Published in final edited form as:

Mayo Clin Proc. 2008 July ; 83(7): 825–846.

Malignant Melanoma in the 21st Century: The Emerging Molecular Landscape

Aleksandar Sekulic, MD, PhD¹, Paul Haluska Jr, MD, PhD⁴, Arlo J. Miller, MD, PhD⁵, Josep Genebriera De Lamo, MD⁵, Samuel Ejadi, MD², Jose S. Pulido, MD, MS, MPH, MBA⁶, Diva R. Salomao, MD⁷, Erik C. Thorland, PhD⁸, Richard G. Vile, PhD⁹, David L. Swanson, MD¹, Barbara A. Pockaj, MD³, Susan D. Laman, MD, MPH¹, Mark R. Pittelkow, MD⁵, and Svetomir N. Markovic, MD, PhD¹⁰ for the Melanoma Study Group of the Mayo Clinic Cancer Center

¹ Department of Dermatology Mayo Clinic, Scottsdale, AZ

² Division of Hematology/Oncology Mayo Clinic, Scottsdale, AZ

³ Department of Surgery, Mayo Clinic, Scottsdale, AZ

⁴ Department of Oncology, Mayo Clinic, Rochester, MN

⁵ Department of Dermatology, Mayo Clinic, Rochester, MN

⁶ Department of Ophthalmology, Mayo Clinic, Rochester, MN

⁷ Division of Anatomic Pathology, Mayo Clinic, Rochester, MN

⁸ Division of Laboratory Genetics, Mayo Clinic, Rochester, MN

⁹ Department of Molecular Medicine, Mayo Clinic, Rochester, MN

¹⁰ Division of Hematology, Mayo Clinic, Rochester, MN

Abstract

Malignant melanoma presents a substantial clinical challenge. Current diagnostic methods are limited in their ability to diagnose early disease and accurately predict individual risk of disease progression and outcome. The lack of adequate approaches to properly define disease subgroups precludes rational treatment design and selection. Better tools are urgently needed to provide more accurate

Address correspondence to Aleksandar Sekulic, MD, PhD, Department of Dermatology, Mayo Clinic, 13400 E Shea Blvd, Scottsdale, AZ 85259 (e-mail: sekulic.aleksandar@mayo.edu). Individual reprints of this article and a bound reprint of the entire Symposium on Solid Tumors will be available for purchase from our Web site www.mayoclinicproceedings.com.

A complete list of the current members of the Melanoma Study Group of the Mayo Clinic Cancer Center appears at the end of this article. A glossary of expanded symbols and abbreviations appears on page 846.

Current Members of the Melanoma Study Group. Jacob B. Allred, BA; Panagiotis Z. Anastasiadis, PhD, MS; Keith H. Baratz, MD; Uldis Bite, MD; Elizabeth A. Bradley; Renee K. Bradshaw, MD; J. Douglas Cameron, MD; Ricky P. Clay, MD; Suzanne M. Connolly, MD; John A. Copland III, PhD; Edward T. Creagan, MD; Gary A. Croghan, MD, PhD; Mark Denis P. Davis, MD; Allan B. Dietz, PhD; John H. Donohue, MD; Lori A. Erickson, MD; David R. Farley, MD; Thomas J. Flotte, MD; Evanthia Galanis, MD; Yolanda I. (Nina) Garces, MD; James A. Garrity, MD; Dennis A. Gastineau, MD; Bobbie S. Gostout, MD; Clive S. Grant, MD; Jennifer L. Hand, MD; James N. Ingle, MD; Amer N. Kalaaji, MD; Jan L. Kasperbauer, MD; Judith S. Kaur, MD; Lisa A. Kottschade, RN; James Keeling, MD; Susan D. Laman, MD; Katherine K. Lim, MD; Noralane M. Lindor, MD; Charles L. Loprinzi, MD; Val Lowe, MD; Joseph Lustgarten, PhD; Leo J. Maguire, MD; William J. Maples, MD; Svetomir N. Markovic, MD, PhD; Colin A. McCannel, MD; Elizabeth S. McDonald, MD, PhD; Robert R. McWilliams, MD; Jane M. Milburn, MBA; Steven L. Moran, MD; Wendy K. Nevala, BS; Kerry D. Olsen, MD; Clark C. Otley, MD; Animesh Pardanani, MBBS, PhD; Galen Perdakis, MD; Mark R. Pittelkow, MD; Jeanette M. Pitts, MD; Barbara A. Pockaj, MD; Karl C. Podratz, MD, PhD; Jose S. Pulido, MD; Michael G. Rock, MD; Randall K. Roenigk, MD; John R. Salassa, MD; Diva R. Salomao, MD; Steven E. Schild, MD; Aleksandar Sekulic, MD, PhD; Thomas C. Shives, MD; Franklin H. Sim, MD; John L. Snow, MD; C. Robert Stanhope, MD; W. P. Daniel Su, MD; Vera J. Suman, PhD; David L. Swanson, MD; Winston Tan, MD; Sarvam P. TerKonda, MD; Nho V. Tran, MD; Celine M. Vachon, PhD; Richard G. Vile, PhD; Stanimir Vuk-Pavlovic, PhD; James C. Waldorf, MD; Roger H. Weenig, MD; Ryan A. Wilcox, MD, PhD; Timothy O. Wilson, MD; Gregory A. Wiseman, MD

and personalized melanoma patient management. Recent progress in the understanding of the molecular aberrations that underlie melanoma oncogenesis will likely advance the diagnosis, prognosis, and treatment of melanoma. The emerging pattern of molecular complexity in melanoma tumors mirrors the clinical diversity of the disease and highlights the notion that melanoma, like other cancers, is not a single disease but a heterogeneous group of disorders that arise from complex molecular changes. Understanding of molecular aberrations involving important cellular processes, such as cellular signaling networks, cell cycle regulation, and cell death, will be essential for better diagnosis, accurate assessment of prognosis, and rational design of effective therapeutics. Defining an individual patient's unique tumor characteristics may lead to personalized prediction of outcomes and selection of therapy. We review the emerging molecular landscape of melanoma and its implications for better management of patients with melanoma.

Malignant melanoma continues to pose a substantial clinical challenge. Our diagnostic and prognostic methods for early disease detection, which rely primarily on microscopic tissue morphology and depth of involvement measured in millimeters, fall short of providing accurate, individualized assessment of risk of disease progression. Similarly, our inability to better classify disease subgroups and rationally select treatments is reflected in the failure to alter substantially the outcome of advanced disease. More accurate, personalized clinical tools are urgently needed.

Advances in genomic strategies during the past decade have exponentially increased our understanding of the molecular underpinnings of melanoma. These insights are likely to affect markedly the diagnosis, prognosis, classification, and treatment of melanoma. We provide an overview of the emerging molecular landscape of melanoma and its implications for our understanding of the disease and for the management of patients with melanoma. We focus on molecular aberrations affecting functionally relevant cellular processes in the oncogenesis of melanoma, such as cell cycle control and cell-signaling mechanisms. Because of distinct biologic and clinical differences, we briefly review the molecular changes in uveal melanoma. Finally, we provide an overview of genome-wide discovery strategies and their contribution to melanoma research and discuss briefly the melanoma stem cell concept and its potential clinical implications. As the ultimate goal of melanoma research is the development of successful treatment strategies, we highlight throughout this review individual molecular aberrations with clear implications for treatment of melanoma.

CELL CYCLE CHANGES IN MELANOMA

The cellular cycle of growth and division (the cell cycle) is a carefully orchestrated sequence of events influenced by many factors, including the general state of cellular differentiation, activation of cell surface receptors, availability of nutrients, and level of cellular stress (eg, DNA damage). Almost all these factors lead to changes in cell-signaling patterns that ultimately converge on a set of molecular regulators of cell cycle progression—cyclins, cyclin-dependent kinases (CDKs), and cell cycle inhibitors (eg, p16^{INK4A} or p14^{ARF} [for expansion of genetic symbols used in article, see page 846]). During the cell cycle, individual cyclins bind to and activate their respective partner CDKs, inducing phosphorylation of downstream target proteins that stimulate cell cycle progression. Conversely, cell cycle inhibitors (eg, p16^{INK4A}) block the activity of cyclin-CDK complexes and oppose growth-promoting phosphorylation events. Dysregulation of the cell cycle and its components can result in uncontrolled proliferation with catastrophic consequences, as can be seen in cancer. Such aberrations in cell cycle control are one of the core mechanisms underlying the oncogenesis of malignant melanoma. Among the most common aberrations are those affecting the *CDKN2A* genetic locus, which ultimately controls RB1 and tumor protein p53 networks (Figure 1).

The *CDKN2A* Genetic Locus

Approximately 10% of all melanomas occur in a familial setting.¹ In addition to recognized heritable traits, such as skin, hair, and eye color, and the presence of large numbers of benign nevi, atypical nevi,^{2,3} or giant congenital nevi,^{4,5} a family history of melanoma is a major risk factor for development of melanoma.^{1,6,7} Early work on the genetics of melanoma linked the susceptibility of familial melanoma to a region on chromosome 9 (9p21).^{1,8} Subsequently, the *CDKN2A* locus in this area was identified as a potential tumor suppressor in cancer cell lines from tissue types,⁹ including melanoma.¹⁰ The mutations in this locus cosegregate with melanoma susceptibility in several melanoma families, thus establishing *CDKN2A* as a major melanoma susceptibility locus in familial melanoma.¹¹ In addition to increasing the susceptibility to melanoma, inactivating germline mutations at the *CDKN2A* locus also increase risk of other cancers, particularly pancreatic adenocarcinoma.¹² It is estimated that *CDKN2A* mutations contribute to between 10% and 40% of familial melanoma cases.^{13–15} The remaining melanoma-prone families likely harbor mutations in other genes, including *CDK4*¹⁶ and unidentified genes on chromosome 1p22¹⁷ and chromosome 20q11.22.¹⁸

The gene structure of the *CDKN2A* locus is very unusual, encoding for 2 overlapping but very disparate proteins, p16^{INK4A} and p14^{ARF} (Figure 1). Although structurally very different, the protein products of both genes act as tumor suppressors by negatively regulating cell cycle progression. The p16^{INK4A} protein regulates the RB1 pathway and p14^{ARF} regulates the p53 pathway, and both pathways act as brakes on the cell cycle. Therefore, genetic or epigenetic changes resulting in a functional loss of p16^{INK4A} and p14^{ARF} ultimately lead to uninhibited cellular growth and proliferation.

The p16^{ink4a} and RB1 Pathway

By controlling the transition from G1 to S phase of the cell cycle (G1/S), RB1 acts as the essential gatekeeper of the cell cycle. In its native, underphosphorylated form, RB1 binds to and sequesters the E2F transcription factor, thereby preventing E2F from inducing the expression of the genes essential for G1/S transition (Figure 1). However, when RB1 is phosphorylated, E2F is released and is able to induce expression of its target genes, leading to progression through G1/S phase interface. This crucial step of RB1 phosphorylation is carried out by an active complex of cyclin D1 (CCND1) and CDK4 or 6. The activity of this catalytic complex is regulated by the relative abundance of p16^{INK4A}. High levels of p16^{INK4A} lead to suppression of CCND1-CDK4/6 complex activity and subsequent reduction of RB1 phosphorylation, sequestration of E2F, and cell cycle arrest at the G1/S phase interface. Conversely, when cellular levels of p16^{INK4A} are low, this regulatory brake is absent and progression through the cell cycle ensues.

Frequent mutations affecting the *CDKN2A* locus in melanoma typically target p16^{INK4A}, suggesting an essential role for this protein in maintenance of cell cycle control in melanocytes. The gene appears to be deleted in approximately 50% of melanomas and to be inactivated by point mutations in approximately 9% of tumors.¹⁹ In addition, the *p16^{INK4A}* gene is frequently silenced by promoter methylation, a process that interferes with gene expression. Hypermethylation has been reported to occur in 20% to 75% of melanomas.^{19,20} These findings are in line with reports demonstrating that lower *p16^{INK4A}* expression levels correlate with disease progression^{21,22} and poor prognosis.²³

In addition to mutations affecting *p16^{INK4A}*, other members of the RB1 pathway are occasionally targeted directly in melanoma. A small fraction of melanoma-prone families carry germline mutations in *CDK4*,^{24–27} with most of these rare mutations changing the arginine at position 24 to cysteine (R24C). This substitution blocks binding of CDK4 to p16^{INK4A}, but preserves the CDK4-CCND1 interaction, resulting in a constitutively active CDK4/CCND1

complex. Notably, familial melanoma patients tend to have either *p16^{INK4A}* mutations or *CDK4^{R24C}* mutations, but not both, suggesting that both events occur in a single pathway and that only one “hit” is necessary for pathway activation.

Aberrations involving CDKs have also been identified in sporadic melanomas. Overexpression of *CDK6*²⁸ and amplifications of *CDK4*^{29,30} have been reported. Similar to the mutual exclusivity of *CDK4* and *p16^{INK4A}* mutations in the familial setting, amplifications of *CDK4* tend to occur in sporadic melanomas without *p16^{INK4A}* deletions.²⁹ In comparison with other subtypes of sporadic melanomas, *CDK4* amplification seems to be relatively more common in acral and mucosal melanomas.^{29,30} Like *CDK4* aberrations, mutations in *CCND1* are rare in melanoma, occurring mainly as amplifications in approximately 4% of tumors.^{29–31}

Inactivating mutations in the *RB1* gene are relatively rare in sporadic melanoma, occurring in approximately 6% of tumors.¹⁹ However, germline mutations in *RB1*, a well-understood risk factor for development of retinoblastoma and various sarcomas, also increase the risk of melanoma development.^{32–34} Interestingly, the risk of melanoma development in these patients seems largely independent of the ionizing radiation factor, unlike the risk of development of retinoblastomas and sarcomas, which is potentiated by exposure to ionizing radiation.

Molecularly targeted therapeutic approaches aimed at restoration of cell cycle control in melanoma are actively being explored. Because restoration of lost tumor suppressor function (eg, *p16^{INK4A}*) is difficult, major efforts have been directed at targeting CDKs as the catalytically active downstream components. Unfortunately, the initial studies of early CDK inhibitors, including a single-agent phase 2 study of flavopiridol (inhibitor of CDK 1/2/4/6), as well as a single-agent, phase 2 study of UCN-01 (7-hydroxy-staurosporine), a CDK2 and protein kinase C inhibitor, have not been promising. As the off-target effects of these older agents can lead to dose-limiting toxicities, renewed efforts to develop and test more specific CDK inhibitors are under way (eAppendix is available at www.mayoclinicproceedings.com, linked to this article).

The p14^{ARF} and p53 pathway

Often called the guardian of the genome, *TP53* is one of the most commonly mutated genes in human cancer. It is activated by many factors, including cellular and oncogenic stress. As a transcription factor, p53 regulates a variety of genes involved in cell cycle arrest, senescence, DNA repair, and cell death. The broad array of cellular functions of p53 is mirrored in the complex network of positive and negative regulators of p53. One of the better-understood negative regulators of p53 function is MDM2, which binds to p53 and leads to ubiquitination and subsequent proteasomal degradation of p53 protein. MDM2 is regulated by p14^{ARF}, which directly binds to and inhibits the function of MDM2, thus leading to stabilization of p53.

Under conditions of substantial cellular stress, such as DNA damage caused by UV radiation (UVR), induction and activation of p53 plays an essential role in halting cellular growth and repairing damaged DNA. An important mediator of the cell cycle–inhibitory function of p53 is the cell cycle inhibitor, p21^{WAF1/CIP1} (Figure 1). As the name suggests, p21 is induced by p53 and inhibits the complex of cyclin E and CDK2 (CCNE1/CDK2). Similar to the inhibition of the CCNE1/CDK4/6 complex by *p16^{INK4A}*, inhibition of CCNE1/CDK2 by p21^{WAF1/CIP1} also leads to decreased phosphorylation of RB1 and, consequently, cell cycle arrest at the G1/S transition point.

