The melanoma revolution: from UV carcinogenesis to a new era in therapeutics

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Abstract

Melanoma, the deadliest form of skin cancer, is an aggressive disease that is rising in incidence. Although melanoma is a historically treatment-resistant malignancy, in recent years unprecedented breakthroughs in targeted therapies and immunotherapies have revolutionized the standard of care for patients with advanced disease. Here we provide an overview of recent developments in our understanding of melanoma risk factors, genomics, and molecular pathogenesis and how these insights have driven advances in melanoma treatment. In addition, we review benefits and limitations of current therapies and look ahead to continued progress in melanoma prevention and therapy. Remarkable achievements in the field have already produced a paradigm shift in melanoma treatment – metastatic melanoma, once considered incurable, can now be treated with potentially curative rather than palliative intent.

Melanoma is among the most aggressive and treatment-resistant human cancers. In 2014, an estimated 76,100 new cases and 9,710 deaths are expected in the United States, with melanoma accounting for 75\% of all skin cancer deaths (1). Although these stark numbers highlight the need for improved prevention strategies and treatments, the explosion of discovery and concrete clinical advances in the melanoma field have brought great optimism in recent years. From identification of cancer genes to successes of new drugs in clinical trials, progress in understanding melanoma is now leading the way for other malignancies.

Cells of origin: melanocytes

Melanomas arise from malignant transformation of melanocytes, the melanin-producing cells of the skin, eye, mucosal epithelia, and meninges that are responsible for pigmentation and photoprotection. Several common subtypes of melanoma are shown in Figure 1. Melanocytes are derived from neural crest progenitors and their development is modulated by the receptor tyrosine kinase (RTK) c-KIT and microphthalmia-associated transcription factor (MITF) (2).

Melanocytes produce two main types of pigment: brown/black eumelanin and red pheomelanin. Eumelanin is the photoprotective pigment that provides ultraviolet radiation (UVR) attenuation. Pigment synthesis is stimulated by binding of α-melanocyte stimulating...
hormone (α-MSH) to melanocortin 1 receptor (MC1R) on melanocytes (Figure 2). MC1R activates cAMP production and CREB-mediated transcriptional activation of MITF. MITF in turn promotes transcription of pigment synthesis genes and melanin production. MC1R is a major determinant of pigmentation, and loss-of-function polymorphisms result in impaired eumelanin production, with the most severe loss-of-function alleles producing red hair and fair skin (2). In addition to basal pigmentation, acquired pigmentation can be elicited by stimuli such as UVR (Figure 4) (3).

Melanoma risk factors

The strongest melanoma risk factors are family history, multiple moles, fair skin, immunosuppression, and UVR. Epidemiologic studies have implicated intense intermittent UVR exposure and severe sunburns during childhood in conferring the highest risk (4). Indoor artificial tanning devices that deliver UVR to the skin have also been linked to dose-dependent melanoma risk (5). UVR has multiple effects in the skin, including genetic changes, induction of reactive oxygen species (ROS), alterations in cutaneous immune function, and production of growth factors (reviewed in (6)). Recent mouse model studies have shown that UVR induces inflammatory responses involving macrophages and neutrophils that can promote melanocytic cell survival, immunoevasion, and perivascular invasion (7, 8).

The red hair/fair skin phenotype, characterized by fair skin, freckling, and inability to tan, is associated with the highest melanoma risk of all pigmentation phototypes (9), an observation traditionally attributed to reduced UVR protection. However, a recent study demonstrated that pheomelanin synthesis contributes to melanogenesis through a UVR-independent mechanism thought to involve elevated ROS (10). Thus, high melanoma susceptibility in red hair/fair skin individuals is likely attributable to intrinsic carcinogenic effects of pheomelanin synthesis as well as UVR.

The mutational landscape of melanoma

Over the past two decades, there have been revolutionary changes in the methodologies used to characterize melanoma genomes. Early insights came from familial melanoma and genome-wide association studies that identified melanoma risk loci such as CDKN2A (11) and pigment-related genes including MC1R and tyrosinase (12). In a 2002 genome-wide screen, BRAF point mutations were discovered at high frequency in melanomas and lower frequencies in other cancers such as those of the thyroid and colon. Most oncogenic BRAF mutations cause valine-to-glutamic acid substitutions at codon 600 (V600E) that constitutively activate the kinase domain (13). In recent years, next-generation sequencing of melanomas has become increasingly accessible through declining costs and improving technologies. Since the first melanoma genome was published in 2010 (14), sequencing studies have identified numerous novel melanoma genes involved in regulation of mitogen-activated protein kinase (MAPK) and other signaling pathways (15–17).

