Genetic Subgrouping of Melanoma Reveals New Opportunities for Targeted Therapy

Keiran S.M. Smalley,1 Katherine L. Nathanson,2,4 and Keith T. Flaherty3,4

1Molecular Oncology Program and Department of Cutaneous Oncology, The Moffitt Cancer Center & Research Institute, Tampa, Florida; and Divisions of Medical Genetics and Hematology-Oncology, Department of Medicine, and 2The Abramson Cancer Center, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

Abstract

The discovery of activating oncogenic BRAF V600E mutations in the majority of melanomas has not yet been translated into more effective therapy. The failure of agents may be due to lack of sufficiently targeted therapies, but is more likely based on the activation of multiple oncogenic pathways in melanomas in addition to the mitogen-activated protein kinase signaling pathway. In contrast, there are groups of melanomas that instead rely on either c-KIT or CRAF signaling that may be amenable to single-agent targeted therapy. In the current review, we discuss how knowledge about these new melanoma subgroups may lead to improved strategies for treating melanomas harboring BRAF V600E mutations.

Background

After decades of negative clinical trials, the new hope for melanoma treatment is targeted therapy, using small-molecule inhibitors directed against the oncogenic mutations responsible for driving tumor progression. Although extensive preclinical studies have validated the BRAF V600E mutation and the downstream mitogen activated protein kinase (MAPK) pathway as excellent therapeutic targets in the majority of melanomas (1, 2), the initial clinical trials of inhibitors of BRAF or MAPK/ extracellular signal-regulated kinase kinase (MEK) have not yet improved treatment for melanoma (3, 4). In part, the lack of response may be due to the nonspecific BRAF inhibitors studied thus far, but it is likely that even they alone will not substantially improve outcome. The reasons for the limitations of single-agent BRAF and MEK inhibitors in melanoma are complex. It is known that metastatic melanomas have signaling activity in many other pathways, such as phospho-inositide-3-kinase (PI3K)/AKT and mammalian target of rapamycin (mTOR; refs. 5, 6), which likely provide compensatory survival signals when BRAF or MEK is inhibited (7). There are also instances where melanomas show amplification or overexpression of key cell cycle and survival pathway components that may limit the effectiveness of molecularly targeted therapies (8–11). Attempts are now under way to molecularly subgroup melanomas according to their patterns of oncogenic mutation and signaling pathway activity, with the hope of matching these with specific therapies. The current review discusses two subgroups of melanomas, those that rely on c-KIT signaling and those that rely on CRAF signaling, as a proof of concept for melanoma targeted therapy. We suggest that a greater understanding of these two subgroups, in which one oncogenic event drives both proliferation and survival, can point to novel strategies for treating BRAF V600E mutated melanomas.

Key Findings

Inhibition of c-KIT modulates both growth and survival. Although a role for c-KIT signaling in melanoma has been long disputed (12, 13), interest in this receptor tyrosine kinase (RTK) was recently rekindled following the identification of subgroups of melanoma harboring activating KIT mutations (14). Melanomas that develop on body sites with little UV exposure, such as the soles of the feet or subungual sites (acral melanomas), or on mucous membranes (mucosal melanomas) have a very low incidence of BRAF mutations, but often harbor activating mutations in KIT (14–16). Within these subgroups of melanoma there also are instances in which c-KIT is amplified in the absence of a mutation, so that the total number of c-KIT aberrations is 39% for mucosal, 36% for acral, and 28% for sun-damaged skin melanomas (14). c-KIT has since been shown to be expressed in 88% of oral mucosal melanomas, with 22% of these lesions harboring KIT mutations (16). Other studies showed that 62% of acral and mucosal melanomas exhibited constitutive c-KIT receptor activity, and that 15% of anal melanomas harbored activating KIT mutations (15, 17).

