Chapter 2
Melanoma Pathogenesis

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Abstract Melanoma is an aggressive and heterogeneous disease with respect to clinical behavior and underlying genomic lesions. Melanoma development is multifactorial, and increased susceptibility is associated with sun exposure, fair pigmentation, family history, and melanocytic nevi. Major advances in our understanding of its molecular pathogenesis include the identification of recurrent mutations and aberrations in key signaling and developmental pathways. BRAF is the most commonly affected gene, with BRAF(V600E) mutations found in half of all melanomas. The discovery and characterization of oncogenic mutations in the MAPK, RB, p53, and MITF pathways have set the stage for clinically meaningful progress in the melanoma field.

Keywords Melanoma · Melanocyte · Pigmentation · Nevus · BRAF · MAPK · MITF · NRAS

2.1 Melanocyte Biology

Melanomas arise from the malignant transformation of melanocytes. Melanocytes are the pigment producing cells of the skin and are derived from neural crest stem cells. Their development is modulated by the receptor tyrosine kinase c-KIT and microphthalmia-associated transcription factor (MITF), two genes that are mutated or amplified in many melanomas [1].

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Melanocytes can produce multiple types of pigment, most obviously dark brown eumelanin and reddish pheomelanin. Pro-pigmentation signaling is initiated by binding of α-melanocyte stimulating hormone (α-MSH) to the melanocortin 1 receptor (MC1R) on the melanocyte cell surface. MC1R is a seven-transmembrane G-protein-coupled receptor that activates adenylate cyclase, leading to increased intracellular cAMP levels and expression of MITF. MITF in turn induces transcription of pigment synthesis genes and production of melanin [2]. Although many loci are involved in human pigmentation, MC1R is a major determinant of pigmentation phenotype. MC1R polymorphisms involving single amino-acid substitutions can reduce MC1R signaling, resulting in impaired eumelanin production and a red hair/fair skin phenotype [3].

In addition to basal pigmentation, acquired pigmentation can occur in response to stimuli such as ultraviolet radiation (UVR). Eumelanin is the pigment that provides UVR attenuation in darkly pigmented skin. The tanning response to UVR has been shown to involve p53 activation in keratinocytes following UV-induced DNA damage, leading to p53-mediated POMC/MSH expression. Secreted MSH stimulates MC1R in neighboring melanocytes and produces cutaneous pigmentation [4].

2.2 Melanoma Risk Factors

Melanoma pathogenesis is driven by both environmental and genetic factors. Epidemiologic studies have linked melanoma to geographic location and sun (UV) exposure, which is believed to be the most important environmental risk factor. In particular, severe sunburns early in life are associated with the highest risk for melanoma [5, 6]. Melanomas occur more frequently on sun-exposed regions of the body. However, unlike most keratinocyte skin cancers which are known to be products of UVR, melanoma primary tumors are not restricted to sun-exposed skin. Although individuals with fair skin are more susceptible, melanomas also arise in darkly pigmented individuals, most often at acral or mucosal sites. These observations suggest that sun exposure does not account for all melanoma risk.

Increased melanoma susceptibility is associated with family history, fair pigmentation phenotypes, and higher numbers of melanocytic nevi. A family history of melanoma confers an estimated twofold increase in melanoma risk, and approximately 10% of melanoma patients have a family history of the disease [7]. Melanoma is considered familial if two first-degree relatives or three individuals in a family, irrespective of relationship, are diagnosed with melanoma. Familial melanoma is most often associated with dysregulation of cell cycle checkpoints due to mutations in cell cycle regulatory genes such as cyclin-dependent kinase 4 (CDK4) [8] and cyclin-dependent kinase inhibitor 2A (CDKN2A), which accounts for 40% of cases and is the most common high-penetrance melanoma susceptibility locus [9]. Germline CDKN2A mutations are responsible for familial atypical multiple mole melanoma (FAMM) syndrome, an autosomal dominant genodermatosis characterized by increased incidence of melanocytic nevi and melanoma, and elevated

risk of other malignancies such as pancreatic cancer in some FAMM kindreds [10, 11]. A less common cause of familial melanoma is the recently reported E318K variant of MITF [12, 13]. This variant exhibited gain-of-function activity for MITF, which is a previously described amplified melanoma oncogene. Individuals carrying the allele exhibited elevated nevus counts and non-blue eye colors, together with increased melanoma risk. The risk was intermediate in nature in the general population (sporadic) and also segregated among many studied melanoma families in multiple continents. The E318K coding variant disrupts a sumoylation site on MITF [14], thereby inhibiting a functionally suppressive post-translational modification on MITF.

Heritable physical characteristics such as fair skin complexion, inability to tan, and blue eyes are associated with elevated melanoma susceptibility. Germline variants of pigmentation genes such as MC1R, agouti signaling protein (ASIP), and tyrosinase (TYR) confer low- or moderate-penetrance melanoma risk [15, 16]. Individuals with non-signaling variants of MC1R have the red hair/fair skin phenotype, characterized by fair pigmentation, freckling, and sun sensitivity, that is associated with the highest risk of melanoma of all pigmentation phenotypes [17]. MC1R coding variants are found in 80 % of individuals with red hair, less than 20 % of individuals with brown or black hair, and less than 4 % of individuals with a robust tanning response [3]. Comparison of melanomas in murine models of different pigmentation phenotypes has demonstrated that the synthesis pathway of the red pigment pheomelanin contributes to melanomagenesis via a UV-independent mechanism [18].

2.3 MAPK and PI3K Pathways

Oncogenic driver mutations have been identified in key signaling and developmental pathways that are involved in survival and proliferation of melanocytes. The most frequently observed recurrent mutations in melanoma occur within the mitogen-activated protein kinase (MAPK) signaling pathway, which promotes cell survival, cell cycle progression, and transformation (Fig. 2.1). In nonmalignant cells, the MAPK pathway is only activated in response to ligand binding to receptor tyrosine kinases or cytokine receptors. Stimulation of receptors leads to activation of RAS family members, monomeric G proteins that act as GTPase switch proteins. RAS-GTP promotes formation of signal-transduction complexes and activates a cascade of serine/threonine kinases culminating in activation of ERK, also known as MAPK. ERK is a serine/threonine kinase that can phosphorylate many targets such as transcription factors.

MAPK signaling is constitutively activated in almost all melanomas. The v-raf murine sarcoma viral oncogene homolog B1 (BRAF) is the dominant genetic target in this pathway, with 40–50 % of melanomas carrying a somatic mutation [19–22]. To a lesser extent, BRAF mutations are also observed in other cancers [23, 24]. BRAF is a serine/threonine kinase directly activated by RAS and is highly expressed in melanocytes, neuronal tissues, testis, and haematopoietic cells. Unlike

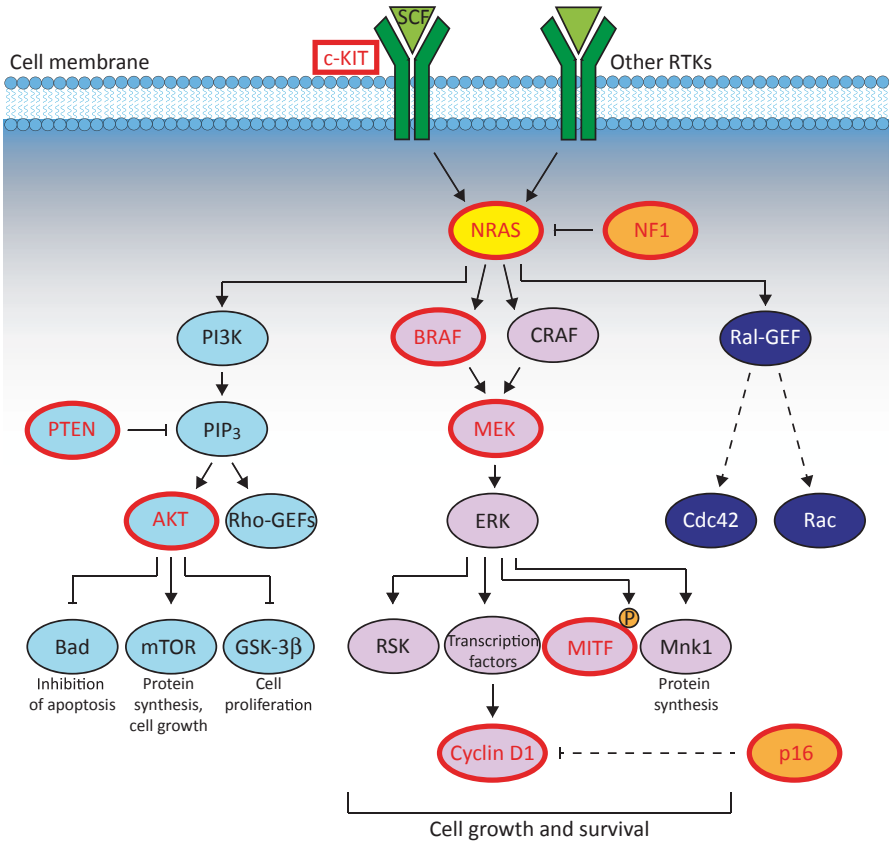

Fig. 2.1 RAS signaling. RAS family members are monomeric G proteins that are activated by receptor tyrosine kinases and signal through direct interaction with effector enzymes including phosphoinositide (PI) 3-kinases, RAF kinases, and Ral-guanine nucleotide exchange factors (*Ral-GEFs*). Although RAS mutations are less common in melanoma than other solid tumors, *NRAS* activating mutations are found in 10–20 % of melanomas. Mitogen-activated protein (MAP) kinase signaling in response to RAF kinase activity promotes cell growth and survival, and the MAPK pathway is constitutively activated in almost all melanomas. *BRAF* is the most frequently mutated gene in melanoma, with activating lesions found in 40–50 % of tumors. Melanoma oncogenes and tumor suppressors are labeled in red. Dotted lines represent omitted pathway components. *NF1* neurofibromatosis 1, *PTEN* phosphatase and tensin homolog, PIP_3 phosphatidylinositol-3,4,5-triphosphate, *mTOR* mammalian target of rapamycin, *GSK-3β* glycogen synthase kinase-3β, *RSK* ribosomal S6 kinase, *Mnk1* MAP kinase-interacting kinase 1, *Cdc42* cell division control protein 42 homolog