Molecular heterogeneity and high mutational loads present major challenges in comprehensive analyses of melanoma genomes. Next-generation sequencing has confirmed that rates of somatic base mutation in melanoma are among the highest of any cancer type.
This elevated mutation rate reduces the statistical power to detect true driver mutations, and creating a complete catalog of melanoma oncogenes and tumor suppressors mutated in at least 2% of patients will require an estimated 5,300 melanoma samples (18).

The high mutation rate in melanoma is largely attributed to mutagenic effects of UVR. Cytosines in UVB-generated cyclobutane pyrimidine dimers are prone to hydrolytic deamination that can result in C>T mutations at the 3’ base of a pyrimidine. Such UVB signature mutations account for most of the elevated mutational burden in melanoma (14). However, not all C>T events are products of UVB damage. A recent computational study of 30 cancer types identified three mutational signatures characterized by C>T transitions in melanoma, each attributable to a distinct source: UVR, age, or chemotherapeutics (19). Although UVB signature mutations dominate melanoma genomes, it is important not to overlook the contribution of UVB-independent processes. The other classic UV signature mutation is G>T transversion resulting from UVA-associated oxidative damage. UVA light is absorbed by endogenous photosensitizers like pheomelanin, leading to ROS amplification and subsequent ROS-induced DNA lesions. The best studied of these is 8-oxoguanine, which mispairs with adenosine and produces G>T mutations (20).

Despite progress in understanding mechanisms of UVR-induced DNA damage, the scope of biologically meaningful UVR-associated mutations remained elusive until the advent of next-generation sequencing. A 2012 analysis of whole-exome sequences found that 46% and 9% of melanoma driver mutations can be attributed to C>T or G>T mutations, respectively (16). Most of these occur in tumor suppressor genes including CDKN2A, PTEN, and TP53. In rarer instances, recurrent UVR signature mutations are found at hotspots in oncogenes such as RAC1 and the telomerase reverse transcriptase (TERT) promoter (16, 17, 21). Consistent with the human data, UVR accelerates melanomagenesis and produces UVB signature mutations in a BRAF^{V600E}-mutant melanoma mouse model (22).

The most common mutation in melanoma, BRAF^{V600E}, is enriched in melanomas of sun-exposed versus sun-shielded skin. This prompted early speculation that BRAF mutation is linked to UVR. Indeed, the rarer BRAF^{V600K} mutation is associated with older age and chronic sun damage and may be attributable to UVB-induced mutagenesis (23). However, most oncogenic BRAF and NRAS mutations are not UVR signature mutations. Moreover, BRAF^{V600E} melanomas are less common in chronically versus intermittently sun-exposed areas (24). These observations suggest that while UVR accounts for many driver mutations, other mutagenic mechanisms are likely important in melanoma development. For instance, ROS are known to produce a host of oxidative DNA base lesions not limited to G>T mutations. While sunscreen has shown significant protection against melanoma (25), the protection it affords may be incomplete, particularly given evidence of UVR-independent melanomagenesis (10). Elucidating UVR-independent mechanisms of mutagenesis has potential implications for improving melanoma prevention.
Common genetic alterations in melanoma

Signaling molecules

The most common genetic targets in melanoma, BRAF and NRAS, are mutated in two-thirds of melanomas and result in MAPK pathway hyperactivation (13, 26). Other recurrent genetic alterations include amplification of AKT3 and loss of PTEN by epigenetic silencing or deletion (27, 28). Both events lead to constitutive phosphatidylinositol 3-kinase (PI3K) signaling (Figure 2).

In some melanomas, MAPK and PI3K pathway dysregulation results from overexpression or hyperactivation of growth factor receptors such as c-KIT, MET, and EGFR (29–31) or inactivating mutations in neurofibromin 1 (NF1), a negative regulator of Ras (32). c-KIT-activating mutations and amplifications are most common in melanomas arising on hairless (acral), mucosal, and chronically sun-damaged skin (31). The dominant genetic lesions in ocular (uveal) melanomas are activating mutations in GNAQ and GNA11, two G protein α-subunits involved in MAPK signaling (33).

Cell cycle and proliferation regulators

The CDKN2A locus encodes two tumor suppressors, p14ARF and p16INK4a, which regulate the p53 and RB pathways. Loss of the p53 cell cycle checkpoint and apoptotic pathway in melanoma can result from deletion or mutations in p14ARF or TP53, as well as rarer amplifications of mouse double minute 2 homolog (MDM2). Loss of the RB pathway can result from p16INK4a lesions, CDK4 point mutations or amplifications, retinoblastoma 1 mutations, and cyclin D1 (CCND1) amplifications (reviewed in (34)). TERT promoter mutations are common and produce MAPK-responsive expression (21).