In contrast to most other cancers, melanomas display a surprisingly low frequency of *TP53* mutations (9%³⁵). Although most other tumors inactivate this pathway directly at the level of

TP53, melanoma appears to rely on inactivation of the pathway at the level of *CDKN2A* and its product, p14^{ARF}. Most *CDKN2A* mutations affect p16^{INK4A}, either alone or in combination with p14^{ARF}, suggesting p16^{INK4A} is the principal susceptibility gene at this locus. However, reports of families with mutations in p14^{ARF} alone raise the possibility that loss of p14^{ARF} could be sufficient to lead to a melanoma phenotype.^{36–38} Although conclusive evidence of independent roles for p16^{INK4A} and p14^{ARF} loss in development of melanoma in humans is still pending, mouse genetics studies suggest that both genes are essential for melanoma suppression. Disruption of either gene alone in mouse germline DNA leads to increased melanoma susceptibility.^{39,40} Interestingly, tumors arising in mice with germline deletion of p19^{ARF} appear to acquire somatic mutations in the p16^{INK4A}/RB1 pathway. Conversely, tumors arising in mice with disrupted p16^{INK4A} tend to develop mutations in the p19^{ARF}/p53 pathway. This complex interplay of genetic alterations provides further support for the essential tumor suppressor role of both genes in mouse models of melanoma.

Several strategies aimed at restoration of p53 function are being developed in preclinical models for treatment of various cancers. These approaches include antagonism of negative regulators of p53, such as MDM2, using small molecules (eg, Nutlin-3a, Roche, Indianapolis, IN),^{41,42} reactivation of mutant p53,^{43,44} and exogenous p53 expression (eg, adenoviral gene therapy).⁴⁵ Currently, it is unclear whether these approaches will prove to be clinically useful in the context of melanoma. Several ongoing clinical trials are evaluating the antagonism of the proteasomal machinery with bortezomib in melanoma and other solid tumors (eAppendix).

More recent data suggest that, in addition to controlling the p53 pathway, the tumor suppressor role of p14^{ARF} in melanoma might also be mediated through other, unidentified p53-independent mechanisms.⁴⁶ This notion might have important clinical implications in light of the ongoing efforts to develop viable p53-activating cancer treatment strategies, as already outlined.⁴⁴ Successful treatment of melanoma may require not only targeting the p53 pathway but also controlling other p53-independent mechanisms.

Genetic Testing for Melanoma

Several laboratories worldwide offer tests for identifying germline mutations in melanoma-associated genes, particularly *CDKN2A* and *CDK4* (www.genetests.org). Most of the current mutational analyses are based on direct sequencing of part of or the entire coding region of a gene. Although very sensitive in detecting mutations, genetic testing should be approached with caution and performed only in select cases. Most families with pedigrees suggestive of familial melanoma have no identifiable mutations in the known genes for which tests are available. Thus, a negative test result does not provide reassurance to the patient or family. Further, an increased frequency of atypical nevi, sometimes seen in *CDKN2A*-normal members of families that carry *CDKN2A* mutations, indicates that these family members might be at higher risk of melanoma despite being noncarriers of the familial mutation. Negative test results might falsely reassure such individuals.¹⁵ Conversely, a positive test result can have considerable psychosocial implications for a patient,⁴⁷ as the penetrance might be less than 100%, and the mutation carrier might never actually develop the disease. Currently, positive test results are unlikely to affect the management recommendations for patients with a family history of melanoma, because prevention and screening approaches would already be fully implemented in these cases on the basis of family history alone. If genetic testing for familial melanoma is considered, appropriate genetic counseling should also be provided to enable the patient to make a fully informed decision.

CELL SIGNALING CHANGES IN MELANOMA

Key signaling pathways that are important in melanoma and that represent potential novel therapeutic targets have been identified, and their regulatory mechanisms are beginning to be

understood. The processes that are important in the development and progression of melanoma can be divided into the following categories: cellular proliferation, prosurvival signaling, growth factor receptor activation, differentiation regulation, cell adhesion, and migration.

Cellular Proliferation

The MAPK (mitogen-activated protein kinase) signal transduction pathway, also known as the RAS/RAF/MEK/MAPK pathway has a key role in the proliferation of most solid tumors, including melanoma.⁴⁸ This pathway links a variety of growth factor receptors to a wide range of cellular effects and processes by way of a series of signal-transducing kinases that sequentially activate downstream kinases (Figure 2). At each level of these signal transduction pathways, several kinase isoforms encoded by separate genes are differentially expressed. The signaling cascade is initiated by growth factor stimulation of membrane-bound receptor tyrosine kinases (see Growth Factor Receptor Activation). Receptor stimulation then activates RAS, a small G protein with 3 isoforms (HRAS, KRAS, and NRAS). On activation of RAS, a complex forms between RAS and one of the RAF serine/threonine protein kinase isoforms: *ARAF*, *BRAF*, and *RAF1* (also known as *CRAF*). Formation of the complex between RAS and RAF leads to activation of RAF. Once RAF is activated, the signal is transduced through phosphorylation of MEK (MAPK/ERK kinase, also known as MAP2K), which is serine/threonine kinase present in 2 isoforms (MEK1 and MEK2). Phosphorylation activates the kinase activity of MEK, which phosphorylates the MAPK (also known as ERK) isoforms, MAPK3 (also known as ERK1) and MAPK1 (also known as ERK2). Of note, while the RAS and RAF isoforms are differentially expressed, both isoforms of MEK and MAPK are usually present and appear to be functionally redundant, supporting their critical role in cell growth.⁴⁹ Because MEK activation appears necessary for MAPK activation through this pathway and is downstream of RAS and RAF, either of which may be constitutively activated, MEK represents a promising target in melanoma.

On activation, MAPK3 and MAPK1 induce a number of mitogenic and survival processes, including proliferation and protection from apoptotic cell death, through their interaction with multiple substrates in the cytosol and subsequent phosphorylation of transcription factors. In the case of normal, nontumorigenic melanocytes and benign melanocytic nevi, activation of this pathway is attenuated and highly regulated and, in the absence of additional oncogenic changes, typically leads to senescence.^{50,51}

Several key changes and aberrations in the MAPK pathway leading to MAPK hyperphosphorylation form the basis for melanoma cell proliferation and survival in as many as 90% of cases.⁵² The most common changes leading to overactivation of the pathway are mutations within *BRAF*, which lead to constitutive activation. Up to 70% of melanomas are characterized by mutations in *BRAF*, whereas mutations in *ARAF* and *RAF1* are not seen.⁵³ In benign nevi, *BRAF* is also mutated at very high frequency, but these pigmented lesions infrequently transform and become malignant, suggesting additional genetic insults are necessary for malignant transformation. In both melanoma and nevi, mutations in *BRAF* most often involve a glutamate for valine substitution at position 600 (V600E). Mutant *BRAF* is essential for melanoma growth and maintenance and stimulates MAPK-mediated proliferation and survival.⁵⁴ Additional promitogenic consequences include regulation of MITF, secretion of angiogenic vascular endothelial growth factor (VEGF), protection against FAS-mediated apoptosis, and promotion of invasion and metastasis.^{55–58}

A number of clinical investigations evaluating the efficacy of RAF inhibition are ongoing, including a phase 3 investigation of the small molecule tyrosine kinase inhibitor sorafenib. Sorafenib is a multikinase inhibitor that targets several tyrosine kinases, including RAF kinases and VEGF receptors. Little efficacy has been shown for sorafenib alone⁵⁹ and in combination with carboplatin and paclitaxel as second-line therapy for metastatic melanoma in patients who

have not benefited from prior therapies.⁶⁰ However, the trial with the latter combination as a first-line treatment in chemotherapy-naïve patients is still ongoing, as are the trials evaluating the combinations of sorafenib with temozolomide or dacarbazine. An ongoing trial combining biweekly bevacizumab, an antibody inhibitor of VEGFR 1 and 2 in combination with carboplatin and weekly paclitaxel has been encouraging regarding progression-free survival.

Because RAF depends on the heat shock protein 90 (hsp90) for proper folding and activity, investigations with the hsp90-disrupting agent 17-(allylamino)-17-demethoxy-geldanamycin (17-AAG) are also ongoing. These investigations are seeking to validate the importance of additional hsp90 client proteins that may be important in melanoma, including AKT and insulinlike growth factor (IGF) 1 receptor, which might also be disrupted by 17-AAG.

Mutations are also common in *NRAS*, which accounts for 15% to 30% of melanoma cases, whereas mutations in other RAS isoforms are rare.^{53,61–63} In cells harboring *NRAS* mutations, the most common aberration is a missense mutation resulting in a substitution of leucine for glutamine at position 61. As with activating *BRAF* mutations, mutant *NRAS* causes constitutive activation of the MAPK pathway. In the absence of the cell cycle inhibitor protein, p16^{INK4A}, oncogenic RAS can induce melanoma and appears to have a critical role in tumor maintenance.^{61,64} Notably, primary melanomas arising on skin without chronic sun exposure typically have mutations in either *NRAS* or *BRAF*, whereas mutation in either gene is uncommon in tumors arising on skin with chronic sun damage and acral or mucosal melanomas, suggesting divergent pathways for these melanoma subtypes.²⁹ Mutations in *NRAS* and *BRAF* rarely coexist, suggesting a redundant or codependent functionality.^{29,53} Because RAS activity depends on post-translational myristylation, clinical investigations in melanoma have focused on farnesyl transferase inhibitors, such as tipifarnib. Current clinical trials include a phase 2 investigation combining tipifarnib with sorafenib in metastatic melanoma.

Prosurvival Signaling

Apoptotic cell death is a key physiologic process that regulates tissue homeostasis and growth. In response to therapies targeting cancer cells, including cytotoxic chemotherapy, radiation, hormonal therapy, and newer biological therapies, programmed cell death is the major mechanism by which these modalities exert their antitumor activity.⁶⁵ Resistance to many of these therapies can be attributed to prosurvival signaling in human cancers, including melanoma, which circumvents apoptotic cell death.^{66–68}

Two major pathways, the intrinsic and extrinsic pathways, converge at various points to result in a final common pathway of apoptosis (Figure 3). Both are subject to aberrant prosurvival signaling that has been implicated in the resistance of melanoma to standard anticancer therapies. The intrinsic (or mitochondrial) pathway of apoptosis involves the permeabilization of the outer mitochondrial membrane through cooperation of proapoptotic members of the *BCL* family of genes, including *BAD*, *BAX*, *BAK*, and *BNIP3L*.⁶⁹ This process is antagonized by the prosurvival *BCL* family members, including *BCL2*, *MCL1*, and *BCL-XL*. Once disrupted, the mitochondria release a number of apoptogenic proteins into the cytosol, including cytochrome C, AIF, HtrA2/Omi, and Smac/DIABLO.⁷⁰ These proteins trigger activation of caspases, a family of cysteine proteases, which cleave several different cytoplasmic and nuclear substrates. As a result of caspase activity, the endonuclease caspase-activated DNase (CAD) becomes activated and cleaves DNA into the characteristic fragmented DNA “ladder.”^{71,72} Additional caspase-dependent and -independent processes result in a number of biological and morphological hallmarks that represent the irreversible and lethal consequences of apoptosis induction, including cell shrinkage and nuclear fragmentation and membrane blebbing.⁷³ The extrinsic pathway, also known as the receptor pathway, involves activation of cell membrane-bound “death receptors” that belong to the tumor necrosis factor (*TNF*) receptor gene family, including CD95 and TRAIL-R1.⁷⁴ On binding of CD95 or TRAIL-

R1 by their activating ligands (Fas ligand or TRAIL, respectively), the receptors aggregate and recruit the adaptor molecule FADD and caspase 8. On caspase 8 activation, apoptosis is initiated by direct cleavage of downstream caspases, including caspase 3.⁷⁵ Additionally, activation of caspase 8 may lead to cleavage and activation of the *BID*, which stimulates BAX to permeabilize the mitochondrial membrane.⁷⁶ The balance between this proapoptotic activation and activation of antiapoptotic proteins, such as BCL2, determines whether apoptotic cell death ensues.

Recently, a multicenter trial using the antisense oligo-nucleotide oblimersen, which binds the the first 6 codons of the *BCL2* mRNA open-reading frame and mediates RNA cleavage by RNase H, in combination with dacarbazine in chemotherapy-naïve patients with metastatic melanoma showed a trend toward improved survival at 24-month minimum follow-up (median, 9.0 vs 7.8 months) and significant increases in progression-free survival (median, 2.6 vs 1.6 months), overall response (13.5% vs 7.5%), complete response (2.8% vs 0.8%), and durable response (7.3% vs 3.6%). A significant interaction between baseline serum lactate dehydrogenase and treatment was observed; oblimersen significantly increased survival in patients whose baseline serum lactate dehydrogenase was not elevated (median overall survival, 11.4 vs 9.7 months).⁷⁷

In melanoma, survival signaling through the PI3K/AKT pathway antagonizes the intrinsic pathway of apoptosis.⁷⁸ Through interactions with activated growth factor receptors, PI3K catalyzes the production of PIP3 by phosphorylating phosphatidylinositol (PI), PIP, and PIP2.⁴⁹ This process is antagonized by the phosphatase PTEN, which removes the phosphate groups. On PIP3 formation, AKT is translocated to the cell membrane where it is activated by PDK1 (also known as PDPK1). The resultant activation of AKT prolongs survival through inactivation of BAD and activation of the forkhead transcription factor FOXO1 (also known as FKHR), which leads to synthesis of prosurvival genes.^{78,79} In melanoma model systems, suppression of an AKT isoform, AKT3, leads to reduced survival of melanoma cells in vitro and in vivo.⁸⁰ Selective activation of AKT3 may be present in as many as 60% of sporadic melanoma cases.⁸⁰ In addition, AKT promotes the vertical growth phenotype in melanoma, increases angio-genesis, enhances glycolytic metabolism, and promotes cell cycle progression through up-regulation of CCND1.^{81,82} Loss of PTEN, which results in dysregulation of AKT activity, has also been implicated in up to 20% of late-stage melanomas.⁸³ Expression of *PTEN* in a murine model of melanoma significantly reduced the development of melanoma tumor formation.⁷⁸ Collectively, these data suggest that blockade of AKT or enhancement of PTEN activity would have therapeutic potential in melanoma. Various agents modulate the activity of the PI3K/AKT pathway, including inhibitors of the mammalian target of rapamycin (mTOR, also known as FRAP1), such as temsirolimus. Multiple phase 1 and 2 trials with mTOR inhibitors are ongoing in combination with inhibitors of RAS, RAF, and VEGF to target multiple key pathways in melanoma. In mammalian cells, mTOR functions as a downstream effector of AKT. Its activation also plays an important role in feedback inhibition of receptor tyrosine kinase activity that feeds back into the PI3K/AKT pathway.^{84,85}

Additional alterations in apoptotic signaling may be important in melanoma survival. The antiapoptotic protein, MCL1, appears to have an important role in chemosensitivity. Knockdown of MCL1 using small interfering RNA sensitizes human melanoma cells in vivo to dacarbazine.⁸⁶ Similarly, inhibition of BCL-XL using a specific antisense oligonucleotide, ISIS 16009, resulted in chemotherapy-induced apoptosis.⁸⁷ The proapoptotic protein NOXA has recently been identified by various groups as a mediator of sensitivity to proteasome inhibition with bortezomib.⁸⁸ This is the first apoptotic protein that has been shown to be induced by proteasome inhibition, representing a novel strategy for targeting melanoma. Although more data are needed to fully understand this phenomenon, the oncogene c-Myc appears to play an important role in regulating bortezomib-induced up-regulation of NOXA.

⁸⁹ As NOXA up-regulation is necessary and predictive of melanoma response to bortezomib, it may be useful as a marker of proteasome inhibition efficacy.

For the extrinsic pathway, the expression of the death receptors TRAIL-R1 and TRAIL-R2 (DR4 and DR5, respectively) may have a role in late melanoma tumor progression. Both DR4 and DR5 are decreased in metastatic melanoma tumors compared with primary melanoma tumors and benign nevi.⁹⁰ Post-translational modification by O-glycosylation of DR4 and DR5 correlates with the sensitivity of 18 melanoma cell lines to TRAIL-induced apoptosis. Melanoma cells with higher levels of peptidyl O-glycosyl-transferase GALNT14 activity had significantly increased sensitivity to apoptosis. Knockdown of GALNT14 in Hs294T cells could induce resistance to TRAIL.⁹¹ Downstream components of death-receptor signaling have been implicated in the attenuation of TRAIL-induced apoptosis in various melanoma cell line models. For instance, down-regulation of initiator caspases 8 and 10 and up-regulation of the antiapoptotic protein cFLIP (also known as CFLAR) have been shown in TRAIL-resistant melanoma cell lines. TRAIL resistance can be reversed by forced expression of these initiator caspases as well as DR4.^{92,93} Early clinical investigations with recombinant TRAIL analogs, such as rhApo2L/TRAIL (Genentech/Amgen, San Francisco, CA), are under way, and these agents may prove useful for clinical investigations in melanoma.

