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Understanding the biology of melanoma and therapeutic implications

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Abstract

From 1976 – 2010, the US FDA approved only two medications for the treatment of metastatic melanoma, dacarbazine and high-dose interleukin 2. Between 2011–13, four agents were approved (ipilimumab, vemurafenib, dabrafenib, trametinib) and other therapies have shown great promise in clinical trials. This startling progress has been made possible by the groundbreaking efforts of basic scientists and the vision and innovation of translational and clinical investigators. Fundamental discoveries such as the identification of oncogenic mutations in the majority of melanomas, the elucidation of the molecular signaling resultant from these mutations, and the revelation that a number of cell surface molecules serve as regulators of immune activation, have all been instrumental to this progress. This chapter provides a summary of the molecular pathogenesis of melanoma by reviewing the relevant melanocyte biology and molecular signaling used by melanoma, describes the current efforts to target oncogene driven signaling, and presents the rationale for combining immune and molecular targeting.

Keywords

BRAF; NRAS; immunotherapy; targeted therapy; MITF

Introduction

Over the course of the past three decades, a number of breakthroughs have greatly expanded what is known about melanocyte biology, relevant oncogenic mutations as well as deletions and amplifications in melanoma, the influence of molecular signaling pathways on melanomagenesis, and the interaction of aberrant signaling pathways with host immune elements. This increased understanding has led to a remarkable number of improvements in

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the diagnosis, classification, and treatment of this disease. This is an amazing achievement with great relevance, as the number of new cases of and deaths from melanoma continues to rise. A hallmark of this recent success is the fact that four therapeutic agents have been granted regulatory approval over the past three years, with at least another six promising agents having just entered or completed Phase III clinical trials.(1–5) Still, the great majority of patients diagnosed with metastatic melanoma will die from their disease within a few years of diagnosis.(6, 7) To achieve the goal of successfully treating metastatic melanoma for nearly all afflicted, continued breakthroughs will be required to provide clinicians diagnostic tools to identify subsets of patients most likely to benefit from a specific line of therapy and improved treatment strategies for these identified subsets. What follows is a review of the relevant discoveries regarding melanocyte and melanoma biology that have been or are beginning to be translated into transformative therapies.

Melanoma Development

Melanocyte formation in development

Melanocytes are neural crest derived cells which develop as a branch of alternative differentiation programs that include the closely related lineages of sympathetic neurons, Schwann cells, or melanocytes. In addition to residing in the basal epidermis and in hair follicles, other melanocyte populations can be found along mucosal surfaces, meninges, in the choroidal layer of the eye, and in the stria vascularis within the cochlea. The pigments produced by melanocytes are comprised of numerous chemical species which have been broadly classified as red/blond pigments (pheomelanin) and brown/black pigments (eumelanin). Whereas brown/black pigment has measureable (albeit modest) UV protective capability, pheomelanin has been associated with increased reactive oxygen species in the skin.(8) Two forms of skin pigmentation exist: constitutive and adaptive. The constitutive or basal skin pigment level is associated with the type of pigment synthesized and the maturation process of the melanin-containing vesicles (called melanosomes). People of varying constitutive pigmentation are thought to contain a constant number of melanocytes, but variations in relative pigment production per cell. The adaptive pigmentation response typically reflects melanin synthesis triggered by ultraviolet radiation. This pigment has been shown to be initiated by UV-induced DNA damage in overlying epidermal melanocytes followed by p53 stabilization and transcriptional activation of the pro-opiomelanocortin (POMC) gene.(9, 10) POMC is post-translationally cleaved into various small peptides, one of which is Melanocyte Stimulating Hormone (MSH) that is secreted and stimulates its receptor (melanocortin receptor 1, MC1R) on underlying melanocytes. Activation of MC1R by MSH peptide results in cAMP induction within melanocytes, followed by stimulation of the gene encoding a transcription factor called MITF, which activates expression of all known pigment-producing enzymes and nearly all of the machinery required for the packaging, maturation, and secretion of pigment-laden melanosomes. It is notable that non-functional polymorphic variants of MC1R are frequently responsible for the redhair/fairskin/freckling phenotype in numerous species including man.

