Genetic Alterations in Signaling Pathways in Melanoma

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Abstract

Alterations in the RAS signaling cascade are almost uniformly present in melanoma. RAS itself is only infrequently mutated in melanoma although downstream of RAS lie BRAF on the mitogen-activated protein kinase pathway and PTEN on the protein kinase B/Akt pathway. These genes are often altered in melanomas; indeed, the most frequent target of mutation in melanomas is BRAF, which is mutated in ~ 60% to 70% of superficial spreading melanomas. These mutations occur in a background that is not normal, with the CDKN2A locus also typically being mutated. We review herein the data that suggest that the distribution of the signaling mutations is important. In general, melanomas carry a mutated NRAS, a mutated BRAF, or concurrent BRAF and PTEN mutations. These data support the hypothesis that the biochemical functions of RAS are portioned by mutations in the pathways lying downstream. Moreover, these mutations have no apparent relationship to the patterns of alteration of CDKN2A and its downstream effectors. Thus, the data also suggest that successful exploitation of mutations in melanoma will be dependent on understanding not only mutations and their frequency but their genetic context as well.

The development of rational treatments for melanoma will depend on our taking advantage of the molecular basis of its clinical features. The necessary understanding of the molecular genetics underlying melanoma is gradually emerging. It has become clear that alterations in two genes and their downstream effectors are almost uniformly present in melanoma. The first of these is the CDKN2A locus, and the second is the pathway that includes RAS. Understanding the role alterations in these pathways play in melanoma and understanding their interactions may provide key insights that will lead to therapeutic interventions that are rationally designed and implemented.

The CDKN2A locus encodes two tumor suppressor genes, CDKN2A/p16 and p14ARF, which play central roles in the control of cell cycle progression and checkpoint control. The p16-CDK4-pRB pathway in melanoma is almost always abnormal. CDKN2A also encodes the p53-controlling p14ARF, and disruption of CDKN2A therefore usually results in both loss of pRB and p53 control. However, altered tumor suppressor loci, with resultant loss of function of the encoded proteins, are problematic therapeutic targets. Efforts to develop small-molecule CDK inhibitors, essentially mimics of p16, are ongoing but progress thus far has been slow.

More important at present in our thinking about targeted therapy for melanoma is the RAS pathway. The RAS gene itself is infrequently mutated in melanoma. However, the RAS protein does several important functions, and downstream of it lie BRAF on the mitogen-activated protein kinase (MAPK) pathway and phosphatase and tensin homologue (PTEN) on the protein kinase B/Akt pathway. These genes are frequently altered in melanomas and it seems that the RAS-PTEN/BRAF axis is almost always abnormal. Mutated targets on this pathway are appealing therapeutic targets.

In this review, we show that the distribution of these mutations is important. We have shown that melanomas carry either a mutated RAS or mutated BRAF and PTEN (1). The lesson is that the latter two abnormalities cooperate in oncogenesis and that this may have important implications for planning therapeutic strategies.

Signaling Mutations in Melanoma

RAS mutations in melanoma. RAS genes are among the most frequently mutated genes in human cancers, but of course different malignancies display different frequencies and spectra of mutations in NRAS, HRAS, and KRAS. Human melanomas carry mutations almost exclusively in NRAS, with 90% of mutations localizing to codon 61 (1). HRAS and KRAS are less frequently mutated. Albino and Fountain (2) report that 24% of cultured metastatic and 12% of noncultured primary and metastatic melanomas carry NRAS alterations, although others report higher frequencies in primary tumors (3) and have suggested that mutations correlate with metastases or disease progression.

RAS controls several biochemical processes. The best understood pathway that involves RAS is the receptor tyrosine kinase-MAPK pathway (4). This pathway includes BRAF and contributes to control of cellular proliferation by RAS, in particular the control of malignant cell proliferation by...
activated RAS. RAS also controls apoptosis (5). This response is controlled through the phosphatidylinositol 3-kinase-PTEN-Akt pathway as RAS directly binds the catalytic subunit of phosphatidylinositol 3-kinase. The downstream consequence of this is phosphorylation of Akt and protection against apoptosis.