CRAF, which can participate in signaling events outside the MAPK pathway, BRAF's only known substrate is MEK/MAP2K. Phosphorylated MEK activates ERK by phosphorylation, leading to pro-growth and transforming effects that are critical in melanoma pathogenesis.

The most common BRAF mutation in melanoma, accounting for 90 % of variants, is a valine to glutamic acid substitution at codon 600 (V600E) in exon 15 [25].

This mutation constitutively activates the kinase domain. Other oncogenic BRAF mutations are found elsewhere in exon 15 or in exon 11, and most of the over 100 rare non-V600E mutations described occur in the glycine-rich loop and activation segment of the kinase domain. Mutations in these regions indirectly activate BRAF by disrupting the normal intramolecular interactions which hold BRAF in an inactive conformation [26].

An alternate oncogenic mechanism in melanoma involves rare BRAF mutants with low kinase activity. Although no CRAF activating mutations have been reported in melanoma [25, 27, 28], mutations such as G469E and D594G produce a BRAF that directly activates CRAF but minimally phosphorylates MEK. Melanoma lines with these low-activity BRAF mutations are dependent on CRAF for survival [29].

BRAF(V600E) mutations are observed much more frequently in melanomas arising in intermittently sun-exposed skin regions than acral or mucosal melanomas, suggesting that BRAF mutations may be linked to sun exposure. However, the thymidine to adenine (T>A) transversion at position 1799 that is responsible for the V600E substitution is not a typical UV-signature DNA mutation. It is possible that the transversion could result from a "non-classic" UV-induced DNA lesion or from secondary effects of UVR exposure such as generation of reactive oxygen species [30].

Mutations that increase RAS activity also promote cell proliferation. In comparison to other solid tumors, RAS mutations occur with relatively low frequency in melanomas. Only 10–20 % of melanomas, most often amelanotic nodular subtypes, carry an activating RAS mutation. NRAS is the most commonly affected RAS family member in melanoma [31, 32], and NRAS activating mutations [33, 34] primarily involve glycine 12, glycine 13, and glutamine 61 and trap NRAS in its active, GTP-bound conformation. While BRAF mutations activate only MAPK signaling, NRAS activating mutations simultaneously activate the MAPK and phosphatidylinositide 3-kinase (PI3K) pathways.

Although oncogenic mutations are usually not stand-alone events in melanoma, some are thought to be mutually exclusive. For example, NRAS and BRAF mutations almost never occur concomitantly [35, 36], suggesting that NRAS and BRAF have overlapping oncogenic activities and either is sufficient for constitutive activation of the MAPK pathway. Both BRAF and NRAS mutations are associated with poorer clinical prognosis. In the rare cases when both BRAF and NRAS mutations are present in melanoma, the BRAF mutation is not the classic V600E substitution [37]. "Acquired" (or selected) NRAS mutations have also been observed simultaneously with BRAF(V600E) in the context of melanomas which initially responded to BRAF-targeted therapy but subsequently became resistant [38].

PI3K signaling results in increased activation of the serine/threonine kinase AKT (also known as protein kinase B), which is a major mediator of cell survival through activation of targets such as mammalian target of rapamycin (mTOR) and inhibition of pro-apoptotic signals. While PI3K itself is rarely mutated in melanoma [39], constitutive activation of NRAS, amplification of AKT3, or loss of the phosphatase and tensin homolog (PTEN) tumor suppressor can lead to dysregulation of the PI3K

pathway. PTEN encodes a lipid and protein phosphatase that negatively regulates signaling pathways which use the cytosolic second messenger phosphatidylinositol-3,4,5-triphosphate (PIP_3), such as the PI3K pathway. Lower levels of intracellular PIP_3 result in less downstream activating phosphorylation of AKT. Thus, loss of PTEN protein or function eliminates a mechanism of negative regulation of AKT and cell survival. Increased phospho-AKT levels are associated with poor melanoma prognosis [40].

PTEN can be lost upon chromosome 10q deletion. 50–60% of melanomas contain hemizygous deletions or point mutations in 10q, while 10% contain homozygous deletion [41]. Epigenetic silencing of PTEN has also been described [36, 42, 43]. Hemizygous PTEN deletions tend to occur with BRAF mutation [42, 44], suggesting that BRAF and PTEN can cooperate in melanomagenesis. This idea is supported by studies of a murine model of melanoma in the setting of BRAF(V600E) and PTEN inactivation [45].

Neoplastic transformation of melanocytes can give rise to benign nevi as well as malignant melanoma, and activating mutations in BRAF and NRAS are implicated in both. Activating BRAF mutations are found in 70–80% of dysplastic nevi [22, 46–48], while NRAS mutations are rare in dysplastic nevi [49, 50] but present in most congenital nevi [50]. Mutation of HRAS is associated with Spitz nevi [51]. The BRAF(V600E) mutation induces nevus formation, involving initial cell proliferation followed by oncogene-induced senescence likely due in part to accumulation of $p16^{INK4A}$ [52]. Mutation of $p16^{INK4A}$ in addition to BRAF leads to transformation of cells *in vitro*, and deletion of PTEN or $p16^{INK4A}$ results in the formation of invasive melanoma in BRAF(V600E) mice [53]. In zebrafish, concomitant BRAF(V600E) mutation and deletion of TP53 leads to the formation of invasive and metastatic melanoma [54].

Given the high incidence of BRAF mutations in nevi, mutation of BRAF was traditionally thought to be a founder event that preceded all other oncogenic events in BRAF mutant melanoma [46]. In this model, senescence induced by BRAF activation is overcome by cooperating genetic lesions such as loss of $p16^{INK4A}$ or PTEN. However, other evidence suggests that the order of melanocytic lesions and relationship between nevi and tumor may be more complex. Although BRAF mutations are found in most nevi and half of vertical growth and metastatic melanomas, they are rare in initial malignant lesions: only 10% of radial growth phase melanomas and 6% of in situ melanomas have mutant BRAF. In addition, many nevi and primary melanomas are polyclonal (contain both BRAF wild type and BRAF mutant cells) while metastatic melanomas are not polyclonal, suggesting that BRAF mutation might occur at later stages of melanomagenesis [25, 46, 55, 56]. In addition, recent data have suggested that a stereotypical mutation in the promoter of the enzyme telomerase reverse transcriptase (TERT) is found in both BRAF mutant or NRAS mutant melanomas, suggesting that it may be an earlier mutation event [57]. This mutation in the TERT promoter occurs at a frequency of approximately 70% in melanomas and is also found in many non-melanoma cancers [57]. Regardless of when BRAF lesions occur, activation of BRAF in invasive melanoma promotes cell growth and dependence on the MAPK signaling pathway [58].

Dysregulation of MAPK signaling in melanoma can alternatively be caused by overexpression or hyperactivation of growth factor receptors such as c-Met, KIT, and epidermal growth factor receptor (EGFR) [59–61]. Mutations in the tumor suppressor neurofibromatosis 1 (NF1), a negative regulator of Ras, were identified in 5 out of 21 tumors without BRAF or NRAS mutations [62]. In the context of BRAF(V600E), NF1 mutations dysregulate the MAPK and PI3K pathways, ultimately suppressing mutant BRAF-induced senescence and promoting melanoma development and proliferation [63]. In some melanomas, inactivating mutations have also been identified in the tumor suppressor neurofibromatosis 2 (NF2) [64]. Germline mutations in NF1 and NF2 are associated with hereditary neurofibromatosis. Recent whole-exome sequencing approaches have identified somatic mutations in downstream MAPK effectors such as MAP3K5, MAP3K9, MEK1, and MEK2 in melanomas [65, 66].