Transcription factors and epigenetic regulators

Although MITF is essential for melanocyte differentiation and pigmentation, it can alternatively act as a lineage-specific oncogene, likely via upregulation of targets that promote cell cycle progression and survival. MITF is amplified in many sporadic melanomas (35) and a germline variant that confers gain-of-function activity is associated with familial melanoma (36). The related oncogene MYC is also occasionally amplified (37). Nuclear β-catenin, a mediator of canonical Wnt pathway activation, is detected in approximately one-third of melanomas (38). Inactivating mutations in BAP1 deubiquitinase are present in half of uveal melanomas and confer increased metastatic risk (39). Loss-of-function mutations in ARID1a and 2, components of the SWI/SNF chromatin-remodeling complex, have been reported in cutaneous melanomas (16).

Targeted therapies in melanoma

The first targeted therapy to demonstrate substantial efficacy against melanoma was vemurafenib, an ATP-competitive BRAF inhibitor (40). In the phase III clinical trial, vemurafenib conferred a survival advantage compared to dacarbazine chemotherapy in patients with BRAFV600E-mutant melanomas, with an overall response rate of 48% (41). Dabrafenib, another BRAF inhibitor, provides similar clinical benefit (42). Vemurafenib and
dabrafenib were FDA-approved for treatment of advanced BRAF-mutant melanoma in 2011 and 2013, respectively (Figure 3).

A remarkable feature of mutant BRAF inhibitors is their ability to trigger paradoxical CRAF hyperactivation in cells containing wildtype BRAF and upstream MAPK pathway activation (43). The emergence of low-grade squamous carcinomas in sun-exposed skin is not uncommon following single-agent vemurafenib or dabrafenib therapy, and is believed to involve drug-induced MAPK pathway hyperactivation in keratinocytes harboring RAS mutations (44). This paradoxical activation provides a unique opportunity for combination of BRAF plus MEK inhibition. When administered together, dabrafenib and the MEK inhibitor trametinib achieve increased clinical benefit with fewer toxicities relative to single-agent treatment (45).

Vemurafenib and dabrafenib are impressive examples of bench to bedside translation in melanoma and produce rapid initial disease stabilization. However, their efficacy is restricted to the subset of patients with BRAF\textsuperscript{V600}-mutant melanomas and has only transient durability. Regressions on BRAF and MEK inhibitors are almost inevitably followed by emergence of drug resistance and disease progression, with median progression-free survival limited to 5–7 months (41, 42, 46).

Mechanisms of resistance to targeted therapy include those that can be predicted from pretreatment analysis and those that are later acquired, or selected. Pretreatment factors that predict early resistance are mediators of apoptosis, growth, and cell cycle progression. They include BCL2A1 expression, PTEN loss, and CCND1 activation (47–49). Most cases of acquired resistance to BRAF inhibitors involve reactivation of MAPK signaling. Such mechanisms include MEK1-activating mutations, COT/MAP3K8 upregulation, and BRAF\textsuperscript{V600E} splicing variants (50–52). NRAS mutation and loss of NF1 have also been described (53, 54). Most MAPK pathway-independent mechanisms of acquired resistance involve activation of PI3K signaling via upregulation of RTKs (53).

Although many mechanisms of resistance to BRAF and MEK inhibitors have been observed in vitro, it is crucial to elucidate which mechanisms are clinically relevant. Results from next-generation sequencing of patient-matched melanoma biopsies before therapy, during response to therapy, and after disease progression are starting to emerge, revealing the kinetics and spectrum of in vivo resistance mechanisms (55). Melanomas with acquired resistance to targeted therapies show evidence of branched evolution and a high degree of genomic heterogeneity, even within single tumor sites. Sequencing has also uncovered new resistance mechanisms including BRAF\textsuperscript{V600E} amplifications and MEK2-activating mutations (55, 56). Comprehensively cataloging the landscape of BRAF inhibitor resistance will enable more accurate prediction of drug response patterns and guide future therapeutic strategies.