Because RAS pathway abnormalities in melanoma occur almost uniformly with CDKN2A alterations, understanding this interaction is crucial. If melanomas carry RAS mutations, do mice engineered to carry an activated RAS develop melanoma? In the Cdkn2a−/− background, the answer is yes (6, 7). This mouse model of melanoma most closely replicates the human genetic findings. There are several other models that are less well characterized but the RAS/Cdkn2a model is the most faithful replica of human data and shows the importance of these pathways in melanoma genesis.

The PTEN tumor suppressor in melanoma. PTEN is another important element in signal transduction altered in human
melanomas. *PTEN* was identified as a tumor suppressor candidate from the region of chromosome 10q23-24 frequently altered in gliomas and melanomas. Cytogenetic evidence shows that 10q loss is an early and frequent event in melanomas (8–11). Although initial work showed no mutations in a small number of melanomas, we showed *PTEN* loss in 30% of melanoma cell lines (12) as did others (13). We have recently reviewed the role of *PTEN* in melanoma in detail (14).

*PTEN* encodes a protein with extensive homology to dual specificity protein phosphatases and, like RAS, it is implicated in the pathways that control apoptosis and signal transduction. Mutations were detected in 66% of melanomas (19) and in a variety of other cancer types. Most mutations occurred through a single substitution (V600E), imparting transforming ability to a variety of other cancer types. Most mutations occurred through a single substitution (V600E), imparting transforming ability to downstream of RAS and effect signal transduction. Mutations found in melanomas occur in *NRAS* (20) but no mutations have been detected in familial melanomas (21) or in uveal melanomas (22).

There are three members of the RAF class: RAF-1, ARAF, and BRAF (23). Each is a cytosolic serine-threonine kinase. BRAF activates the MAPK/extracellular signal–regulated kinase B/Akt (14). The latter protein, when phosphorylated, has several activities but functions mainly to antagonize apoptosis. Several groups have shown that loss of *PTEN* induces phosphorylation of *PTEN* in inhibition of protein kinase B/Akt phosphorylation (16–18). This places *PTEN* effectively downstream of RAS as RAS controls the same function via its interaction with phosphatidylinositol 3-kinase.

**BRAF and melanoma.** The most important mutations yet discovered in melanomas occur in *BRAF*. RAF family proteins lie downstream of RAS and effect signal transduction. Mutations were detected in 66% of malignant melanomas (19) and in a variety of other cancer types. Most mutations occurred through a single substitution (V600E), imparting transforming ability to the protein. Of note, ~80% of benign nevi carry this mutation (20) but no mutations have been detected in familial melanomas (21) or in uveal melanomas (22).

Finding these mutations downstream of RAS has led to excitement and intensive study. Importantly, the mutations suggest the ability to specifically target BRAF therapeutically, and inhibitors of RAF are already in clinical trials (26–29). The first suggestion in melanoma that *BRAF* phosphorylates and negatively regulates *BRAF* (24, 25) but further details about the control of apoptosis by BRAF are lacking.

Patterns of Signaling Mutations in Melanoma

**RAS mutations in human melanoma.** Because RAS genes are the most commonly mutated oncogenes in melanomas, we examined the status of RAS in our melanoma cell lines. Tsao et al. (1) evaluated 53 cutaneous melanoma cell lines and 17 uncultured metastases for mutations in codons 12, 13, and 61 of NRAS, KRAS, and HRAS. They found a total of 11 NRAS mutations [11 of 53 (21%); 1 at codon 12 and 10 at codon 61] in our cell lines. From our uncultured samples, we found two NRAS mutations (2 of 17; 12%) in codon 61. The authors found no mutations in the other RAS genes.

