Systemic therapies for advanced melanoma have lagged behind treatments for most other cancers, both in the metastatic and earlier-stage, adjuvant setting. Metastatic melanoma continues to be largely refractory to available therapies, with a small percentage of patients responding to either chemotherapies or immunotherapies such as dacarbazine and interleukin-2. In the adjuvant setting, high-dose interferon remains the treatment standard. However, this therapy is not as effective at preventing recurrence as adjuvant therapies for several other cancer types, and it is associated with significant toxicity burdens.

The treatment of some other cancers has been revolutionized by evolving molecular understanding of signal transduction within cancer cells and of how mutations in key

From the Editors

In the coming years, study of the human genome is likely to culminate in discovery of multiple genes related to melanoma. These discoveries will influence how we identify individuals at high risk of the disease. In fact, genetic testing for the presence or absence of one such high-risk melanoma gene, CDKN2A, is now commercially available.

In this issue of *The Melanoma Letter*, Bradley Bloom and Dr. David Polsky identify another high-risk gene called MDM2, which when mutated, and in the presence of estrogen, may increase a woman’s propensity for developing melanoma, especially at younger ages. If their findings are substantiated, it may help explain why younger women have higher melanoma incidence than younger men.

Although some melanoma-related genes may express themselves phenotypically (e.g., red hair, multiple nevi, etc.), other genes’ phenotypic expression may be minimal. Nonetheless, depending on environmental influences, these genes may also lead to melanoma. As new high-risk melanoma genes are discovered one by one, it will be interesting to observe whether targeted screening of high-risk patients will eventually shift from the current phenotypic-centric identification to genotypic-centric identification.

Another benefit of studying the human genome is identifying specific genes involved in the cell cycle. The knowledge gained from this may help researchers manipulate these genes to our advantage therapeutically. The possibility of designing drugs that attenuate or nullify the effects of a given mutated gene opens the door for targeted therapy. It is now well established that each melanoma subtype has its own unique set of mutations, which impart their influence on melanocyte proliferation, differentiation, survival, and apoptosis. Examples include c-Kit and GNAQ mutations in mucosal and ocular melanoma, respectively. Patients with melanomas expressing c-kit or GNAQ mutations may benefit therapeutically from targeted therapy. One example is the administration of Imatinib to target melanomas expressing c-kit mutations.

Another mutation frequently found in melanomas that develop on intermittently sun-exposed skin involves BRAF. It has been known for some time that the BRAF pathway is important in melanoma. However, only recently were specific BRAF mutations identified in melanoma. This finding led to the development of a drug called PLX4032, designed to target V600E, a specific BRAF mutation. Dr. Flaherty highlights the preliminary but promising results obtained with PLX4032 in the treatment of patients with metastatic melanoma.

Based on the articles in this issue of *The Melanoma Letter*, it is compelling to contemplate just when clinical practice will evolve from the current phenotype-based screening and disease-based therapy to molecular/genetic-based targeted screening and therapy.

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genes that encode signaling molecules underlie the development of these malignancies. The advent of targeted therapies that block the signaling function of such “oncogenes” has provided far more effective and tolerable therapy for patients with chronic myelogenous leukemia, gastrointestinal stromal tumors, non-small cell lung cancer, breast cancer, colon cancer, renal cell carcinoma, and glioblastoma multiforme. Several of these tumors share with melanoma the same resistance to conventional cytotoxic chemotherapy, yet have proven susceptible to oncogene-targeted therapy.

Great advances have recently been made in understanding the oncogenes and signal transduction pathways that contribute to melanoma formation and dissemination. The gene that has garnered most attention in recent years is BRAF, a constituent of the so-called MAP (mitogen-activated protein) kinase signaling pathway, in which activating mutations occur in 50-60 percent of melanomas. Laboratory evidence has consistently shown that targeting BRAF can be an effective strategy for slowing growth and inducing cell death in melanoma cells that harbor BRAF mutations, though these tumor cells also harbor a host of additional mutations in other key signaling molecules. However, it has taken seven years from the time of initial discovery of BRAF mutations to establish that a drug can successfully target BRAF in humans with metastatic melanoma.

Especially with the recent early success using PLX4032, we have new evidence that changes the landscape of clinical research in melanoma and will hopefully soon change the treatment paradigm for this traditionally refractory disease.

BRAF Biology

Raf kinases are components of the MAP kinase pathway, a signal transduction pathway that normally controls cell growth and division downstream of activated cell surface growth factor receptors. RAF1, formerly known as CRAF, is the most ubiquitously expressed of the RAF family members, and previously was the most extensively studied. However, activating mutations in RAF1 are known to be very rare. BRAF is expressed most highly in neuronal tissues and melanocytes (both of neural crest origin), as well as testis and hematopoietic cells. Based on several large genetic screening studies, BRAF appears to be mutated in approximately 7 percent of all cancers, but most commonly in melanoma. (See Figure 1.)