Although there has been significant progress in the melanoma targeted therapy field, a major challenge continues to be forestalling the emergence of resistant disease. Combination of BRAF and MEK inhibitors provide some delay in disease progression, but most patients still
Melanoma immunotherapies

The importance of immune responses in melanoma has long been appreciated, with reports of spontaneous melanoma regressions published over 50 years ago (57). The cancer immunosurveillance hypothesis, which posits that adaptive immunity can prevent cancer development and progression, was supported by observation of higher melanoma incidence in immunosuppressed patients (58). Early discovery of immune infiltrates and tumor-specific antibodies as positive prognostic factors provided additional evidence of immune interactions with melanoma (59, 60).

Although immunotherapy has historically been an active area of research, major successes were elusive until recently. Melanoma vaccines and treatments with non-specific immune stimulants like Bacillus Calmette-Guerin have thus far failed to deliver predictable major clinical benefit. High-dose interleukin-2 (IL-2), a type I cytokine that activates cytotoxic T cells, became the first non-chemotherapeutic FDA-approved therapy for advanced melanoma in 1998. While its use is limited by high toxicity and low response rates, IL-2 can produce long-term remissions in a small subset of patients (61).

Another treatment that can yield durable responses is adoptive cell transfer with autologous T cells, in which lymphocytes are harvested from patient tumors, expanded and activated ex vivo, and re-infused (62). Combination with complementary modalities such as dendritic cell immunization and BRAF inhibition are under investigation. There is also interest in the related technologies of transgenic T-cell receptor therapy and chimeric antigen receptor therapy, which involve adoptive transfer of peripheral T cells genetically modified to target specific tumor-associated antigens.

The most successful immunotherapy approach to date has been immune checkpoint inhibition. T cell activation requires T-cell receptor recognition of an antigenic peptide/MHC on the surface of an antigen-presenting cell (APC), and a costimulatory interaction between the T cell and APC. The second costimulation step can be mediated by either stimulatory or inhibitory receptor-ligand pairs, known as “immune checkpoints” (63). Two of the best-studied checkpoints involve cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death-1 (PD-1), two coinhibitory T cell receptors that mediate immune tolerance. Interestingly, activated T cells can induce upregulation of PD-1 ligands PD-L1 and PD-L2 in many peripheral tissues and APCs. One important example of tumor-mediated immune evasion is PD-L1 upregulation on cells in inflammatory tumor microenvironments (64).

Ipilimumab, a humanized CTLA-4 blocking monoclonal antibody, was the first treatment to prolong survival in advanced melanoma and was FDA-approved in 2011 (Figure 3). Although ipilimumab responses were limited to 11% of patients in the phase III trial, many of these responses were durable (65). Monoclonal antibodies that antagonize PD-1 or PD-L1 have been even more impressive in clinical studies, with higher response rates and fewer autoimmune toxicities (66, 67). The PD-1 blocking antibody pembrolizumab won FDA
approval this year and approval of another PD-1 antibody, nivolumab, is expected in the near future.

With the successes of ipilimumab, nivolumab, pembrolizumab, and several additional checkpoint inhibitors in the pipeline, the past few years have been a time of accelerated progress in immunotherapy. Striking durable responses and curative outcomes have established immune checkpoint inhibitors as the most promising treatments available for metastatic melanoma. Efforts are now focused on increasing the proportion of patients who respond to checkpoint blockade, with great interest in potential synergy of combinatorial therapies.

One immunotherapy combination under investigation is coinhibition of CTLA-4 and PD-1/PD-L1, as the two checkpoints are known to play non-redundant roles in regulation of immune responses (68). Another compelling strategy is combination of immune checkpoint inhibitors with BRAF or MEK inhibitors. Preclinical data suggest that MAPK pathway inhibition may enhance immune cell function and increase melanocytic antigen expression (69). Although the phase I trial of ipilimumab plus vemurafenib was closed due to severe hepatotoxicity (70), several trials with other combinations of targeted therapies, checkpoint inhibitors, and cytokines are ongoing.

Future prospects

Although skin is the most common site of cancer, its malignancies are likely to be among the most preventable. A well-established carcinogen, UVR, is responsible for a large fraction of melanomas and can be limited or avoided to dramatically reduce melanoma risk. Thus it is especially concerning that melanoma incidence is on the rise (1). Despite convincing evidence that indoor tanning increases skin cancer risk (5), tanning devices are increasingly popular in many countries and the FDA estimates that they are used by more than 30 million Americans every year.

In addition to aesthetic preference for tanned skin (71), an emerging factor believed to contribute to the popularity of tanning is its addictive potential. Many tanners meet CAGE and DSM-IV criteria for substance abuse with respect to UVR (72), and a recent study reported that UVR-induced β-endorphin production in skin mediates addiction to UV light in mice (73) (Figure 4). Biological addiction may help to explain the risky UVR-associated behaviors of some melanoma survivors. In a 2012 study, less than half of survivors reported regular sunscreen use, and 6% continued to indoor tan after their melanoma diagnosis (74). Future skin cancer prevention efforts may benefit from considering UVR-seeking behavior in the context of biological addiction.