Role of UV

Ultraviolet radiation is deeply implicated in the formation of common forms of cutaneous melanoma in man. UV wavelengths residing within the UVB portion of the spectrum produce stereotypical nucleotide adducts known as cyclobutane pyrimidine dimers, which in turn result in formation of pyrimidine dimer mutations, in which a cytosine located in a dipyrimidine sequence becomes mutated to thymidine. These “UV signature mutations” are easily recognizable within irradiated DNA. Exomic deep sequencing of human melanomas has been carried out in recent years, and clearly reveals a striking abundance of such UV derived genomic mutations within melanomas.(11) Still, there are features of melanoma that suggest contributions in addition to UV, such as red/blond pigment, that may provide new opportunities to improve prevention strategies.(8)

Role of MITF

As described above, MITF is a transcriptional regulator of the pigment pathway in melanocytes. However MITF deficiency affects melanocytes more profoundly than purely affecting pigment synthesis. MITF deficiency produces Waardenburg Syndrome Type 2A in man and complete absence of the melanocyte lineage in numerous species.(12) This vital role for MITF in melanocyte development has led to its being dubbed a “master regulator of melanocyte development.” In contrast to consequences of MITF deficiency, more recent studies demonstrated that genomic amplification of MITF, a recurrent activating germline point mutation, functions to activate melanoma oncogenes in man.(13, 14) The recurrent point mutation in MITF was identified in cases of familial melanoma, and resulted in disruption of a SUMO modification consensus sequence, thereby modestly increasing transcriptional activity of MITF protein. The means through which MITF dysregulation contributes to melanocytic transformation to malignancy is still being determined. It is unlikely that MITF’s oncogenic activity is related to its production of eumelanin (indeed, dark eumelanin pigment is likely to be protective against melanoma and nonmelanoma skin cancers). However MITF has also been seen to directly regulate expression of anti-apoptotic genes (BCL2, BCL2A1), the cell cycle regulator CDK2, the metabolic regulator PGC1 α , and numerous others.(15–17) MITF’s role as an active regulator of melanoma proliferation/survival is likely to occur in most melanomas regardless of whether it is also an amplified or mutated oncogene, due to its role as a lineage survival factor. MITF’s direct phosphorylation by MAPK and subsequent ubiquitin-dependent proteolysis link MITF activity to the common melanoma oncogenes BRAF and NRAS.(18, 19)

Molecular signaling in melanoma

MAPK signaling

The mitogen activated protein kinase (MAPK) signaling pathway is critical to the pathobiology of a number of malignancies including melanoma.(20) Canonical signaling (summarized in Figure 1) through the pathway begins with ligand-receptor, either receptor tyrosine kinase (RTK) or G-protein coupled receptor activation (GPCR), engagement at the cell surface that ultimately triggers recruitment and activation of one of three (H-, K-, and N-) RAS isoforms.(21) Once activated, RAS binds to the RAS binding domain on one of three (A-, B-, C-) RAF isoforms triggering RAF homo- or heterodimerization and activation

of its serine-threonine kinase domain.(22–24) Phosphorylation of MAPK kinase (MEK) by RAF leads its activation that then leads to the activation of its only known substrate, MAPK otherwise known as the extracellular signal-regulated kinase (ERK).(25, 26) The activation of ERK then leads to a host of downstream events that promote cell cycle progression and cell survival.

