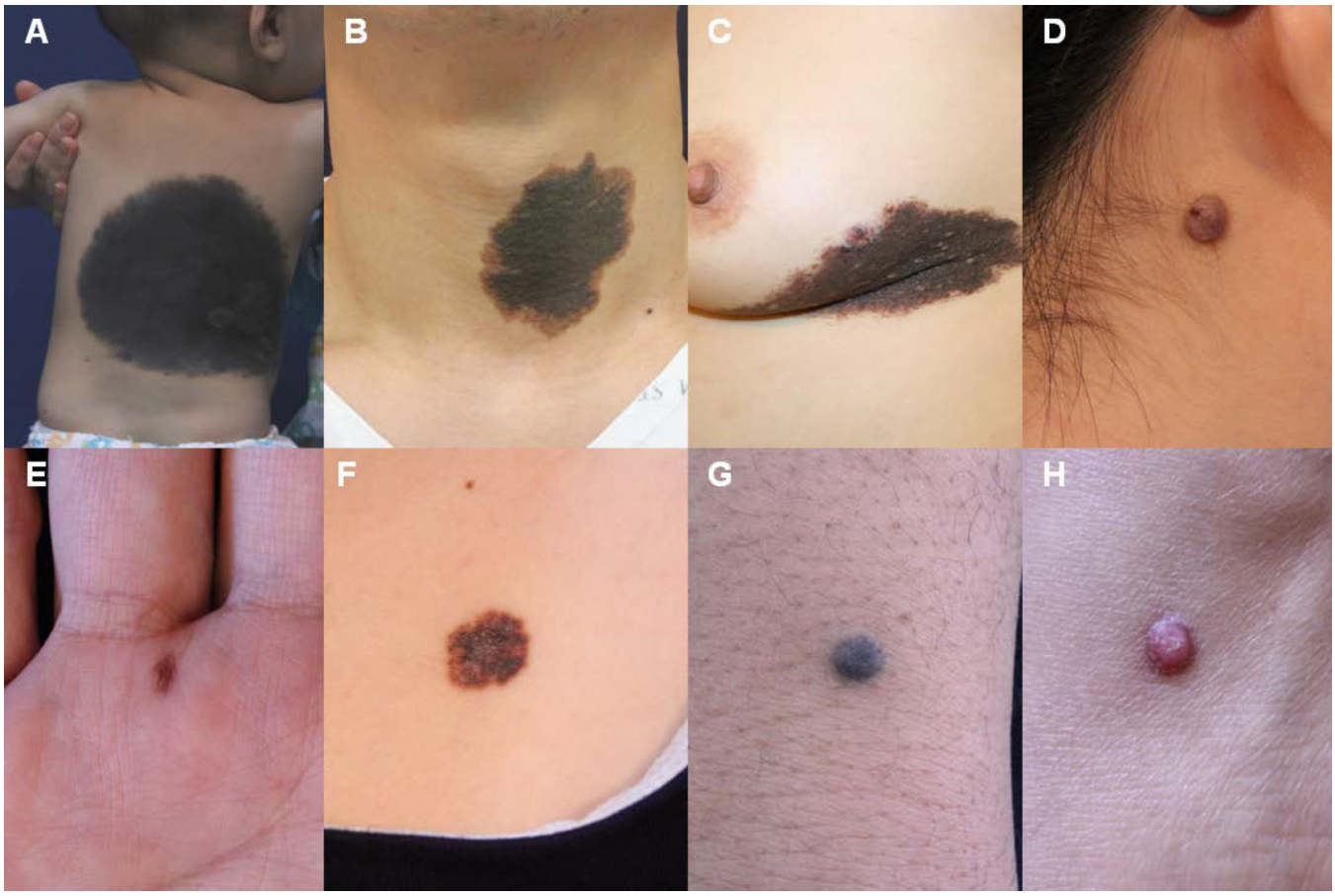

Figure 2.
Clinical images of nevi. Subtypes of nevi include large CMN (A), medium CMN (B), melanoma arising in CMN (C), intradermal nevus (D), acral junctional nevus (E), dysplastic nevus (F), blue nevus (G), and Spitz nevus (H).

Table 1

Studies evaluating the mutation status of *BRAF* and *NRAS* in congenital melanocytic nevi.