2.4 RB and p53 Pathways

The retinoblastoma (RB) and TP53 tumor suppressor pathways are dysregulated in many sporadic and familial melanomas, and all known inherited high-risk melanoma susceptibility loci are genes in the RB pathway. However, loss or lesions of RB and TP53 occur much less frequently in sporadic melanomas than in most other solid tumors. Instead, genetic alterations in CDKN2A can eliminate upstream signaling in these pathways in melanoma. The CDKN2A locus at chromosome 9p21 encodes four exons, and alternative splicing yields two distinct tumor suppressors that share a common second exon: $p16^{INK4a}$ and $p14^{ARF}$ [67]. Mutations in $p16^{INK4A}$ functionally inactivate the RB pathway while mutations in $p14^{ARF}$ functionally inactivate the p53 pathway. The most common CDKN2A lesions in melanoma are point mutations, which are found as germline lesions in 25–40% of familial melanomas and as sporadic alterations in 10% of non-familial melanomas [67]. CDKN2A point mutations are also associated with dyplastic nevi. As with PTEN loss, CDKN2A mutation tends to coincide with BRAF mutation [21].

The RB pathway regulates the G1/S cell cycle checkpoint. During normal cell cycle progression, the RB tumor suppressor is phosphorylated by mammalian G1 cyclin-CDK complexes. Hyperphosphorylation of RB triggers release of E2F family members, transcription factors that activate expression of genes important for entry into S phase and DNA synthesis. $p16^{INK4a}$ binds and inhibits cyclin-dependent kinases 4 and 6 (CDK4/6) from inappropriately phosphorylating the RB protein [68]. Thus, loss of $p16^{INK4a}$ facilitates RB phosphorylation and subsequent re-entry into the cell cycle. Point mutations or transcriptional silencing are responsible for loss of $p16^{INK4a}$ expression in 30–70% of melanomas, leading to increased cellular proliferation and escape from oncogene-induced senescence.

Activating mutations in CDK4 are found in a small number of melanomas. CDK4 germline mutations always occur at a conserved arginine residue, R24, that is necessary for regulatory inhibition of CDK4 by $p16^{INK4a}$ [8, 69]. 5% of melanomas

contain somatic CDK4 point mutation or amplification [70]. $p16^{INK4a}$ and CDK4 mutations are mutually exclusive [29, 71, 72].

While TP53 mutations are found in 5% of melanomas [73], the p53 apoptotic pathway is more often deficient due to loss of $p14^{ARF}$ function in melanomas [74]. $p14^{ARF}$ binds and inhibits the mouse double minute 2 homolog (MDM2). MDM2 encodes an E3 ubiquitin ligase that inhibits p53 transcriptional activity and targets p53 for proteasomal degradation. Inactivating $p14^{ARF}$ mutations permit the p53-antagonizing activity of MDM2 and subsequent genomic instability [75–77]. In rare cases, amplification of MDM2 without alterations in CDKN2A sequence or expression has been observed in melanoma [70].

2.5 MITF

MITF is the master lineage regulator of melanocyte development and survival. It serves as the transcription factor for differentiation and pigmentation genes such as TYR, tyrosinase-related protein 1 (TYRP1), dopachrome tautomerase (DCT), melanoma antigen recognized by T-cells 1 (MART1, also known as gp100), and premelanosome protein (PMEL17, also known as SILV). Although MITF is essential for melanocyte differentiation, it can alternatively promote malignant behavior in some melanomas. The most common genetic alteration of MITF is amplification, which occurs in 15–20% of melanomas with a higher prevalence among metastatic melanomas [78]. MITF amplification is thought to usually occur as a late event in melanoma progression and was associated with poorer 5 year survival in the pre-vemurafenib and ipilimumab era [79]. Many melanomas continue to depend on MITF expression for survival, and suppression of MITF *in vitro* is lethal to most melanoma cell lines [80, 81].

The transcriptional targets of MITF that mediate its oncogenic activity as distinct from its regulation of pigmentation and differentiation are not fully characterized. However, MITF is known to enhance expression of genes involved in cell cycle progression, cell proliferation, and cell survival. For example, MITF is a transcription factor for cell cycle kinase CDK2 [81], CDK inhibitors $p16^{INK4a}$ [82] and p21 [83], and anti-apoptotic mitochondrial membrane protein B-cell lymphoma 2 (BCL-2) [84] as well as its related family member BCL2A1 [85]. In melanomas with elevated MITF activity, increased expression of these MITF targets likely contributes to growth, invasion, and survival of melanoma cells.

MITF is known to cooperate with BRAF in melanoma transformation *in vitro* [78] and *in vivo* [86]. MAPK pathway activation, which is found in the majority of melanomas, results in MITF phosphorylation at Ser73 by ERK2 [87]. Phosphorylation at Ser73 affects MITF regulation in two ways: enhanced recruitment of p300, an MITF transcriptional coactivator and histone acetyltransferase, and increased ubiquitination of MITF [88, 89]. Because Ser73 phosphorylation ultimately accelerates proteasomal degradation of MITF, MAPK signaling in melanomas can reduce

expression of many MITF targets. BRAF inhibitors may enhance immunotherapy by stabilizing MITF and upregulating transcription of targets like MART1 and other antigens that are recognized by the immune response to melanoma [90].

Other post-translational modifications of MITF include phosphorylation by ribosomal S6 kinase (RSK), glycogen synthase kinase-3β (GSK-3), and p38 and sumoylation by protein inhibitor of the activated STAT3 (PIAS3) [91–94]. MITF is also a substrate for proteolytic degradation by caspase 3 [95]. Protein kinase C interacting protein 1 and PIAS3, which preferentially binds Ser73-phosphorylated MITF, inhibit MITF binding to DNA [14, 96–98]. Sumoylation of MITF reduces transcription of a subset of MITF targets whose promoters contain multiple MITF binding sites [99, 100]. In light of this observation and the complexity of MITF regulation, it is tempting to speculate that post-translational modifications determine MITF target gene specificity in response to cell context. By such mechanisms, MITF may be able to switch between its two recognized functions of regulating melanocytic differentiation/pigmentation and modulating survival/proliferation effects capable of producing an oncogenic transcriptional program in melanoma.

Germline loss-of-function mutation of MITF in humans causes Waardenburg syndrome type IIA, an autosomal dominant inherited condition characterized by lack of melanocytes in the eye, forelock, and inner ear [101]. Melanocyte deficiencies in individuals with Waardenburg syndrome result in deafness, white forelock (unpigmented hair in the midline), and eye color variability [102]. In contrast, increased numbers of nevi and darker eye colors are associated with the gain-of-function mutation conferred by a germline missense mutation in codon 318 of MITF. As previously discussed, this mutation abrogates a sumoylation site, resulting in altered transcription of some MITF targets and elevated melanoma susceptibility [12, 13] (Fig. 2.2).

2.6 Acral and Mucosal Melanomas

KIT mutations and amplifications are the most common genetic alterations in melanomas arising in acral, mucosal, and chronically sun-damaged skin. Although KIT mutations are found in only 1% of all melanomas, they are reported in 10% of acral and 10% of mucosal melanomas [103]. Less than 10% of KIT mutant melanomas contain BRAF or NRAS mutations.

c-KIT encodes the receptor tyrosine kinase for stem cell factor. In response to ligand binding, KIT activates signaling of pathways such as RAS. The most commonly observed KIT variant in melanoma, found in a third of KIT-mutant melanomas, is L576P [55]. Activating mutations such as L576P promote melanocyte growth and survival by causing constitutive stimulation of MAPK and PI3K/AKT signaling. KIT mutations are associated with poorer clinical outcomes in acral and mucosal melanoma [119].