Deregulation of cell cycle progression is a critical consequence of MAPK activation in melanoma. This can occur through genomic mutations and amplifications (discussed below) or through hyperactivation of ERK. ERK phosphorylation and cyclin D1 expression, measured by immunohistochemistry (IHC) staining, has been correlated in analysis of primary melanomas.(27) Additionally, in both uveal and acral melanoma, increased MAPK signaling has been shown to be associated with cyclin D expression in the absence of oncogenic mutations of either BRAF or NRAS.(28, 29) In the setting of oncogenic BRAF mutations, the cyclin dependent kinase (CDK) inhibitor p27^{KIP1} is downregulated and cyclin D1, is constitutively activated, both in an ERK-dependent manner, leading to enhanced proliferation.(30, 31) In the setting of oncogenic NRAS mutations, cyclin D1 also appears to be regulated in through ERK-phosphorylation and plays an important role in driving proliferation.(32, 33)

MAPK signaling also leads to inhibition of apoptosis in melanoma cells. This is likely through a number of mechanisms, though most of its effects appear to be in the regulation of various pro- and anti-apoptotic B-cell lymphoma 2 (BCL-2) family members including BIM, BAD, BCL-2, MCL-1, BAX, NOXA, and PUMA.(34–39) The preponderance of evidence suggests that the most critical effects of MAPK hyperactivation, particularly via oncogenic BRAF, on apoptosis are exerted through inhibition/down-regulation of the pro-apoptotic, BH3-protein BIM.(34, 36, 38) This may occur through several mechanisms including decreased RNA expression, increased anti-apoptotic BCL2 family member expression, or alterations in the BIM splicing machinery.(40)

PI3K signaling

The phosphoinositol-3-kinase (PI3K) pathway is the second, major signaling pathway involved in melanoma pathogenesis.(41) Canonical activation of the PI3K pathway begins, much like the MAPK pathway activation, with cell surface receptor-ligand engagement, thereby triggering conversion of PIP2 to PIP3 that then binds to and activates AKT. Activated AKT leads to a number of cellular processes that promote cell proliferation, cell survival, and angiogenesis. In melanoma, the PI3K pathway may be activated via a number of mechanisms including NRAS activation, either through mutation or loss of neurofibromatosis 1 (NF1) function, which can activate PI3K in a receptor independent mechanism, loss of function of the PI3K regulator, phosphatase tensin homologue (PTEN), or oncogenic mutation of AKT3.(20, 42–45) One of the key downstream events of AKT activation is the activation of the mammalian target of rapamycin complexes 1 and 2 (TORC1, 2) through the inhibition of the tuberous sclerosis complex 1 and 2 (TSC1/TSC2), a tumor suppressor protein that regulates mTOR. TORC1 and TORC2 activation then lead to a number of cellular events, often hallmarked by the phosphorylation of the ribosomal

protein S6 (phospho-S6), that lead to proliferation, angiogenesis, and resistance to apoptosis.

TSC1/TSC2 expression of a number of molecules including AMP kinase 1 (AMPK1), which is in turn activated by increased liver kinase B1 (LKB1). This pathway appears to be important in melanoma, and in particular BRAF-mutant melanoma, as oncogenic BRAF is associated with downregulation of LKB1 and LKB1 expression increases in the setting of BRAF inhibitor therapy.(46) This serves as a critical connection between the MAPK and PI3K signaling pathways that may have prognostic and therapeutic implications. Specifically, a recent analysis of patients treated with BRAF inhibitors showed that outcome to therapy was significantly improved when on-treatment phospho-sS6 was absent than when it was preserved.(47) It is conceivable that incorporating an “early look” at phospho-S6 expression in patients treated with BRAF inhibitors could help clinicians switch to a broader molecular targeted regimen that incorporates PI3K pathway inhibition.

Oncogenic mutations

The transformation of melanocytes into melanoma cells is a complex process that involves the acquisition of multiple molecular aberrations. These include genetic mutations and amplifications of well-described oncogenes that lead to hyperactivation of signal transduction pathways that promotes cell growth, angiogenesis, survival, and immune evasion. Interestingly, the pattern of mutations varies depending upon where the melanoma arises. (Figure 2)