The endothelin (EDN) receptor pathway also appears to be important for the development of normal melanocytes and transformation into melanoma (Figure 4). The pathway consists of peptide ligands, EDN 1, 2, and 3 (also known as ET 1, 2, and 3), which can activate their cognate G protein-coupled receptors, EDNR A and B. Mounting data suggest that EDN1 and possibly EDN3 may exert prosurvival effects on melanoma cells in a paracrine fashion.^{94,95}

Through mechanisms that are not well understood, activation of EDNRB induces *SOX10*-dependent transcription of *MITF*.⁹⁶ In support of these data, EDN1 decreases the apoptotic rate of 5 melanoma cell lines, including SK-Mel-19, as well as normal melanocytes. Blockage of EDNRB with the specific antagonists BQ123, BQ788, and A192621 has been shown to inhibit proliferation and tumor cell invasion in a number of melanoma cell models.^{97,98} Because EDN1 has been shown to induce up-regulation of melanoma cell adhesion molecule, a melanoma invasion promoter, in normal human melanocytes, it may also have a role in cell adhesion and metastasis.⁹⁹ Activation of the EDN receptor β by EDN1 also down-regulates E cadherin, which functions as a melanoma invasion suppressor.

Cooperation of RAS-RAF-MAPK and PI3K-AKT Pathways

Signaling through the RAS/RAF/MAPK pathway is not exclusively proliferative, and signaling through the PI3K/AKT pathway is not entirely antiapoptotic. In melanoma, the interaction between these two signaling pathways enhances tumorigenesis, growth, chemoresistance, invasion, migration, and cell cycle dysregulation.

In addition to PI3K regulation, PTEN has a role in regulating growth factor-stimulated MAPK signaling and arrests cell cycle progression at G1/S through up-regulation of the CDK inhibitor, p27^{KIP1} (also known as CDKN1B).⁷⁹ Oncogenic RAS can also bind and activate PI3K, resulting in increased AKT activity. These data suggest that loss of PTEN and mutated/ oncogenic RAS are redundant. This hypothesis is supported by the finding that *PTEN* loss-of-function mutations and *NRAS*-activating mutations are uncommon in the same cell line.¹⁰⁰

Recent data have suggested that IGFBP7 is a regulator of *BRAF*-mediated proliferation in normal melanocytes. However, in the presence of the constitutively active mutant *BRAF*, melanoma cells lose IGFBP7 expression, enabling uncontrolled proliferation. However, on addition of exogenous IGFBP7, melanoma cells with mutant *BRAF* undergo apoptosis.¹⁰¹ These exciting data begin to explain how *BRAF* mutations, which occur at a similar frequency

in benign nevi and melanoma, can induce either senescence or malignancy, depending on the cellular context.¹⁰²

Growth Factor Receptor Activation

Signal transduction through the RAS/RAF/MEK/MAPK and PI3K/AKT pathways are initiated through activation of growth factor receptors that are stimulated by their cognate ligands (Figure 2). Although highly regulated in normal cellular physiology, these proteins may become dysregulated by a variety of mechanisms in melanoma. Autocrine-loop and paracrine overstimulation of receptor tyrosine kinases, including C-MET (also known as MET), fibroblast growth factor (FGF) receptor, and IGF1, are important mechanisms by which both the RAS/RAF/MAPK and PI3K/AKT pathways can be overactivated without RAS or RAF mutations.¹⁰³ Clinical investigations targeting individual receptors and multiple tyrosine kinases in melanoma with both small molecule kinase inhibitors and monoclonal antibody inhibitors are under way.

Although the role of IGF1R and its stimulatory ligands is still being investigated, existing data are intriguing. For instance, in normal melanocytes, IGF1 is critical for the growth of nevus cells and early melanoma cells. Also, IGF1 and IGF1R have been shown to be expressed by all melanocytic cells.¹⁰⁴ Although the presence of the IGF1 ligand enhances survival, growth, and migration in early melanoma lesions, it does not appear to be produced in late primary or metastatic lesions.¹⁰⁵ Nonetheless, knockdown of the IGF1R using small interfering RNA has resulted in apoptosis of melanoma cell lines harboring wild-type or mutant forms of RAS or RAF. These data suggest that targeting of the IGF1R may be an attractive therapy in patients with melanoma, irrespective of their *BRAF* or *RAS* mutational status.¹⁰⁶ Angiogenic signaling, which has a critical role in melanoma, is mediated by growth factor receptors, including the VEGF pathway, platelet-derived growth factor pathway, and basic FGF paracrine and autocrine effects. Although conflicting data also exist for this family of receptors, VEGF activation of VEGFR1, VEGFR2, and VEGFR3 likely has an important role in melanoma growth, survival, and invasiveness through autocrine loop formation.^{107,108} However, in the melanoma model cell line WM164, antisense inhibition of basic *FGF* expression, but not *VEGF* expression, inhibited colony formation in vitro and tumor growth in vivo, suggesting that VEGF does not have a universally essential role in melanoma.¹⁰⁹ Using a dominant negative FGFR expression model, FGF has been shown to be a powerful mediator of proliferation and survival signaling through autocrine activation of its receptor.^{110,111}

The C-KIT (also known as KIT) receptor is necessary for melanocyte survival; however, its expression is lost in nevi. Recently, activating *KIT* mutations were identified in a small minority (2 out of 100) of melanoma cases. Upon identification of strong immunohistochemical staining for C-KIT from paraffin-embedded tissue, direct DNA sequencing revealed mutations in exon 11 (G469V and G469R) in these 2 cases.¹¹² Mutations of *C-KIT* in exon 11 (L576P) have also been described in 3 out of 20 cases of anal melanoma. In these tumors, the mutated *C-KIT* was highly expressed (as measured by immunohistochemistry), and transformants harboring the *L576P* mutation were sensitive to the small molecule inhibitors dasatinib and imatinib.¹¹³ Amplification of *C-KIT* has also been identified in 7 of 102 primary melanomas, all of which were in chronic sun-damaged melanoma and none of which had coincident *BRAF* or *NRAS* mutations.¹¹⁴ In uveal melanoma, *C-KIT* expression is present in nearly 80% of cases, suggesting that (at least in these specific situations) the efficacy of therapy targeting C-KIT should be investigated.¹¹⁵ On the basis of these encouraging data, multiple phase 2 investigations with imatinib or dasatinib, alone or in combination with other agents, have been begun.

Several transgenic models of melanocyte development have established hepatocyte growth factor (HGF) activation of C-MET receptor as a key mechanism through interactions with

MITF. Until recently, the mechanism by which this occurred was unknown. Recent investigations using a dominant-negative MITF adenovirus construct showed that HGF-dependent matrix invasion is dependent on MITF-directed transcription of C-MET. By this mechanism, HGF stimulation of melanocytes through MITF up-regulates C-MET.¹¹⁶ Up-regulation of C-MET through α -MSH activation of MITF also stimulates *C-MET* expression, promoting HGF-mediated protection of melanocytes and melanoma cells from apoptosis.¹¹⁷

Regulation of Differentiation

The melanocyte transcription factor MITF is essential for the development, differentiation, and maintenance of melanocytes.^{118,119} In melanocyte differentiation, it functions downstream of several pathways, including melanocortin, HGF, and C-KIT (Figure 4). These pathways have each been shown to have important effects on melanocyte survival, migration, and pigmentation and require MITF function to produce these effects. In the melanocortin pathway, α -MSH, a gene product of the *POMC* (proopiomelanocortin) gene that also encodes adrenocorticotrophic hormone, binds to its cognate G protein-coupled receptor MC1R and activates adenylate cyclase. One of the downstream consequences of MC1R activation is increased transcription of MITF. However, to achieve its full transcriptional activity, MITF must be phosphorylated. At this step, crosstalk occurs with other signaling pathways important for melanocyte development and melanoma progression. Activation of C-KIT and C-MET receptors by their cognate ligands, stem cell factor (SCF), and HGF leads to activation of MAPK, which in turn phosphorylates MITF. This phosphorylation leads to increased transcription of MITF-dependent genes, many of which are involved in pigmentation, including the family of pigmentation enzymes, such as TYR, TYRP1, and DCT.^{120–123} In addition, MITF regulates expression of several melanoma markers, including SILV/gp100/HMB45,^{124,125} MLANA/MART1,¹²⁴ and AIM1,¹²⁶ as well as the prognostically relevant melanoma marker TRPM1.^{127,128}

Through its regulation of the *BCL2* gene, a key anti-apoptotic factor, MITF is crucial for melanocyte survival.¹²⁹ Deficiencies in MITF-BCL2 interaction cause the death of melanocytes through the loss of the melanocyte progenitor cells in the hair follicle.¹³⁰ In melanoma cell lines, forced reduction of *BCL2* expression also leads to cell death, suggesting that the survival of transformed melanocytes also depends on BCL2.¹³¹ In melanoma, the progression from nevus to frank melanoma is frequently associated with the loss or decreased expression of several melanoma markers regulated by MITF, including HMB45, MLANA, and melanin itself.^{132–135} However, the expression of MITF is maintained in nearly all melanomas,^{136–138} suggesting a necessary role. In a subset of melanomas, most frequently in tumors with a worse prognosis, MITF was recently found to be amplified and to be associated with increased resistance to chemotherapy, suggesting MITF expression likely enhances tumor growth.¹³⁹ Several novel therapeutic agents with potential to affect the MITF pathway are currently under development or in clinical trials. These include C-KIT inhibitors (eg, imatinib and dasatinib), C-MET inhibitors (eg, SGX 523; SGX Pharmaceuticals, San Diego, CA) as well as agents targeting *BCL2* (eg, oblimersen).

Cell Adhesion and Invasion

As in all solid tumors, the actual morbidity and mortality of melanoma are a direct consequence of local invasion and metastatic spread. The metastatic phenotype represents alterations in cell adhesion that normally control cell migration, tissue organization, and organogenesis¹⁴⁰ but participate in tumor invasion, tumor-stroma interactions, and tumor-cell signaling. In melanocytes, cell surface cadherins have been well studied as direct mediators of cell adhesion with surrounding cells as well as components of cell-signaling pathways that influence differentiation. The extracellular domain of cadherins binds to like cadherins on other cells in regions of cell contacts called adherens junctions, and alterations in cadherin expression affect

the interaction of cells with their environment. In particular, expression of E cadherin on melanocytes mediates their association with keratinocytes,¹⁴¹ thereby restraining melanocyte proliferation and maintaining melanocyte differentiation through a variety of signals.¹⁴² The progression from the radial to the vertical growth phase of melanoma is characterized by loss of E cadherin, with subsequent breakdown in melanocyte-keratinocyte interaction, and gain of N cadherin expression, which is often expressed aberrantly by invasive carcinomas.^{143–145}

Cadherin expression introduces additional complexity because the intracellular domain of these molecules is associated with a large protein complex including β -catenin that forms structural links with bundles of actin filaments and participates in cell signaling (Figure 4). Decreased E cadherin expression increases β -catenin signaling,¹⁴⁶ presumably by freeing it from the actin cytoskeleton. In addition, the association between E cadherin and β -catenin can be regulated by signaling from such growth factors as WNTs, leading to the phosphorylation of β -catenin.¹⁴⁷ The WNTs are secreted proteins with important developmental functions, especially in neural crest cells such as melanocytes. When WNT proteins bind their receptors, they inactivate GSK3B, which normally phosphorylates β -catenin and targets it for proteasomal degradation.^{148,149} Thus, WNT signaling leads to increased levels of β -catenin, activation of the TCF-LEF transcriptional complex, and the expression of downstream target genes including *MITF*¹⁵⁰ and *CCND1*.¹⁵¹

Photosensitivity, UVR, and Melanoma

Ultraviolet radiation is the most thoroughly understood environmental factor contributing to skin cancer risk of all types. Its effects on the skin include immune suppression, genetic damage, and the generation of reactive oxygen species.^{2,152} However, individual biologic response to UVR can differ markedly. Notably, the ability of skin to tan is closely associated with cutaneous cancer risk.^{153,154}

Much of the molecular basis of this phenotype is related to the *MC1R* locus. People with specific polymorphisms in this gene differ in both their abilities to tan and their risk of developing melanoma. At the molecular level, UV exposure leads to increased pigmentation after UVR-induced DNA damage in keratinocytes, resulting in increased p53 activity, transcription of α -MSH and its secretion by keratinocytes, and stimulation of intracellular signaling responses in melanocytes that, in turn, lead to increased expression of melanin-producing enzymes.^{155–157} Light-skinned and redheaded people often carry germline polymorphisms in *MC1R*^{158,159} that reduce the activity of the receptor¹⁶⁰ and increase the risk of melanoma by 2.7 to 16.0 fold.¹⁶¹ Mice carrying certain *MC1R* polymorphisms cannot tan in response to UVR exposure; this phenotype can be overcome with the delivery of a compound that stimulates MITF activity, decreasing susceptibility to subsequent cancers.

A recent conundrum in melanoma genetics has been the finding that melanomas occurring in areas without chronic sun damage have the highest rates of *BRAF* mutation, although *BRAF* mutations do not occur in mucosal melanomas that have received no UV exposure. Recently, it was found in humans that specific *MC1R* polymorphisms increase the propensity toward development of *BRAF* mutations.^{162,163} Although *BRAF* mutations do not bear the typical C>T (cytosine to thymidine) signature of UVR-induced mutations, they occur only in UV-exposed skin and their incidence is increased in those with UVR susceptibility. These findings suggest a model in which the *MC1R* polymorphism predisposes to *BRAF* mutation in the skin areas with intermittent sun exposure.

THE GENOMICS OF MELANOMA

Recent advances in high-resolution genome-wide molecular techniques have greatly increased our ability to examine and understand molecular changes in cancer, including melanoma. The

essence of these methods lies in the inherent property of DNA strands to form a duplex when two complementary DNA strands meet. This quality was exploited for design of DNA array platforms capable of measuring global changes in gene expression levels (ie, gene expression profiling), changes in gene copy number (eg, comparative genomic hybridization [CGH]), as well as changes in allelic balance through detection of single-nucleotide polymorphism (ie, SNP arrays). Although these methods are mainly used in experimental settings, they are rapidly entering the clinical arena as methods of patient selection in clinical trials and as predictive tests for selection of treatment in some malignancies (eg, Oncotype DX; Genomic Health, Redwood City, CA; MammaPrint, Agendia, Amsterdam, Netherlands). A discussion of several genomic techniques follows.

Gene expression profiling evaluates simultaneously the expression levels of numerous genes across the genome. Once isolated from the sample of interest (eg, melanoma), RNA is labeled with a fluorophore and hybridized to a prefabricated microarray containing oligonucleotide sequences complementary to all genes to be examined. The array is scanned, and the color intensity for each array spot, representing a gene, is measured. A change in color intensity correlates with a change in expression level of the corresponding gene. Such data can be used to identify both global patterns of gene expression as well as changes in expression of individual genes.

Array-based CGH aims to detect changes in gene copy number at the DNA level. Loss and gain of genetic material are frequently seen in cancer, the former as deletion of tumor suppressors and the latter as amplification of oncogenes. As its name suggests, array CGH relies on comparative hybridization of genomic DNA; a DNA of interest (eg, melanoma DNA) and a control genomic DNA are labeled with different fluorophores and hybridized to a DNA array platform with sequences complementary to all genetic regions to be examined. On scanning, the relative abundance of two colors on the array is analyzed, allowing identification of areas that might be lost or gained in the genome of interest (eg, melanoma).

Single-nucleotide polymorphism arrays detect DNA sequence variations commonly seen in the healthy population. Such polymorphisms can be used to track maternal and paternal copies of a gene but also to quantify the gene copy number, similar to array CGH. Genomic DNA is isolated, labeled using a fluorophore, and hybridized to an array containing sequences corresponding to the individual polymorphic variants to be examined. Scanning of the array allows identification and quantification of polymorphisms in the genomic DNA of interest.