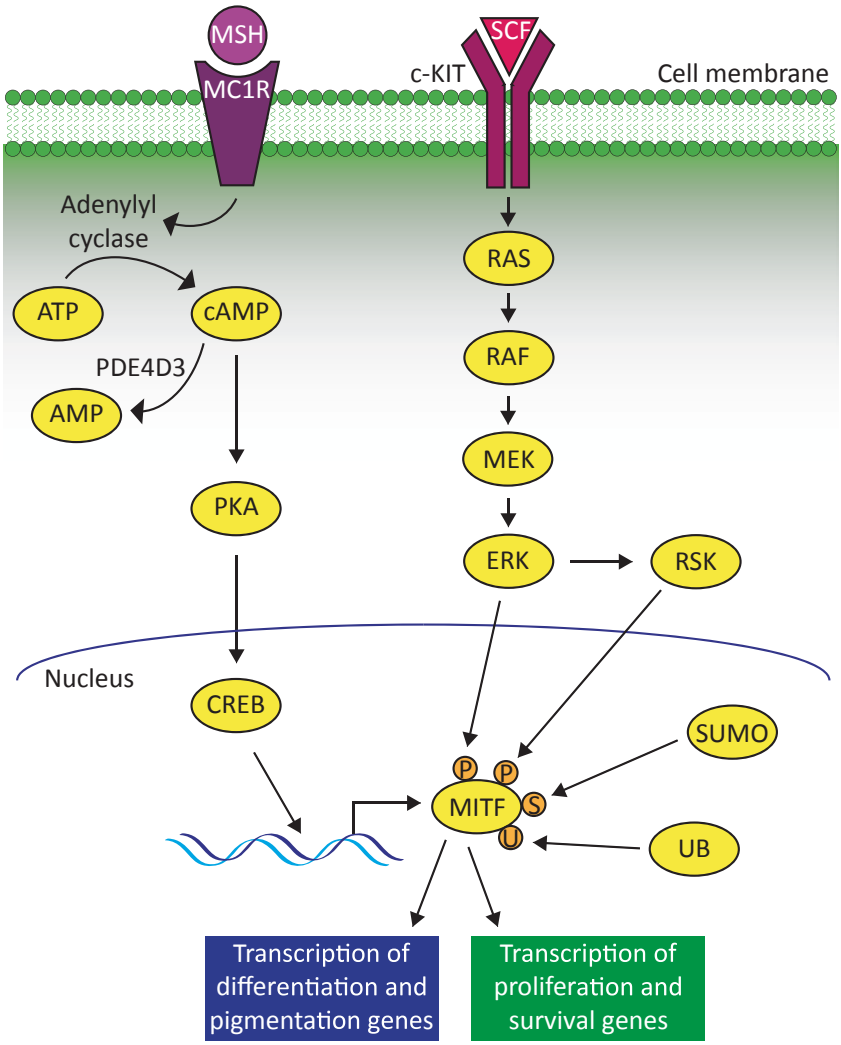

Fig. 2.2 The MITFaxis. In melanocytes, microphthalmia-associated transcription factor (*MITF*) is expressed in response to melanocortin 1 receptor (*MC1R*) signaling upon binding of melanocyte-stimulating hormone (*MSH*). Non-signaling variants of *MC1R* are associated with the red hair/fair skin phenotype and increased melanoma susceptibility. *MITF* activity is modulated by phosphorylation, sumoylation, and ubiquitination. *MITF* target genes include regulators of differentiation and pigmentation as well as proliferation and survival. *c-KIT* signaling is essential for melanocyte development. *c-KIT*, *NRAS*, *BRAF*, and *MITF* are known melanoma oncogenes in the *c-KIT* pathway. *SCF* stem cell factor, *cAMP* cyclic AMP, *PKA* protein kinase A; *CREB* cAMP-responsive element-binding protein

2.7 Uveal Melanoma

Unlike other clinical subtypes of melanoma, uveal melanomas rarely if ever involve mutations in BRAF, NRAS, or KIT. The dominant genetic alterations observed in uveal melanomas are somatic activating mutations in one of two heterotrimeric G protein α-subunits: GNAQ and GNA11. These mutations are almost never concomitant and are exclusively found in 80% of uveal melanomas, with GNAQ and GNA11 each affected in 40% of uveal melanomas. GNAQ and GNA11 mutations are also commonly found in proliferations of dermal melanocytes called blue nevi. In contrast, GNAQ and GNA11 mutations were only found in 1 of 273 (0.4%) of extraocular melanomas [104, 105].

G protein α-subunits are GTPases that serve as molecular switches for the G protein, which is active in its GTP-bound state and inactive in its GDP-bound state. In uveal melanoma, GNAQ and GNA11 mutations are restricted to codon R183 in exon 4 and codon Q209 in exon 5 [105], and their effect is to trap GNAQ and GNA11 in their active, GTP-bound states [106, 107]. As a result, GNAQ and GNA11 mutations contribute to uveal melanomagenesis by activating signaling of numerous pathways regulated by GPCRs including the MAPK pathway [105].

Interestingly, when taken together, the incidence of GNAQ and GNA11 mutations is not higher in uveal melanoma metastases than in primary uveal melanomas. However, in one study of 187 patients GNAQ mutations were proportionally more common in primary uveal melanomas while GNA11 mutations were found in a greater fraction of metastases, suggesting that stratifying by affected G protein α-subunit may be clinically useful [105].

Loss of the tumor suppressor BRCA1-associated protein (BAP1) on chromosome 3 is associated with metastatic uveal melanoma. BAP1 encodes a deubiquitinase that is a component of Polycomb-repressive complexes. Loss of BAP1 in uveal melanoma is thought to most often result from loss of one chromosome 3 allele combined with somatic mutation in the other BAP1 allele. Complete or partial monosomy of chromosome 3 occurs in about 25% of uveal melanomas [108].

While uveal melanoma may be diagnosed at relatively early stages due to visual symptoms, the disease has a striking propensity to metastasize to the liver. BAP1 mutation predicts poor clinical outcome and is particularly associated with risk of metastatic disease: in one study BAP1 was mutated in 84% of uveal melanomas from patients at high risk for metastasis but only 4% of tumors from patients at low risk for metastasis [109].

Germline BAP1 mutation or loss predisposes individuals to malignancy, with familial uveal melanoma accounting for 2–5% of all uveal melanoma cases. However, penetrance of disease is relatively low in these families, perhaps because inactivation of BAP1 occurs as a late event in melanoma progression [110].

2.8 Melanoma Genomics

Recently, improving technologies, robust bioinformatics platforms, and declining costs of sequencing have made comprehensive analysis of melanoma mutations accessible. These analyses are complicated by tumor heterogeneity and the high mutation rate associated with melanoma. Genome sequencing has revealed that the rates of base mutation are higher in melanoma than in other solid tumors [111]. The elevated mutational load is almost entirely attributable to cytidine to thymidine (C>T) transitions, which can be induced by UVR exposure. Traditionally, C>T mutations at dipyrimidine sequences in the context of melanoma are considered UVB signature mutations while G>T mutations are attributed to oxidative damage mediated by UVA. However, many recurrent mutations in melanoma, including oncogenic BRAF and NRAS lesions, do not involve C>T or G>T base changes, suggesting that alternate mutagenic mechanisms may be involved.

The high somatic mutation rate in melanoma is an important challenge when discriminating between true driver mutations, which confer a fitness advantage to the tumor cell during melanomagenesis, and passenger mutations. A recent statistical approach to sequence analysis refined the predicted background passenger mutation rate to be heterogeneous rather than genome-uniform by allowing for variations associated with transcriptional status and location relative to exons. This approach infers positive selection at each locus based on the exon/intron distribution of mutations and predicted functional consequences of mutations. By this analysis, 46 and 9% of melanoma driver mutations can be attributed to C>T or G>T mutations, respectively, accounting for two-thirds of all non-BRAF or NRAS driver mutations [62].

Since the first genome of a melanoma cell line was published in 2010 [111], exome and whole-genome sequencing of patient tumors has identified multiple novel melanoma genes. In studies sampling up to 25 tumors, recurrent somatic mutations were identified in the downstream MAPK pathway components MAP3K5, MAP3K9, MEK1, and MEK2 [65, 66], ionotropic glutamate receptor GRIN2A [112], and the phosphatidylinositol 3,4,5-trisphosphate RAC exchange factor PREX2 [113].

In one report, GRIN2A mutations were found in one quarter of melanomas [112]. Although GRIN2A has not been functionally validated as an oncogene, glutamate receptor pathway dysregulation was previously implicated in melanoma in studies of another glutamate receptor, GRM3 [114]. Activated GRM3 is an accessory to MAPK signaling and can itself be mutated in melanomas [115]. PREX2 has been shown to negatively regulate PTEN in breast cancer and was mutated in 23 out of 107 melanomas in another study [113].

Whole-exome sequencing of larger melanoma cohorts, including 147 and 121 tumors respectively, identified novel melanoma genes including RAC1 and PPP6C [62, 116]. Recurrent mutations in both RAC1 and PPP6C result from C>T transitions. Somatic gain-of-function mutations in RAC1 were found in 5–10% of melanomas. These mutations destabilize Rac1's inactive GDP-bound state and result in

increased Rac1 activation, promoting cell proliferation and migration [116]. PPP6C encodes a serine/threonine phosphatase that was mutated in approximately 10% of melanomas. PPP6C acts as a tumor suppressor by negatively regulating levels of cyclin D1 (CCND1) during the G1 phase of the cell cycle. Thus, PPP6C loss-of-function mutations likely dysregulate cell cycle and mitosis in some melanomas.

2.9 Conclusion

Although melanoma is a highly heterogeneous disease with respect to clinical behavior, histology, and underlying genomic aberrations and mutations, several themes have emerged in our understanding of its molecular pathogenesis. The MAPK pathway is the key signaling pathway, with activating mutations in BRAF, NRAS, KIT, GNAQ, or GNA11 found in almost all melanomas. The RB and p53 pathways are also frequently dysregulated in melanoma and are implicated in many familial cases. Given the important role of MAPK, RB, and p53 signaling in other malignancies, understanding abnormalities of these pathways may have broad implications for research and treatment of many cancers.

Lineage-specific activity is known to contribute to melanomagenesis as well, with amplification and dysregulation of MITF found in 20% of melanomas. Other genes, which are less commonly affected, have been identified by analysis of large exome sequence datasets and other methods. In the future, intron and UTR sequence data from whole-genome sequencing will allow further refinement of algorithms and increased statistical power to find low frequency driver mutations in melanoma.