BRAF—Oncogenic mutations in the kinase domain of the serine/threonine kinase BRAF are present in nearly 50% of melanomas.(48, 49) These mutations most commonly involve the valine at the 600 position (V600) whereby this is exchanged for a glutamic acid (V600E) or an arginine (V600K). Other mutations have also been described in BRAF, but the V600 mutations dominate, representing the great majority (over 95%) of the BRAF mutations identified in melanoma, and are associated with constitutive activation of the mitogen activated protein kinase (MAPK) pathway.(49) Importantly, in the setting of oncogenic BRAF mutations, RAS-RAF engagement and RAF dimerization is not required for RAF induced activation of MEK and ERK.(50, 51)

BRAF^{V600E} mutations are the most common BRAF mutations and occur more commonly in females, younger patients, and non-sun exposed areas.(52) In contrast, BRAF^{V600K} mutations occur more frequently in older patients and men on sun-exposed areas.(53) These mutations appear to be more frequent in geographic locations with more intense sun exposure, Australia and Texas, and less commonly in the Northeast. Both mutations lead to a constitutively activated kinase that signals in monomeric form and is susceptible to inhibition with small molecules. Three such agents, vemurafenib, dabrafenib, and LGX818 have shown significant clinical activity with both vemurafenib and dabrafenib having achieved regulatory approval for the treatment of BRAF^{V600E/K}-mutant melanoma patients. (1, 3, 54)

NRAS/NF1—The N-isoform of RAS is mutated in 15–25% of patients with melanoma and appears to be represented in similar frequencies in all anatomic subsets of the disease.(20)

Importantly, NRAS mutations appear to be mutually exclusive to BRAF mutations in the great majority of cases (dual positivity is seen at an incidence of less than 0.5%) and direct oncogenic signaling through both the MAPK and phosphatidylinositol-3-kinase (PI3K) pathways.(43, 55) In addition, a potent regulator of NRAS, the tumor suppressor gene neurofibromatosis 1 (NF1) is dysfunctional in another 10–15% of cases, which leads to upregulation of NRAS signaling.(45) These recently identified loss of function mutations of NF1 likely are associated with similar activation of both the MAPK and PI3K pathways as activating NRAS mutations and may be susceptible to the same treatment approaches.

CKIT—The tyrosine kinase receptor, CKIT, provides growth pathway activation following interaction with and activation by its ligand stem cell factor. This is a critical pathway in a number of normal cellular processes including melanocyte pigment production.(18, 56) However, mutations, and genomic amplifications, in the kinase or transmembrane domains lead to constitutive activation of the receptor and are associated with a number of malignancies including melanoma and gastrointestinal stromal tumor (GIST).(57, 58) In melanoma, mutations typically are seen in the transmembrane domains coded in exons 11 and 13, are more commonly found in acral/lentiginous and mucosal melanomas, and may be sensitive to small molecule inhibition with CKIT inhibitors such as imatinib.(58–61)

GNAQ/GNA11—G-protein coupled receptors (GPCRs) are seven-transmembrane domain receptors that transduce signals from a number of types of ligands including hormones, growth factors, cytokines, and chemokines. Mutations in the one of two g-proteins, GNAQ and GNA11, have been identified in the great majority of patients with uveal melanoma.(62, 63) In this setting, these constitutively activated proteins lead to the activation of protein kinase C that triggers the MAPK pathway activity through MEK phosphorylation, and renders these cells susceptible to MEK and PKC inhibitor therapy.(64–69)

Other mutations—While mutations of BRAF, NRAS, CKIT, GNAQ/GNA11, and NF1 mutations serve as primary mutations that are typically mutually exclusive of each other, a number of other mutations or genetic aberrations help the oncogenic phenotype in melanoma.

Cell Cycle Regulation: Dysregulation of the cell cycle at the genomic level is common in melanoma.(11, 70) The most commonly aberrant cell cycle regulating gene in melanoma is CDKN2A, which codes for p16^{INK4A}, a cyclin dependent kinase (CDK) inhibitor, and is either deleted or mutated in at least 50–60% of tumors.(71, 72) Additionally, loss of p16 can occur through promoter methylation in up to 10–20% of cases; thus up to 60–80% of patients may have disruption of p16^{INK4A} expression or function.(73) These aberrations lead to deregulation and overexpression of CDK4, which contributes to oncogenesis in the setting of other oncogenic drivers such as NRAS and BRAF mutations.(70)

Notably, the great majority of melanomas are sporadic, however germline mutations of CDKN2A are the most commonly inherited mutation associated with hereditary melanoma.(74–77) Affected individuals have an approximately two-thirds lifetime risk of developing melanoma. A second, heritable form of melanoma involves germline-activating mutations of CDK4.(78) While these are not as common as CDKN2A mutations, they similarly are

associated with multiple nevi, early-onset melanoma, and the development of multiple primary melanomas.