Genome-wide analytical methods offer several advantages over the more traditional, focused approaches for the study of cancer. The capacity for robust data collection at a single gene resolution across the entire genome makes genomic methods ideal for detection of novel cancer-specific changes, even when they could not have been suspected on the basis of current knowledge. The genome-wide nature of these approaches allows detection of global patterns that can be used for disease classification, prognosis, and even selection of appropriate therapy, as recently demonstrated with gene expression profiling in several human cancers.^{164,165} Finally, integration of genomic data sets from different platforms, such as gene copy number and expression profiling data, represents a powerful method for identification of functionally relevant molecular aberrations.¹⁶⁶ However, genomic techniques do not supplant more targeted analytical methods. The complementary use of both these approaches will be essential to identify and exploit molecular changes in cancer for improved diagnosis and treatment.

Early analyses of melanoma genomes through cytogenetics have uncovered an astounding level of structural complexity and have identified several areas of melanoma-specific chromosomal gains and losses,^{167–169} with the most common changes affecting chromosomes 1, 6, 7, 9, and 10.^{29,169} The high frequency of the more common chromosomal aberrations in various

subtypes of melanoma suggest chromosome 6p gains and 6q losses as early events in cutaneous melanoma; in contrast, chromosome 3 losses predominate as early events in ocular melanoma.¹⁶⁹ Subsequent higher-resolution genomic approaches, including array CGH, have provided an increasingly detailed view of such gene copy number changes.

A recent landmark study of gene copy number alterations in primary melanomas by array CGH has identified distinct subclasses of melanoma on the basis of anatomic site, extent of UV exposure, and mutational status of several genes, including *NRAS* and *BRAF*.²⁹ Melanomas from intermittently sun-exposed skin tend to harbor an activated form of *BRAF*, whereas melanomas from chronically sun-exposed skin, acral sites, or mucosal surfaces do not. Curtin et al²⁹ were able to use these patterns of genomic alterations and *BRAF* mutational status to derive an algorithm that could be used to correctly identify clinical subgroups of melanoma relying only on the observed molecular changes.²⁹ Such melanoma-specific genomic alterations could provide a basis for distinguishing melanomas from benign nevi in cases with challenging histology. Indeed, a fluorescent in situ hybridization diagnostic test based on these findings is in development.¹⁷⁰

The sheer number of gene aberrations identified by high-resolution genomic approaches can pose sizeable challenges when attempting to isolate functionally and therapeutically relevant changes. Evaluation of the co-occurrence patterns among the individual aberrations can help identify groups of genes affected in a nonrandom fashion. Using this approach, Curtin et al²⁹ analyzed co-occurrence patterns of genetic aberrations in primary melanomas and noted a clear mutual exclusivity of the alterations involving the members of the single signaling pathway.²⁹ Similar to the mutual exclusivity of *BRAF* and *NRAS* mutations already outlined in the previous section, tumors with activating mutations in *NRAS* or *BRAF* do not contain amplifications of downstream target genes, such as *CDK4* or *CCND1*, suggesting that aberration at any of several levels is sufficient to activate the pathway and drive tumor progression.^{29,31} In addition to elucidating the molecular mechanism of tumorigenesis in melanoma, such findings are likely to provide the basis for therapy selection. Similarly, an observation of frequent co-occurrence of *BRAF* activation and *PTEN* inactivation or deletion indicates that, in addition to *BRAF* activation, melanomas likely require PI3K pathway activation.³¹ Interestingly, *NRAS*-activating mutations do not co-occur with *PTEN* mutations, consistent with the notion that, unlike *BRAF*, which cannot induce PI3K activation, *NRAS* alone stimulates both the *BRAF*/MAPK and PI3K pathways.³¹ Effective anti-melanoma therapy likely requires simultaneous inhibition of both the MAPK and PI3K pathways.

Recurrent genomic aberrations in cancer often affect larger DNA segments that contain numerous genes, thus obscuring the identity of the genes targeted by such aberrations. Integration of complementary genomic methods addresses this issue and provides a robust approach for candidate gene identification. Lin et al¹⁶⁶ have recently used such an approach to study melanoma cell lines and short-term cultures by merging the gene copy number data with expression data from the same set of samples. Focusing on statistically significant gene expression changes, they prioritized the candidate genes within the common DNA copy number alterations. They identified several genes with a known or suspected role in the pathogenesis of melanoma (eg, *PTEN*), as well as several novel genes not previously implicated in melanoma. This type of approach could be used to identify novel therapeutic targets for treatment of melanoma. However, development of flexible and robust computational approaches for such integrated genomic analyses will be essential for continued progress in human genomics.

UVEAL MELANOMA

Uveal melanomas (melanomas of the eye) are the most common primary intraocular malignancy. Melanomas of the eye can be divided into four predominant types: ciliary and choroidal melanomas, which account for most uveal melanomas, and iris and conjunctival melanomas, which are less common. This section will focus mainly on ciliary and choroidal melanomas, ie, uveal melanomas, and specific molecular characteristics of iris and conjunctival melanoma will be highlighted for comparison.

Uveal melanoma differs markedly from other melanoma subtypes in both its clinical behavior and its molecular makeup. Occurring most frequently in elderly people, it is fatal in up to 50% of patients, even when the primary tumor is successfully treated.¹⁷¹ Unlike most cutaneous melanoma lesions, which tend to metastasize through lymphatic spread, uveal melanomas favor hematogenous spread, with the liver and lung being the more common metastatic sites.¹⁷² These clinical differences are reflected in disparate molecular changes observed in cutaneous vs ocular melanoma.

In addition to general aneuploidy,¹⁷³ several specific and recurrent cytogenetic changes have been observed in uveal melanomas, including monosomy 3 and 8q gains, both of which appear to be associated with worse prognosis.^{174–178} However, the exact genes affected by these chromosomal alterations have not been identified. Initial efforts aimed at more detailed, gene-focused analysis of uveal melanoma have been difficult. Because uveal melanomas rarely occur in a familial setting, traditional linkage analysis for identification of susceptibility genes has not been successful. Unlike familial cases of cutaneous melanomas, *CDKN2A* appears to be an infrequent germline mutation in the rare, uveal melanoma-prone families.¹⁷⁹ Targeted examination of cancer-related genes, including *RB1*, *TP53* and *BRAF*, has failed to show significant frequency of mutations in these genes in uveal melanoma.^{180–184} However, dysregulation of the p53 and RB1 pathway at transcriptional (eg, increased levels of MDM2)¹⁸⁰ and post-translational levels (eg, increased RB1 phosphorylation)¹⁸¹ has been observed. In contrast to ciliary and choroidal melanomas, melanomas of the iris and conjunctiva do not harbor chromosome 3 monosomy but instead (reminiscent of some cutaneous melanomas) have an appreciable frequency of *BRAF* mutations.^{184–187}

In addition to the described cytogenetic alterations, global analyses of gene expression patterns in uveal melanoma have been particularly informative. On the basis of gene expression patterns, uveal melanomas seem to cluster naturally in 2 classes (classes 1 and 2). These classes seem to have a clinical prognostic relevance; patients with class 1 tumors rarely die of metastases, whereas patients with class 2 tumors have a high rate of metastatic death.^{188,189} Interestingly, class 2 tumors also display previously identified poor prognostic factors, including monosomy 3, epithelioid cell type, looping extracellular matrix patterns,¹⁹⁰ loss of melanocytic markers, and increased expression of E cadherin.¹⁹¹ One of the top differentiating genes, *ID2*, a transcriptional target of β -catenin, normally expressed in uveal melanocytes, was significantly down-regulated in type 2 tumors. In functional studies, anchorage-independent growth seen in the class 2 phenotype could be recapitulated by elimination of *ID2* from class 1 uveal melanoma cells. Similarly, overexpression of *ID2* was capable of turning the class 2 cells into the less aggressive class 1 cell type.¹⁹¹ This duality of phenotype in uveal melanoma might hold an important clue for future clinical trial design and for development of molecularly targeted therapies.

Very recent and exciting findings point to the potential involvement of the protein kinase C (PKC) pathway in uveal melanoma.¹⁹² Mouse genetic studies have recently found that mutations in *GNAQ*, a heterotrimeric G protein α subunit, are associated with a dark skin phenotype and increased melanocyte numbers in mouse skin.¹⁹³ Analysis of human tumors

for similar mutations revealed an oncogenic mutation at codon 209 in *GNAQ* in approximately 50% of uveal melanomas and 78% of blue nevi; this mutation was only infrequently observed in cutaneous melanomas.¹⁹² Interestingly, mutations at codon 209 of *GNAQ* were also detected in nevus of Ota, a congenital melanocytic proliferation similar to blue nevi that confers an increased risk of development of ocular melanoma. The MAPK pathway is stimulated by *GNAQ* via PKC and (reminiscent of *BRAF* mutations) *GNAQ* mutations and *KIT*, *BRAF*, and *NRAS* mutations appear to be mutually exclusive. Functional studies confirm the transforming nature of oncogenic *GNAQ* in immortalized melanocytes. Similarly, knockdown of oncogenic *GNAQ* in uveal melanoma cell lines resulted in decreased proliferation and cell death.¹⁹² Thus, *GNAQ* is the first oncogene found to be mutated frequently in ocular melanoma. This finding might have important therapeutic implications, because it suggests that PKC could be a target for treatment of ocular melanomas harboring the activating *GNAQ* mutations.

MELANOMA STEM CELLS

The concept of cancer stem cells, or cancer-initiating cells, has gained considerable momentum in recent years. Although the identification of cancer stem cell-specific molecular aberrations is still in its infancy, the extent of the changes documented thus far and their potential implications for cancer therapy warrant a brief review.

Normal adult stem cells can be found in most somatic tissues, where they participate in renewal, repair, and maintenance of normal tissues. This essential function is particularly important in perpetually regenerating tissues, such as blood, intestinal epithelium, and skin.¹⁹⁴ Two properties essential for the role of normal tissue stem cells are the ability to self renew and the ability to give rise to a spectrum of tissue-specific cell types (ie, pluripotency). Normal adult stem cells are relatively rare in tissues and are typically quiescent or slowly dividing. This slow pace of division contrasts that of their numerous daughter cells, often referred to as *transient amplifying cells*, which divide rapidly and then differentiate to form the individual tissue components. Interestingly, normal stem cells display a higher degree of resistance to radiation or chemotherapy treatments than their differentiated progeny, perhaps because of the relative quiescence of stem cells,¹⁹⁵ higher levels of ABC transporter expression,¹⁹⁶ and activation of prosurvival mechanisms.¹⁹⁷ Other phenotypic characteristics of normal stem cells include the expression of genes involved in self-renewal, including hedgehog, Notch, and WNT.¹⁹⁸ These self-renewal pathways seem to be tightly regulated through interaction of stem cells with their microenvironment, ie, the *stem cell niche*.¹⁹⁹

Important similarities exist between normal and cancerous tissues. Akin to normal tissue stem cells, tumorigenic cancer cells are capable of tissue renewal and can give rise to phenotypically heterogeneous cell populations in a tumor. The high proliferative capacity and plasticity seen in both settings has led to development of a “cancer stem cell” theory, which proposes existence of a stem cell-like population in cancer. Indeed, a subpopulation of cancer cells possessing characteristics typically associated with adult tissue stem cells has been identified in hematopoietic malignancies²⁰⁰ and several solid tumors,^{201–203} including melanoma.²⁰⁴ These cells are highly tumorigenic, in contrast to the remaining bulk of cancer cells, which do not form tumors in various experimental systems.²⁰⁵ Although multiple cell surface markers have been used to isolate cancer stem cells (eg, CD133 or CD44), such markers are not specific for cancer stem cells. Rather, they appear to enrich for the population in which the cells with high tumorigenic potential are found. This important distinction further stresses the need to establish better functional assays and molecular markers for isolation and accurate identification of cancer stem cell populations.

Mounting evidence supports the existence of melanoma stem cells. Melanomas show phenotypic heterogeneity, suggesting that they originate from a cell capable of multi-lineage

differentiation.^{206,207} Melanoma cells retain their morphologic and biological plasticity after repeated cloning²⁰⁶ and tend to express developmental genes and stem cell markers, including Notch receptors,²⁰⁸ WNTs,²⁰⁹ CD133, CD34, C-KIT and nestin,^{206,210} and bone morphogenic protein.^{211,212} Global expression profiles of melanoma resemble stem cell signatures.^{206,213}

Probably the single most therapeutically relevant property of cancer stem cells, including melanoma stem cells, is their resistance to drugs and toxic treatments, reminiscent of that observed in normal stem cells.¹⁹⁵ Indeed, “complete responses” after chemotherapeutic treatments in a variety of cancers are frequently followed by tumor relapse. This observation is consistent with a notion of a therapy-resistant subpopulation within the tumor that is capable of reinitiating tumor growth (Figure 5). If this notion is broadly confirmed, it will greatly affect our approach to and selection of cancer therapies, leading to the targeting of both the bulk of the highly proliferative tumor mass, as well as the quiescent, highly drug-resistant cancer stem cell population.

Recent identification of ABCB5 as a potential marker of melanoma stem cells brings together the functional and phenotypic characteristic of this cell population.²⁰⁴ A human ABC transporter and P-glycoprotein family member that mediates chemotherapeutic drug resistance in malignant melanoma,²¹⁴ ABCB5 has been shown to be a major efflux mediator of doxorubicin resistance in melanomas.²¹⁵ Moreover, ABCB5 was preferentially expressed on a distinct subset of chemoresistant CD133⁺ tumor cells, indicating that this ABC transporter may mark more primitive melanoma cells.²¹⁵ Melanoma cells that are ABCB5⁺ possess greater tumorigenic capacity than ABCB5[−] cells and have significantly higher capacity for self-renewal and differentiation.²⁰⁴ Expression of ABCB5 appears to increase with disease progression. Systemic melanoma therapy with antibodies targeting ABCB5 in a mouse model resulted in a significant inhibition of melanoma growth and accompanying tumor death.²⁰⁴

Continuing the theme of drug resistance genes as functional markers of melanoma stem cells, Keshet et al²¹⁶ recently reported identification of a subpopulation of human melanoma cells expressing *MDR1* (also known as *ABCB1*). This subpopulation coexpresses the ABC transporters, ABCB5 and ABCC2, in addition to the stem cell markers, nanog and human telomerase reverse transcriptase. Cells that were MDR1⁺ exhibited increased potential for clonogenicity, self-renewal, and anchorage independence, key cancer-related functions not seen in MDR1[−] cells.²¹⁶ Future studies confirming and further characterizing functional markers of melanoma stem cells are eagerly awaited and may facilitate development of new therapies specifically targeted at elimination of this unique self-renewing cell population within melanoma tumors.

TARGETED MOLECULAR THERAPY

Despite decades of investigation, no systemic treatment that improves overall survival in most patients with un-resectable metastatic melanoma has been developed. Occasional durable complete responses do occur in patients with metastatic disease who receive high-dose interleukin 2, biochemotherapy, and even chemotherapy alone; however, these occur in only 1% to 6% of patients, and no definitive tests exist to reliably predict these outcomes. During the past decade, only one agent has been approved by the Food and Drug Administration as a treatment for unresectable metastatic melanoma—the cytokine interleukin 2 (approved in January 1998). In addition, only one agent has been approved by the Food and Drug Administration for adjuvant therapy of high-risk stage II and III melanoma—the cytokine interferon α -2b (approved in December 1995). Unfortunately, for a variety of reasons, 90% of new anticancer agents designed in the laboratory never make it into routine clinical use.²¹⁷ Some patients may obtain substantial benefit from investigational agents during trials; this is not an uncommon occurrence in patients with metastatic melanoma. However, because the

overall response rates are often considered too low, most of the tested agents are abandoned as ineffective. However, 2 essential questions remain unanswered. Are common features shared by responders to a given therapy? Could such markers be used to identify patients who are likely to respond to a given treatment?

The reliability of early in vitro tests designed to predict resistance to chemotherapeutic agents through exposure of surgically resected tumor cells to suprapharmacologic doses of conventional agents was controversial at best. However, the application of genomic technology holds promise for improved outcomes in treatment of melanoma patients.²¹⁸ Genomic signatures obtained from melanoma cells, surrounding stromal cells, or circulation may help us better understand factors that determine resistance and sensitivity to various treatment modalities such as chemotherapy,^{219–222} immunotherapy,^{223,224} and radiation therapy.²²⁵ Molecular signatures may also serve as predictive biomarkers of sensitivity or resistance to these various treatments, opening doors to individualized therapy.

