Mutational Analysis of Selected Genes in the TGFβ, Wnt, pRb, and p53 Pathways in Primary Uveal Melanoma

Scott C. Edmunds, David P. Kelsell, John L. Hungerford, and Ian A. Cree

PURPOSE. It is known that the pRb pathway cell-cycle inhibitor p16INK4A plays a significant role in cutaneous melanoma and that alteration of p16INK4A, which resides within the 9p21-22 locus that also contains p15INK4B and p14ARF, may occur in up to one third of uveal melanomas. The absence of TGFβ responsiveness noted in cultured uveal melanoma cells also suggests that the TGFβ pathway plays a role in the formation of this tumor. Therefore, mutational screening was performed in several key genes in tumor-suppressor pathways that are known to be altered in some uveal melanomas.

METHODS. Using denaturing high-performance liquid chromatography (DHPLC) analysis and DNA sequencing, a series of 67 uveal melanomas were screened for inactivating mutations in the TGFβ pathway members Smad4 and TGFβ receptor type 2 (TGFβR2), the downstream cell-cycle inhibitor p15INK4B, and the cell-cycle inhibitors p14ARF and p16INK4A. p16INK4A was also investigated for promoter hypermethylation. Mutational analysis was also performed on the Wnt pathway gene β-catenin, known to be mutated in approximately one quarter of cutaneous melanoma cell lines.

RESULTS. Polymorphisms in p16INK4A were detected in 3 of 50 samples, but no inactivating mutations were detected in any of the genes screened. Promoter hypermethylation of p16INK4A was detected in 5 of 55 tumors, and loss of heterozygosity of the p16INK4A locus was detected in 5 of 16 tumors.

CONCLUSIONS. Most primary uveal melanomas do not appear to contain somatic mutations in Smad4, TGFβR2, p14ARF, p15INK4B, p16INK4A, or β-catenin. However, methylation of the p16INK4A promoter and loss of heterozygosity of the p14ARF-p16INK4A locus occurs in some tumors. (Invest Ophthalmol Vis Sci. 2002;43:2845–2851)

Uveal melanoma is the most frequently occurring primary intraocular tumor in white adults, having an annual incidence rate of 0.7 per 100,000 persons. The eye is the commonest site for noncutaneous melanomas, accounting for approximately 80% of such lesions and accounting for 13% of all deaths caused by melanoma, which has a very high mortality rate. This tumor carries up to a 50% 5-year mortality, depending on the tumor size.

Both uveal and cutaneous melanoma originate from the melanocyte, but little is known about the underlying molecular pathogenesis of uveal melanoma, in contrast to cutaneous melanoma, for which there have been more substantial advances in detecting mutations. Both tumors differ significantly in their origins, with UV light appearing to play little or no part in the causation of uveal melanoma, unlike cutaneous melanoma. Unlike cutaneous melanoma, no genes or tumor-suppressor pathways have so far been convincingly linked to uveal melanoma. Uveal melanoma spreads hematogenously, leading to liver metastasis, whereas cutaneous melanoma spreads mainly through the lymphatics, with skin metastases a more common problem. Various chromosomal abnormalities have been noted in uveal melanoma—predominantly, nonrandom alterations in chromosomes 3, 6, and 8. This is in contrast to cutaneous melanoma, which has only chromosome 6 alterations in common with uveal melanoma, and with further common cytogenetic changes in chromosomes 1, 7, 9, and 10.

Transforming growth factor (TGF)β is known to suppress the growth of normal human melanocytes, but this response is lost by approximately two thirds of ocular melanoma cells. This loss of TGFβ responsiveness suggests that an abnormality of the TGFβ pathway or of downstream components could be a cause of formation of these tumors. In the adult eye, TGFβ is produced by the ciliary body and retina and mediates a variety of effects, including immune suppression and modulation of fibrosis in response to injury. Secreted TGFβ binds to two different types of serine/threonine kinase receptors, known as TGFβ type 1 and type 2 (TGFβR1 and TGFβR2; for a review see Ref. 11). TGFβ binding activates various biochemical pathways through intracellular signaling molecules known as Smads. TGFβ stimulation is known to upregulate a number of cell-cycle inhibitors, including p15INK4B and p27KIP1.