Another major opportunity lies in understanding mechanisms of UVR-independent or UVR-synergistic melanoma susceptibility. Elucidating such processes, perhaps involving oxidative stress, could provide novel targets for intervention. In combination with protection from UVR, these additional strategies may achieve optimal melanoma prevention in vulnerable populations.
Despite challenges remaining in melanoma prevention, the past three years have been a time of remarkable achievement. Five drugs have gained FDA approval for the treatment of advanced melanoma, with approval of several others anticipated in the near future. The arrival of these therapeutics has been accompanied by major strides in our understanding of the molecular events in melanomagenesis. Ongoing deep-sequencing projects are uncovering new information about not only genomic alterations, but also epigenomic and transcriptomic changes that underlie melanoma development, progression, and drug resistance. These discoveries have profound implications for the development of novel targeted therapies that may complement existing treatment modalities.

Interestingly, the preponderance of UVR-induced mutations that is implicated in melanomagenesis offers a unique immunotherapeutic opportunity. Somatic mutations can produce altered proteins that, in the context of immune surveillance, are distinct from self proteins and are not protected by central tolerance. These new mutant “neoantigens” can potentially serve as targets of immune responses, and studies have reported that some melanoma neoantigens are recognized by tumor infiltrating lymphocytes (75). Efforts are underway to target melanoma neoantigens through personalized approaches such as vaccination.

Although identifying strategies to overcome resistance to targeted therapies and increase the proportion of responders to immunotherapies remain important next steps in melanoma research, recent breakthroughs have already produced a paradigm shift in melanoma treatment. Metastatic melanoma, which was once considered incurable, can now be treated with potentially curative rather than palliative intent. Moving forward, complementary insights from melanoma and immunology research will continue to drive development of novel melanoma therapies and help to determine optimal therapeutic sequences and combinatorial strategies, likely relevant to many malignancies in man.

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References and Notes


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Fig. 1.
Clinical images of melanomas. Subtypes of melanoma include superficial spreading melanoma (A), amelanotic melanoma (B), nodular melanoma (C), acral lentiginous melanoma (D), and uveal melanoma (E). Images courtesy of H. Tsao, C.H. Won, and I. Kim.
Fig. 2.
Signaling pathways in melanoma. MAPK signaling promotes cell growth and survival and is constitutively active in most melanomas. RAS family members are activated by RTKs and signal through effector proteins including PI3K, RAF kinases, and Ral-GEFs. Oncogenic BRAF and NRAS are found in 40–60% and 10–30% of melanomas, respectively. c-KIT signaling is essential for melanocyte development and is associated with melanomas arising on acral, mucosal, and chronically sun-damaged skin. Mutations in GNAQ and GNA11, two G protein α-subunits involved in MAPK signaling, are the dominant genetic lesions in uveal melanomas. MITF, the master transcriptional regulator in melanocytes and lineage-specific oncogene, is expressed in response to MC1R signaling. Loss-of-function variants of MC1R are associated with the red hair/fair skin phenotype and increased melanoma susceptibility. Known melanoma oncogenes and tumor suppressors are labeled in red. Dotted lines represent omitted pathway components.
Fig. 3. Timeline of FDA regulatory approval for metastatic melanoma. Between 1976 and 2011, dacarbazine chemotherapy (1976) and high-dose IL-2 (1998) were the only approved agents for the treatment of advanced melanoma. The number of approved agents more than tripled in the last three years with the approvals of ipilimumab (anti-CTLA-4 antibody) and vemurafenib (BRAF inhibitor) in 2011, dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) in 2013, and pembrolizumab (anti-PD-1 antibody) in 2014. FDA approval of nivolumab (anti-PD-1 antibody) is anticipated in the near future. Targeted therapies labeled in green; immunotherapies labeled in purple.
Cutaneous response to UVR. Tanning involves p53 activation in keratinocytes in response to UVR-induced DNA damage, leading to p53-mediated upregulation of proopiomelanocortin (POMC). Post-translational cleavage of POMC produces β-endorphin and α-MSH. Secreted α-MSH stimulates MC1R on adjacent melanocytes, resulting in melanin synthesis and eventual transfer of melanin-containing vesicles (melanosomes) to keratinocytes. Chronic UVR results in elevated circulating β-endorphin levels, leading to analgesia and physical dependence.