Molecular signatures may also help identify novel drug targets for treatment of melanoma. As already discussed, the application of early genomic technology has already confirmed ubiquitously disrupted molecular pathways in melanoma cells. Similar aberrations in functional pathways in stromal or immune cells are also being identified as potential targets for drug intervention. As a consequence, many small-molecule drugs that target these pathways have been created and are currently in preclinical assessment, as well as in phase 1 and phase 2 clinical trials. The molecular targets and corresponding drugs are listed in the eAppendix along with the mechanism of action and the current clinical trials for each agent. Because the status of drugs may change, readers should consult suggested resources for updates. New agents targeting the cells of the immune system are not included in this review.

Recent clinical observations suggest that very few single-agent therapies lead to substantial clinical benefits.²²⁶ Given the multiplicity of disrupted molecular pathways and the redundant compensatory pathways in the more common solid organ cancers such as melanoma, these agents will likely require concurrent or sequential treatment with other agents or modalities to produce meaningful clinical benefit for patients. In addition, proper molecular classification of tumors might prove essential for selection of effective therapy. Molecular patterns identified by global approaches, such as expression profiles, CGH, and/or proteomic analysis, may provide the necessary tools to achieve these goals. To this end, it will be as important to establish effective and consistent tissue collection and procurement methods as to develop standardized analytical approaches addressing the current issues facing the genomics of cancer.^{227,228}

CONCLUSION

Our understanding of the molecular basis of melanoma has grown exponentially during the past decade. These scientific advances are likely to greatly affect the practice of medicine, including the diagnosis, prognosis, and treatment of melanoma. The enormous molecular complexity of melanoma and the realization that a clinical diagnosis of melanoma likely covers a spectrum of molecularly and mechanistically distinct entities highlight the need for better molecular disease classification and point the way for development of individually tailored therapeutic strategies. Better understanding of this heterogeneity on the molecular level will likely lead to reevaluation of the basic concepts underlying melanoma therapeutics development and clinical trial design. The low overall response rates observed in clinical trials that rely on clinical disease features for patient selection might simply reflect a relatively low percentage of patients with the disease susceptible to a given therapeutic agent or combination. If such is the case, patient selection for clinical trials and selection of therapy on the basis of individual molecular attributes might be necessary to improve response rates to any therapy.

This further highlights the need for well-designed, standardized collection and analysis of tissues before and perhaps during treatment as an integral component of clinical trial design. These important concepts mark the beginning of a new era of rational therapy design and selection for treatment of malignant melanoma.

Glossary

CDK	cyclin-dependent kinase
CGH	comparative genomic hybridization
EDN	endothelin
ERK	extracellular signal–regulated kinase
FGF	fibroblast growth factor
HGF	hepatocyte growth factor
IGF	insulinlike growth factor
MAPK	mitogen-activated protein kinase
MEK	MAPK/ERK kinase
TNF	tumor necrosis factor
UVR	UV radiation
VEGF	vascular endothelial growth factor

Glossary

ABC	adenosine triphosphate–binding cassette
ABCB5	ABC subfamily B (MDR/TAP) member 5
AIF	apoptosis-inducing factor
AIM1	absent in melanoma 1
AKT	

	v-akt murine thymoma viral oncogene homolog
APAF1	apoptotic peptidase-activating factor 1
ARAF	v-raf murine sarcoma 3611 viral oncogene homolog
BAD	<i>BCL2</i> antagonist of cell death
BAK	<i>BCL2</i> homologous antagonist/killer
BAX	<i>BCL2</i> -associated X protein
BCL2	B cell chronic lymphocytic leukemia/lymphoma 2
BID	BH3-interacting domain death agonist
BNIP3L	<i>BCL2</i> /adenovirus E1B 19kDa interacting protein 3-like
BRAF	v-raf murine sarcoma viral oncogene homolog B1
CAD	caspase-activated DNase
cAMP	cyclic adenosine monophosphate
Casp	caspase
CCND1	cyclin D1
CCNE1	cyclin E1
CD95	APO (apoptosis-inducing protein)-1/Fas
CDKN1B	CDK inhibitor 1B
CDKN2A	CDK inhibitor 2A
cFLIP (also know as CFLAR)	cellular FLICE-inhibitory protein
CFOS	v-fos FBJ murine osteosarcoma viral oncogene homolog

C-KIT (also known as KIT)

v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog

C-MET (also known as MET)

met proto-oncogene (hepatocyte growth factor) receptor

CREB1

cyclic adenosine monophosphate responsive element-binding protein

DCT

dopachrome tautomerase

DR4 (also known as TNFRSF10A)

tumor necrosis factor receptor superfamily, member 10a

EDNR

endothelin receptor

ELK1

member of ETS oncogene family

ERK

extracellular signal-regulated kinase

FADD

Fas-associated death domain

FAS

Fas (TNF receptor superfamily, member 6)

FGFR

fibroblast growth factor receptor

FOXO1

forkhead box O1

G

gap phase

G protein

guanine nucleotide-binding protein

GSK3Bglycogen synthase kinase 3 β **HRAS**

v-Ha-ras Harvey rat sarcoma viral oncogene homolog

HtrA2/Omi

high-temperature requirement protein A2

ID2

inhibitor of DNA binding 2

IGFBP7

IGF binding protein 7

IGF1R

IGF1 receptor

KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
LEF1	lymphoid enhancer-binding factor 1
M	mitosis
MAPK1 (also known as ERK2)	mitogen-activated protein kinase 1
MAPK3 (also known as ERK1)	mitogen-activated protein kinase 3
MCL1	myeloid cell leukemia sequence 1
MC1R	melanocortin 1 receptor
MDM2	murine double minute 2 protein
MDR1	multidrug resistance gene product 1
MEK (also known as MAP2K)	MAPK/ERK kinase
MITF	microphthalmia-associated transcription factor
MLANA (also known as MART1)	melan-A
α-MSH	α melanocyte-stimulating hormone
mTOR (also known as FRAP1)	mammalian target of rapamycin
NOXA	PMAIP1, phorbol-12-myristate-13-acetate-induced protein 1
NRAS	neuroblastoma RAS viral oncogene homolog
p14^{ARF}	p14, alternative reading frame
p16^{INK4A}	p16, inhibits CDK4
p19^{ARF}	a mouse ortholog of human p14 ^{ARF}
p21^{WAF1/CIP1}	wild-type p53-activated fragment 1/CDK-interacting protein

p53	tumor protein 53
PAX3	paired box 3
PDK1 (also known as PDPK1)	phosphoinositide-dependent kinase 1
PI	phosphatidylinositol
PI3K	phosphatidylinositol 3-kinase
PIP	phosphatidylinositol-4-phosphate
PIP2	phosphatidylinositol-4,5-bisphosphate
PIP3	phosphatidylinositol-3,4,5-triphosphate
PKC	protein kinase C
PTEN	phosphatase and tensin homolog
RAF1	v-raf-1 murine leukemia viral oncogene homolog 1
RAS	rat sarcoma viral oncogene homolog
RB1	retinoblastoma 1
S	DNA synthesis phase
SCF	stem cell factor (KITLG, KIT ligand)
SHC	Src homology 2 domain– containing protein
SILV	silver homolog (mouse)
Smac/DIABLO	second mitochondria-derived activator of caspases/direct IAP-binding protein with low PI
SOX10	sex-determining region Y–box 10
TCF	

	T-cell factor
TP53	tumor protein p53 (gene)
TRAIL	tumor necrosis factor–related apoptosis-inducing ligand-receptor
TRAIL-RI	TRAIL receptor 1
TRPM1	transient receptor potential cation channel, subfamily M, member 1
TYR	tyrosinase
TYRP1	TYR-related protein 1
UUU	poly-ubiquitin
VEGFR (also known as KDR)	VEGF receptor
WNT	wingless-type MMTV (mouse mammary tumor virus) integration site family

References

1. Meyer LJ, Zone JH. Genetics of cutaneous melanoma. *J Invest Dermatol* 1994;103(5 suppl):112S–116S. [PubMed: 7963671]
2. Gilchrest BA, Eller MS, Geller AC, Yaar M. The pathogenesis of melanoma induced by ultraviolet radiation. *N Engl J Med* 1999;340(17):1341–1348. [PubMed: 10219070]
3. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. *Eur J Cancer* 2005;41(1):28–44. [PubMed: 15617989]
4. Tannous ZS, Mihm MC Jr, Sober AJ, Duncan LM. Congenital melanocytic nevi: clinical and histopathologic features, risk of melanoma, and clinical management. *J Am Acad Dermatol* 2005;52(2):197–203. [PubMed: 15692463]
5. Watt AJ, Kotsis SV, Chung KC. Risk of melanoma arising in large congenital melanocytic nevi: a systematic review. *Plast Reconstr Surg* 2004;113(7):1968–1974. [PubMed: 15253185]
6. Cho E, Rosner BA, Feskanich D, Colditz GA. Risk factors and individual probabilities of melanoma for whites. *J Clin Oncol* 2005;23(12):2669–2675. [PubMed: 15837981]
7. Greene MH, Clark WH Jr, Tucker MA, Kraemer KH, Elder DE, Fraser MC. High risk of malignant melanoma in melanoma-prone families with dysplastic nevi. *Ann Intern Med* 1985;102(4):458–465. [PubMed: 3977193]
8. Cannon-Albright LA, Meyer LJ, Goldgar DE, et al. Penetrance and expressivity of the chromosome 9p melanoma susceptibility locus (MLM). *Cancer Res* 1994;54(23):6041–6044. [PubMed: 7954442]
9. Kamb A, Gruis NA, Weaver-Feldhaus J, et al. A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 1994;264(5157):436–440. [PubMed: 8153634]
10. Kamb A, Shattuck-Eidens D, Eeles R, et al. Analysis of the p16 gene (CDKN2) as a candidate for the chromosome 9p melanoma susceptibility locus. *Nat Genet* 1994;8(1):23–26. [PubMed: 7987388]
11. Hussussian CJ, Struwing JP, Goldstein AM, et al. Germline p16 mutations in familial melanoma. *Nat Genet* 1994;8(1):15–21. [PubMed: 7987387]

12. Rulyak SJ, Brentnall TA, Lynch HT, Austin MA. Characterization of the neoplastic phenotype in the familial atypical multiple-mole melanoma-pancreatic carcinoma syndrome. *Cancer* 2003;98(4):798–804. [PubMed: 12910525]
13. Eliason MJ, Larson AA, Florell SR, et al. Population-based prevalence of CDKN2A mutations in Utah melanoma families. *J Invest Dermatol* 2006;126(3):660–666. [PubMed: 16397522]
14. Goldstein AM, Chan M, Harland M, et al. 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 Feb;44(2):99–106. [PubMed: 16905682]Epub 2006 Aug 11
15. Newton Bishop JA, Gruis NA. Genetics: what advice for patients who present with a family history of melanoma? *Semin Oncol* 2007;34(6):452–459. [PubMed: 18083368]
16. Goldstein AM, Chidambaram A, Halpern A, et al. Rarity of CDK4 germline mutations in familial melanoma. *Melanoma Res* 2002;12(1):51–55. [PubMed: 11828258]
17. Gillanders E, Juo SH, Holland EA, et al. Melanoma Genetics Consortium. Localization of a novel melanoma susceptibility locus to 1p22. *Am J Hum Genet* 2003 Aug;73(2):301–313. [PubMed: 12844286]Epub 2003 Jul 3
18. Brown KM, MacGregor S, Montgomery G, et al. Common sequence variants on 20q11.22 confer melanoma susceptibility. *Nat Genet*. 2008Epub 18 May 2008
19. Bennett DC. How to make a melanoma: what do we know of the primary clonal events? *Pigment Cell Melanoma Res* 2008;21(1):27–38. [PubMed: 18353141]
20. Marini A, Mirmohammadsadegh A, Nambiar S, Gustrau A, Ruzicka T, Hengge UR. Epigenetic inactivation of tumor suppressor genes in serum of patients with cutaneous melanoma. *J Invest Dermatol* 2006;126(2):422–431. [PubMed: 16374457]
21. Keller-Melchior R, Schmidt R, Piepkorn M. Expression of the tumor suppressor gene product p16INK4 in benign and malignant melanocytic lesions. *J Invest Dermatol* 1998;110(6):932–938. [PubMed: 9620301]
22. Grover R, Chana JS, Wilson GD, Richman PI, Sanders R. An analysis of p16 protein expression in sporadic malignant melanoma. *Melanoma Res* 1998;8(3):267–272. [PubMed: 9664149]
23. Straume O, Sviland L, Akslen LA. Loss of nuclear p16 protein expression correlates with increased tumor cell proliferation (Ki-67) and poor prognosis in patients with vertical growth phase melanoma. *Clin Cancer Res* 2000;6(5):1845–1853. [PubMed: 10815907]
24. Zuo L, Weger J, Yang Q, et al. Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. *Nat Genet* 1996;12(1):97–99. [PubMed: 8528263]
25. Soufir N, Avril MF, Chompret A, et al. French Familial Melanoma Study Group. Prevalence of p16 and CDK4 germline mutations in 48 melanoma-prone families in France [published correction appears in *Hum Mol Genet*. 1998;7(5):941]. *Hum Mol Genet* 1998;7(2):209–216. [PubMed: 9425228]
26. Wolfel T, Hauer M, Schneider J, et al. A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science* 1995;269(5228):1281–1284. [PubMed: 7652577]
27. Tsao H, Benoit E, Sober AJ, Thiele C, Haluska FG. Novel mutations in the p16/CDKN2A binding region of the cyclin-dependent kinase-4 gene. *Cancer Res* 1998;58(1):109–113. [PubMed: 9426066]
28. Tang L, Li G, Tron VA, Trotter MJ, Ho VC. Expression of cell cycle regulators in human cutaneous malignant melanoma. *Melanoma Res* 1999;9(2):148–154. [PubMed: 10380937]
29. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. *N Engl J Med* 2005;353(20):2135–2147. [PubMed: 16291983]
30. 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(6):2632–2642. [PubMed: 17363583]
31. Jonsson G, Dahl C, Staaf J, et al. Genomic profiling of malignant melanoma using tiling-resolution arrayCGH. *Oncogene* 2007 Jul 12;26(32):4738–4748. [PubMed: 17260012]Epub 2007 Jan 29
32. Sanders BM, Jay M, Draper GJ, Roberts EM. Non-ocular cancer in relatives of retinoblastoma patients. *Br J Cancer* 1989;60(3):358–365. [PubMed: 2789942]
33. Draper GJ, Sanders BM, Kingston JE. Second primary neoplasms in patients with retinoblastoma. *Br J Cancer* 1986;53(5):661–671. [PubMed: 3718823]