The cell cycle inhibitor p15INK4B is known to be part of the pRb tumor-suppressor pathway that regulates the G1-S checkpoint of the cell cycle and is one of the most often deregulated pathways in cancer. p16INK4A is the main target for inactivation in this pathway by germ line mutation in some cutaneous melanomas and in other cancers. The 9p21 INK4A-ARF locus that contains the p16INK4A gene undergoes allelic loss in between one quarter to one third of cutaneous and uveal melanoma tumors. The INK4A-ARF locus contains two tumor-suppressor genes, p16INK4A and p14ARF (lying 20 kb apart), which have different first exons (1α and 1β, respectively) and share exons 2 and 3, although p14ARF uses an alternative reading frame, so that the two proteins share no amino acid sequence homology. p14ARF is another cell regulator protein, but acts through the p53 pathway rather than the pRb pathway.

In addition, p15INK4B maps in the 9p21-22 region. This protein having a very similar if not identical function in the cell cycle by inhibiting cyclin-dependent-kinase (CDK)-4, which phosphorylates and inactivates pRb protein. Because of the proximity of the two genes, the high number of 9p21 alterations in many tumors can often overlap both genes. The
p15INK4B gene is known to be deleted along with p16INK4A in nearly 10% of cutaneous melanoma tumors.5,10 p15INK4B mutations have been found in a number of cancers, particularly leukemias (up to 30% of some forms).16 Rare point mutations of the p14ARF (see Ref. 20) and p15INK4B (see Ref. 19) genes have been detected in a few cases of cutaneous melanoma tumors.

The Wnt/wingless pathway gene β-catenin was also screened for mutations, because it has been shown to be mutated in approximately one quarter of cutaneous melanoma cell lines.21 The Wnt pathway is mediated by β-catenin, which activates members of the LEF1/TCF transcription factor family—in turn, activating the transcription of Wnt target genes such as cyclin D1.

In our study, we describe analysis of key members of the TGFβ, pRb, and Wnt pathways for somatic mutations in sporadic uveal melanoma. We specifically examined key components of these pathways that have previously been shown to be mutated or methylated in cutaneous melanoma and other cancers. Mutational analysis was confined to exons that contain known mutational hot spots. Denaturing high-performance liquid chromatography (DHPLC; Transgenomic, Crewe, UK) was used to look for mutations, because of its high throughput and sensitivity. Genes screened include the TGFβ pathway member TGFβR2 (exons 3, 5, and 7), the mutation of which is known to play a role in the formation of colon and gastric cancers.22 Another TGFβ pathway member investigated was Smad4 (exons 8–11), because it is known to be mutated in pancreatic cancers.23 The exon 3 region of the β-catenin gene is known to be mutated in numerous cancers and was sequenced in many of our tumors. Allelic loss of the 9p21-22 p16INK4A locus has been observed in approximately 25% of both uveal5,24 and cutaneous25 melanoma tumors, and we therefore decided to look at this region in our series of tumors (Fig. 1). We also screened for mutations in the cell-cycle regulatory genes mapping to the 9p21 locus: p16INK4A (exons 1a and 2), p14ARF (exon 1β), and p15INK4B (exon 2).

**Materials and Methods**

**Melanomas**

A total of 66 primary uveal melanomas collected from enucleations at Moorfields eye hospital were used in this study, along with a uveal melanoma liver metastasis. The median age of the patients was 61 years, and there was a small bias toward male specimens (56%). These tumors were predominantly choroidal (74% of tumors), with smaller numbers of ciliary body (12%)– and iris–stromal (14%) tumors. All tumors were fixed in 10% formalin and embedded in paraffin for sectioning. The median age of the patients was 61 years, and there was a small bias toward male specimens (56%). These tumors were predominantly choroidal (74% of tumors), with smaller numbers of ciliary body (12%)– and iris–stromal (14%) tumors. All tumors were fixed in 10% formalin and embedded in paraffin for sectioning. However, characteristic versus noncharacteristic melanomas were not well defined.