Initial clinical studies on the c-KIT inhibitor imatinib in groups of melanoma patients unselected for c-KIT status were negative (18). The validity of c-KIT as a therapeutic target in melanoma was recently shown by two clinical case reports, in which melanoma patients with activating mutations in c-KIT showed remarkable responses to imatinib (19, 20). It is currently unclear whether overexpression of c-KIT or the presence of an activating KIT mutation predicts response to imatinib. There are cases where high expression of c-KIT, in the absence of an activating mutation, leads to constitutive signaling activity. We recently identified a group of melanoma cell lines that exhibit high levels of c-KIT receptor expression, lack KIT mutations, and respond to imatinib in three-dimensional organotypic cell cultures and xenograft models (21). These results were subsequently confirmed in other melanoma cell lines harboring either an activating KIT mutation or an SCF/c-KIT autocrine loop (15, 22). However, c-KIT receptor expression is not always predictive of response, as shown by the lack of imatinib response seen in melanoma cell lines that maintained expression of c-KIT in the presence of BRAF V600E mutation (23). In this instance, the melanoma cell lines lacked phospho-c-KIT expression, indicating a lack of reliance of the cell lines on c-KIT signaling for their survival. Likewise, expression of c-KIT receptor in the absence of downstream signaling activity was not predictive of imatinib response in a recent clinical trial of uveal melanoma (24).
The maximal benefit of imatinib treatment is likely to be seen in melanomas in which c-KIT activity constitutes the major signaling node involved in survival and growth. In mucosal melanoma cell lines harboring an activating c-KIT mutation, imatinib treatment led to the simultaneous inhibition of the MAPK, PI3K/AKT, and JAK/STAT signaling pathways (22). The combined suppression of these three pathways led to cell cycle arrest and apoptosis, associated with the down-regulation of Bcl-2, survivin, and Mcl-1 expression (21, 22). These marked imatinib-induced cytotoxic effects are also seen in clinical studies, wherein treatment of a c-KIT–expressing melanoma led to significant levels of apoptosis both in the tumor and in the surrounding endothelial cells (25). The studies on melanomas with KIT mutations suggest that preclinical and clinical benefit can be observed when multiple signaling pathways are simultaneously inhibited, leading to both cytostasis and cytotoxicity.

Targeting CRAF has dual effects on proliferation and survival. Although nearly all melanoma cell lines have constitutive MAPK activity, it is not always mediated through BRAF signaling. A significant group of melanomas rather have constitutive levels of MAPK activity as a consequence of activation of the closely related serine/threonine kinase CRAF (Raf-1; refs. 23, 26). Although activating mutations of CRAF are virtually unknown in melanoma, recent studies have identified at least two distinct melanoma subgroups that rely on CRAF (23, 26, 27). Unlike BRAF, which seems to signal exclusively through the MAPK pathway, CRAF has multiple other downstream targets as well, such as MST-2, ASK-1, and nuclear factor κB. In addition to localizing at the plasma membrane, CRAF also associates with the mitochondria and directly controls apoptosis through the regulation of phospho-BAD and Bcl-2 (23). This interaction is likely to be significant because high expression of Bcl-2 is known to be an important mediator of survival in melanoma (9).

Melanomas harboring activating NRAS mutations are known to rely on CRAF to induce MAPK pathway activity (26). In normal melanocytes, RTK-induced activation of Ras leads to the stimulation of both BRAF and CRAF (26). Under these conditions, activation of the MAPK pathway only proceeds via BRAF, as constitutive protein kinase A (PKA) activity leads to the phosphorylation and inactivation of CRAF. In melanomas with NRAS mutations, the cyclic AMP/PKA system is deregulated, so that PKA no longer suppresses CRAF, allowing CRAF-mediated MAPK activation to occur (26).

Although most work to date has focused on the BRAF V600E mutation, at least 70 other BRAF mutations have been identified (27). Many of these show reduced BRAF kinase activity relative to the V600E mutant form in isolated kinase assays and have been classified as “low-activity” mutants. However, when low-activity BRAF mutant proteins are transfected into COS-1 cells, they are able to activate the MAPK pathway by directly binding to CRAF, leading to its phosphorylation and transactivation (27). Thus, it is likely that CRAF is a valid therapeutic target in melanomas with low-activity BRAF mutations. A Food and Drug Administration–approved agent with selectivity for CRAF over BRAF is the kinase inhibitor sorafenib (28). Treatment of D594G and G469E mutant cell lines with either sorafenib or a shRNA directed against CRAF induced apoptosis leading to the down-regulation of Bcl-2 and phospho-BAD expression (23). In this instance, the effects of sorafenib were found to be independent of MEK inhibition because treatment of the low-activity mutant cell lines with the MEK inhibitor U0126 did not affect Bcl-2/BAD expression or induce apoptosis. Based on its roles in proliferation and apoptosis, as well as BRAF inhibitor resistance, CRAF inhibition may prove to be a suitable therapeutic target in subgroups of melanomas with activating NRAS mutations, those with low-activity mutations in BRAF, or those that become BRAF inhibitor resistant (Fig. 1; ref. 29).