Mutations in the tumor suppressor p53 are very common in human malignancies, though are infrequently seen in patients with melanoma.(11, 79) With that said, amplification and overexpression of MDM2, a protein which ubiquitinates p53 therapy leading to its jettisoning from the nucleus and proteasomal degradation, is present in approximately over 10% and 50% of patients with melanoma, respectively.(80, 81)

PTEN loss: As described above, PTEN is a tumor suppressor gene/protein and the major regulator of PI3K activation. Mutations leading to a truncated and/or nonfunctional protein and deletion of PTEN (so-called PTEN loss) are seen in approximately 25% of patients with melanoma.(11) These are most commonly seen concomitant with BRAF mutations, though rarely occur in the setting of NRAS mutations or neither BRAF or NRAS mutations.(11, 42, 43, 82) In the context of oncogenic BRAF mutations, PTEN loss is associated with PI3K pathway signaling and reduced effectiveness of BRAF inhibitors.(83, 84) Recent data suggests that PTEN loss is specifically associated with PI3K-beta signaling, and the first PI3K beta inhibitors currently are being tested in clinical trial (NCT01673737).(85) It is expected that dual targeting of the MAPK pathway and PI3K-beta will be explored early in the development of these agents.

Oncogene-directed treatments

The identification of targetable mutations in melanoma, as with other malignancies such as chronic myelogenous leukemia, non-small cell lung cancer, and gastrointestinal stromal tumor, has led to a robust clinical development effort of small molecule inhibitors for the treatment of melanoma. As Figure 3 depicts, there are a number of potential types of agents that are being tested in patients with molecularly defined subtypes of melanoma. The most success to date has been with inhibitors of BRAF and MEK in BRAF-mutant melanoma and to a lesser extent MEK and KIT inhibitors in NRAS-mutant and CKIT-mutant melanoma, respectively. While the clinical data from these studies will be addressed in other chapters of this collection, there are two key principles of targeted therapy development in melanoma that will be addressed in this review.

The first principle of targeted therapy drug development is to use effective inhibitors of the target. With the identification of oncogenic BRAF mutations in a near majority of patients, inhibitors of RAF generally and BRAF specifically were brought quickly to the clinic. (49) The first two RAF inhibitors to make it to early phase clinical trials were sorafenib and RAF265. Both are pan-RAF inhibitors, meaning they inhibit BRAF and CRAF, as well the mutant forms of BRAF.(86, 87) In preclinical studies, these agents had broad activity against both BRAF mutant and wild-type cell lines.(86–91) Though there was some initial enthusiasm of sorafenib in combination with chemotherapy, it has been proven to be ineffective as a single-agent and in combination with chemotherapy in numerous phase I, II, and III trials.(92–98) Additionally, RAF265 has been shown to be similarly ineffective.(99) With each agent, both on and off-target toxicity limited drug exposures predicted for efficient RAF inhibition and likely explains the relative ineffectiveness of these agents

compared to the more selective BRAF inhibitors that have either been approved or are in clinical development.(93, 99) The first of these to demonstrate effectiveness in patients with melanoma was vemurafenib, a more selective RAF inhibitor that has nearly 10-fold increased inhibition of mutant BRAF versus wild-type BRAF.(100) Interesting in the dose-escalation of this agent, drug exposure was lower than predicted and not dose dependent; treatment with this formulation was associated with minimal activity.(101) With reformulation, drug exposure was dose dependent and the majority of patients with BRAF mutations had disease regression.(101) Clinical activity was confirmed in both phase II and III trials, leading to its approval by the FDA in 2011.(1, 102)