**PTEN mutations in human melanomas.** We also screened for mutations in exon 1 through 9 of *PTEN* in 45 melanoma cell lines, 17 paired cutaneous melanoma and peripheral blood samples, and germ-line DNA from 28 melanoma patients with a family history of melanoma. Table 1 illustrates some of our findings (12). *PTEN* alterations were shown in 13 of 45 lines initially tested (9 homozygous deletions and 4 mutations). Intragenic homozygous deletion clearly identifies *PTEN* as the target of chromosome 10 loss. We also showed *PTEN* mutations in 3 of 17 uncultured melanomas and 1 homozygous deletion of *PTEN*.

These studies helped to establish an important role for the involvement of *PTEN* in the pathogenesis of melanoma. Additional authors have made similar observations (13, 31–35). Our initial studies on both these genes also established a relationship between mutations in them both. Cell lines or uncultured tumors carry a mutation in NRAS, *PTEN*, or neither, but not in both. A total of 51% of cell lines carried mutations in NRAS (21%) or *PTEN* (30%); only one had both. This was the first suggestion in melanoma that RNAs and *PTEN* had overlapping functions. The relationship with *BRAF* mutations (discussed next) sheds further light on this finding.

**Mutations in BRAF.** The observation that *BRAF* mutations in melanoma distributed reciprocally to NRAS mutations raised the possibility that *BRAF* activation and *PTEN* inactivation cooperate. To directly test this hypothesis, we screened for *BRAF* mutations in our series of melanoma samples and found evidence of possible concurrence between *BRAF* and *PTEN* mutation. We used PCR-single-strand conformation polymorphism and confirmed abnormalities by sequencing. *BRAF* was mutated in 28 of 47 (60%) cell lines and 9 of 16 (56%) uncultured metastases analyzed.

**Relationship between mutations in NRAS, PTEN, and BRAF.** Table 1 lists mutations shown in melanoma cell lines and their distribution. A similar analysis was done on uncultured melanomas and primary tumors.

In general, the data show that melanomas fall into one of three classes. They carry a mutation in NRAS alone, concurrent *PTEN* and *BRAF* mutation, or *BRAF* mutation alone. Overall, 40 of 47 (85%) melanoma cell lines and 11 of 16 (69%) uncultured melanoma metastases had mutations in NRAS, *BRAF*, or *PTEN*. NRAS was exclusively mutated in 9 of 47 (19%) cell lines and 2 of 16 (13%) metastases whereas *BRAF* was solely mutated in 28 of 47 (60%) cell lines and 9 of 16 (56%) metastases. In the 12 of the 15 melanoma cell lines (80%) and both melanoma metastases with *PTEN* alterations, *BRAF* was also mutated. Recently, Goel et al. (36) examined a series of 69 primary melanomas for alterations in the same genes. We showed in this analysis that *BRAF* mutation occurs irrespective of primary Breslow thickness but that *PTEN* loss is correlated with increasing Breslow depth and tumor progression. These data support the hypothesis that the biochemical functions of RAS in melanoma can be partitioned (30). The genetic data also suggest that concurrent *BRAF/PTEN* mutations function like NRAS mutation (Fig. 1).
Relationship of signaling mutations to alteration in p16 and p53.

We characterize a subset of our melanomas for alterations in the p16 and p53 pathways. It has previously been shown that in cell lines that lack detectable alterations in CDKN2A, mutations in CDK4 occur frequently (37), and it has been suggested that alterations in this pathway are essentially uniform in melanoma cell lines. A compendium of the genetic data is given in Table 2.

Several features are apparent from this analysis. First, virtually all the lines carry alterations in the pRB pathways through CDKN2A or CDK4. The alterations are generally reciprocal; only two lines carry mutations in both. The two proteins, p16 and CDK4, interact functionally in the control of RB1 phosphorylation; thus, mutations in both would be redundant.