Despite substantial progress in our understanding of melanoma pathogenesis, several important observations remain unexplained. Sun exposure is the leading environmental risk factor for melanoma, but the most common oncogenic mutations (in BRAF and NRAS) are not caused by known UV-related mechanisms. Sunscreens confer protection against cutaneous squamous cell carcinoma and have been shown to diminish melanoma incidence in certain contexts, but less so (or not at all) in other studies, suggesting a complexity that is poorly understood [117, 118]. Moreover, a recent study reported that the red hair/fair skin pigmentation phenotype is associated with elevated melanoma risk independent of UV exposure [18]. Elucidating the molecular basis for UV-independent melanoma susceptibility and genetic lesions will provide the framework for progress in melanoma prevention.

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References

1. Chin L, Garraway LA, Fisher DE. Malignant melanoma: genetics and therapeutics in the genomic era. Genes Dev. 2006;20:2149–82.
2. Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. Nature. 2007;445:843–50.
3. Valverde P, Healy E, Jackson I, Rees JL, Thody AJ. Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. Nat Genet. 1995;11:328–30.
4. Cui R, Widlund HR, Feige E, Lin JY, Wilensky DL, Igras VE, D'Orazio J, Fung CY, Schanbacher CF, Granter SR. Central role of p53 in the suntan response and pathologic hyperpigmentation. Cell. 2007;128:853.
5. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. J Photochem Photobiol B: Biol. 2001;63:8–18.
6. Whiteman DC, Whiteman CA, Green AC. Childhood sun exposure as a risk factor for melanoma: a systematic review of epidemiologic studies. Cancer Causes Control. 2001;12:69–82.
7. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Zanetti R, Masini C, Boyle P, Melchi CF. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. Eur J Cancer. 2005;41:2040–59.
8. Zuo L, Weger J, Yang Q, Goldstein AM, Tucker MA, Walker GJ, Hayward N, Dracopoli NC. Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. Nat Genet. 1996;12:97–9.
9. Goldstein AM, Chan M, Harland M, Hayward NK, Demenais F, Bishop DT, Azizi E, Bergman W, Bianchi-Scarra G, Bruno W, Calista D, Albright LAC, Chaudru V, Chompret A, Cuellar F, Elder DE, Ghiorzo P, Gillanders EM, Gruis NA, Hansson J, Hogg D, Holland EA, Kanetsky PA, Kefford RF, Landi MT, Lang J, Leachman SA, MacKie RM, Magnusson V, Mann GJ, Bishop JN, Palmer JM, Puig S, Puig-Butille JA, Stark M, Tsao H, Tucker MA, Whitaker L, Yakobson E. Lund melanoma study group, melanoma genetics consortium (GenoMEL): features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. J Med Genet. 2007;44:99–106.
10. Hussussian CJ, Struewing JP, Goldstein AM, Higgins PA, Ally DS, Sheahan MD, Clark WH, Tucker MA, Dracopoli NC. Germline p16 mutations in familial melanoma. Nat Genet. 1994;8:15–21.
11. Bergman W, Gruis N. Familial melanoma and pancreatic cancer. N Engl J Med. 1996;334:471–2.
12. Yokoyama S, Woods SL, Boyle GM, Aoude LG, MacGregor S, Zismann V, Gartside M, Cust AE, Haq R, Harland M, Taylor JC, Duffy DL, Holohan K, Dutton-Regester K, Palmer JM, Bonazzi V, Stark MS, Symmons J, Law MH, Schmidt C, Lanagan C, O'Connor L, Holland EA, Schmid H, Maskiell JA, Jetann J, Ferguson M, Jenkins MA, Kefford RF, Giles GG, Armstrong BK, Aitken JF, Hopper JL, Whiteman DC, Pharoah PD, Easton DF, Dunning AM, Newton-Bishop JA, Montgomery GW, Martin NG, Mann GJ, Bishop DT, Tsao H, Trent JM, Fisher DE, Hayward NK, Brown KM. A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. Nature. 2011;480:99–103.
13. Bertolotto C, Lesueur F, Giuliano S, Strub T, de Lichy M, Bille K, Dessen P, d'Hayer B, Mohamdi H, Remenieras A, Maubec E, la Fouchardière de A, Molinié V, Vabres P, Dalle S, Poulalhon N, Martin-Denavit T, Thomas L, Andry-Benzaquen P, Dupin N, Boitier F, Rossi A, Perrot J-L, Labeille B, Robert C, Escudier B, Caron O, Brugières L, Saule S, Gardie B, Gad S, Richard S, Couturier J, Teh BT, Ghiorzo P, Pastorino L, Puig S, Badenas C, Olsson H, Ingvar C, Rouleau E, Lidereau R, Bahadoran P, Vielh P, Corda E, Blanché H, Zelenika D, Galan P, French Familial Melanoma Study Group, Aubin F, Bachollet B, Becuwe C, Berthet P, Bignon YJ, Bonadona V, Bonafe J-L, Bonnet-Dupeyron M-N, Cambazard F, Chevrant-Breton J, Coupier I, Dalac S, Demange L, d'Incan M, Dugast C, Faivre L, Vincent-Fétita L, Gauthier-Villars M, Gilbert B, Grange F, Grob J-J, Humbert P, Janin N, Joly P, Kerob D, Lasset C, Leroux D, Levang J, Limacher J-M, Livideanu C, Longy M, Lortholary A, Stoppa-

Lyonnet D, Mansard S, Mansuy L, Marrou K, Matéus C, Maugard C, Meyer N, Nogues C, Souteyrand P, Venat-Bouvet L, Zattara H, Chaudru V, Lenoir GM, Lathrop M, Davidson I, Avril M-F, Demenais F, Ballotti R, Bressac-de Paillerets B. A Sumoylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. Nature. 2011;480:94–8.
14. Miller AJ, Levy C, Davis IJ, Razin E, Fisher DE. Sumoylation of MITF and its related family members TFE3 and TFEB. J Biol Chem. 2005;280:146–55.
15. Bishop DT, Demenais F, Iles MM, Harland M, Taylor JC, Corda E, Randerson-Moor J, Aitken JF, Avril M-F, Azizi E, Bakker B, Bianchi-Scarra G, Bressac-de Paillerets B, Calista D, Cannon-Albright LA, Chin-A-Woeng T, Debniak T, Galore-Haskel G, Ghiorzo P, Gut I, Hansson J, Hocevar M, Höiom V, Hopper JL, Ingvar C, Kanetsky PA, Kefford RF, Landi MT, Lang J, Lubiński J, Mackie R, Malvehy J, Mann GJ, Martin NG, Montgomery GW, van Nieuwpoort FA, Novakovic S, Olsson H, Puig S, Weiss M, van Workum W, Zelenika D, Brown KM, Goldstein AM, Gillanders EM, Boland A, Galan P, Elder DE, Gruis NA, Hayward NK, Lathrop GM, Barrett JH, Bishop JAN. Genome-wide association study identifies three loci associated with melanoma risk. Nat Genet. 2009;41:920–5.
16. Gudbjartsson DF, Sulem P, Stacey SN, Goldstein AM, Rafnar T, Sigurgeirsson B, Benediktsdottir KR, Thorisdottir K, Ragnarsson R, Sveinsdottir SG, Magnusson V, Lindblom A, Kostulas K, Botella-Estrada R, Soriano V, Juberías P, Grasa M, Saez B, Andres R, Scherer D, Rudnai P, Gurzau E, Koppova K, Kiemeney LA, Jakobsdottir M, Steinberg S, Helgason A, Gretarsdottir S, Tucker MA, Mayordomo JI, Nagore E, Kumar R, Hansson J, Olafsson JH, Gulcher J, Kong A, Thorsteinsdottir U, Stefansson K. ASIP and TYR pigmentation variants associate with cutaneous melanoma and basal cell carcinoma. Nat Genet. 2008;40:886–91.
17. Rees JL. Genetics of hair and skin color. Annu Rev Genet. 2003;37:67–90.
18. Mitra D, Luo X, Morgan A, Wang J, Hoang MP, Lo J, Guerrero CR, Lennerz JK, Mihm MC, Wargo JA, Robinson KC, Devi SP, Vanover JC, D'Orazio JA, McMahon M, Bosenberg MW, Haigis KM, Haber DA, Wang Y, Fisher DE. An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. Nature. 2012;491:449–53.
19. Chong H, Guan K-L. Regulation of Raf through phosphorylation and N terminus-C terminus interaction. J Biol Chem. 2003;278:36269–76.
20. Beeram M, Patnaik A, Rowinsky EK. Raf: a strategic target for therapeutic development against cancer. J Clin Oncol. 2005;23:6771–90.
21. Daniotti M, Oggionni M, Ranzani T, Vallacchi V, Campi V, Di Stasi D, Torre GD, Perrone F, Luoni C, Suardi S, Frattini M, Pilotti S, Anichini A, Tragni G, Parmiani G, Pierotti MA, Rodolfo M. BRAF alterations are associated with complex mutational profiles in malignant melanoma. Oncogene. 2004;23:5968–77.
22. Kumar R, Angelini S, Snellman E, Hemminki K. BRAF mutations are common somatic events in melanocytic nevi. J Invest Dermatol. 2004;122:342–8.
23. Ciampi R, Nikiforov YE. Alterations of the BRAF gene in thyroid tumors. Endocr Pathol. 2005;16:163–72.
24. Young J, Barker MA, Simms LA, Walsh MD, Biden KG, Buchanan D, Buttenshaw R, Whitehall VLJ, Arnold S, Jackson L, Kambara T, Spring KJ, Jenkins MA, Walker GJ, Hopper JL, Leggett BA, Jass JR. Evidence for BRAF mutation and variable levels of microsatellite instability in a syndrome of familial colorectal cancer. Clin Gastroenterol Hepatol. 2005;3;254–63.
25. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W. Mutations of the BRAF gene in human cancer. Nature. 2002;417:949–54.
26. Wan PTC, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, Marshall CJ, Springer CJ, Barford D, Marais R. Cancer genome project: mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004;116:855–67.
27. Fischer A, Hekman M, Kuhlmann J, Rubio I, Wiese S, Rapp UR. B- and C-RAF display essential differences in their binding to Ras: the isotype-specific N terminus of B-RAF facilitates Ras binding. J Biol Chem. 2007;282:26503–16.
28. Tran NH, Wu X, Frost JA. B-Raf and Raf-1 are regulated by distinct autoregulatory mechanisms. J Biol Chem. 2005;280:16244–53.

