Loss of Heterozygosity on Chromosomes 3, 9, 13, and 17, Including the Retinoblastoma Locus, in Uveal Melanoma

Andrea G. M. Scholes, Triantafillos Liloglou, Paul Maloney, Suzanne Hagan, Janice Nunn, Paul Hiscott, Bertil E. Damato, Ian Grierson, and John K. Field

PURPOSE. To identify tumor-suppressor loci that may contribute to the pathogenesis of uveal melanoma.

METHODS. Multiplex fluorescence microsatellite assays were performed on 27 uveal melanomas using markers at 3p25-p26, 3p14.2, 9p21-p23, 13q14, 13q12.3-q13, and 17p13, close to or within the von Hippel Lindau (VHL), fragile histidine triad (FHIT), p16/cyclin-dependent kinase inhibitor 2 (CDKN2A), retinoblastoma (RB1), breast cancer 2 (BRCA2), and p53 tumor suppressor loci, respectively. Further markers on chromosomes 3 and 9 were analyzed individually.

RESULTS. Loss of heterozygosity (LOH) was identified in 63% of tumors, most frequently on chromosome 3 (52%), in association with epithelioid cells (P = 0.0002) and microvascular loops (P = 0.0008). In the majority of cases, LOH on chromosome 3 was detected at all informative markers. The second most common alteration was LOH at an RB1 intragenic marker (21% tumors), with retention of a more centromeric 13q marker (near BRCA2). The pattern of LOH on chromosome 9p was consistent with the involvement of a region telomeric to CDKN2A. LOH at TP53 was infrequent.

CONCLUSIONS. In the majority of cases, chromosome 3 LOH involves an entire chromosome homologue, which hampers identification of the relevant suppressor loci. This LOH correlates with the presence of microvascular loops and epithelioid cells, two of the recognized histologic indicators of poor prognosis. Data for chromosomes 13 and 9 support a role for RB1 in the pathogenesis of uveal melanoma but also raise the possibility of the involvement of additional loci close to RB1 and CDKN2A. (Invest Ophtalmol Vis Sci. 2001;42:2472–2477)

Uveal melanoma is the most common primary intraocular malignancy in adults, although the incidence, approximately six per million per year in whites, is relatively low compared with that of cutaneous melanoma. Overall mortality remains high (approximately 50%), however, due to the propensity of uveal melanoma to metastasize to the liver. After clinical diagnosis of hepatic metastases, life expectancy is extremely poor, and the median survival time is only 7 months.

Cytogenetic analyses of uveal melanoma have identified loss of an entire chromosome 3 homologue (monosomy 3) and increased chromosome 8q copy number, often coexisting, in approximately 50% of cases. These alterations show a significant correlation with metastasis and decreased survival, and appear to be better prognostic indicators than selected clinical and histopathologic criteria, at least in the short-term. Altered imbalance on chromosome 3 has been found at high frequency in many tumor types, and it has been proposed that at least three distinct regions on the chromosome’s p arm contain tumor-suppressor genes (TSGs). The presence of monosomy 3 in uveal melanoma suggests a requirement for inactivation of multiple suppressor genes, but there have been few molecular genetic studies of uveal melanoma, and the involvement of known or putative TSGs on this chromosome has not been identified.

We addressed the question of whether LOH at specific TSG loci is associated with prognostic indicators in uveal melanoma using microsatellite assays. Similar molecular mechanisms may be operative in the development and progression of many types of neoplasia, in that there is considerable overlap among the chromosomal imbalance profiles of tumors from different tissues. Thus, the markers chosen initially for this study of uveal melanoma were located close or intragenic to known or putative TSGs involved in other tumor types: von Hippel Lindau (VHL), fragile histidine triad (FHIT), p16/cyclin-dependent kinase inhibitor 2 (CDKN2A), retinoblastoma (RB1), breast cancer 2 (BRCA2), and p53. The VHL and FHIT genes were of particular interest due to their localization on chromosome 3, the loss of which is associated with a poor prognosis in uveal melanoma. Further chromosome 3 markers were also analyzed individually, to determine the extent of the detected chromosome 3 LOH and to identify other putative uveal melanoma loci. The CDKN2A (p16) gene on chromosome 9p21 has been implicated in the pathogenesis of uveal melanoma. We used a range of chromosome 9p markers to determine whether additional loci are involved, as suggested in other tumor types. The BRCA2 TSG on chromosome 13q was included, because the incidence of uveal melanoma appears to be increased in families with BRCA2-related breast cancer. This gene lies centromeric to prototype ocular TSG, RB1. Both RB1 and TP53 have been implicated in the pathogenesis of a large range of cancers.

MATERIALS AND METHODS

Patients and Samples

Twenty-seven consecutive patients treated for uveal melanoma by enucleation or local resection (as the primary treatment) were included in this study. Informed consent was obtained from all patients, according to the tenets of the Declaration of Helsinki, and tumor and...
blood samples were collected for experimental purposes. Part of each tumor was snap-frozen in liquid nitrogen, and the remainder was formalin-fixed and paraffin-embedded. Histologic features, including confirmation of diagnosis, tumor cell type and the presence or absence of microvascular loops, were recorded from hematoxylin and eosin, and periodic acid-Schiff–stained sections of paraffin-embedded tumors. Clinical details were recorded in a computerized patient database at the time of examination.