Second, virtually all cell lines carry alterations in the p53 pathway. The observation that the p53 mutation rate was low in melanoma has been puzzling. We now understand this to be explained by the fact that CDKN2A encodes two proteins, the p16 cyclin-dependent kinase inhibitor and p14, which interacts with murine double minute-2 on the p53 pathway. Thus, homozygous deletion of CDKN2A deletes both p16 and p14. Again, p14 and p53 mutations are reciprocal, and the presence of CDKN2A (and thus p14) loss renders p53 mutation redundant. In turn, this model predicts that in tumors that lack p16 alterations, p53 should be mutated. In fact, the only detectable p53 mutations in our series to date are in cell lines with normal CDKN2A and mutated CDK4. Why this should occur specifically in melanoma, however, is not yet clear. In pancreatic cancer, for example, the rate of both p16 loss and p53 mutation is ~60%. Third, and most significantly, because the p16 and p53 pathways are uniformly abrogated in our samples, the mutant PTEN/BRAF and mutant NRAS genotypes both cooperate with these loci in vivo in contributing to melanoma oncogenesis. PTEN/BRAF can cooperate with either loss of CDKN2A or mutation of CDK4 and p53 (Fig. 2).

Implications for Melanoma Therapy

The best examples of successful rational drug design in the last 5 years have involved the targeting of overexpressed or mutated signaling molecules by small-molecule inhibitors or antibodies. The overexpression of Her2/neu in breast cancer has been successfully exploited by therapy with trastuzumab (38). c-KIT mutations in gastrointestinal stromal tumors have been exploited using imatinib (39) and epidermal growth factor receptor mutation and overexpression in non–small cell lung cancer enable these tumors to be treated with erlotinib and gefitinib (40, 41). What are the prospects for extending this paradigm of inhibition of genetic targets to melanoma?

On initial examination, it would seem that the high incidence of BRAF mutation in melanoma suggests a biology similar to that operative in the aforementioned malignancies.

### Table 2. p16 and p53 pathway alterations in melanoma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>PTEN</th>
<th>BRAF</th>
<th>RAS</th>
<th>CDKN2A</th>
<th>p14 or p16</th>
<th>CDK4</th>
<th>p53</th>
</tr>
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<tr>
<td>MGH-BO-1</td>
<td>Del exon 2</td>
<td>V599E</td>
<td>WT</td>
<td>HD</td>
<td>Both</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>SK-Mel-131</td>
<td>IVSS+2T-A</td>
<td>V599E</td>
<td>WT</td>
<td>173del8/X</td>
<td>Both</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>NM455</td>
<td>Del exon 6</td>
<td>V599E</td>
<td>WT</td>
<td>HD</td>
<td>Both</td>
<td>WT</td>
<td>Not done</td>
</tr>
<tr>
<td>G-mel</td>
<td>Del exon 1</td>
<td>V599E</td>
<td>WT</td>
<td>HD</td>
<td>Both</td>
<td>WT</td>
<td>Not done</td>
</tr>
<tr>
<td>UACC903</td>
<td>Y76X</td>
<td>V599E</td>
<td>WT</td>
<td>HD</td>
<td>Both</td>
<td>WT</td>
<td>Not done</td>
</tr>
<tr>
<td>SK-Mel-37</td>
<td>Del exon 2</td>
<td>V599E</td>
<td>WT</td>
<td>WT</td>
<td>Neither</td>
<td>R24H</td>
<td>R175H</td>
</tr>
<tr>
<td>SK-Mel-39</td>
<td>546insA/Ter</td>
<td>V599E</td>
<td>WT</td>
<td>WT</td>
<td>Neither</td>
<td>K22Q</td>
<td>WT</td>
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<tr>
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<td>T167A</td>
<td>V599E</td>
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<td>Neither</td>
<td>R24C</td>
<td>L145R</td>
</tr>
<tr>
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<td>Del exons 2–6</td>
<td>Not done</td>
<td>WT</td>
<td>HD</td>
<td>Both</td>
<td>R24C</td>
<td>WT</td>
</tr>
<tr>
<td>MGH-PO-1</td>
<td>WT</td>
<td>WT</td>
<td>Q61K</td>
<td>HD</td>
<td>Both</td>
<td>R24H</td>
<td>WT</td>
</tr>
<tr>
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<td>WT</td>
<td>Q61K</td>
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</tr>
<tr>
<td>MelJuso</td>
<td>WT</td>
<td>WT</td>
<td>Q61I</td>
<td>HD</td>
<td>Both</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>MGH-RO-1</td>
<td>WT</td>
<td>WT</td>
<td>Q61R</td>
<td>HD</td>
<td>Both</td>
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<td>WT</td>
</tr>
<tr>
<td>SK-Mel-30</td>
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<td>Q61K</td>
<td>P114L</td>
<td>p16 only</td>
<td>WT</td>
<td>Not done</td>
</tr>
<tr>
<td>Mel-Swift</td>
<td>WT</td>
<td>WT</td>
<td>Q61K</td>
<td>R58X</td>
<td>p16 only</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>MGH-KO-1</td>
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<td>WT</td>
<td>HD</td>
<td>Both</td>
<td>WT</td>
<td>WT</td>
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<tr>
<td>MGH-MC-1</td>
<td>WT</td>
<td>V599E</td>
<td>WT</td>
<td>HD</td>
<td>Both</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>MGH-NA</td>
<td>WT</td>
<td>Not done</td>
<td>WT</td>
<td>HD</td>
<td>Both</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>MGH-OQ</td>
<td>WT</td>
<td>Not done</td>
<td>WT</td>
<td>HD</td>
<td>Both</td>
<td>WT</td>
<td>WT</td>
</tr>
</tbody>
</table>