**Polymerase Chain Reaction**

PCR reactions were of 50-μL volume, with a commercial PCR buffer (Bioline, London, UK), 1 mM MgCl₂, 200 μM dNTP, 20 to 100 ng genomic DNA template, and 0.5 U Taq polymerase, unless otherwise specified. All PCR assays were run on a commercial system (Hybaid, Ashford, UK). PCR primer sequences were taken from previously published reports (see Table 1 for references for all exons screened) or designed from genomic sequences to exons of interest. These primer pairs either spanned the entire exon and intron–exon junction or were split into two overlapping PCR products if the fragments were too long for DHPLC analysis and sequencing. Exons that were too large and had to be split were p16INK4A exon 2 and p14ARF exon 1β. PCR products were then used for mutational analysis by DHPLC. Exon 3 of β-catenin did not produce a PCR fragment suitable for DHPLC analysis, and PCR was therefore undertaken on 10 tumors and the products sequenced. β-Catenin exon 3 and p15INK4B exon 2 were not covered fully by these PCR fragments, but the exon 3 fragment had 88.5% of the exon sequence including all the key glycogen synthase kinase-3β phosphorylation site,25 and the p15INK4B exon 2 fragment represented more than 80% of the exon sequence. The p15INK4B exon 2 primers were a gift from Nadem Z. Soufi (Laboratoire de Biochimie B, Hormonologie et Genetique, Hopital Bichat-Claude-Bernard, Paris).

**Heteroduplex Formation and DHPLC Analysis**

Heteroduplex formation was performed by heating the PCR products for 5 minutes at 94°C, followed by cooling to 4°C at a rate of 0.03°C per second, and the PCR products were then analyzed by DHPLC. The optimum temperatures for the analysis for each fragment were calculated by computer (WAVE-Maker software; Transgenomic). Any variants detected were then purified and sequenced.50 In addition, for each exon screened PCR products for 10 tumors were also sequenced to confirm the data generated by DHPLC.

**PCR Product Purification and Sequence Analysis**

PCR products were purified, using a PCR purification kit (QiAquick; Qiagen, Crawley, UK). Purified PCR products are then directly sequenced (BigDye terminator chemistry; Applied Biosystems, Warrington, UK) and analyzed (model 377 automated sequencer; Applied Biosystems).
TABLE 1. Primer Sequences Used, with References

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Primer Sequence</th>
<th>Annealing Temperature</th>
<th>Reference</th>
</tr>
</thead>
</table>
| TGFβR2        | 3     | F: TGCAATGAATCTCTTGACTC  
               |       | R: CCCACCTTTATTAGACAGA  
               |       | 55°C                  | 22        |
|               | 5     | F: GGCAGCTGAAATTTAAATGATGGGC  
               |       | R: TGCTGAAAGCAACACATG  
               |       | 55°C                  | 22        |
|               | 7     | F: CCACACATGGCTGGCCCTTTTG  
               |       | R: CTCTGGGACATGCCCAGCTTG  
               |       | 55°C                  | 22        |
| Smad4         | 8     | F: TGTTTTGTGGTGTCTTTCATTCTC  
               |       | 54°C                  | 26        |
|               | 9     | F: TATTTTTTTAAGTACCTTTCGTGAC  
               |       | R: GTTTAAGCGTCTTACATATCTG  
               |       | 54°C                  | 26        |
|               | 10    | F: AGGACATTGTGTTTTAATGTTG  
               |       | R: CTGCTCAAAGAAACTAATCAAC  
               |       | 57°C                  | 26        |
|               | 11    | F: CAAAAGTGGTGACGTGGTTG  
               |       | R: CAGTTTCTGTCTGCTAGGAG  
               |       | 57°C                  | 26        |
| p14ARF        | 1β—1  | F: TCAGGAGGGGGGCGGTTGGCG  
               |       | R: GCCGGGGAGTGACAGCAACCA  
               |       | 60°C                  | 16        |
|               | 1β—2  | F: GCCCGGAGTGAAGGGTTTT  
               |       | R: CACCCGGGTTATCTCCCTC  
               |       | 60°C                  | 16        |
| p15INK4B      | 2     | F: GCCCTCCTGACTGCTGCT  
               |       | R: GTGGGCGGGCTGGGCGAGGCC  
               |       | 64°C                  | Gift from N. Soufir |
| p16INK4A      | 1     | F: GGGAGGCGATGAGCGCGCGCG  
               |       | 63°C                  | 27        |
|               | 2A    | F: AGCTCTCTTCTCCGTATCG  
               |       | R: GCAGACACACAGCGCGTG  
               |       | 55°C                  | 27        |
|               | 2—2   | F: GCCGCCCGCACCTCTCACCC  
               |       | R: GTGCTGAAAGTTAGGTCCTG  
               |       | 55°C                  | 28        |
| β-Catenin     | 3     | F: ATGGAGCACAGCAAAAGGC  
               |       | R: GCTACTGTGTCTGAGTGAG  
               |       | 56°C                  | 25        |
| Nested methylation PCR  
inner and outer primer pairs) | p16A | — | F: GTTGGTTTATAGGAGGGTGGG  
|               |       | R: CTTCCACCITAACTATCTCAC  
|               | p16B | — | 64°C                  | 29        |