While there are numerous types of mutations in BRAF, the T1796A point mutation accounts for 97 percent of the mutations, resulting in substitution of glutamic acid for valine at the 600 position of the amino acid sequence \( V600E \). This mutation locks BRAF in the active signaling conformation so that it drives signaling through the MAP kinase pathway regardless of activation of receptor tyrosine kinases or other inputs. The first evidence that blocking \( V600E \) BRAF activity could have potential therapeutic value in melanoma came from laboratory experiments in which \( V600E \) BRAF was eliminated from cells using a sequence of nucleotides that neutralize the messenger RNA encoding BRAF. However, this is very different from demonstrating an effect with a drug administered systemically to humans. That milestone had to await the successful discovery and development of far more potent and specific inhibitors of BRAF.

BRAF Inhibitors in Clinical Trials

Sorafenib

Sorafenib (BAY 43-9006, Nexavar®) was first selected for development as an inhibitor of RAF1, the ubiquitously expressed but rarely mutated Raf isoform. Sorafenib is tenfold less potent against BRAF, and apparently even less potent against \( V600E \) BRAF. In the laboratory setting, sorafenib is able to block \( V600E \) BRAF and downstream activation of the MAP kinase pathway. However, the concentrations of the drug needed to induce cell death also kill melanoma cells that lack a BRAF mutation. This is concerning in that BRAF appears to be an important component of MAP kinase pathway signaling and a cell survival factor only when it is mutated. Therefore, sorafenib’s effect on melanoma cells may not relate to BRAF inhibition. Furthermore, the concentrations required to kill melanoma cells were sufficiently high that similar concentrations may not be achievable in humans.

At the maximum tolerated dose, sorafenib was not found to have significant single-agent activity in melanoma. Mechanistic investigations have been limited, but suggest that sorafenib does not effectively inhibit BRAF in human tumors, as measured by ERK (extracellular signal-regulated kinases) activation before and during treatment, whereas significant changes in tumor vascular permeability (an established surrogate for VEGF signaling) were noted. Based on this evidence, it appeared that sorafenib was unable to exert a sufficient effect on BRAF to test the value of BRAF as a therapeutic target in melanoma. Several trials were conducted investigating the possibility that sorafenib might enhance the efficacy of chemotherapy. Although single-arm phase II trials suggested a benefit, randomized trials comparing the efficacy of chemotherapy
selected patients underwent biopsy of superficial tumors to confirm target inhibition. At the same doses with which significant tumor regression was noted in patients, the MAP kinase pathway appeared to be almost completely inhibited.\(^3\)

It is remarkable that early responses to the drug are so commonly seen, despite the diversity of additional genetic alterations in these tumors. As patients’ tumors begin to progress following initial response, it will be of great interest to know what the drivers of treatment resistance might be, but at this time, no data are available to shed light on this question. In the context of the ongoing phase II and phase III trials with single-agent PLX4032, many more patients will be treated, and further insight into this issue should be gained.

Future Directions

Two clinical trials are now under way to further characterize the efficacy of PLX4032 in metastatic melanoma. Hopefully, these trials will serve as the basis for regulatory approval and incorporation of this highly targeted therapy as standard treatment for metastatic melanoma patients whose tumors harbor BRAF mutations. A single-arm phase II trial is being conducted to evaluate the objective response rate and duration of response in a larger cohort of V600E BRAF-mutated metastatic melanoma patients than were included in the original phase I/II trial. The second trial, a larger, randomized, phase III trial, is comparing PLX4032 to the reference standard therapy for metastatic melanoma, dacarbazine, in patients with previously untreated metastatic melanoma. The goal of this trial is to show an improvement in overall survival, providing definitive proof that this agent alters the natural history of metastatic disease, rather than merely inducing short-term regression.

There are numerous possibilities to consider in the effort to improve single-agent BRAF inhibition. The next critical goal is to enhance longevity of response or obtain complete responses. It is possible that the greatest disease control will come from sequencing targeted therapies aimed at intercepting the mechanism of escape/resistance following BRAF inhibition. Combination strategies are also being considered, since concomitant inhibition of signaling pathways known to be activated in concert with BRAF mutations is theorized to induce greater degrees of cell death and potentially delay the emergence of resistance.