34. Fletcher O, Easton D, Anderson K, Gilham C, Jay M, Peto J. Lifetime risks of common cancers among retinoblastoma survivors. *J Natl Cancer Inst* 2004;96(5):357–363. [PubMed: 14996857]
35. Petitjean A, Mathe E, Kato S, et al. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum Mutat* 2007;28(6):622–629. [PubMed: 17311302]
36. Rizos H, Darmanian AP, Holland EA, Mann GJ, Kefford RF. Mutations in the INK4a/ARF melanoma susceptibility locus functionally impair p14ARF. *J Biol Chem* 2001 Nov 2;276(44):41424–41434. [PubMed: 11518711]Epub 2001 Aug 22
37. Harland M, Taylor CF, Bass S, et al. Intronic sequence variants of the CDKN2A gene in melanoma pedigrees. *Genes Chromosomes Cancer* 2005;43(2):128–136. [PubMed: 15761864]
38. Randerson-Moor JA, Harland M, Williams S, et al. A germline deletion of p14(ARF) but not CDKN2A in a melanoma-neural system tumour syndrome family. *Hum Mol Genet* 2001;10(1):55–62. [PubMed: 11136714]
39. Krimpenfort P, Quon KC, Mooi WJ, Loonstra A, Berns A. Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. *Nature* 2001;413(6851):83–86. [PubMed: 11544530]
40. Sharpless NE, Bardeesy N, Lee KH, et al. Loss of p16Ink4a with retention of p19Arf predisposes mice to tumorigenesis. *Nature* 2001;413(6851):86–91. [PubMed: 11544531]
41. Efeyan A, Ortega-Molina A, Velasco-Miguel S, Herranz D, Vassilev LT, Serrano M. Induction of p53-dependent senescence by the MDM2 antagonist nutlin-3a in mouse cells of fibroblast origin. *Cancer Res* 2007;67(15):7350–7357. [PubMed: 17671205]
42. Vassilev LT. MDM2 inhibitors for cancer therapy. *Trends Mol Med* 2007 Jan;13(1):23–31. [PubMed: 17126603]Epub 2006 Nov 28
43. Bullock AN, Fersht AR. Rescuing the function of mutant p53. *Nat Rev Cancer* 2001;1(1):68–76. [PubMed: 11900253]
44. Joerger AC, Fersht AR. Structural biology of the tumor suppressor p53. *Annu Rev Biochem*. 2008;77:1146–1146. [published online ahead of print April 14, 2008]
45. Chada S, Menander KB, Bocangel D, Roth JA, Ramesh R. Cancer targeting using tumor suppressor genes. *Front Biosci* 2008;13:1959–1967. [PubMed: 17981683]
46. Ha L, Ichikawa T, Anver M, et al. ARF functions as a melanoma tumor suppressor by inducing p53-independent senescence. *Proc Natl Acad Sci U S A* 2007 Jun 26;104(26):10968–10973. [PubMed: 17576930]Epub 2007 Jun 19
47. Kasparian NA, Meiser B, Butow PN, Job RF, Mann GJ. Better the devil you know? High-risk individuals' anticipated psychological responses to genetic testing for melanoma susceptibility. *J Genet Couns* 2006;15(6):433–447. [PubMed: 17103321]
48. Fecher LA, Amaravadi RK, Flaherty KT. The MAPK pathway in melanoma. *Curr Opin Oncol* 2008;20(2):183–189. [PubMed: 18300768]
49. Meier F, Schitteck B, Busch S, et al. The RAS/RAF/MEK/ERK and PI3K/AKT signaling pathways present molecular targets for the effective treatment of advanced melanoma. *Front Biosci* 2005;10:2986–3001. [PubMed: 15970553]
50. Braig M, Schmitt CA. Oncogene-induced senescence: putting the brakes on tumor development. *Cancer Res* 2006;66(6):2881–2884. [PubMed: 16540631]
51. Clark WH Jr, Elder DE, Guerry D IV, Epstein MN, Greene MH, Van Horn M. A study of tumor progression: the precursor lesions of superficial spreading and nodular melanoma. *Hum Pathol* 1984;15(12):1147–1165. [PubMed: 6500548]
52. Cohen C, Zavala-Pompa A, Sequeira JH, et al. Mitogen-activated protein kinase activation is an early event in melanoma progression. *Clin Cancer Res* 2002;8(12):3728–3733. [PubMed: 12473582]
53. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature* 2002 Jun 27;417(6892):949–954. [PubMed: 12068308]Epub 2002 Jun 9
54. Gray-Schopfer VC, da Rocha Dias S, Marais R. The role of B-RAF in melanoma [published correction appears in *Cancer Metastasis Rev*. 2005;24(2):367]. *Cancer Metastasis Rev* 2005;24(1):165–183. [PubMed: 15785879]
55. Smalley KS. A pivotal role for ERK in the oncogenic behaviour of malignant melanoma? *Int J Cancer* 2003;104(5):527–532. [PubMed: 12594806]

56. Trisciuoglio D, Iervolino A, Zupi G, Del Bufalo D. Involvement of PI3K and MAPK signaling in bcl-2-induced vascular endothelial growth factor expression in melanoma cells. *Mol Biol Cell* 2005 Sep;16(9):4153–4162. [PubMed: 15987743]Epub 2005 Jun 29
57. Widlund HR, Fisher DE. Microphthalmia-associated transcription factor: a critical regulator of pigment cell development and survival. *Oncogene* 2003;22(20):3035–3041. [PubMed: 12789278]
58. Wilson DJ, Alessandrini A, Budd RC. MEK1 activation rescues Jurkat T cells from Fas-induced apoptosis. *Cell Immunol* 1999;194(1):67–77. [PubMed: 10357882]
59. Eisen T, Ahmad T, Flaherty KT, et al. Sorafenib in advanced melanoma: a phase II randomised discontinuation trial analysis. *Br J Cancer* 2006 Sep 4;95(5):581–586. [PubMed: 16880785]Epub 2006 Aug 1
60. Agarwala SS, Keilholz U, Hogg D, et al. Randomized phase III study of paclitaxel plus carboplatin with or without sorafenib as second-line treatment in patients with advanced melanoma [abstract 8510]. *J Clin Oncol* 2007;25(1 suppl):18s.
61. Ackermann J, Fruttschi M, Kaloulis K, McKee T, Trumpp A, Beermann F. Metastasizing melanoma formation caused by expression of activated N-RasQ61K on an INK4a-deficient background. *Cancer Res* 2005;65(10):4005–4011. [PubMed: 15899789]
62. Gray-Schopfer V, Wellbrock C, Marais R. Melanoma biology and new targeted therapy. *Nature* 2007;445(7130):851–857. [PubMed: 17314971]
63. 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(3):285–290. [PubMed: 8120410]
64. Chin L, Tam A, Pomerantz J, et al. Essential role for oncogenic Ras in tumour maintenance. *Nature* 1999;400(6743):468–472. [PubMed: 10440378]
65. Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene* 2006;25(34):4798–4811. [PubMed: 16892092]
66. Glinsky GV, Glinsky VV. Apoptosis and metastasis: a superior resistance of metastatic cancer cells to programmed cell death. *Cancer Lett* 1996;101(1):43–51. [PubMed: 8625281]
67. Glinsky GV, Glinsky VV, Ivanova AB, Hueser CJ. Apoptosis and metastasis: increased apoptosis resistance of metastatic cancer cells is associated with the profound deficiency of apoptosis execution mechanisms. *Cancer Lett* 1997;115(2):185–193. [PubMed: 9149123]
68. Valente P, Fassina G, Melchiori A, et al. TIMP-2 over-expression reduces invasion and angiogenesis and protects B16F10 melanoma cells from apoptosis [published correction appears in *Int J Cancer*. 1999;80(3):485]. *Int J Cancer* 1998;75:246–253. [PubMed: 9462715]
69. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. *Science* 2004;305(5684):626–629. [PubMed: 15286356]
70. Saelens X, Festjens N, Vande Walle L, van Gurp M, van Loo G, Vandenabeele P. Toxic proteins released from mitochondria in cell death. *Oncogene* 2004;23(16):2861–2874. [PubMed: 15077149]
71. Degterev A, Boyce M, Yuan J. A decade of caspases. *Oncogene* 2003;22(53):8543–8567. [PubMed: 14634618]
72. Nagata S. Apoptotic DNA fragmentation. *Exp Cell Res* 2000;256(1):12–18. [PubMed: 10739646]
73. Hengartner MO. The biochemistry of apoptosis. *Nature* 2000;407(6805):770–776. [PubMed: 11048727]
74. Walczak H, Krammer PH. The CD95 (APO-1/Fas) and the TRAIL (APO-2L) apoptosis systems. *Exp Cell Res* 2000;256(1):58–66. [PubMed: 10739652]
75. Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. *Science* 2002;296(5573):1634–1635. [PubMed: 12040173]
76. Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. *Nat Rev Cancer* 2002;2(9):647–656. [PubMed: 12209154]
77. Bedikian AY, Millward M, Pehamberger H, et al. Bcl-2 antisense (oblimersen sodium) plus dacarbazine in patients with advanced melanoma: the Oblimersen Melanoma Study Group. *J Clin Oncol* 2006 Oct 10; 24(29):4738–4745. [PubMed: 16966688]Epub 2006 Sep 11
78. Stahl JM, Cheung M, Sharma A, Trivedi NR, Shanmugam S, Robertson GP. Loss of PTEN promotes tumor development in malignant melanoma. *Cancer Res* 2003;63(11):2881–2890. [PubMed: 12782594]

79. Wu H, Goel V, Haluska FG. PTEN signaling pathways in melanoma. *Oncogene* 2003;22(20):3113–3122. [PubMed: 12789288]
80. Stahl JM, Sharma A, Cheung M, et al. Deregulated Akt3 activity promotes development of malignant melanoma. *Cancer Res* 2004;64(19):7002–7010. [PubMed: 15466193]
81. Govindarajan B, Sligh JE, Vincent BJ, et al. Overexpression of Akt converts radial growth melanoma to vertical growth melanoma. *J Clin Invest* 2007 Mar;117(3):719–729. [PubMed: 17318262]Epub 2007 Feb 22
82. Miller AJ, Mihm MC Jr. Melanoma. *N Engl J Med* 2006;355(1):51–65. [PubMed: 16822996]
83. 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(2):337–341. [PubMed: 15009714]
84. Granville CA, Memmott RM, Gills JJ, Dennis PA. Handicapping the race to develop inhibitors of the phosphoinositide 3-kinase/Akt/mammalian target of rapamycin pathway. *Clin Cancer Res* 2006;12(3 pt 1):679–689. [PubMed: 16467077]
85. O'Reilly KE, Rojo F, She QB, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 2006;66(3):1500–1508. [PubMed: 16452206]
86. Thallinger C, Wolschek MF, Wacheck V, et al. Mcl-1 antisense therapy chemosensitizes human melanoma in a SCID mouse xenotransplantation model. *J Invest Dermatol* 2003;120(6):1081–1086. [PubMed: 12787138]
87. Heere-Ress E, Thallinger C, Lucas T, et al. Bcl-X(L) is a chemoresistance factor in human melanoma cells that can be inhibited by antisense therapy. *Int J Cancer* 2002;99(1):29–34. [PubMed: 11948488]
88. Fernandez Y, Verhaegen M, Miller TP, et al. Differential regulation of noxa in normal melanocytes and melanoma cells by proteasome inhibition: therapeutic implications. *Cancer Res* 2005;65(14):6294–6304. [PubMed: 16024631]
89. Nikiforov MA, Riblett M, Tang WH, et al. Tumor cell-selective regulation of NOXA by c-MYC in response to proteasome inhibition. *Proc Natl Acad Sci U S A* 2007 Dec 4;104(49):19488–19493. [PubMed: 18042711]Epub 2007 Nov 27
90. Zhuang L, Lee CS, Scolyer RA, et al. Progression in melanoma is associated with decreased expression of death receptors for tumor necrosis factor-related apoptosis-inducing ligand. *Hum Pathol* 2006 Oct;37(10):1286–1294. [PubMed: 16949935]Epub 2006 Jul 18
91. Wagner KW, Punnoose EA, Januario T, et al. Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. *Nat Med* 2007 Sep;13(9):1070–1077. [PubMed: 17767167]Epub 2007 Sep 2
92. Kurbanov BM, Fecker LF, Geilen CC, Sterry W, Eberle J. Resistance of melanoma cells to TRAIL does not result from upregulation of antiapoptotic proteins by NF- β B but is related to downregulation of initiator caspases and DR4. *Oncogene* 2007 May 17;26(23):3364–3377. [PubMed: 17160022]Epub 2006 Dec 11
93. Geserick P, Drewniok C, Hupe M, et al. Suppression of cFLIP is sufficient to sensitize human melanoma cells to TRAIL- and CD95L-mediated apoptosis [published online ahead of print Dec 17, 2007]. *Oncogene* 2008;27:3211–3220.10.1038/sj.onc.1210985 [PubMed: 18084329]
94. Eberle J, Fecker LF, Orfanos CE, Geilen CC. Endothelin-1 decreases basic apoptotic rates in human melanoma cell lines. *J Invest Dermatol* 2002;119(3):549–555. [PubMed: 12230494]
95. Tang L, Su M, Zhang Y, et al. Endothelin-3 is produced by metastatic melanoma cells and promotes melanoma cell survival. *J Cutan Med Surg* 2008;12(2):64–70. [PubMed: 18346402]
96. Lahav R. Endothelin receptor B is required for the expansion of melanocyte precursors and malignant melanoma. *Int J Dev Biol* 2005;49(2–3):173–180. [PubMed: 15906230]
97. Bagnato A, Rosano L, Spinella F, Di Castro V, Tecce R, Natali PG. Endothelin B receptor blockade inhibits dynamics of cell interactions and communications in melanoma cell progression. *Cancer Res* 2004;64(4):1436–1443. [PubMed: 14973117]
98. Rosano L, Spinella F, Genovesi G, Di Castro V, Natali PG, Bagnato A. Endothelin-B receptor blockade inhibits molecular effectors of melanoma cell progression. *J Cardiovasc Pharmacol* 2004;44(suppl 1):S136–S139. [PubMed: 15838263]
99. Mangahas CR, dela Cruz GV, Schneider RJ, Jamal S. Endothelin-1 upregulates MCAM in melanocytes. *J Invest Dermatol* 2004;123(6):1135–1139. [PubMed: 15610525]

100. Tsao H, Zhang X, Fowlkes K, Haluska FG. Relative reciprocity of NRAS and PTEN/MMAC1 alterations in cutaneous melanoma cell lines. *Cancer Res* 2000;60(7):1800–1804. [PubMed: 10766161]
101. Wajapeyee N, Serra RW, Zhu X, Mahalingam M, Green MR. Oncogenic BRAF induces senescence and apoptosis through pathways mediated by the secreted protein IGFBP7. *Cell* 2008;132(3):363–374. [PubMed: 18267069]
102. Pollock PM, Harper UL, Hansen KS, et al. High frequency of BRAF mutations in nevi. *Nat Genet* 2003 Jan;33(1):19–20. [PubMed: 12447372]Epub 2002 Nov 25
103. Satyamoorthy K, Li G, Gerrero MR, et al. Constitutive mitogen-activated protein kinase activation in melanoma is mediated by both BRAF mutations and autocrine growth factor stimulation. *Cancer Res* 2003;63(4):756–759. [PubMed: 12591721]
104. Kanter-Lewensohn L, Dricu A, Wang M, Wejde J, Kiessling R, Larsson O. Expression of the insulin-like growth factor-1 receptor and its anti-apoptotic effect in malignant melanoma: a potential therapeutic target. *Melanoma Res* 1998;8(5):389–397. [PubMed: 9835451]
105. Satyamoorthy K, Li G, Vaidya B, Patel D, Herlyn M. Insulin-like growth factor-1 induces survival and growth of biologically early melanoma cells through both the mitogen-activated protein kinase and β -catenin pathways. *Cancer Res* 2001;61(19):7318–7324. [PubMed: 11585772]
106. Yeh AH, Bohula EA, Macaulay VM. Human melanoma cells expressing V600E B-RAF are susceptible to IGF1R targeting by small interfering RNAs. *Oncogene* 2006 Oct 26;25(50):6574–6581. [PubMed: 16715137]Epub 2006 May 22
107. Graells J, Vinyals A, Figueras A, et al. Overproduction of VEGF concomitantly expressed with its receptors promotes growth and survival of melanoma cells through MAPK and PI3K signaling. *J Invest Dermatol* 2004;123(6):1151–1161. [PubMed: 15610528]
108. Lacal PM, Ruffini F, Pagani E, D'Atri S. An autocrine loop directed by the vascular endothelial growth factor promotes invasiveness of human melanoma cells. *Int J Oncol* 2005;27(6):1625–1632. [PubMed: 16273219]
109. Graeven U, Rodeck U, Karpinski S, Jost M, Philippou S, Schmiegel W. Modulation of angiogenesis and tumorigenicity of human melanocytic cells by vascular endothelial growth factor and basic fibroblast growth factor. *Cancer Res* 2001;61(19):7282–7290. [PubMed: 11585767]
110. Ozen M, Medrano EE, Ittmann M. Inhibition of proliferation and survival of melanoma cells by adenoviral-mediated expression of dominant negative fibroblast growth factor receptor. *Melanoma Res* 2004;14(1):13–21. [PubMed: 15091189]
111. Yayon A, Ma YS, Safran M, Klagsbrun M, Halaban R. Suppression of autocrine cell proliferation and tumorigenesis of human melanoma cells and fibroblast growth factor transformed fibroblasts by a kinase-deficient FGF receptor 1: evidence for the involvement of Src-family kinases. *Oncogene* 1997;14(25):2999–3009. [PubMed: 9223663]
112. 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. *Hum Pathol* 2005;36(5):486–493. [PubMed: 15948115]
113. Antonescu CR, Busam KJ, Francone TD, et al. L576P KIT mutation in anal melanomas correlates with KIT protein expression and is sensitive to specific kinase inhibition. *Int J Cancer* 2007;121(2):257–264. [PubMed: 17372901]
114. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. *J Clin Oncol* 2006 Sep 10;24(26):4340–4346. [PubMed: 16908931]Epub 2006 Aug 14
115. Pereira PR, Odashiro AN, Marshall JC, Correa ZM, Belfort R Jr, Burnier MN Jr. The role of c-kit and imatinib mesylate in uveal melanoma. *J Carcinog* 2005;4:19.10.1186/1477–3163–4–19 [PubMed: 16236162]
116. McGill GG, Haq R, Nishimura EK, Fisher DE. c-Met expression is regulated by Mitf in the melanocyte lineage. *J Biol Chem* 2006 Apr 14;281(15):10365–10373. [PubMed: 16455654]Epub 2006 Feb 2
117. Beuret L, Flori E, Denoyelle C, et al. Up-regulation of MET expression by α -melanocyte-stimulating hormone and MITF allows hepatocyte growth factor to protect melanocytes and melanoma cells from apoptosis. *J Biol Chem* 2007 May 11;282(19):14140–14147. [PubMed: 17371876]Epub 2007 Mar 19