The relatively low occurrence of low-activity BRAF mutant melanomas in the general patient population has made clinical testing for them difficult. Thus, the essentially negative phase II trials with single-agent sorafenib in unselected melanoma patients failed to address the potential value of this agent in low-activity BRAF mutant tumors (3). Clearly, further clinical evaluation of sorafenib in patients presenting with low-activity BRAF mutations is warranted.

Current status of therapy for BRAF V600E mutated melanomas. As a proof of concept of the importance of matching the appropriate targeted therapy with genetic subgroup, single-agent trials with c-KIT inhibitors in c-KIT–dependent melanomas and sorafenib (as a CRAF inhibitor) in low-activity BRAF mutant melanomas are now under way. However, these two subgroups may account for no more than 5% of advanced melanomas. For the vast majority of patients, strategies will need to be defined for melanomas harboring either NRAS or BRAF V600E mutations.

BRAF V600E mutated tumors make up the single largest subgroup of defined melanomas, and have been the subject of intensive preclinical and clinical study by our group and others. These tumors are also likely to be the most genetically diverse, with subgroups of BRAF V600E mutated melanomas being identified with alterations in PTEN, cyclin D1, CDK2, CDK4, MITF, and AKT3 (8, 10, 11, 30, 31). In the phase II trial investigating a selective MEK inhibitor (AZD6244) among patients with metastatic melanoma harboring V600E mutations, 12% of patients experienced significant, but incomplete, regression (4). This relatively modest activity is likely a consequence of compensatory activity from other intracellular signaling pathways and is consistent with preclinical data showing that BRAF/MEK inhibition is mostly cytostatic in V600E-mutated melanoma cells (32–34). It remains to be determined if selective BRAF inhibitors will only be active in a restricted subgroup of melanomas, as we are still conducting dose escalation trials with the first two selective BRAF inhibitors (RAF-265 and PLX4032). Melanomas with BRAF V600E mutations, which are unresponsive to BRAF/MEK inhibitors, may be sensitive to dual inhibition of the MAPK/PI3K pathways, or the MAPK/mTOR pathways, as has been shown preclinically (35–37). It is likely that the combined inhibition of these pathways will lead to the desired dual cytotoxic and cytostatic activity, similar to that observed when KIT-dependent melanomas are treated with imatinib or CRAF-dependent melanomas are treated with sorafenib. Beyond rapamycin-analogue mTOR inhibitors, potent and selective PI3K and AKT inhibitors are just now emerging from single-agent phase I trials. The availability of these drugs sets the stage for rigorous clinical testing of this hypothesis.

To maximize the probability of success, future targeted therapy trials must be conducted in patient populations prospectively analyzed for the presence of the relevant somatic genetic changes. With this new paradigm established, we are at the threshold of a new era of therapeutic discovery for melanoma.

Conclusion

While trials using targeted BRAF inhibitors are currently under way, single-agent BRAF/MEK inhibitor treatments are unlikely
to lead to long-term clinical responses. There is already preclinical evidence of emerging resistance to BRAF inhibitors following prolonged treatment (29). Efforts are ongoing to determine the optimal combination of signaling pathways to target in melanoma to induce both growth arrest and cell death. Preclinical and clinical evidence suggests that the selection of the correct molecular target, such as CRAF or c-KIT, in defined molecular subgroups of melanomas can lead to the desired cytotoxic effects.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 11/11/08; revised 1/27/09; accepted 2/1/09; published OnlineFirst 4/7/09.

Grant support: Melanoma Research Foundation and the American Cancer Society Institutional Research Grant #93-032-13 (K.S.M. Smalley), and National Institutes of Health grants R01 CA117881 and P50 CA093372 (K.L. Nathanson).

References


Genetic Subgrouping of Melanoma Reveals New Opportunities for Targeted Therapy

Keiran S.M. Smalley, Katherine L. Nathanson and Keith T. Flaherty


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-08-4305

Cited articles
This article cites 37 articles, 18 of which you can access for free at:
http://cancerres.aacrjournals.org/content/69/8/3241.full#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/69/8/3241.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/69/8/3241.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.