29. Smalley KSM, Xiao M, Villanueva J, Nguyen TK, Flaherty KT, Letrero R, Van Belle P, Elder DE, Wang Y, Nathanson KL, Herlyn M. CRAF inhibition induces apoptosis in melanoma cells with non-V600E BRAF mutations. Oncogene. 2009;28:85–94.
30. Wang H-T, Choi B, Tang M-S. Melanocytes are deficient in repair of oxidative DNA damage and UV-induced photoproducts. Proc Natl Acad Sci USA. 2010;107:12180–5.
31. Ball NJ, Yohn JJ, Morelli JG, Norris DA, Golitz LE, Hoeffler JP. Ras mutations in human melanoma: a marker of malignant progression. J Invest Dermatol. 1994;102:285–90.
32. van't Veer LJ, Burgering BM, Versteeg R, Boot AJ, Ruiter DJ, Osanto S, Schrier PI, Bos JL. N-ras mutations in human cutaneous melanoma from sun-exposed body sites. Mol Cell Biol. 1989;9:3114–6.
33. Stevens CW, Manoharan TH, Fahl WE. Characterization of mutagen-activated cellular oncogenes that confer anchorage independence to human fibroblasts and tumorigenicity to NIH 3T3 cells: sequence analysis of an enzymatically amplified mutant HRAS allele. Proc Natl Acad Sci U S A. 1988;85:3875–9.
34. Yuasa Y, Gol RA, Chang A, Chiu IM, Reddy EP, Tronick SR, Aaronson SA. Mechanism of activation of an N-ras oncogene of SW-1271 human lung carcinoma cells. Proc Natl Acad Sci U S A. 1984;81:3670–4.
35. Tsao H, Goel V, Wu H, Yang G, Haluska FG. Genetic interaction between NRAS and BRAF mutations and PTEN/MMAC1 inactivation in melanoma. J Invest Dermatol. 2004;122:337–41.
36. Goel VK, Lazar AJF, Warneke CL, Redston MS, Haluska FG. Examination of mutations in BRAF, NRAS, and PTEN in primary cutaneous melanoma. J Invest Dermatol. 2006;126:154–60.
37. Heidorn SJ, Milagre C, Whittaker S, Nourry A, Niculescu-Duvas I, Dhomen N, Hussain J, Reis-Filho JS, Springer CJ, Pritchard C, Marais R. Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell. 2010;140:209–21.
38. Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, Chen Z, Lee M-K, Attar N, Sazegar H, Chodon T, Nelson SF, McArthur G, Sosman JA, Ribas A, Lo RS. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature. 2010;468:973–7.
39. Omholt K, Kröckel D, Ringborg U, Hansson J. Mutations of PIK3CA are rare in cutaneous melanoma. Melanoma Res. 2006;16:197–200.
40. Dai DL, Martinka M, Li G, Dai DL. Prognostic significance of activated Akt expression in melanoma: a clinicopathologic study of 292 cases. J Clin Oncol. 2005;23:1473–82.
41. Stark, M., Hayward, N. Genome-wide loss of heterozygosity and copy number analysis in melanoma using high-density single-nucleotide polymorphism arrays. Cancer Res. 2007;67:2632–42.
42. Lin WM, Baker AC, Beroukhim R, Winckler W, Feng W, Marmion JM, Laine E, Greulich H, Tseng H, Gates C, Hodi FS, Dranoff G, Sellers WR, Thomas RK, Meyerson M, Golub TR, Dummer R, Herlyn M, Getz G, Garraway LA. Modeling genomic diversity and tumor dependency in malignant melanoma. Cancer Res. 2008;68:664–73.
43. Zhou XP, Gimm O, Hampel H, Niemann T, Walker MJ, Eng C. Epigenetic PTEN silencing in malignant melanomas without PTEN mutation. Am J Pathol. 2000;157:1123–8.
44. Bastian BC. Understanding the progression of melanocytic neoplasia using genomic analysis: from fields to cancer. Oncogene. 2003;22:3081–6
45. Dankort D, Curley DP, Cartlidge RA, Nelson B, Karnezis AN, Damsky WE, You MJ, DePinho RA, McMahon M, Bosenberg M. Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. Nat Genet. 2009;41:544–52.
46. Pollock PM, Harper UL, Hansen KS, Yudt LM, Stark M, Robbins CM, Moses TY, Hostetter G, Wagner U, Kakareka J, Salem G, Pohida T, Heenan P, Duray P, Kallioniemi O, Hayward NK, Trent JM, Meltzer PS. High frequency of BRAF mutations in nevi. Nat Genet. 2003;33:19–20.

47. Yazdi AS, Palmedo G, Flaig MJ, Puchta U, Reckwerth A, Rütten A, Mentzel T, Hügel H, Hantschke M, Schmid-Wendtner M-H, Kutzner H, Sander CA. Mutations of the BRAF gene in benign and malignant melanocytic lesions. J Invest Dermatol. 2003;121:1160–2.
48. Saldanha G, Purnell D, Fletcher A, Potter L, Gillies A, Pringle JH. High BRAF mutation frequency does not characterize all melanocytic tumor types. Int J Cancer. 2004;111:705–10.
49. Jafari M, Papp T, Kirchner S, Diener U, Henschler D, Burg G, Schiffmann D. Analysis of ras mutations in human melanocytic lesions: activation of the ras gene seems to be associated with the nodular type of human malignant melanoma. J Cancer Res Clin Oncol. 1995;121:23–30.
50. Papp T, Pemsel H, Zimmermann R, Bastrop R, Weiss DG, Schiffmann D. Mutational analysis of the N-ras, p53, p16INK4a, CDK4, and MC1R genes in human congenital melanocytic naevi. J Med Genet. 1999;36:610–4.
51. Bastian BC, LeBoit PE, Pinkel D. Mutations and copy number increase of HRAS in Spitz nevi with distinctive histopathological features. Am J Pathol. 2000;157:967–72.
52. Michaloglou C, Vredeveld LCW, Soengas MS, Denoyelle C, Kuilman T, van der Horst CMAM, Majoor DM, Shay JW, Mooi WJ, Peeper DS. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature. 2005;436:720–4.
53. Dhomen N, Reis-Filho JS, Da Dias SR, Hayward R, Savage K, Delmas V, Larue L, Pritchard C, Marais R. Oncogenic Braf induces melanocyte senescence and melanoma in mice. Cancer cell. 2009;15:294–303.
54. Patton EE, Widlund HR, Kutok JL, Kopani KR, Amatruda JF, Murphey RD, Berghmans S, Mayhall EA, Traver D, Fletcher CDM, Aster JC, Granter SR, Look AT, Lee C, Fisher DE, Zon LI. BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. Curr Biol. 2005;15:249–54.
55. Willmore-Payne C, Holden JA, Tripp S, Layfield LJ. Human malignant melanoma: detection of BRAF- and c-kit-activating mutations by high-resolution amplicon melting analysis. Human Pathology. 2005;36:486–93.
56. Poynter JN, Elder JT, Fullen DR, Nair RP, Soengas MS, Johnson TM, Redman B, Thomas NE, Gruber SB. BRAF and NRAS mutations in melanoma and melanocytic nevi. Melanoma Res. 2006;16:267–73.
57. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013.
58. Sharma A, Trivedi N, Zimmerman M, Tuveson D, Smith C, Robertson G, Sharma A. Mutant V599EB-Raf regulates growth and vascular development of malignant melanoma tumors. Cancer Res. 2005;65:2412–21.
59. Furge KA, Kiewlich D, Le P, Vo MN, Faure M, Howlett AR, Lipson KE, Vande Woude GF, Webb CP. Suppression of Ras-mediated tumorigenicity and metastasis through inhibition of the Met receptor tyrosine kinase. Proc Natl Acad Sci U S A. 2001;98:10722–7.
60. Bardeesy N, Kim M, Xu J, Kim R, Shen Q. Role of epidermal growth factor receptor signaling in RAS-driven melanoma. Mol Cell Biol. 2005;25(10):4176–88.
61. Monsel G, Ortonne N, Bagot M, Bensussan A, Dumaz N. c-Kit mutants require hypoxia-inducible factor 1alpha to transform melanocytes. Oncogene. 2010;29: 227–36.
62. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat J-P, Nickerson E, Auclair D, Li L, Place C, DiCara D, Ramos AH, Lawrence MS, Cibulskis K, Sivachenko A, Voet D, Saksena G, Stransky N, Onofrio RC, Winckler W, Ardlie K, Wagle N, Wargo J, Chong K, Morton DL, Stemke-Hale K, Chen G, Noble M, Meyerson M, Ladbury JE, Davies MA, Gershenwald JE, Wagner SN, Hoon DSB, Schadendorf D, Lander ES, Gabriel SB, Getz G, Garraway LA, Chin L. A landscape of driver mutations in melanoma. Cell. 2012;150;251–63.
63. Maertens O, Johnson B, Hollstein P, Frederick DT, Cooper ZA, Messaien L, Bronson RT, McMahon M, Granter S, Flaherty KT, Wargo JA, Marais R, Cichowski K. Elucidating distinct roles for NF1 in melanomagenesis. Cancer Discov. 2012.
64. Bianchi AB, Hara T, Ramesh V, Gao J, Klein-Szanto AJ, Morin F, Menon AG, Trofatter JA, Gusella JF, Seizinger BR. Mutations in transcript isoforms of the neurofibromatosis 2 gene in multiple human tumour types. Nat Genet. 1994;6:185–92.