Conclusion

BRAF appears to represent an important new target in melanoma. Melanoma has been the disease context in which the most extensive clinical evaluation has been conducted with RAF inhibitors. The clinical trials currently under way for metastatic melanoma represent the first opportunity for these therapies to be established as standard in the treatment of cancer. However, melanoma is an aggressive tumor, associated with a large array of genetic alterations beyond mutations in BRAF. We anticipate that some tumors will be more or less sensitive to treatment based on the constellation of other genetic changes, and these will be defined as predictive markers. Understanding the network of signal transduction pathways and how they may adapt to BRAF will point to the next generation of clinical trials investigating rational sequences of therapy and combination regimens.

References


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Melanoma in Women Under Age 50: Can Genetics Work with Estrogen To Boost Risk?

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A key goal of oncology is to detect and remove cancers at an early stage, when they are localized to the primary site and malignant cells have not metastasized to other organs. Screening the entire population for early cancers, however, is costly and often of low yield. The yield could be increased if we focused primarily on those patients who are at increased risk of disease; however, other than age-related recommendations for tests such as mammography or colonoscopy, or recommendations specifically for patients in highly cancer-prone families, we do not yet have accurate enough tools to select high-risk patients for cancer screening.

Currently, several groups have been conducting genome-wide association studies to identify inherited genetic variations that might identify individuals at increased risk for cancer, including melanoma. These gene discovery efforts are not based on pre-existing hypotheses regarding the role of any particular gene or genetic pathway in the disease of interest, but they potentially can produce important breakthroughs in our understanding of various diseases, including cancers such as melanoma. These discoveries may lead to genetic tests to identify high-risk individuals.

An alternative approach to identify individuals at high risk for melanoma begins with a hypothesis to explain epidemiologic observations, such as the link between sunlight, fair skin, and melanoma, and investigates known genes that may play a role in the disease process. One epidemiologic observation that has been of great interest to our group is the difference in age-related melanoma incidence rates between men and women. Investigating this observation using the tools of genetic epidemiology led to our preliminary discovery of a genetic marker associated with increased risk of melanoma among women under 50 years of age.

Incidence Differs Markedly in Men and Women

Melanoma incidence rates display a unique profile with respect to age and gender. For nearly all the other major forms of adult cancer, incidence rates increase exponentially with increasing age and in parallel fashion for men and women. Cancer rates are also generally higher among men than women across all age groups. In contrast, melanoma incidence is greater among women than men between age 20 and age 40, but greater among men than women at ages greater than 50. Although the age-related melanoma incidence rate among men displays the same exponential increase as is seen in other cancers, melanoma incidence in women never exhibits this exponential pattern of increase with increasing age. (See Figure 1.)

These differences raise many intriguing questions. Why is melanoma incidence greater in young women than in young men? Why doesn’t the incidence curve in women rise sharply with age as it does in men? Why doesn’t it resemble the curve for women with other common cancers such as lung and colon? Among the possible explanations for these trends is that women generally have more melanomas removed at the in-situ stage, which is not counted in the epidemiologic statistics, and this prevents the later development of invasive melanomas; or that age-related sun exposure patterns differ between men and women. It is possible that women become more aware of the risks of ultraviolet (UV) exposure and avoid the sun as they age, thereby reducing the incidence of sunlight/UV-driven melanomas among older women. Although these are interesting hypotheses, we are unaware of data to support or refute them.

An alternate explanation is that some melanomas in women are related to estrogen signaling. This hypothesis is based in part on the observation that the average age of menopause in the United States is 51, about the age at which melanoma incidence becomes greater in men than in women. The idea is that estrogen combines with excessive UV exposure and certain inherited genetic factors to transform benign melanoma precursor cells into malignant melanoma cells. When estrogen levels decline after menopause, the risk of melanoma also declines in women who have inherited the genetic factors that cooperate with estrogen to produce melanoma.

MMD2 SNP309 and Melanoma Risk in Women

A single nucleotide polymorphism (SNP) at position 309 of the MMD2 gene (SNP309) was found to be associated with the onset of several different cancers such as soft tissue sarcomas, diffuse large B-cell lymphoma, colorectal cancer, and non–small cell lung cancer in women under age 51. MMD2 is a key negative regulator of the tumor suppressor gene p53. In certain human tumors, overexpression of MMD2 is oncogenic, that is, associated with accelerated cancer progression due to excessive inactivation of p53. In addition, laboratory studies have demonstrated that the estrogen receptor complex activates MMD2 expression by binding to the SNP309 site, especially when the site is occupied by the G nucleotide. This leads to higher levels of expression of MMD2 and suppression of...
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p53. These observations formed the basis for the hypothesis that estrogen signaling could be involved in cancer development related to the SNP309 G allele.