118. Lerner AB, Shiohara T, Boissy RE, Jacobson KA, Lamoreux ML, Moellmann GE. A mouse model for vitiligo. *J Invest Dermatol* 1986;87(3):299–304. [PubMed: 3525691]
119. Steingrimsson E, Moore KJ, Lamoreux ML, et al. Molecular basis of mouse microphthalmia (mi) mutations helps explain their developmental and phenotypic consequences. *Nat Genet* 1994;8(3):256–263. [PubMed: 7874168]
120. Bentley NJ, Eisen T, Goding CR. Melanocyte-specific expression of the human tyrosinase promoter: activation by the microphthalmia gene product and role of the initiator. *Mol Cell Biol* 1994;14(12):7996–8006. [PubMed: 7969139]
121. Hemesath TJ, Steingrimsson E, McGill G, et al. Microphthalmia, a critical factor in melanocyte development, defines a discrete transcription factor family. *Genes Dev* 1994;8(22):2770–2780. [PubMed: 7958932]
122. Yasumoto K, Yokoyama K, Shibata K, Tomita Y, Shibahara S. Microphthalmia-associated transcription factor as a regulator for melanocyte-specific transcription of the human tyrosinase gene [published correction appears in *Mol Cell Biol*. 1995;15(3):1833]. *Mol Cell Biol* 1994;14(12):8058–8070. [PubMed: 7969144]
123. Goding CR. Mitf from neural crest to melanoma: signal transduction and transcription in the melanocyte lineage. *Genes Dev* 2000;14(14):1712–1728. [PubMed: 10898786]
124. Du J, Miller AJ, Widlund HR, Horstmann MA, Ramaswamy S, Fisher DE. MLANA/MART1 and SILV/PMEL17/GP100 are transcriptionally regulated by MITF in melanocytes and melanoma. *Am J Pathol* 2003;163(1):333–343. [PubMed: 12819038]
125. Baxter LL, Pavan WJ. Pmel17 expression is Mitf-dependent and reveals cranial melanoblast migration during murine development. *Gene Expr Patterns* 2003;3(6):703–707. [PubMed: 14643677]
126. Du J, Fisher DE. Identification of Aim-1 as the underwhite mouse mutant and its transcriptional regulation by MITF. *J Biol Chem* 2002;277(1):402–406. [PubMed: 11700328]Epub 2001 Nov 7
127. Miller AJ, Du J, Rowan S, Hershey CL, Widlund HR, Fisher DE. Transcriptional regulation of the melanoma prognostic marker melastatin (TRPM1) by MITF in melanocytes and melanoma. *Cancer Res* 2004;64(2):509–516. [PubMed: 14744763]
128. Zhiqi S, Soltani MH, Bhat KM, et al. Human melastatin 1 (TRPM1) is regulated by MITF and produces multiple polypeptide isoforms in melanocytes and melanoma. *Melanoma Res* 2004;14(6):509–516. [PubMed: 15577322]
129. McGill GG, Horstmann M, Widlund HR, et al. Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. *Cell* 2002;109(6):707–718. [PubMed: 12086670]
130. Nishimura EK, Granter SR, Fisher DE. Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. *Science* 2005 Feb 4;307(5710):720–724. [PubMed: 15618488]Epub 2004 Dec 23
131. Banerjee D. Genasense (Genta Inc). *Curr Opin Investig Drugs* 2001;2(4):574–580.
132. Hofbauer GF, Kamarashev J, Geertsens R, Boni R, Dummer R. Melan A/MART-1 immunoreactivity in formalin-fixed paraffin-embedded primary and metastatic melanoma: frequency and distribution. *Melanoma Res* 1998;8(4):337–343. [PubMed: 9764809]
133. Salti GI, Manougian T, Farolan M, Shilkaitis A, Majumdar D, Das Gupta TK. Microphthalmia transcription factor: a new prognostic marker in intermediate-thickness cutaneous malignant melanoma. *Cancer Res* 2000;60(18):5012–5016. [PubMed: 11016620]
134. Seiter S, Monsurro V, Nielsen MB, et al. Frequency of MART-1/MelanA and gp100/PMel17-specific T cells in tumor metastases and cultured tumor-infiltrating lymphocytes. *J Immunother* 2002;25(3):252–263. [PubMed: 12000867]
135. Takeuchi H, Kuo C, Morton DL, Wang HJ, Hoon DS. Expression of differentiation melanoma-associated antigen genes is associated with favorable disease outcome in advanced-stage melanomas. *Cancer Res* 2003;63(2):441–448. [PubMed: 12543800]
136. King R, Weilbaecher KN, McGill G, Cooley E, Mihm M, Fisher DE. Microphthalmia transcription factor: a sensitive and specific melanocyte marker for MelanomaDiagnosis. *Am J Pathol* 1999;155(3):731–738. [PubMed: 10487831]

137. Miettinen M, Fernandez M, Franssila K, Gatalica Z, Lasota J, Sarlomo-Rikala M. Microphthalmia transcription factor in the immunohistochemical diagnosis of metastatic melanoma: comparison with four other melanoma markers. *Am J Surg Pathol* 2001;25(2):205–211. [PubMed: 11176069]
138. Granter SR, Weilbaecher KN, Quigley C, Fisher DE. Role for microphthalmia transcription factor in the diagnosis of metastatic malignant melanoma. *Appl Immunohistochem Mol Morphol* 2002;10(1):47–51. [PubMed: 11893035]
139. Garraway LA, Widlund HR, Rubin MA, et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature* 2005;436(7047):117–122. [PubMed: 16001072]
140. Johnson JP. Cell adhesion molecules in the development and progression of malignant melanoma. *Cancer Metastasis Rev* 1999;18(3):345–357. [PubMed: 10721489]
141. Hsu M, Andl T, Li G, Meinkoth JL, Herlyn M. Cadherin repertoire determines partner-specific gap junctional communication during melanoma progression. *J Cell Sci* 2000;113(pt 9):1535–1542. [PubMed: 10751145]
142. Valyi-Nagy IT, Hirka G, Jensen PJ, Shih IM, Juhasz I, Herlyn M. Undifferentiated keratinocytes control growth, morphology, and antigen expression of normal melanocytes through cell-cell contact. *Lab Invest* 1993;69(2):152–159. [PubMed: 8350597]
143. Danen EH, de Vries TJ, Morandini R, Ghanem GG, Ruiter DJ, van Muijen GN. E-cadherin expression in human melanoma. *Melanoma Res* 1996;6(2):127–131. [PubMed: 8791270]
144. Hsu MY, Wheelock MJ, Johnson KR, Herlyn M. Shifts in cadherin profiles between human normal melanocytes and melanomas. *J Invest Dermatol Symp Proc* 1996;1(2):188–194.
145. Scott RA, Lauweryns B, Snead DM, Haynes RJ, Mahida Y, Dua HS. E-cadherin distribution and epithelial basement membrane characteristics of the normal human conjunctiva and cornea. *Eye* 1997;11(pt 5):607–612. [PubMed: 9474304]
146. Gottardi CJ, Wong E, Gumbiner BM. E-cadherin suppresses cellular transformation by inhibiting β -catenin signaling in an adhesion-independent manner. *J Cell Biol* 2001;153(5):1049–1060. [PubMed: 11381089]
147. Brembeck FH, Schwarz-Romond T, Bakkers J, Wilhelm S, Hammerschmidt M, Birchmeier W. Essential role of BCL9–2 in the switch between β -catenin's adhesive and transcriptional functions. *Genes Dev* 2004 Sep 15;18(18):2225–2230. [PubMed: 15371335]Epub 2004 Sep 1
148. Bienz M. β -Catenin: a pivot between cell adhesion and Wnt signalling. *Curr Biol* 2005;15(2):R64–R67. [PubMed: 15668160]
149. Gottardi CJ, Gumbiner BM. Adhesion signaling: how β -catenin interacts with its partners. *Curr Biol* 2001;11(19):R792–R794. [PubMed: 11591340]
150. Widlund HR, Horstmann MA, Price ER, et al. β -Catenin-induced melanoma growth requires the downstream target *Microphthalmia*-associated transcription factor. *J Cell Biol* 2002 Sep 16;158(6):1079–1087. [PubMed: 12235125]Epub 2002 Sep 16
151. Shutterman M, Zhurinsky J, Simcha I, et al. The cyclin D1 gene is a target of the β -catenin/LEF-1 pathway. *Proc Natl Acad Sci U S A* 1999;96(10):5522–5527. [PubMed: 10318916]
152. Thompson JF, Scolyer RA, Kefford RF. Cutaneous melanoma. *Lancet* 2005;365(9460):687–701. [PubMed: 15721476]
153. MacKie RM. Risk factors for the development of primary cutaneous malignant melanoma. *Dermatol Clin* 2002;20(4):597–600. [PubMed: 12380046]
154. Marks R. Epidemiology of melanoma. *Clin Exp Dermatol* 2000;25(6):459–463. [PubMed: 11044179]
155. Cui R, Widlund HR, Feige E, et al. Central role of p53 in the suntan response and pathologic hyperpigmentation. *Cell* 2007;128(5):853–864. [PubMed: 17350573]
156. D'Orazio JA, Nobuhisa T, Cui R, et al. Topical drug rescue strategy and skin protection based on the role of Mc1r in UV-induced tanning. *Nature* 2006;443(7109):340–344. [PubMed: 16988713]
157. Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. *Nature* 2007;445(7130):843–850. [PubMed: 17314970]
158. Naysmith L, Waterston K, Ha T, et al. Quantitative measures of the effect of the melanocortin 1 receptor on human pigmentary status. *J Invest Dermatol* 2004;122(2):423–428. [PubMed: 15009725]

159. 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(3):328–330. [PubMed: 7581459]
160. Frandberg PA, Doufexis M, Kapas S, Chhajlani V. Human pigmentation phenotype: a point mutation generates nonfunctional MSH receptor. *Biochem Biophys Res Commun* 1998;245(2):490–492. [PubMed: 9571181]
161. Kennedy C, ter Huurne J, Berkhout M, et al. Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color. *J Invest Dermatol* 2001;117(2):294–300. [PubMed: 11511307]
162. Landi MT, Bauer J, Pfeiffer RM, et al. MC1R germline variants confer risk for BRAF-mutant melanoma. *Science* 2006 Jul 28;313(5786):521–522. [PubMed: 16809487]Epub 2006 Jun 29
163. Fargnoli MC, Pike K, Pfeiffer RM, et al. MC1R Variants increase risk of melanomas harboring BRAF mutations. *J Invest Dermatol*. 2008;10.1038/jid.2008.67[published online ahead of print March 27, 2008]
164. Jaeger J, Koczan D, Thiesen HJ, et al. Gene expression signatures for tumor progression, tumor subtype, and tumor thickness in laser-microdissected melanoma tissues. *Clin Cancer Res* 2007;13(3):806–815. [PubMed: 17289871]
165. Potti A, Nevins JR. Utilization of genomic signatures to direct use of primary chemotherapy. *Curr Opin Genet Dev*. 2008;10.1016/j.gde.2008.01.018.[published online ahead of print March 11, 2008]
166. Lin WM, Baker AC, Beroukhi R, et al. Modeling genomic diversity and tumor dependency in malignant melanoma. *Cancer Res* 2008;68(3):664–673. [PubMed: 18245465]
167. Trent JM. Cytogenetics of human malignant melanoma. *Cancer Metastasis Rev* 1991;10(2):103–113. [PubMed: 1873851]
168. Mertens F, Johansson B, Hoglund M, Mitelman F. Chromosomal imbalance maps of malignant solid tumors: a cytogenetic survey of 3185 neoplasms. *Cancer Res* 1997;57(13):2765–2780. [PubMed: 9205089]
169. Hoglund M, Gisselsson D, Hansen GB, et al. Dissecting karyotypic patterns in malignant melanomas: temporal clustering of losses and gains in melanoma karyotypic evolution. *Int J Cancer* 2004;108(1):57–65. [PubMed: 14618616]
170. Bastian BC. Integrating genotypes and phenotypes of melanocytic neoplasia [abstract]. *Pigment Cell Res* 2007;20(6):513. [PubMed: 17935493]
171. Eskelin S, Pyrhonen S, Summanen P, Hahka-Kemppinen M, Kivela T. Tumor doubling times in metastatic malignant melanoma of the uvea: tumor progression before and after treatment. *Ophthalmology* 2000;107(8):1443–1449. [PubMed: 10919885]
172. Didolkar MS, Elias EG, Barber NA, Moore RH. Biologic behavior of ocular malignant melanoma and comparison with melanoma of the head and neck. *Am J Surg* 1980;140(4):522–526. [PubMed: 7425236]
173. Ehlers JP, Worley L, Onken MD, Harbour JW. Integrative genomic analysis of aneuploidy in uveal melanoma. *Clin Cancer Res* 2008;14(1):115–122. [PubMed: 18172260]
174. Tschentscher F, Prescher G, Zeschnigk M, Horsthemke B, Lohmann DR. Identification of chromosomes 3, 6, and 8 aberrations in uveal melanoma by microsatellite analysis in comparison to comparative genomic hybridization. *Cancer Genet Cytogenet* 2000;122(1):13–17. [PubMed: 11104026]
175. Cross NA, Ganesh A, Parpia M, Murray AK, Rennie IG, Sisley K. Multiple locations on chromosome 3 are the targets of specific deletions in uveal melanoma. *Eye* 2006;20(4):476–481. [PubMed: 15920570]
176. Damato B, Eleuteri A, Fisher AC, Coupland SE, Taktak AF. Artificial neural networks estimating survival probability after treatment of choroidal melanoma. *Ophthalmology*. 2008;10.1016/j.optha.2008.01.032[published online ahead of print March 13, 2008]
177. Shields CL, Ganguly A, Materin MA, et al. Chromosome 3 analysis of uveal melanoma using fine-needle aspiration biopsy at the time of plaque radiotherapy in 140 consecutive cases. *Arch Ophthalmol* 2007;125(8):1017–1024. [PubMed: 17698747]