Abbreviations: WT, wild type; HD, homozygous deletion.
and that one might predict that therapy aimed at inhibiting \textit{BRAF} would lead to similar success. However, a more complete understanding of the genetic data suggests several problems to be anticipated.

First, the successful examples of targeted therapy listed herein all involve inhibition of targets that lie on the upstream limbs of their respective signaling pathways. All biochemical pathways branch and the functional implications of digressing pathways as they are followed downstream are not always clear. Inhibition of signaling at its inception, at the ligand or receptor, obviates any consideration of which of the pathway tributaries is most central to oncogenesis. This has been accomplished in the other diseases cited. In melanoma, in contrast, the most frequent mutations, those in \textit{BRAF}, lie several steps down-stream of the receptor-ligand interaction, which is itself unknown. This suggests that inhibition of \textit{BRAF} alone would not be sufficient for therapeutic effect.

Consistent with this hypothesis is the observation that acquired nevi have nearly the same frequency of mutations in \textit{BRAF} as do malignant melanomas. \textit{BRAF} mutation alone is not sufficient to cause melanoma; therefore, reversal of \textit{BRAF} activity would likely be insufficient to cause clinical regression.

Also consistent with this hypothesis are the observed patterns of melanoma mutations described herein. \textit{NRAS} mutation occurs reciprocally with \textit{BRAF} and \textit{PTEN} mutations in a subset of cases. This strongly suggests that \textit{BRAF} and \textit{PTEN} functions recapitulate that of \textit{RAS} and that other mutations must occur concomitant to \textit{BRAF} mutation when \textit{PTEN} is normal. It also suggests that other mutations, either upstream of \textit{RAS} or downstream altogether, will be discovered in cases that completely lack \textit{NRAS} or \textit{BRAF} mutations. Analysis of the prevalence of \textit{NRAS} and \textit{BRAF} mutations (~70% total) suggests that as many as 30% of melanomas may have other signaling mutations.