65. Nikolaev SI, Rimoldi D, Iseli C, Valsesia A, Robyr D, Gehrig C, Harshman K, Guipponi M, Bukach O, Zoete V, Michielin O, Muehlethaler K, Speiser D, Beckmann JS, Xenarios I, Halazonetis TD, Jongeneel CV, Stevenson BJ, Antonarakis SE. Exome sequencing identifies recurrent somatic MAP2K1 and MAP2K2 mutations in melanoma. Nat Genet. 2011;44:133–39.
66. Stark MS, Woods SL, Gartside MG, Bonazzi VF, Dutton-Regester K, Aoude LG, Chow D, Sereduk C, Niemi NM, Tang N. Frequent somatic mutations in MAP3K5 and MAP3K9 in metastatic melanoma identified by exome sequencing. Nat Genet. 2011;44:165–9.
67. Chin L. The genetics of malignant melanoma: lessons from mouse and man. Nat Rev Cancer. 2003;3:559–70.
68. Koh J, Enders GH, Dynlacht BD, Harlow E. Tumour-derived p16 alleles encoding proteins defective in cell-cycle inhibition. Nature. 1995;375:506–10.
69. Soufir N, Avril MF, Chompret A, Demenais F, Bombled J, Spatz A, Stoppa-Lyonnet D, Bénard J, Bressac-de Paillerets B. Prevalence of p16 and CDK4 germline mutations in 48 melanoma-prone families in France. Hum Mol Genet. 1998;7:209–16.
70. Muthusamy V, Hobbs C, Nogueira C, Cordon-Cardo C, McKee PH, Chin L, Bosenberg MW. Amplification of CDK4 and MDM2 in malignant melanoma. Genes Chromosom Cancer. 2006;45:447–54.
71. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho K-H, Aiba S, Bröcker E-B, LeBoit PE, Pinkel D, Bastian BC. Distinct sets of genetic alterations in melanoma. N Engl J Med. 2005;353:2135–47.
72. Smalley KSM, Lioni M, Dalla Palma M, Xiao M, Desai B, Egyhazi S, Hansson J, Wu H, King AJ, Van Belle P, Elder DE, Flaherty KT, Herlyn M, Nathanson KL. Increased cyclin D1 expression can mediate BRAF inhibitor resistance in BRAF V600E-mutated melanomas. Mol Cancer Ther. 2008;7:2876–83.
73. Soussi T, Kato S, Levy PP, Ishioka C. Reassessment of the TP53 mutation database in human disease by data mining with a library of TP53 missense mutations. Hum Mutat. 2005;25:6–17.
74. Yang G, Rajadurai A, Tsao H. Recurrent patterns of dual RB and p53 pathway inactivation in melanoma. J Invest Dermatol. 2005;125:1242–51.
75. Kamijo T, Weber JD, Zambetti G, Zindy F, Roussel MF, Sherr CJ. Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. Proc Natl Acad Sci U S A. 1998;95:8292–7.
76. Stott FJ, Bates S, James MC, McConnell BB, Starborg M, Brookes S, Palmero I, Ryan K, Hara E, Vousden KH, Peters G. The alternative product from the human CDKN2A locus, p14(ARF), participates in a regulatory feedback loop with p53 and MDM2. EMBO J. 1998;17:5001–14.
77. Zhang Y, Xiong Y, Yarbrough WG. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. Cell. 1998;92:725–34.
78. Garraway LA, Widlund HR, Rubin MA, Getz G, Berger AJ, Ramaswamy S, Beroukhim R, Milner DA, Granter SR, Du J, Lee C, Wagner SN, Li C, Golub TR, Rimm DL, Meyerson ML, Fisher DE, Sellers WR. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. Nature. 2005;436:117–22.
79. Ugurel S, Houben R, Schrama D, Voigt H, Zapatka M, Schadendorf D, Bröcker EB, Becker JC. Microphthalmia-associated transcription factor gene amplification in metastatic melanoma is a prognostic marker for patient survival, but not a predictive marker for chemosensitivity and chemotherapy response. Clin Cancer Res. 2007;13:6344–50.
80. McGill GG, Horstmann M, Widlund HR, Du J, Motyckova G, Nishimura EK, Lin Y-L, Ramaswamy S, Avery W, Ding H-F, Jordan SA, Jackson IJ, Korsmeyer SJ, Golub TR, Fisher DE. Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. Cell. 2002;109:707–18.
81. Du J, Widlund HR, Horstmann MA, Ramaswamy S, Ross K, Huber WE, Nishimura EK, Golub TR, Fisher DE. Critical role of CDK2 for melanoma growth linked to its melanocyte-specific transcriptional regulation by MITF. Cancer Cell. 2004;6:565–76.
82. Loercher AE, Tank EMH, Delston RB, Harbour JW. MITF links differentiation with cell cycle arrest in melanocytes by transcriptional activation of INK4A. J Cell Biol. 2005;168:35–40.