To investigate this hypothesis, we conducted a pilot study to examine the relationship between age at melanoma diagnosis and SNP309 genotypes for men and women. We examined a population of 227 newly diagnosed primary melanoma patients prospectively enrolled in the Interdisciplinary Melanoma Cooperative Group at the New York University Langone Medical Center from August 2002 to November 2006. The population was 41 percent female and 59 percent male, with a median age at diagnosis of 57 years. We found that the age of diagnosis among women with two copies of the G allele (GG genotype) was 13 years earlier (median 46 years) compared to women with either the TG or TT genotype (median 59 years); however, this difference was not statistically significant. Of note, by age 50, 11 of 21 women (52.4 percent) with the GG genotype were diagnosed with melanoma, compared to 15 of 68 women (22.1 percent) with either the TG or TT genotype (p=0.01).

We did observe a statistically significant increase in the odds ratios for a melanoma diagnosis in the GG genotype when we analyzed the patients by age groups (Table 1). Specifically, women with the GG genotype had an odds ratio of 3.89 for a melanoma diagnosis at ages under 50 years, and this increased to 4.62 for those under age 40. There was no significant age association found using a cutpoint of 60 years and older, and no significant association for any age cutpoint among men. These data demonstrate that women with the SNP309 GG genotype may be more likely to be diagnosed with melanoma at ages under 50 compared to women with either the TG or TT genotype. Taken together with the epidemiologic incidence data and our knowledge regarding the average age of menopause, these findings suggest that estrogen may play a role in the etiology of melanoma among women with a genetic predisposition such as MDM2 SNP309.

Important Caveats

It is important to point out that this initial study has several limitations. We evaluated a relatively small number of patients, and all the individuals studied had been diagnosed with melanoma. As such, these findings suggest that MDM2 SNP309 must be cooperating with other genetic and environmental factors that contribute to melanoma. Indeed, analysis of the SNP309 polymorphism is complicated by a relatively high frequency of the GG genotype in the population. Definite population frequency data are not available through the National Center for Biotechnology Information, but frequency estimates in various published reports range from 15 to 30 percent of individuals. This is clearly much higher than the percentage of individuals who will develop melanoma, so SNP309 cannot be used as a sole marker of genetic risk for melanoma among young women. Based on this high frequency of the GG genotype, it is likely that analyzing melanoma risk using a study that compares melanoma patients with unaffected controls will yield lower odds ratios that may not be statistically significant. For example, no association between SNP309 and melanoma risk was found in a study using subjects enrolled in the Nurses Health Study, a large prospective cohort study that included research subjects with and without melanoma. It is difficult to compare the Nurses Health Study data with ours; however, because the average age of melanoma diagnosis in the Nurses Health Study was 63 compared to 57 in our group, their population had a much lower prevalence of the GG genotype (13 percent, vs. 24 percent in our cohort), and the investigators did not present an estimate of the risk of melanoma by age group and genotype. In addition, there may be other as yet unknown differences between our study population and theirs that may affect the association between SNP309 and melanoma risk.

Future Directions

Nevertheless, the unique pattern of age-related melanoma incidence among women remains intriguing and will be the subject of additional studies. As the current model of melanomagenesis is based on the cooperation of both genetic and environmental factors in the transformation of benign melanoma precursor cells into malignant melanoma, it is likely that inherited genetic variations such as SNP309 will play a role in explaining why some women develop melanoma before age 50 and others do not. Ultimately, a more sophisticated melanoma risk assessment model will need to include many genes, probably at least 10, in combination with clinical risk factors such as mole pattern and an assessment of sun and tanning bed exposure. It is possible that a woman’s SNP 309 genotype may be one of these important factors. With such a model in place, we would be able to recommend more intensive melanoma surveillance to those at high risk, hopefully intervening in the disease process at an early, curable stage.

Table 1: Odds ratios for initial diagnosis of melanoma at ages less than those shown above, for the MDM2 SNP309 GG genotype compared to TG and TT genotypes

<table>
<thead>
<tr>
<th>Age cutpoint</th>
<th>Women (n=89) OR (95% CI)</th>
<th>Men (n=127) OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40 vs ≥40</td>
<td>4.62 (1.23, 16.86)</td>
<td>2.64 (0.68, 9.55)</td>
</tr>
<tr>
<td>&lt;50 vs ≥50</td>
<td>3.89 (1.22, 12.31)</td>
<td>0.89 (0.31, 2.39)</td>
</tr>
<tr>
<td>&lt;60 vs ≥60</td>
<td>1.33 (0.44, 4.08)</td>
<td>0.76 (0.31, 1.86)</td>
</tr>
<tr>
<td>&lt;70 vs ≥70</td>
<td>1.15 (0.34, 4.60)</td>
<td>0.53 (0.19, 1.54)</td>
</tr>
<tr>
<td>&lt;80 vs ≥80</td>
<td>1.09 (0.19, 11.61)</td>
<td>0.47 (0.05, 5.89)</td>
</tr>
</tbody>
</table>

References

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