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New Horizons in Melanoma Treatment: Targeting Molecular Pathways

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INTRODUCTION

Melanoma is the deadliest type of skin cancer. Over the past several decades, the incidence of melanoma has been increasing rapidly, especially in the southern and western United States (the so-called Sun Belt) and in subtropical countries such as Australia settled by fair-skinned peoples.[1] In 2009, it was estimated that 60,000 patients would be diagnosed as having melanoma and 7,000 would die of this disease in the United States.[2] Melanoma arises from transformed melanocytes, long-lived pigment-producing cells that reside within the basal epidermis. Melanocyte progenitors arise from the neural crest and migrate into the basal epidermis during embryonic development guided by c-KIT/stem cell factor signaling. Over the past few years, significant advances in our understanding of melanocyte biology and the role of protein kinases in melanoma have given rise to a wealth of therapeutic targets that are now in preclinical or clinical evaluation. These targets are different from the more traditional immunologic manipulations and cytotoxic chemotherapy that have been used to treat melanoma over the past 4 decades.[3]

THE MOLECULAR PATHOGENESIS OF MELANOMA

Melanoma develops through the complex interaction of molecular pathways controlling cell proliferation, senescence, and apoptosis.[4] The RAS-RAF-MAP kinase pathway seems to be the major signaling cascade involved in cell growth and proliferation in melanocytes. Growth factor receptors activate RAS, which in turn activates the downstream kinases RAF, MEK, and ERK (MAP kinase), leading to gene transcription and cellular proliferation.[5] In melanoma, mutations in *RAS* occur almost exclusively in *NRAS*.[6] These occur in approximately 20% of melanomas that develop on skin without evidence of sun-induced damage.[7] Mutations in *BRAF* are common in melanoma and occur in 66% of cases.[8] In addition, RAS activates phosphoinositide-3 kinase (PI3K), which leads to the formation of the second messenger PIP3, leading to the activation of the protein AKT.[9] The activation of AKT stimulates cell cycle progression, survival, metabolism, and migration through the phosphorylation of various downstream targets.[10] *PTEN* is an important tumor suppressor that has a role in regulating the PI3K signaling cascade.[11] PTEN dephosphorylates PIP3 and blocks the activation of AKT, suppressing signaling from growth factors. Activation of the PI3K pathway in melanoma is evidenced by the increasing expression of AKT as normal nevi progress to severely dysplastic nevi and melanoma.[12] Most commonly, the aberrations in this pathway are the result of alterations in PTEN, and mutations or deletions of *PTEN* occur in about one-half of melanomas, while epigenetic silencing is found in a smaller proportion.[13–16] Mutations in *AKT* have also been found in a small proportion of melanoma cell lines,[17] and increased expression of activated AKT is associated with tumor progression and shorter survival.[12,18]

CDKN2A is an important gene involved in melanoma pathogenesis. By alternative splicing, it encodes for 2 different proteins (INK4A and ARF).[19] Through their interaction with cyclin D, cyclin-dependent kinases (CDKs) 4 and 6 inactivate the retinoblastoma (Rb) protein. Active hypophosphorylated Rb inhibits the growth factor E2F, blocking the expression of genes required for the progression of the cell from the G1 phase to the S1 phase. INK4A inhibits CDK4/6. Therefore, inhibition of INK4A allows cyclin D–CDK4/6 to inactivate Rb, leading to increased cell cycle activity. The second protein encoded by *CDKN2A* is ARF, which inhibits the mouse double minute 2 (MDM2) protein. In response to DNA damage, MDM2 inactivates and leads to the degradation of the p53 protein. This protein is important in the activation of DNA repair, cell cycle arrest, and apoptosis. Therefore,

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in the absence of ARF, p53 levels are decreased, and the cellular response to DNA damage is blunted. In North America, germline mutations in *CDKN2A* occur in 45% of families that are prone to melanoma, and it was calculated that 67% of mutation carriers will develop melanoma by age 80 years.[20] *CDK4* is mutated in a small proportion of familial melanoma among families lacking a mutation in *CDKN2A*.[21] *CDKN2A* is the most commonly deleted gene in nonfamilial primary melanoma, and cyclin D and CDK4 are amplified in specific subsets of melanoma.[7]