The second principle of effective drug development is careful patient selection. The development of the KIT inhibitor imatinib for the treatment of melanoma serves as an excellent example of this principle. In fact, the clinical development of imatinib in melanoma nearly was permanently derailed by two, phase II trials(103) performed in patients with stage IV melanoma.(104) The genesis of these trials was that KIT expression, measured by IHC testing, was seen in the majority of patients with melanoma; though in these trials of 18 and 29 patients, no responses were seen, survival was poor, toxicity high, and though IHC expression was seen in only greater than 50% of samples that were available, this did not appear to correlate to outcome. Since the completion of these trials, mutations and amplifications of CKIT have been identified (as described above), and in three phase II trials enriched with patients with either CKIT mutation or amplification, response rates have ranged from 17–29% in all patients and 30–54% in patients with mutation or amplification.(58–61) This example nicely highlights the concept that an effective inhibitor will only be effective when given to the patients most likely to benefit. As targeted therapy development continues in melanoma, appropriate definition of molecular subsets remains one of the most critical factors to get right.

Interaction of immunology and molecular signaling

Therapies that modulate the immune system to overcome tumor-mediated immune anergy have been used to treat melanoma for decades. Initially, this consisted of using non-specific immunogenic agents such as Cooley's toxin and Bacillus Calmette-Guerin (BCG), the latter delivered via direct tumor injection.(105, 106) The next generation of immunotherapies involved the use of a number of different cytokines, including interferon-alpha and interleukin 2 (IL-2) that received FDA-approval for high-risk Stage II/III and Stage IV melanoma respectively.(107, 108) While these treatments help only a subset of patients, they provide an important "proof-of-principle" that immunotherapy can lead to potential cures in a predictable percentage of patients. For example, the use of high-dose IL-2 is associated with durable remission in 5–10% of patients with Stage IV melanoma.(109) More recently, a number of newer agents that inhibit checkpoints of T-cell activation and regulation, such as cytotoxic T-lymphocyte antigen 4 (CTLA4) and programmed death 1 (PD1) and its ligands (PDL1 and PDL2), have been shown to have amazing clinical activity. Included among these agents are the anti-CTLA4 antibody ipilimumab, which is associated with a doubling of survival at two and three years and has been approved by the FDA since 2011, and the anti-PD1 antibodies nivolumab and MK3475, which have been associated with response

rates in the 30–40% range with 1-year survival rates ranging between 60–80%.(4, 5, 110, 111)

With the concomitant development of oncogene-targeted therapies, there has been a major push to combine small molecule target therapies with immunotherapy in an attempt to get the best of both worlds – frequent (oncogene-targeted therapy) and durable responses (immunotherapies). In melanoma, there is emerging evidence that combining these treatment modalities is not only practical (due to the availability of these agents) but also makes sound biologic sense.(112) In particular, oncogenic BRAF mutations reduce MITF activity and BRAF/MAPK inhibition is associated with increased MITF activity.(15) A number of groups now have shown, in paired patient samples, that BRAF inhibitor therapy is associated with increased tumor expression of melanocyte-derived antigens (MDAs) and leads to an increase in CD-8, cytotoxic T-lymphocyte (CTL) in tumors.(113–115) Ex vivo studies of these tumor-infiltrating lymphocytes suggest that these CTL are able to recognize MDAs.(113) Finally, while MEK inhibitor therapy appears to impair lymphocyte function whereas treatment with a BRAF inhibitor, if anything, enhances lymphocyte function.(114, 116, 117) Based on this data, it is expected that combined modality treatment with a BRAF inhibitor and an immunotherapy, such as IL-2 or immune checkpoint inhibitors, may be associated with better outcomes. To date, the results from only one such trial, the combination of vemurafenib and ipilimumab, has been reported.(118) In this study, the degree of hepatic toxicity was very high and precluded further development of the combination. It is unclear if this is a sign of things to come with combined targeted and immunotherapy or just a particular problem with these specific agents. A number of other studies are ongoing combining BRAF (with or without MEK inhibitors) with a number of cytokines (interferon and IL2) and checkpoint inhibitors. (NCT01940809; NCT01767454; NCT01943422; NCT01683188; NCT01656642; NCT01959633; NCT01659151; NCT01603212; NCT01754376)