F, forward primer; R, reverse primer.

Methylation Analysis

Approximately 100 to 200 ng DNA from each tumor was sodium bisulfite modified. The DNA was denatured in 0.3 M NaOH in a volume of 20 μL for 15 minutes at 37°C. A solution of 120 μM 5.6 M sodium bisulfite and 0.6 mM hydroquinone was added, and the samples were cycled for 5 hours (30 seconds at 95°C, 15 minutes at 50°C for 40 cycles) in a PCR apparatus (Hybaid). The DNA was then desalted with a DNA purification resin (Wizard; Promega, Southampton, UK) and desulfonated by the addition of 5 M NaOH to 0.3 M and incubated at room temperature for 5 minutes. The DNA was neutralized with glacial acetic acid and then precipitated with two volumes of ethanol in the presence of yeast transfer (t)RNA and 0.01 M MgCl₂. DNA was then resuspended in 30 to 40 μL sterile water and amplified by nested PCR with Taq polymerase (Red-Hot Taq; ABgene, Epsom, UK) and buffers.²⁹

Loss-of-Heterozygosity Analysis and Microsatellite Markers

Dye-labeled PCRs of highly polymorphic microsatellite markers positioned around the 9p21-22 p16INK4A locus (D9S1748 and D9S1749) were used for loss-of-heterozygosity (LOH) analysis. This region is known to show allelic loss in numerous cancers, particularly in cutaneous melanoma. The reported heterozygosity of these markers was 0.87 and 0.94 for D9S1748 and D9S1749, respectively. PCR was performed under standard cycling conditions on 18 extracted blood and tumor pairs. Microsatellite profiles were then visualized and analyzed in an automated sequencer (Li-Cor 4200 GeneReadIR; MWG, Milton Keynes, UK). The data were collected automatically and analyzed on computer (GeneImageIR RFLPscan Plus ver. 3.0; Scanalytics, Fairfax, VA). LOH was quantitatively assessed according to the method of Okabe et al.¹¹ This method calculates the LOH index, defined as the allele ratio in normal tissue divided by the allele ratio of the tumor tissue. The allele ratio was calculated as the peak height of the smaller allele divided by the peak height of the larger allele. Allelic loss was defined by an LOH index of less than 0.5 or more than 2.0.

RESULTS

Mutational Analysis

No mutations were identified in any of our tumor samples in any of the exons screened. Screening of p16INK4A exon 2 produced variant DHPLC profiles (distinct doublet peaks) in three samples, with the sequencing showing that they were all heterozygous for a previously reported Ala148Thr polymorphism.²² (Fig. 2).

LOH Analysis

This series of tumors had previously undergone LOH analysis for the 3p22 TGFβR2 locus, finding allelic loss in approximately 30% of the tumors.⁷ There was then enough DNA remaining in 18 blood and tumor pairs to allow for LOH analyses to be performed on a few more loci (Table 2, Fig. 3). Of these 18 pairs of samples analyzed for 9p21-22 p16INK4A LOH, 2 (11.1%) samples were uninformative at both loci, 5 (27.8%) were informative for both loci, and 11 (61.1%) were
informative at only one locus. Of the 16 samples that were informative, 5 showed LOH in at least one of the loci (31%). Of these samples showing LOH, two displayed LOH at one locus only. Some samples could not be analyzed for technical reasons (labeled in Table 2 as ND, no data). After repeating analyses of most of these samples, we did not have enough DNA for further loci to be examined.

**Methylation Analysis**

Evidence of methylation of the p16INK4A promoter was detected in 5 (9.1%) of 55 of the tumors examined. Only the reverse-sequenced strand produced good enough quality sequencing (Fig. 4). Because of the small number of samples screened for LOH, it was not possible to determine whether there was any association between the samples that had p16INK4A methylation and 9p21-22 p16INK4A LOH.