The microphthalmia-associated transcription factor (MITF) is a key controller of the melanocyte lineage and seems to have an important role in melanoma.[22] Melanocortin (α-MSH) acts on its receptor, MC1R (a G protein–coupled receptor), leading to G protein–mediated activation of adenylate cyclase. This promotes the intracellular formation of cyclic adenosine monophosphate, leading to MITF transcription. In turn, MITF activates the transcription of proteins that are important in melanin production. A large-scale search for genomic changes in melanoma through single-nucleotide polymorphism analysis found that MITF amplification was more prevalent in metastatic disease and was correlated with decreased patient survival.[23] Inducing overexpression of MITF with mutant BRAF led to the transformation of human melanocytes. Inducing interference of MITF function in vitro led to increased chemosensitivity to these melanoma cell lines, suggesting that MITF is a potential target for treatment.

MUTATIONS BASED ON LOCATION OF PRIMARY MELANOMA

A critical insight into melanoma biology by Curtin and colleagues[7] showed that melanomas tend to have specific mutations based on the site of the primary tumor. They found that mutations in *RAF* and *RAS* seemed to be mutually exclusive. Mutations in *BRAF* were significantly more common in melanomas that were on skin without chronic sun-induced damage (assessed by the degree of solar elastosis on histopathologic examination). Most melanomas on skin without chronic sun-induced damage had mutations in *BRAF*. On the other hand, melanomas arising in chronically sun-damaged skin, mucosal surfaces, and acral skin had wild-type *BRAF* and *RAS*. This suggests that patients with melanoma that develops on areas only intermittently exposed to the sun may have an increased susceptibility to UV exposure, leading to a higher probably of acquiring *BRAF* mutations. On the other hand, patients who develop melanomas on chronically sun-exposed areas may have melanocytes that are less susceptible to mutations from UV light, requiring higher cumulative doses to induce melanoma. Curtin and colleagues went on to identify *KIT* (an essential gene for melanocyte survival and development)[24,25] as an important oncogene in melanomas on chronically sun-damaged skin, the mucosa, and acral skin.[26] Aberrations in *KIT* (mutations or copy number increases) were identified in 39% of mucosal melanomas, 36% of acral melanomas, 28% of melanomas on skin with chronic sun-induced damage, and 0% of melanomas on skin without chronic sun-induced damage.

Uveal melanomas arise from melanocytes in the uveal tract, including the iris, ciliary body, and choroid. Although accounting for only 5% of melanomas, uveal melanoma is an aggressive malignant neoplasm with a 5-year disease-specific survival rate of approximately 75%.[27] Clinically, differences in behavior of uveal melanoma compared with cutaneous melanoma are evident. Unlike cutaneous melanoma, in which the most common site of distant metastases is the lung, the liver is the most commonly involved organ in uveal melanoma.[28] Systemic treatments for metastatic uveal melanoma are ineffective, and the median survival is less than 6 months.[29] Frequent somatic mutations in *GNAQ*, a gene encoding a G protein α subunit, have been demonstrated in uveal melanomas.[30] None of these showed mutations in *BRAF* or *NRAS*. Our colleague Boris Bastian (personal communication, November 2009) identified *GNA11*, a second gene encoding another G protein α subunit, as frequently mutated in uveal melanoma and occurring mutually exclusively of *GNAQ* mutations. Altogether, mutations in *GNAQ* or *GNA11* seem to occur in approximately 80% of uveal melanomas. The G protein–coupled receptors represent the largest family of cell surface molecules involved in signal transduction and have an important role in cell regulation, including proliferation, survival, and motility.[31] *GNAQ* and *GNA11* represent potential targets for uveal melanoma therapy.

THERAPIES TARGETING MOLECULAR SIGNALING

The management of metastatic melanoma is challenging, and few patients experience durable responses to systemic treatment.[32] The poor efficacy of available treatments has spurred the search for new modalities. Many of the therapies under development are based on a new understanding of the molecular pathways that govern melanoma pathogenesis and target specific steps in these pathways.