Finally, consistent with this suggestion is the observation that the small-molecule inhibitor of \textit{BRAF}, sorafenib, presently in clinical trials has little single-agent activity against melanoma (27–29). A possible explanation for this is that the agent may be insufficiently potent. However, we favor the biological explanation suggested by the genetic data (Fig. 3). Explaining this lack of activity is one of the important current challenges to the field.

Another major impediment to melanoma therapy, as well as to therapy of other malignancies, is the importance of tumor suppressor mutations. It has been shown here that essentially all of the melanoma samples analyzed have alterations in both the p16-pRB and p53 pathways. At this time, we have no therapeutic tools to address these abnormalities in cell cycle and checkpoint control. Even if one chooses to concentrate only on the signal transduction pathways and granted that the most narrow interpretation of the genetic data presented is correct (that \textit{BRAF} plus \textit{PTEN} mutation equals \textit{RAS} mutation), one still is left without a drug development strategy for replacement of lost \textit{PTEN} activity. Replacing loss-of-function alterations will likely be a requisite step in successful melanoma targeted therapy.

In summary, our understanding of the genetic alterations found in melanoma will likely provide the foundation for successful therapies in the future. Important additional information is emerging for the understanding of the role of microphthalmia-associated transcription factor in the development of melanoma. Taken together, this handful of genes, which are currently the focus of intense investigation, will likely guide the way to new therapy for patients with melanoma.

Open Discussion

Dr. Atkins: What do we know about the relationship between melanoma sun exposure and biology?

Dr. Haluska: Very little. Some data suggest that there is a relationship between \textit{BRAF} mutation on sun-exposed skin and the type of sun exposure. In other words, multiple intermittent exposures give rise to higher \textit{BRAF} mutation rates or to \textit{BRAF} mutation rates that are observed in non-sun-exposed skin. But the mutations you see are not typical UV-generated thymidine dimers, which is true in p16 as well. There clearly is an interplay between solar exposure and what these genes do, but it is probably not at the level of sun’s causing a mutation in the gene. We don’t talk much about xeroderma pigmentosum, which really is the most florid melanoma predisposition syndrome. Xeroderma pigmentosum is due to mutations in a series of genes that function in DNA damage control, and those patients are exquisitely sensitive to sun. In xeroderma
pigmentosum patients, we understand the development of melanoma, but in the more common somatic changes, we don’t have much of an understanding.

Dr. Elder: There are some interesting but incomplete data linking melanoma risk to pheomelanin presence in melanocytes. In dysplastic nevi compared with normal skin, pheomelanin is overexpressed. In patients who express more pheomelanin compared with those who express less, their melanoma risk is generally increased. One theory is that pheomelanin not only is a poor sunscreen but also generates free radicals on exposure to UV. This has hardly been followed up, but it would be another mechanism whereby sun exposure could directly cause mutations that would not be thymidine dimers.

Dr. Haluska: What we all think is that melanin is protective against sun and that more heavily pigmented populations have reduced risk of melanoma, but albino patients don’t get melanoma at very high rates. Thus, melanin may protect against other sun-related cancers, such as basal cell and squamous cell carcinoma, but in some cases may give rise to melanoma.

Dr. Sondak: Albinos do get melanoma.

Dr. Haluska: Very rarely though.

Dr. Flaherty: Considering that BRAF mutations are prevalent in benign nevi and recognizing that therapeutic target versus tumor promoter (oncogene) in tumor progression are two potentially separate issues, there are a few lines of evidence that suggest that BRAF is a “weak” oncogene. What does that make you think about BRAF as a driver in melanoma?

Dr. Haluska: Understanding on a biochemical basis what the term “weak oncogene” means is important. Part of that may mean that it is downstream and that it must collaborate with other genes to cause malignant transformation, so the epidemiologic observation that BRAF mutation is a frequent early event implies that the development of melanoma requires a concomitant genetic change. We don’t know what that change is yet.