83. Carreira S, Goodall J, Aksan I, La Rocca SA, Galibert M-D, Denat L, Larue L, Goding CR. Mitf cooperates with Rb1 and activates p21Cip1 expression to regulate cell cycle progression. Nature. 2005;433:764–9.
84. Levy C, Khaled M, Fisher DE. MITF: master regulator of melanocyte development and melanoma oncogene. Trends Mol Med. 2006;12:406–14.
85. Haq R, Yokoyama S, Hawryluk EB, Jönsson GB, Frederick DT, McHenry K, Porter D, Tran T-N, Love KT, Langer R, Anderson DG, Garraway LA, Duncan LM, Morton DL, Hoon DSB, Wargo JA, Song JS, Fisher DE. BCL2A1 is a lineage-specific antiapoptotic melanoma oncogene that confers resistance to BRAF inhibition. Proc Natl Acad Sci USA. 2013;110:4321–6.
86. Jané-Valbuena J, Widlund HR, Perner S, Johnson LA, Dibner AC, Lin WM, Baker AC, Nazarian RM, Vijayendran KG, Sellers WR, Hahn WC, Duncan LM, Rubin MA, Fisher DE, Garraway LA. An oncogenic role for ETV1 in melanoma. Cancer Res. 2010;70:2075–84.
87. Hemesath TJ, Price ER, Takemoto C, Badalian T, Fisher DE. MAP kinase links the transcription factor Microphthalmia to c-Kit signalling in melanocytes. Nature. 1998;391:298–301.
88. Price ER, Ding HF, Badalian T, Bhattacharya S, Takemoto C, Yao TP, Hemesath TJ, Fisher DE. Lineage-specific signaling in melanocytes. C-kit stimulation recruits p300/CBP to microphthalmia. J Biol Chem. 1998;273:17983–6.
89. Wellbrock C, Rana S, Paterson H, Pickersgill H, Brummelkamp T, Marais R. Oncogenic BRAF regulates melanoma proliferation through the lineage specific factor MITF. PLoS ONE. 2008;3:e2734.
90. Boni A, Cogdill AP, Dang P, Udayakumar D, Njauw C-NJ, Sloss CM, Ferrone CR, Flaherty KT, Lawrence DP, Fisher DE, Tsao H, Wargo JA. Selective BRAFV600E inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function. Cancer Res. 2010;70:5213–9.
91. Wu M, Hemesath TJ, Takemoto CM, Horstmann MA, Wells AG, Price ER, Fisher DZ, Fisher DE. c-Kit triggers dual phosphorylations, which couple activation and degradation of the essential melanocyte factor Mi. Genes Dev. 2000;14:301–12.
92. Weilbaecher KN, Motyckova G, Huber WE, Takemoto CM, Hemesath TJ, Xu Y, Hershey CL, Dowland NR, Wells AG, Fisher DE. Linkage of M-CSF signaling to Mitf, TFE3, and the osteoclast defect in Mitf(mi/mi) mice. Mol Cell. 2001;8:749–58.
93. Takeda K, Takemoto C, Kobayashi I, Watanabe A, Nobukuni Y, Fisher DE, Tachibana M. Ser298 of MITF, a mutation site in Waardenburg syndrome type 2, is a phosphorylation site with functional significance. Hum Mol Genet. 2000;9:125–32.
94. Mansky KC, Sankar U, Han J, Ostrowski MC. Microphthalmia transcription factor is a target of the p38 MAPK pathway in response to receptor activator of NF-kappa B ligand signaling. J Biol Chem. 2002;277:11077–83.
95. Larribere L, Hilmi C, Khaled M, Gaggioli C, Bille K, Auberger P, Ortonne JP, Ballotti R, Bertolotto C. The cleavage of microphthalmia-associated transcription factor, MITF, by caspases plays an essential role in melanocyte and melanoma cell apoptosis. Genes Dev. 2005;19:1980–5.
96. Levy C, Nechushtan H, Razin E. A new role for the STAT3 inhibitor, PIAS3: a repressor of microphthalmia transcription factor. J Biol Chem. 2002;277:1962–6.
97. Levy C, Sonnenblick A, Razin E. Role played by microphthalmia transcription factor phosphorylation and its Zip domain in its transcriptional inhibition by PIAS3. Mol Cell Biol. 2003;23:9073–80.
98. Razin E, Zhang ZC, Nechushtan H, Frenkel S, Lee YN, Arudchandran R, Rivera J. Suppression of microphthalmia transcriptional activity by its association with protein kinase C-interacting protein 1 in mast cells. J Biol Chem. 1999;274:34272–6.
99. Murakami H, Stroke NI, Arnheiter H. Sumoylation modulates transcriptional activity of MITF in a promoter-specific manner. Pigment Cell Research. 2005;18:265–77.
100. Miller AJ, Levy C, Davis IJ, Razin E, Fisher DE, Miller AJ. Sumoylation of MITF and its related family members TFE3 and TFEB. J Biol Chem. 2005;1–10.

101. Tassabehji M, Newton VE, Read AP. Waardenburg syndrome type 2 caused by mutations in the human microphthalmia (MITF) gene. Nat Genet. 1994;8:251–5.
102. Price ER, Fisher DE. Sensorineural deafness and pigmentation genes: melanocytes and the Mitf transcriptional network. Neuron. 2001;30:15.
103. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol. 2006;24:4340–6.
104. Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, Simpson EM, Barsh GS, Bastian BC. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. Nature. 2009;457:599–602.
105. Van Raamsdonk CD, Griewank KG, Crosby MB, Garrido MC, Vemula S, Wiesner T, Obenauf AC, Wackernagel W, Green G, Bouvier N, Sozen MM, Baimukanova G, Roy R, Heguy A, Dolgalev I, Khanin R, Busam K, Speicher MR, O'Brien J, Bastian BC. Mutations in GNA11 in uveal melanoma. N Engl J Med. 2010;363:2191–9.
106. Sondek J, Lambright DG, Noel JP, Hamm HE, Sigler PB. GTPase mechanism of Gproteins from the 1.7-A crystal structure of transducin alpha-GDP-AIF-4. Nature. 1994;372:276–9.
107. Kalinec G, Nazarali AJ, Hermouet S, Xu N, Gutkind JS. Mutated alpha subunit of the Gq protein induces malignant transformation in NIH 3T3 cells. Mol Cell Biol. 1992;12:4687–93.
108. Shields CL, Ganguly A, Bianciotto CG, Turaka K, Tavallali A, Shields JA. Prognosis of uveal melanoma in 500 cases using genetic testing of fine-needle aspiration biopsy specimens. Ophthalmology. 2011;118:396–401.
109. Harbour JW, Onken MD, Roberson EDO, Duan S, Cao L, Worley LA, Council ML, Matatall KA, Helms C, Bowcock AM. Frequent mutation of BAP1 in metastasizing uveal melanomas. Science. 2010;330:1410–3.
110. Harbour JW. The genetics of uveal melanoma: an emerging framework for targeted therapy. Pigment Cell Melanoma Res. 2012;25:171–81.
111. Pleasance ED, Cheetham RK, Stephens PJ, McBride DJ, Humphray SJ, Greenman CD, Varela I, Lin M-L, Ordóñez GR, Bignell GR, Ye K, Alipaz J, Bauer MJ, Beare D, Butler A, Carter RJ, Chen L, Cox AJ, Edkins S, Kokko-Gonzales PI, Gormley NA, Grocock RJ, Haudenschild CD, Hims MM, James T, Jia M, Kingsbury Z, Leroy C, Marshall J, Menzies A, Mudie LJ, Ning Z, Royce T, Schulz-Trieglaff OB, Spiridou A, Stebbings LA, Szajkowski L, Teague J, Williamson D, Chin L, Ross MT, Campbell PJ, Bentley DR, Futreal PA, Stratton MR. A comprehensive catalogue of somatic mutations from a human cancer genome. Nature. 2010;463:191–6.
112. Wei X, Walia V, Lin JC, Teer JK, Prickett TD, Gartner J, Davis S, Stemke-Hale K, Davies MA, Gershenwald JE, Robinson W, Robinson S, Rosenberg SA, Samuels Y. Exome sequencing identifies GRIN2A as frequently mutated in melanoma. Nat Genet. 2011;43:442–6.
113. Berger MF, Hodis E, Heffernan TP, Deribe YL, Lawrence MS, Protopopov A, Ivanova E, Watson IR, Nickerson E, Ghosh P, Zhang H, Zeid R, Ren X, Cibulskis K, Sivachenko AY, Wagle N, Sucker A, Sougnez C, Onofrio R, Ambrogio L, Auclair D, Fennell T, Carter SL, Drier Y, Stojanov P, Singer MA, Voet D, Jing R, Saksena G, Barretina J, Ramos AH, Pugh TJ, Stransky N, Parkin M, Winckler W, Mahan S, Ardlie K, Baldwin J, Wargo J, Schadendorf D, Meyerson M, Gabriel SB, Golub TR, Wagner SN, Lander ES, Getz G, Chin L, Garraway LA. Melanoma genome sequencing reveals frequent PREX2 mutations. Nature. 2012;485:502–6.
114. Pollock PM, Cohen-Solal K, Sood R, Namkoong J, Martino JJ, Koganti A, Zhu H, Robbins C, Makalowska I, Shin S-S, Marin Y, Roberts KG, Yudt LM, Chen A, Cheng J, Incao A, Pinkett HW, Graham CL, Dunn K, Crespo-Carbone SM, Mackason KR, Ryan KB, Sinsimer D, Goydos J, Reuhl KR, Eckhaus M, Meltzer PS, Pavan WJ, Trent JM, Chen S. Melanoma mouse model implicates metabotropic glutamate signaling in melanocytic neoplasia. Nat Genet. 2003;34;108–12.
115. Prickett TD, Wei X, Cardenas-Navia I, Teer JK, Lin JC, Walia V, Gartner J, Jiang J, Cherukuri PF, Molinolo A, Davies MA, Gershenwald JE, Stemke-Hale K, Rosenberg SA, Margulies EH, Samuels Y. Exon capture analysis of G protein-coupled receptors identifies activating mutations in GRM3 in melanoma. Nat Genet. 2011;43:1119–26.

116. Krauthammer M, Kong Y, Ha BH, Evans P, Bacchiocchi A, McCusker JP, Cheng E, Davis MJ, Goh G, Choi M, Ariyan S, Narayan D, Dutton-Regester K, Capatana A, Holman EC, Bosenberg M, Sznol M, Kluger HM, Brash DE, Stern DF, Materin MA, Lo RS, Mane S, Ma S, Kidd KK, Hayward NK, Lifton RP, Schlessinger J, Boggon TJ, Halaban R. Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma. Nat Genet. 2012;44:1006–14.
117. Green A, Williams G, Logan V, Strutton G. Reduced melanoma after regular sunscreen use: randomized trial follow-up. Clin Oncol. 2011;29:257–63.
118. Huncharek M, Kupelnick B. Use of topical sunscreens and the risk of malignant melanoma: a meta-analysis of 9067 patients from 11 case-control studies. Am J Public Health. 2002;92:1173–7.
119. Kong Y, Si L, Zhu Y, Xu X, Corless CL, Flaherty KT, Li L, Li H, Sheng X, Cui C, et al. Large-scale analysis of KIT aberrations in Chinese patients with melanoma. Clin Cancer Res. 2011;17:1684–91.