**DISCUSSION**

In a previous study, we examined the expression of members of the TGFβ pathway by immunocytochemistry, and found that the expression of Smads 2, 3, and 4; p27; and TGFβR2 was absent in a large percentage of uveal melanoma tumors, along with a loss of TGFβ responsiveness. In the current study, we investigated whether this loss of expression was due to somatic mutation of key members of these pathways—events that would be consistent with the absence of TGFβ responsiveness and with the potential abrogation of this and other pathways. Because uveal melanoma tumors have no obvious major inherited component we concentrated on finding somatic mutations in these pathways. We also screened for genetic alterations several genes known to be altered in cutaneous melanoma—such as p14ARF, p15INK4B, p16INK4A, and

**TABLE 2.** Table of LOH and p16INK4A Methylation Results for 18 Blood-Tumor Pairs

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Age at Enucleation</th>
<th>Site of Tumor</th>
<th>Cell Type</th>
<th>p16 Methylated</th>
<th>P16 LoH: 9p21-22</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>D9S1748</td>
</tr>
<tr>
<td>1</td>
<td>96/3</td>
<td>40</td>
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<td>ND</td>
<td>NI</td>
</tr>
<tr>
<td>2</td>
<td>96/5</td>
<td>73</td>
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<td>Epithelioid</td>
<td>No</td>
<td>NI</td>
</tr>
<tr>
<td>3</td>
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<td>39</td>
<td>CB/Choroid</td>
<td>Spindle</td>
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<td>NI</td>
</tr>
<tr>
<td>4</td>
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<td>79</td>
<td>CB</td>
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<td>–</td>
</tr>
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<td>5</td>
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<tr>
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<td>Spindle</td>
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</tbody>
</table>

ND, no data; NT, not tested; NI, not informative; +, LOH; –, no LOH; CB, ciliary body.

*Tumors were shown to be methylated, but were not tested for LOH.*

**FIGURE 2.** (A) A positive DHPLC trace, showing a wild-type sequence with a wild-type DHPLC profile and a p16INK4A exon 2 variant with a variant DHPLC profile and a distinct doublet peak. (B) Sequence showing the p16INK4A exon 2Ala148Thr polymorphism, with the wild-type sequence shown above the tumor’s heterozygous sequence.
β-catenin—and we found it unlikely that mutation of these important members of the pRb, TGFβ, and Wnt pathways are responsible for disregulation in these pathways. It is known that DHPLC analysis has difficulty in resolving C-to-G transversions and also has difficulty analyzing high-melting domains embedded in low-melting DNA regions, such as β-catenin exon 3. Apart from these slight problems, DHPLC analysis is considered to be very accurate, with a sensitivity and specificity thought to be higher than 96%. In a study screening 113 amplicons containing 14 different BRCA1 mutations DHPLC resolved 100% of the alterations, compared with 96 using single-strand conformation polymorphism (SSCP) analysis. Most mutations that were not detected by DHPLC would have been expected to be detected from the sequencing of each exon, especially in that we concentrated on codons with published mutations. Not all the exons were screened, and very little of the surrounding intronic sequence was examined; therefore, some mutations could have been missed by this analysis.

There were other genes in these pathways that have had mutations noted in certain cancers, Smad2 being a notable example, but many of these published mutations are thought to be very rare. There is also a possibility the mutations are present in unscreened exons of these genes (e.g., Smad4 exons 1–7, p16INK4A exon 3). However, these regions do not have the same degree of structural conservation across the species and are without the same frequency of published mutations in other cancers as the mutational hot spots analyzed in this study.

A minority of these uveal melanoma tumors displayed methylation of p16INK4A promoter (10%) and allelic loss of the 9p21 p16INK4A locus (31%), although not in the same tumors. Because of the small number of samples and particularly the small number of positive samples, no significant correlations could be made between these DNA abnormalities and any clinical or histologic features. Ideally, we would have liked to examine further loci; but, unfortunately, DNA was limited. The level of 9p21 LOH observed in this study (31%) was consistent with previous molecular studies of uveal melanoma, which have found levels of p16INK4A LOH of between 24% and 32%. Other studies have also detected p16INK4A homozygous deletions in approximately 12% of tumors and intronic p16INK4A mutations. The alterations noted may all contribute to lower levels of expression of many of these genes, and if there are enough key members of these pathways with reduced expression, these may act as contributory factors to the development of melanoma.