178. Young TA, Burgess BL, Rao NP, Gorin MB, Straatsma BR. High-density genome array is superior to fluorescence in-situ hybridization analysis of monosomy 3 in choroidal melanoma fine needle aspiration biopsy. *Mol Vis* 2007;13:2328–2333. [PubMed: 18199974]
179. Goldstein AM, Stacey SN, Olafsson JH, et al. CDKN2A Mutations and melanoma risk in the Icelandic population. *J Med Genet* 2008 May;45(5):284–289. [PubMed: 18178632]Epub 2008 Jan 4
180. Brantley MA Jr, Harbour JW. Deregulation of the Rb and p53 pathways in uveal melanoma. *Am J Pathol* 2000;157(6):1795–1801. [PubMed: 11106551]
181. Brantley MA Jr, Harbour JW. Inactivation of retinoblastoma protein in uveal melanoma by phosphorylation of sites in the COOH-terminal region. *Cancer Res* 2000;60(16):4320–4323. [PubMed: 10969768]
182. Cohen Y, Goldenberg-Cohen N, Parrella P, et al. Lack of BRAF mutation in primary uveal melanoma. *Invest Ophthalmol Vis Sci* 2003;44(7):2876–2878. [PubMed: 12824225]
183. Kilic E, Bruggenwirth HT, Verbiest MM, et al. The RAS-BRAF kinase pathway is not involved in uveal melanoma. *Melanoma Res* 2004;14(3):203–205. [PubMed: 15179189]
184. Spendlove HE, Damato BE, Humphreys J, Barker KT, Hiscott PS, Houlston RS. BRAF mutations are detectable in conjunctival but not uveal melanomas. *Melanoma Res* 2004;14(6):449–452. [PubMed: 15577314]
185. Henriquez F, Janssen C, Kemp EG, Roberts F. The T1799A BRAF mutation is present in iris melanoma. *Invest Ophthalmol Vis Sci* 2007;48(11):4897–4900. [PubMed: 17962436]
186. Gear H, Williams H, Kemp EG, Roberts F. BRAF mutations in conjunctival melanoma. *Invest Ophthalmol Vis Sci* 2004;45(8):2484–2488. [PubMed: 15277467]
187. Goldenberg-Cohen N, Cohen Y, Rosenbaum E, et al. T1799A BRAF mutations in conjunctival melanocytic lesions. *Invest Ophthalmol Vis Sci* 2005;46(9):3027–3030. [PubMed: 16123397]
188. Onken MD, Worley LA, Ehlers JP, Harbour JW. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. *Cancer Res* 2004;64(20):7205–7209. [PubMed: 15492234]
189. Tschentscher F, Husing J, Holter T, et al. Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities. *Cancer Res* 2003;63(10):2578–2584. [PubMed: 12750282]
190. Onken MD, Lin AY, Worley LA, Folberg R, Harbour JW. Association between microarray gene expression signature and extravascular matrix patterns in primary uveal melanomas. *Am J Ophthalmol* 2005;140(4):748–749. [PubMed: 16226537]
191. Onken MD, Ehlers JP, Worley LA, Makita J, Yokota Y, Harbour JW. Functional gene expression analysis uncovers phenotypic switch in aggressive uveal melanomas. *Cancer Res* 2006;66(9):4602–4609. [PubMed: 16651410]
192. Bastian, B. Frequent somatic mutations of GNAQ in uveal melanoma and intradermal melanocytic proliferations. *Proceedings of the Annual Meeting of the American Association for Cancer Research*; San Diego, CA. April 12–16, 2008; [abstract]
193. Van Raamsdonk CD, Fitch KR, Fuchs H, de Angelis MH, Barsh GS. Effects of G-protein mutations on skin color. *Nat Genet* 2004 Sep;36(9):961–968. [PubMed: 15322542]Epub 2004 Aug 22
194. Fuchs E. Scratching the surface of skin development. *Nature* 2007;445(7130):834–842. [PubMed: 17314969]
195. Harrison DE, Lerner CP. Most primitive hematopoietic stem cells are stimulated to cycle rapidly after treatment with 5-fluorouracil. *Blood* 1991;78(5):1237–1240. [PubMed: 1878591]
196. Zhou S, Schuetz JD, Bunting KD, et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med* 2001;7(9):1028–1034. [PubMed: 11533706]
197. Peters R, Leyvraz S, Perey L. Apoptotic regulation in primitive hematopoietic precursors. *Blood* 1998;92(6):2041–2052. [PubMed: 9731062]
198. Molofsky AV, Pardal R, Morrison SJ. Diverse mechanisms regulate stem cell self-renewal. *Curr Opin Cell Biol* 2004;16(6):700–707. [PubMed: 15530784]
199. Scadden DT. The stem-cell niche as an entity of action. *Nature* 2006;441(7097):1075–1079. [PubMed: 16810242]

200. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997;3(7):730–737. [PubMed: 9212098]
201. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells [published correction appears in *Proc Natl Acad Sci U S A*. 2003;100(7):6890]. *Proc Natl Acad Sci U S A* 2003 Apr 1;100(7):3983–3988. [PubMed: 12629218] Epub 2003 Mar 10
202. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res* 2003;63(18):5821–5828. [PubMed: 14522905]
203. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007 Jan 4;445(7123):106–110. [PubMed: 17122772] Epub 2006 Nov 19
204. Schatton T, Murphy GF, Frank NY, et al. Identification of cells initiating human melanomas. *Nature* 2008;451(7176):345–349. [PubMed: 18202660]
205. Wang Y, Armstrong SA. Cancer: inappropriate expression of stem cell programs? *Cell Stem Cell* 2008;2(4):297–299. [PubMed: 18397746]
206. Hendrix MJ, Seftor EA, Hess AR, Seftor RE. Molecular plasticity of human melanoma cells. *Oncogene* 2003;22(20):3070–3075. [PubMed: 12789282]
207. Fang D, Nguyen TK, Leishear K, et al. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 2005;65(20):9328–9337. [PubMed: 16230395]
208. Balint K, Xiao M, Pinnix CC, et al. Activation of Notch1 signaling is required for β -catenin-mediated human primary melanoma progression. *J Clin Invest* 2005 Nov;115(11):3166–3176. [PubMed: 16239965] Epub 2005 Oct 20
209. Weeraratna AT, Jiang Y, Hostetter G, et al. Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell* 2002;1(3):279–288. [PubMed: 12086864]
210. Klein WM, Wu BP, Zhao S, Wu H, Klein-Szanto AJ, Tahan SR. Increased expression of stem cell markers in malignant melanoma. *Mod Pathol* 2007 Jan;20(1):102–107. [PubMed: 17143262] Epub 2006 Nov 24
211. Rothhammer T, Poser I, Soncin F, Bataille F, Moser M, Bosserhoff AK. Bone morphogenic proteins are overexpressed in malignant melanoma and promote cell invasion and migration. *Cancer Res* 2005;65(2):448–456. [PubMed: 15695386]
212. Dou J, Pan M, Wen P, et al. Isolation and identification of cancer stem-like cells from murine melanoma cell lines. *Cell Mol Immunol* 2007;4(6):467–472. [PubMed: 18163959]
213. Bittner M, Meltzer P, Chen Y, et al. Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature* 2000;406(6795):536–540. [PubMed: 10952317]
214. Chen KG, Szakacs G, Annereau JP, et al. Principal expression of two mRNA isoforms (ABCB⁵ α and ABCB⁵ β) of the ATP-binding cassette transporter gene ABCB 5 in melanoma cells and melanocytes. *Pigment Cell Res* 2005;18(2):102–112. [PubMed: 15760339]
215. Frank NY, Margaryan A, Huang Y, et al. ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma. *Cancer Res* 2005;65(10):4320–4333. [PubMed: 15899824]
216. Keshet GI, Goldstein I, Itzhaki O, et al. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008 Apr 18;368(4):930–936. [PubMed: 18279661] Epub 2008 Feb 3
217. Von Hoff DD. There are no bad anticancer agents, only bad clinical trial designs—twenty-first Richard and Hinda Rosenthal Foundation Award Lecture. *Clin Cancer Res* 1998;4(5):1079–1086. [PubMed: 9607564]
218. Chudnovsky Y, Khavari PA, Adams AE. Melanoma genetics and the development of rational therapeutics. *J Clin Invest* 2005;115(4):813–824. [PubMed: 15841168]
219. Potti A, Dressman HK, Bild A, et al. Genomic signatures to guide the use of chemotherapeutics [published correction appears in *Nat Med*. 2007;13(11):1388]. *Nat Med* 2006 Nov;12(11):1294–1300. [PubMed: 17057710] Epub 2006 Oct 22
220. Györfy B, Surowiak P, Kiesslich O, et al. Gene expression profiling of 30 cancer cell lines predicts resistance towards 11 anticancer drugs at clinically achieved concentrations. *Int J Cancer* 2006;118(7):1699–1712. [PubMed: 16217747]

221. Staunton JE, Slonim DK, Collier HA, et al. Chemosensitivity prediction by transcriptional profiling. *Proc Natl Acad Sci U S A* 2001;98(19):10787–10792. [PubMed: 11553813]
222. Bonnefoi H, Potti A, Delorenzi M, et al. Validation of gene signatures that predict the response of breast cancer to neoadjuvant chemotherapy: a substudy of the EORTC 10994/BIG 00–01 clinical trial. *Lancet Oncol* 2007 Dec;8(12):1071–1078. [PubMed: 18024211]Epub 2007 Nov 19
223. Wang E, Miller LD, Ohnmacht GA, et al. Prospective molecular profiling of melanoma metastases suggests classifiers of immune responsiveness. *Cancer Res* 2002;62(13):3581–3586. [PubMed: 12097256]
224. Marincola FM, Wang E, Herlyn M, Seliger B, Ferrone S. Tumors as elusive targets of T-cell-based active immunotherapy. *Trends Immunol* 2003;24(6):335–342. [PubMed: 12810110]
225. Amundson SA, Do KT, Vinikoor LC, et al. Integrating global gene expression and radiation survival parameters across the 60 cell lines of the National Cancer Institute Anticancer Drug Screen. *Cancer Res* 2008;68(2):415–424. [PubMed: 18199535]
226. Smalley KS, Haass NK, Brafford PA, Lioni M, Flaherty KT, Herlyn M. Multiple signaling pathways must be targeted to overcome drug resistance in cell lines derived from melanoma metastases. *Mol Cancer Ther* 2006;5(5):1136–1144. [PubMed: 16731745]
227. Brafford P, Herlyn M. Gene expression profiling of melanoma cells—searching the haystack [editorial]. *J Transl Med* 2005;3(1):2. [PubMed: 15649323]
228. Hoek KS. DNA microarray analyses of melanoma gene expression: a decade in the mines. *Pigment Cell Res* 2007;20(6):466–484. [PubMed: 17935490]

FIGURE 1. The *CDKN2A* locus and cell cycle control

The *CDKN2A* locus on chromosome 9p21 has an unusual structure because it encodes for 2 overlapping but very distinct proteins: p16^{INK4A} and p14^{ARF}. This is accomplished through selective use of an alternative first exon (exon E1a in p16^{INK4A} and exon E1b in p14^{ARF}). Although structurally very different, both protein products act as negative regulators of cell cycle progression. The p16^{INK4A} protein inhibits the activation of CDK4 and CDK6 by cyclin D1 (CCND1), thereby preventing the subsequent phosphorylation of RB1. Underphosphorylated RB1 sequesters the transcription factor E2F and prevents it from inducing the progression from G1 to S phase of the cell cycle. The absence of functional p16^{INK4A}, therefore, leads to hyperphosphorylation of RB1 with resulting release of E2F and

uninhibited cell cycle progression. In contrast, p14^{ARF} regulates tumor protein 53 (p53) activity by inhibiting MDM2, a ubiquitin ligase that otherwise targets p53 for degradation by proteasome. High levels of p14^{ARF} stabilize p53, permitting it to induce p21^{WAF1/CIP1}, a cell cycle inhibitor that blocks CDK2/cyclin E (CCNE1)–mediated phosphorylation of RB1. In the absence of functional p14^{ARF}, uncontrolled ubiquitination and degradation of p53 removes this important cell cycle brake, leading ultimately to hyperphosphorylation of RB1 and cell cycle progression. P = phosphate. For expansion of other abbreviations, see Glossary on page 846.

FIGURE 2. The MAPK and PI3K/AKT pathways

Activation of various receptor tyrosine kinases (VEGFR, C-KIT, C-MET, FGFR, IGF1R, and others) by their cognate ligands (VEGF, SCF, HGF, FGF, and IGF1/2, respectively) leads to proliferation and survival of melanoma cells through two interacting signal transduction pathways. Ligand availability through autocrine loops (eg, VEGF), paracrine secretion (eg, IGF1), as well as IGF1R can modulate activation of these pathways. Within the MAPK pathway, activated receptors lead to SHC-mediated activation of RAS and propagation of signaling through RAF, MEK (also known as MAP2K), and MAPK (also known as ERK). Activated MAPK transduces signals that regulate multiple cell processes including proliferation, differentiation, angiogenesis, and survival. Isolated activation of the MAPK

pathway by oncogenic stimuli, such as mutated *BRAF*, results in expression and secretion of IGFBP7, which, in turn, suppresses MAPK activity and induces senescence, as seen in benign nevi. Melanomas seem to circumvent this negative feedback loop by blocking the expression of IGFBP7 through yet unidentified mechanisms. The second essential signaling pathway stimulated by receptor tyrosine kinases is the PI3K/AKT pathway. PI3K, a lipid kinase, catalyzes the phosphorylation of phosphatidylinositol (PI). Triple phosphorylation of PI is necessary for the association of AKT with the cell membrane and its subsequent activation by PDK1. The key regulatory component is PTEN, a phosphatase that regulates PI3K and SHC phosphorylation. RAS may also regulate PI3K activity. Through activated AKT, a number of key mitogenic processes are activated including inhibition of apoptosis, survival gene transcription, cell cycle progression, protein translation, and cell growth and proliferation. The balance between AKT activation and PTEN regulatory activity is a key determinant in cell cycle progression. FGF = fibroblast growth factor; HGF = hepatocyte growth factor; IGF = insulinlike growth factor; P = phosphate; VEGF = vascular endothelial growth factor; ? = mechanism unknown. For expansion of other abbreviations, see Glossary on page 846.

FIGURE 3. Apoptosis pathway

Apoptotic cell death can be initiated through two main pathways: the death receptor (extrinsic) pathway and the mitochondrial (intrinsic) pathway. The death receptor pathway is activated by the binding of FAS or TRAIL ligands to their receptors (DR4 or DR5), stimulating receptor aggregation. This aggregation stimulates recruitment of FADD and caspase (casp) 8 activation, which is regulated by cFLIP (also known as CFLAR) and growth factor signaling through the MAPK pathway. Casp 8 activation leads to casp 3 cleavage, which initiates multiple proapoptotic processes, including CAD stimulation of DNA cleavage. Through the mitochondrial pathway, prosurvival signaling through AKT activation stimulates phosphorylation of BAD, which allows BCL2 to exert its antiapoptotic effects by blocking proapoptotic proteins NOXA and BAX. However, dephosphorylated BAD blocks BCL2 by heterodimerization (BAD/BCL2), which allows proapoptotic proteins to form pores in the mitochondria. This process then releases apoptogenic factors from the mitochondrial intermembrane space, including cytochrome C (Cyt C), APAF1, and caspase 9. These factors form the so-called apoptosome, which stimulates apoptosis through caspase 3 cleavage. Recent data have suggested that the proteasome regulates the activity of the proapoptotic protein, NOXA, as proteasomal inhibition with bortezomib induces NOXA up-regulation and apoptosis in

melanoma cell lines. P = phosphate. For expansion of other abbreviations, see Glossary on page 846.

FIGURE 4. MITF signaling

MITF plays the important role of a master regulator of melanocyte-specific transcription. Several pathways affect the level of MITF expression by acting on the MITF promoter. Endothelin signaling is currently the least well characterized. Better understood are the WNT and α -MSH pathways, which independently are capable of increasing MITF transcriptional activity. Specifically, α -MSH binds to the G protein–coupled MC1R, which acts through adenylate cyclase to stimulate binding of CREB1 to the MITF promoter. The activity of MITF protein is affected by several growth factors including C-KIT (also known as KIT) and hepatocyte growth factor (HGF), which stimulate MAPK-mediated MITF phosphorylation. In the phosphorylated state, MITF has higher transcriptional activity. Both increased expression of MITF and its activation by phosphorylation stimulate the transcription of MITF target genes, including those involved in melanin production (*TYR*, *TRP1*, and *DCT*), genes important for survival (*BCL2*), and numerous markers important in melanoma (*MLANA*, *TRPM1*, and

SILV). **E cadherin and β -catenin signaling.** β -Catenin functions at the cell membrane forming a link between E cadherin and the actin cytoskeleton as well as in the cytoplasm where it participates in WNT signaling. The WNT acts through the G protein-coupled receptor Frizzled to suppress GSK3B, leading to decreased β -catenin phosphorylation, which ordinarily leads to β -catenin degradation. Such WNT signaling thus increases β -catenin levels, which causes β -catenin translocation to the nucleus, where it interacts with members of the TCF/LEF family and activates transcription of target genes, including MITF. P = phosphate; ? = mechanism unknown. For expansion of other abbreviations, see Glossary on page 846.

FIGURE 5. Stem cells in melanoma

Melanoma stem cells, also known as tumor-initiating cells, are thought to be a subpopulation of tumor cells that display many attributes characteristic of normal tissue stem cells. This cellular population grows slowly, is capable of self-renewal, and is relatively resistant to drugs and toxic treatments. These attributes have potentially critical implications for cancer therapy. Current therapies, which aim to interfere with rapid cell proliferation, would likely be ineffective at destroying the melanoma stem cell compartment. As illustrated in the Figure, classic chemotherapy treatment, when successful, would primarily target the bulk of the tumor composed of rapidly proliferating daughter cells but would not eradicate slowly cycling, drug-

resistant melanoma stem cells. Subsequent repopulation of the rapidly proliferating daughter cells thus leads to renewed tumor growth with clinical melanoma relapse.