Dr. Gajewski: The mutant mouse models are informative. Even active RAS as a transgene with a tyrosinase promoter alone doesn’t cause melanoma in mice at a reasonable frequency. Active RAS in conjunction with a p16 mutation does. Part of the reason is probably because active RAS in primary cells induces a cell cycle arrest and this then has to be overcome for tumor progression. Together it might argue that with mutation of downstream signaling molecules, even like RAS, which is so central, other mutations are going to be required for the malignant phenotype. If melanoma is dominantly driven by concerted interaction between multiple mutations, then it’s going to be extremely difficult and maybe even impossible to treat melanoma with agents that target individual pathways.

Dr. Sosman: Depending on the mutation you have, the tumor cells are wired differently. Whether the cells are NRAS mutated or BRAF mutated, agents can block the MAPK pathway differently depending on where the mutation is. Looking at both cell cultures and different cell lines, there is so much difference in terms of how the tumor cells are wired that an agent could seem either very active or completely inactive depending on where that mutation is located.

Dr. Atkins: Do the moles and the melanomas from patients with the dysplastic nevus syndrome have a BRAF mutation, RAS mutation, or a combination of a PTEN mutation and RAF mutation? What about patients with p16 mutations?

Dr. Haluska: My understanding is that they have BRAF mutations. We have not seen PTEN mutations in either preneoplastic or thin melanomas. They are typically in deeper lesions. RAS mutations are occasionally seen and relatively infrequent. The p16 families don’t necessarily have the dysplastic nevus phenotype.

Dr. Slingluff: I’ve seen patients who don’t look like melanoma candidates who get melanoma. At least some of them have an Irish background. Is something linked with this Irish background?

Dr. Haluska: This argues that in those populations, there is something that is more Mendelian and by that I mean single locus rather than multifactorial. I have a disproportionate number of patients with melanoma who do not have the typical skin type that you’d expect. This suggests that it’s more than just skin type.

Dr. Elder: What is clear is that p16, not necessarily through mutation, is actually progressively lost from nevi, as they progress to melanoma. So one can paint a picture of loss of the suppressor gene function freeing up the oncogene, however weak, and then requiring additional oncogenes to become an aggressive melanoma.

Dr. Keilholz: There are several ways to measure the activity of all those compounds inhibiting certain pathways. You can look at the inhibition of the downstream signaling, the effect on proliferation of cells, or the plating efficiency in culture and systems. Can you determine from the literature which of these assays has been most promising for reflecting the in vivo situations in animals?

Dr. Mier: The in vitro cultures are poorly predictive. Just allowing the cells to adopt a topology has little effect on the viability of these cells whereas allowing them to flatten out against a polystyrene surface renders them exquisitely sensitive to drugs that otherwise they would be highly resistant to. There are gene expression data in the literature depicting what happens to melanocytes when they’re simply cocultivated. There are all sorts of growth-associated genes that are induced by either secreted factors like fibroblast growth factor or perhaps even cell-to-cell contact, just as a consequence of being in a heterogenous milieu. One issue is that in a living tumor you have stromal elements that are probably there for a reason—to benefit the tumor. All of these things have to be considered when you’re trying to extrapolate into a clinical model.

Dr. Essner: How about studying the tumors themselves?

Dr. Haluska: We’ve looked at a variety of primaries and metastases. We have ~100 cell lines in the laboratory, but I can’t tell you whether they come from mucosal tumors or not. The genetics they display are similar to what we see in primary mucosal tumors, so I think one subset does and one subset doesn’t have the mutations that we observed. Now let me add one final thing. The study of predisposition is much more developed in breast cancer and colon cancer and in some of the more epidemiologic prevalent solid tumors than ours. This is a misfortune for our patients. The American Society of Clinical Oncology provided guidelines several years ago that basically said that p16 testing should be done in a research setting. The general understanding among clinical geneticists is that a family history of melanoma mandates requisite screening, and knowing that the patient has p16 mutation doesn’t change that recommendation. Understanding the predisposition to melanoma deserves our research, and especially for our patients it deserves attention in the field.
References