RAF

Of the RAF proteins, only BRAF is commonly mutated in cancer, including melanoma. More than 90% of mutations in *BRAF* result in a substitution of

valine in place of glumatic acid in amino acid position 600, resulting in the BRAF V600E mutant kinase.[8] This mutation confers increased kinase activity and can lead to melanocyte proliferation and senescence.[33] Surprisingly, the BRAF V600E mutation is also commonly found in benign nevi.[34] Overexpression of BRAF in mice leads to widespread melanocytic hyperplasia and progression to melanoma only on further loss of *CDKN2A*[35] or *PTEN*.[36] Over the past decade, several RAF inhibitors have been developed in the expectation of targeting this kinase in the treatment of melanoma. Sorafenib is a potent inhibitor of CRAF and to a lesser extent BRAF.[37] In addition, sorafenib inhibits the vascular endothelial growth factor receptor (VEGFR) and KIT. A phase II trial of sorafenib alone showed little or no efficacy in patients with advanced unresectable or metastatic melanoma.[38] Trials of sorafenib in combination with other agents have been likewise disappointing.[39,40] This may result from weak BRAF inhibition by sorafenib at clinically achievable concentrations. PLX-4032 is a selective inhibitor of activated BRAF that translates to greatly increased selectivity for the BRAF V600E mutant kinase. This drug has shown great promise in a recent phase I trial, with 5 of 7 patients having melanoma who were positive for the mutation displaying tumor regression.[41] A phase II trial of PLX-4032 in previously treated patients has completed accrual,[42] and a randomized phase III trial comparing PLX-4032 with dacarbazine as a first-line treatment is recruiting patients.[43]

MEK

MEK is a downstream target of RAF in its signaling cascade, and there have been attempts to target this kinase in the treatment of melanoma. A phase I trial with the MEK inhibitor AZD6244 showed some promise in melanoma, with 6 of 11 patients showing tumor shrinkage.[44] A subsequent phase II trial showed no benefit compared with temozolomide, a conventional first-line oral alkylating agent.[45] In addition, there was no benefit in the *BRAF* mutant subgroup. However, of 6 patients with partial responses to the MEK inhibitor, 5 had a mutation in *BRAF*. In contrast, of 9 patients with partial responses to temozolomide, only 3 had *BRAF* mutations. This may suggest different efficacies in melanomas with different oncogenic alterations. Newer MEK inhibitors such as GSK1120212 (undergoing phase II evaluation)[46] in BRAF mutant melanoma are also showing great promise.

Mammalian Target of Rapamycin

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that is regulated by nutrients and growth factors and functions in the control of protein translation, ribosome formation, autophagy, and metabolism.[47] mTOR is thought to have a role in AKT activation, and increased activation of mTOR was found in 73% of melanoma cell lines.[48] mTOR can be inhibited through its rapamycin binding domain, and several rapamycin analogues are in clinical trials. Sirolimus (CC1-779) has been tested in a melanoma phase II clinical trial with disappointing results.[49] BEZ23, a molecule with dual mTOR and PI3K inhibition, has shown greater activity in preclinical melanoma models and is in phase I testing.[50]

KIT

Imatinib was the earliest *KIT* inhibitor tested in clinical trials. Two different phase II trials of imatinib yielded no treatment responses.[51,52] Neither of these trials screened for mutations in *KIT* as part of the study inclusion protocol. The tumors in most of these patients showed minimal or no expression of KIT by immunohistochemistry. In a third more recent trial, the single partial responder who was treated with imatinib also happened to have the highest expression of KIT among all patients.[53] Although a mutation in *KIT* was not found, the patient's primary melanoma was located on an acral surface, an area with frequent aberrations in KIT, including copy number increases. In 2 case reports, patients having metastatic melanoma with *KIT* mutations displayed dramatic responses to imatanib.[54,55] A phase II trial of imatinib in patients screened for mutations or amplifications in *KIT* is ongoing.[56] At the time of interim report, of 12 patients who received treatment (all with mutations in *KIT*), 2 had complete responses, 2 had partial responses, and 6 had stable disease, with disease progressing in only 2 patients. These encouraging data suggest that therapies may be ineffective in unselected patients but may be active in specific subsets of patients.