Previous studies have found constitutional p16INK4A mutations in a substantial proportion of familial cutaneous melanoma (>40%) and in up to 75% of cutaneous melanoma cell lines, but not in the small number of families predisposed to uveal melanoma, although it is probable that most familial cases of uveal melanoma represent an aggregation of sporadic cases rather than true Mendelian inheritance. The number of sporadic cutaneous melanoma cases involving the p16INK4A mutation is much lower, varying between 3.3%, 24% and 25%. The frequency of p16INK4A methylation found (10%) is consistent with levels in cutaneous melanoma (10%) and is between levels recorded in previous studies in uveal melanoma (6% to 32%). The higher frequency recorded by van der Velden et al. was obtained by using the more sensitive technique of methylation-specific PCR, which can detect tumors with only partial methylation.

Samples were regarded as methylated when the level of each methylated CG dinucleotide was greater than the unmethylated dinucleotide (TG), suggesting greater than 50% methylation of the target DNA. There were a small number of samples that were unmethylated but had very low levels of CG (<5%), but we could not be sure whether this was due to methylation or to background unincorporated dye terminators in the sequencing trace. Low levels of methylation of this magnitude and even levels too small to be seen in the sequence trace are theoretically detected by the alternative technique used by van der Velden et al. Because of the purity of the uveal melanoma tumor DNA samples, the lower levels of methylation noted are unlikely to be due to contamination from normal tissues. Contamination from partially bisulfite-modified DNA is also unlikely, because the primers were designed so that unmodified DNA would not amplify.

The absence of expression of Smads 2, 3, and 4; p27; and TGFβR2 noted in a large percentage of uveal melanoma tumors without any obvious inactivating mutations discovered, could be explained by promoter hypermethylation of these genes. However Smad4, p27, and TGFβR2, have been screened for promoter hypermethylation in a small number of tumors.

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**FIGURE 3.** Electropherogram, showing LOH assessment on the chromosome 9p21-22 locus. Blood is compared with tumor tissue, and the density units of each allele are compared (y-axis). The lower (smaller) allele is deleted in the tumor.

**FIGURE 4.** Examples of bisulfite-modified DNA sequence from uveal melanoma tumors showing methylated and unmethylated sequence of the p16[INK4A] promoter. In the unmethylated sequence, all cytosines are converted by the bisulfite modification step and PCR to thymidine, whereas, in the methylated sequence, all 5-methylcytosines are resistant to modification and remain cytosines. This DNA was extracted from a nonpigmented, spindle-cell choroidal tumor from a 48-year-old woman (sample 98/35, Table 2).
types, with no Smad4 methylation discovered to date.41 Some promoter hypermethylation has been detected in a small number of cancer cell lines in the p2742 and TGFβR2 genes,43 although not in any significant amounts in primary tumors.44,45 That there were no significant amounts of methylation of these genes in other tumors does not discount it as a plausible mechanism in uveal melanoma. This should be investigated further.

Uveal melanoma has a low cellular proliferation rate and is different than cutaneous melanoma in behavior and response to chemotherapy.46 These findings further highlight the molecular differences between uveal and cutaneous melanoma and suggests that in uveal melanoma, methylation may play a more important role than somatic mutation.

In summary, no genetic mutations were detected in any of the Wnt, pRb, and TGFβ pathway genes that we screened, although in this series approximately 10% of the tumors had high levels of p16INK4A promoter methylation and approximately 31% of the tumors also showed loss of heterozygosity of the 9p21 p16INK4A locus. Because of the limited availability of samples, protein expression studies of could not be performed. Thus, further studies are needed to relate the molecular alterations in the 9p21 p16INK4A locus with p16 INK4A protein expression. Mutations in Smad4, TGFβR2, β-catenin, p16INK4A, p14ARF, and p14ARF are unlikely to be frequent contributors to uveal melanoma. So far, non-cancer-causing genes have been found to undergo inactivating mutations in most uveal melanomas, suggesting that there may be other mechanisms of tumorigenesis in uveal melanomas that are yet to be discovered.

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References