Study	Size of CMN analyzed*	*BRAF* mutations	*NRAS* mutations	Median age (years) at biopsy/excision	Documented presence at birth
(Carr and Mackie, 1994)	Small	--	12/43(27.9%)	28	Yes
(Papp et al., 1999)†	Small	--	1/2(50%)		--
	Medium	--	9/16(56.2%)	12	--
(Pollock et al., 2003)	--	6/7(85.7%)	2/7(28.6%)	--	--
(Yazdi et al., 2003)	--	6/13(46.2%)	--	--	--
(Papp et al., 2005)†	Small	1/2(50%)	--		--
	Medium	6/16(37.5%)	--	12	--
(De Raeve et al., 2006)	Giant	0/9	--	<1	Yes
(Ichii-Nakato et al., 2006)	Small	37/42(88.1%)	--	26 (mean)	Yes
	Medium	6/20(30%)	9/20(45%)	19 (mean)	Yes
(Bauer et al., 2007)	Small#	20/28(71.4%)	7/28(25%)	40	--
	Medium/Large	0/32	26/32(81.2%)	1.33	Yes
(J. Wu et al., 2007)	Small#	20/25(80%)	--	--	--
	Large	6/9(66.7%)	--	--	Yes
(Dessars et al., 2009)	Medium	1/3(33.3%)	1/3 (33.3%)	--	--
	Large	3/24(12.5%)▪	18/24(75%)	--	--
(Phadke et al., 2011)	Small/Medium	7/16(43.8%)	1/16(6.2%)	4	--
	Giant	2/27(7.4%)	12/27(44.4%)		--
(Qi et al., 2011)	--	61/104(58.7%)	2/104(1.9%)	--	Yes
(D. Wu et al., 2011)	Medium	9/37(24.3%)	10/37(27%)	10 (mean)	Yes
	Giant	0/18	3/18 (16.7%)	7.9 (mean)	Yes
(Kinsler et al., 2013)	Medium/Large/Giant	--	10/13(76.9%)	8.3	--
(Charbel et al., 2014)	Small/Medium	6/20(30%)	14/20(70%)	4.17	Yes
	Large/Giant	1/19(5.3%)	18/19(94.7%)	0.66	Yes

*Studies used different classification schemes to define medium, large, and giant CMN

† Same CMN samples used in both studies by Papp et al.

"congenital pattern nevi"

▪ Two *BRAF* wild-type CMN showed chromosomal translocations affecting *BRAF* loci, with suspected oncogene activation.

Table 2

Studies evaluating the mutation status of *BRAF* and *NRAS* in acquired melanocytic nevi

Study	**Histology of nevi analyzed***	***BRAF* mutations**	***NRAS* mutations**
(Jafari et al., 1995)	Benign nevi	--	0/5 (0%)
(Pollock et al., 2003)	Intradermal	37/42(88%)	--
	Compound	16/23(70%)	--
(Dong et al., 2003)	Benign melanocytic nevi	17/24(70.8%)	0/24(0%)
(Saldanha et al., 2004)	Common acquired nevi	14/16(87.5%)	2/11(18.2%)
(Poynter et al., 2006)	Benign melanocytic nevi	42/51(82.3%)	3/51(5.9%)
(Ichii-Nakato et al., 2006)	Common acquired nevi	105/120(87.5%)	--
(Uribe et al., 2006)	Common acquired nevi	16/24(66.7%)	--
(Bloethner et al., 2007)	Benign melanocytic nevi	18/30(60%)	--
(J. Wu et al., 2007)	Common acquired nevi	83/101(82.2%)	--
(Venesio et al., 2008)	Compound nevi	6/13(46.2%)	0/13(0%)
	Intradermal nevi	3/6(50%)	0/6(0%)
	Junctional nevi	2/3(66.7%)	0/3(0%)
(Tschandl et al., 2013)	Control nevi	14/25(56%)	3/21(14.3%)

Table 3

Studies evaluating the mutation status of *BRAF* in dysplastic nevi

Study	**Nevi included**	***BRAF* mutations**	***NRAS* mutations**
(Pollock et al., 2003)	Dysplastic nevi	4/5(80%)	--
(Papp et al., 2005)	Dysplastic nevi	4/18(22%)	--
(Uribe et al., 2006)	Atypical nevi	13/21(61.9%)	--
(Saroufim et al., 2014)	Dysplastic nevi	63/125(55.8%)	--

Table 4

Studies evaluating the genetic alteration status of *BRAF*, *NRAS*, and *HRAS* in Spitz nevi.

Study	**Histological Subtypes**	***BRAF* mutations**	***NRAS* mutations**	***HRAS* alterations**
(Bastian et al., 1999)	Spitz Nevi	--	--	4/17(23.5%)*
(Bastian et al., 2000)	Spitz Nevi	--	--	12/102(12%)*
(Yazdi et al., 2003)	Spitz Nevi	0/69(0%)	--	--
(Palmedo et al., 2004)	Spitz Nevi	0/21(0%)	--	--
(Mihic-Probst et al., 2004)	Spitz Nevi	0/20(0%)	--	--
(Saldanha et al., 2004)	Spitz Nevi	0/26(0%)	1/16(6.25%)	--
(Gill et al., 2004)	Spitz Nevi	0/30(0%)	0/30(0%)	0/30(0%)
(Turner et al., 2005)	Spitz Nevi	0/24(0%)	--	--
(van Dijk et al., 2005)	Spitz Nevi and Atypical Spitz Nevi	0/30(0%)	0/30(0%)	6/30(20%)
(Fullen et al., 2006)	Spitz Nevi	10/48(20.8%)	1/48(2.08%)	--
(La Porta et al., 2006)	Spitz Nevi	8/8(100%)	--	--
(Takata et al., 2007)	Spitz Nevi	0/12(0%)	0/12(0%)	0/12(0%)
(Da Forno et al., 2009)	Spitz Nevi and Atypical Spitz Nevi	2/22(9.1%)	2/22(9.1%)	2/19(10.5%)
(Emley et al., 2010)	Spitz Nevi and Atypical Spitz Nevi	1/20(5%)	0/20(0%)	--
(van Engen-van Grunsven et al., 2010)	Spitz Nevi	--	--	24/93(25.8%)

* These studies reported frequency of copy number gain, not mutation