It is important to note that the mere presence of a BRAF mutation does not appear to be associated with outcome with immunotherapy. Specifically, in retrospective studies of IL-2 and ipilimumab, patients with BRAF mutants had similar response rates to patients without BRAF mutations.(119, 120) However, emerging evidence suggests that NRAS mutations may be associated with higher response rates compared to patients with BRAF mutations and patients who have BRAF and NRAS wild-type melanoma. In a retrospective analysis of 208 consecutive patients treated with IL-2 in whom 103 had BRAF and NRAS typing, the 15 patients with NRAS mutations had a higher response rate (45%) than patients treated with either a BRAF mutation (22%) or no identifiable mutation in BRAF or NRAS (12%). (119) This has been followed up by a retrospective analysis of 173 patients treated with either high-dose IL-2, ipilimumab, the PD1 antibodies MK3475 or nivolumab, or the anti-PDL1 antibody MPDL3280A. In this series, patients treated with NRAS mutations (59/173, 34%) had a two-fold higher response rate to any immunotherapy than patients without a BRAF or NRAS mutation (114/173; 66%); the response rate for BRAF mutant patients was not calculated for this study, since a large number of these patients were treated after BRAF inhibitor therapy (a scenario associated with a poor response to immunotherapy).(121) While the mechanism of this association has not been determined, it stands to reason that it involves molecular signaling pathways mediated by NRAS mutations. If this is true, then it

stands to reason that pharmacological manipulation of molecular signaling pathways may improve immunotherapy outcomes.

Conclusions

The elucidation of melanoma biology has led to the identification of therapeutic targets and the development of effective treatments. It is expected that continued laboratory and translational research efforts focused on describing the interplay between molecular biology, immunology, and melanoma will lead to further advances and ultimately transformative therapies.

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Key points

1. Melanomagenesis is a complex process that involves carcinogenic exposure (e.g. UVB radiation) and genetic predisposition (MCR1 polymorphisms).
2. MITF, the “master regulator of melanocyte development”, functions as an oncogene when dysregulated, leading to the upregulation of cell cycle progression and favorable metabolic changes, as well as the inhibition of apoptosis.
3. Mutations of oncogenes (BRAF, NRAS, CKIT, GNAQ, GNA11) and a tumor suppressor gene (NF1) are present in the great majority of melanomas, though the rate of each type of mutations varies by anatomic site subset (e.g. mucosal v cutaneous v uveal).
4. The MAPK pathway is upregulated in nearly all melanomas and is susceptible to small molecule inhibition.
5. Interplay between the MAPK pathway and tumor antigen expression and immune infiltration predicts therapeutic synergy with combined molecular and immune targeting.

Figure 1.
Canonical Mitogen Activated Protein Kinase (MAPK) pathway signaling.

Figure 2. Estimated oncogenic driving mutation rate by anatomic subtype

Color Key: Blue (BRAF^{V600}), Green (NRAS), Yellow (NF1 inactivating mutation), Orange (CKIT), Red (GNAQ/GNA11), Purple (unknown), Red patterned (GNAQ), Orange patterned (GNA11), Purple patterned (unknown). CSD = cutaneous, chronic sun damaged skin; non-CSD = cutaneous, non-chronic sun damaged skin.

Figure 3. Targeted therapy in melanoma

The effects of the major oncogenic driving mutations on the MAPK pathway are summarized above on the left (A). Secondary molecular targets are shaded in purple and anti-tumor proteins are shaded in gray (B). In each instance, inhibitors of these secondary targets are either approved (e.g. the MEK inhibitor for BRAF mutant melanoma) or in clinical development. *Additionally, agents that promote apoptosis are in development.