Epidermal Growth Factor Receptor Family

The epidermal growth factor receptor (EGFR) family consists of the following 4 receptors: EGFR (ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4).[57] Several different ligands (including EGF-like molecules, transforming growth factor α, and neuregulins) activate the receptors, which then relay signals to the MAPK and PI3K pathways. ErbB1 is expressed in up to 96% of primary melanomas and in 90% of metastatic tumors.[58] There are gains in chromosome 7 (where *EGFR* resides) in about 50% of melanomas, and increased copy number of chromosome 7 has been associated with poor prognosis in some studies.[59,60] ErbB3 is also frequently expressed in melanoma and has been associated with tumor progression and a worse

prognosis.[61–63] Evidence of the importance of EGFR signaling has been seen in melanoma cell lines[61] and in animal models.[64] A screen for somatic mutations in *ErbB4* revealed that 19% of metastatic tumors harbored this mutation.[65]

The ErbB1 inhibitor erlotinib hydrochloride has been evaluated in a phase II trial of metastatic melanoma and showed no objective responses, but 4 of 14 patients had stable disease.[66] The ErbB1/B2 inhibitor gefitinib was tested, and only 2 of 50 evaluable patients had a partial response.[67] A trial of erlotinib hydrochloride in combination with the vascular endothelial growth factor A inhibitor bevacizumab showed greater efficacy, with 2 of 23 patients having partial responses lasting less than 6 months and 5 patients having stable disease lasting greater than 6 months.[68] Toxic effects were greater with this combination, with 1 patient each experiencing myocardial infarction and bowel perforation.

MET

MET is a receptor tyrosine kinase that is activated by its ligand hepatocyte growth factor (HGF). MET is essential for normal development and is important in cell migration, growth, survival, differentiation, and angiogenesis.[69] In normal skin, MET is expressed on epithelial cells and melanocytes, whereas HGF is produced mainly by mesenchymal cells and interacts with MET in a paracrine manner.[70] c-MET overexpression correlates with the invasive growth of melanoma cells, and many melanomas also secrete HGF, which can induce sustained activation of MET in an autocrine fashion.[71] The c-MET inhibitor XL184 is in a phase II trial among patients with various solid tumors, including melanoma.[72] XL880, a second MET inhibitor that also inhibits VEGFR2, has shown activity in patients with melanoma.[73]

CONCLUSION

Many exciting developments in melanoma therapy are underway. The realization that dysregulated protein kinases have a critical role in melanoma development and that inhibition of these can drastically increase the effectiveness of melanoma therapy is perhaps the greatest recent advance in melanoma research. Because distinct subtypes of melanoma show distinct patterns of kinase alterations, the first step in the emerging world of melanoma therapy is to accurately genotype a specimen, preferably from a metastatic lesion. Once the subtype of melanoma has been determined, BRAF or MEK inhibition may be the optimal choice for patients with BRAF V600E mutations. Those with *NRAS* mutations may benefit from MEK inhibitors, as may those with *GNAQ* and *GNA11* mutations. Patients with *KIT* mutations are probably best treated with KIT inhibitors. The observed brief responses to the kinase inhibitors suggest that combinations of inhibitors—either vertically (such as BRAF plus MEK), horizontally (BRAF plus AKT), or in combination with chemotherapy—need to be investigated. For example, in vitro data suggest that resistance to *BRAF* inhibitors may develop as a result of the emergence of downstream mutations in *MEK*.[74] Combined exposure to both a BRAF inhibitor and a MEK inhibitor suppressed the emergence of resistant colonies. Also, the role of VEGF inhibitors and CDK inhibitors in combination with MAP kinase pathway inhibitors needs to be evaluated and defined.

Questions remain regarding melanomagenesis in the approximately 40% of patients without known mutations in the known protein kinase signaling cascades, but research is under way in an attempt to identify kinase mutations in these patients as well. Although these developments have engendered great expectation in the melanoma community, the long-term outcome and safety profile of these drugs have not been demonstrated. For example, PLX4032 has been associated with secondary nonmelanoma skin cancers. In addition, notable advances in gene therapy, activated lymphocyte infusions, and novel immunomodulating monoclonal antibodies have brightened the horizon for melanoma therapy. It is anticipated that melanoma therapy in the future will be substantially more successful than it has been to date.

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