**Microsatellite Analysis**

DNA was extracted from blood and 10-μm frozen tumor sections using standard procedures. A 5-μm section from each tumor was stained with hematoxylin and eosin to confirm the histology and to ensure that the specimen was composed of at least 90% tumor cells.

Fluorescence microsatellite assays and analysis on an automatic sequencer were used to detect LOH. Microsatellite markers were chosen from a mapping set (Linkage Mapping Set V2.0; PE-Applied Biosystems, Warrington, UK). Seven markers were chosen, located within or close to known or putative TSGs: D3S1300 (3p14.2; intragenic FHIT), D3S157 (9p23-p22), D9S161 (9p21; CDKN2A/p16), D9S157 (9p23-p22), D13S171 (13q21-q31; BRCA2), D13S153 (13q14.2; intragenic RB1), and D17S2179E (17p13; intragenic TP53). These seven microsatellites were amplified simultaneously in multiplex reactions. The reaction mixtures contained the microsatellite primers at various concentrations (80–145 nM), buffer (GeneAmp Buffer II; PE-Applied Biosystems), 350 μM dNTPs, 2.66 mM MgCl₂, and 3.5 U polymerase (AmpliTaq Gold; PE-Applied Biosystems). Thermo-cycling conditions were as follows: initial denaturation for 12 minutes, followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with a final extension step at 72°C for 20 minutes. 

PCR products were diluted 2.5-fold in deionized distilled (dd)H₂O and mixed with loading buffer (size marker ROX-350, dextran blue, and formamide in a ratio of 1:1:5). After denaturation at 95°C for 5 minutes, samples were analyzed on an automatic sequencer (Prism 377, using Genescan and Genotyper software; PE-Applied Biosystems). 

For heterozygous samples a reduction of at least 23% (allelic imbalance factor [IF] of 0.77) in the peak area of one allele in the tumor (normalized against the retained allele) was used to score LOH. In each case the IF was determined by calculating the ratio of alleles for both the normal (N) and tumor (T) sample, and then the tumor ratio was divided by the normal ratio: T1/T2/N1:N2. If the value obtained was greater than 1.00, the reciprocal 1/(T1:T2/N1:N2) was used (to give a range of 0.00 to 1.00). Samples with 0.65 to 0.77 on initial analysis were subjected to another assay and scored as LOH only if a second result less than 0.77 was obtained. The cut-off level used (IF 0.77) was determined by calculating interassay variation using normal DNA. To assess reproducibility, 24 normal blood DNA samples were subjected to multiplex assays, with each sample repeated four times. Comparison of allelic ratios of these normal samples showed a SD of 0.076, indicating that the reaction-to-reaction variation was reasonably small and that allelic imbalance carried by at least 23% of the cells in the sample could be detected with an extremely high confidence. This cut-off was also confirmed by mixing normal and tumor DNA in various ratios.

Further microsatellite assays were undertaken using 10 chromosome 3p and 3q markers (Research Genetics, Huntsville, AL): D3S1038 (3p26.1-p25.2), D3S1283 (3p25-p24.2), D3S1619 (3p24.2-p22), D3S1029 (3p21.3-p21.2), D3S1210 (3p14.1-p12), D3S1271 (3cen-q13), D3S1589 (3q21), D3S1605 (3q25.1-25.2), D3S1580 (3q27), and D3S1311 (3q27-qter). An additional seven markers located on chromosome 9p23-p21 were also analyzed: D9S156, D9S162, D9S1846, D9S1749, D9S1748, D9S1679, and D9S171 (Research Genetics). Amplification products were analyzed on silver-stained polyacrylamide gels, and LOH was recorded for informative markers if the intensity of a tumor allele was reduced by at least 30% relative to the corresponding normal DNA. This change in allele intensity was obvious visually (see Fig. 3).

**Results**

**Clinicalopathology**

The relevant clinical and histopathologic details of the uveal melanomas studied are shown in Table 1. All tumors originated in the choroid or ciliary body, and comprised 8 spindle cell, 16 mixed cell, and 3 epithelioid cell tumors. The largest basal tumor diameter ranged from 10 to 20 mm (mean, 15.8 mm). Microvascular loops were detected in 17 (63%) of 27 tumors.

<table>
<thead>
<tr>
<th>Tumor Number</th>
<th>Tumor Site of Origin</th>
<th>Tumor Diameter*</th>
<th>Cell Type</th>
<th>Microvascular Loops</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ch</td>
<td>17</td>
<td>S</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Ch</td>
<td>15</td>
<td>E</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Ch</td>
<td>13</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Ch</td>
<td>20</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Ch</td>
<td>19</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>Ch</td>
<td>19</td>
<td>S</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Ch</td>
<td>16</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>Ch</td>
<td>15</td>
<td>S</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Ch</td>
<td>10</td>
<td>M</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>Ch</td>
<td>16</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>CB</td>
<td>20</td>
<td>E</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>Ch</td>
<td>16</td>
<td>S</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>Ch</td>
<td>11</td>
<td>M</td>
<td>No</td>
</tr>
<tr>
<td>14</td>
<td>CB</td>
<td>18</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>Ch</td>
<td>18</td>
<td>S</td>
<td>No</td>
</tr>
<tr>
<td>16</td>
<td>Ch</td>
<td>20</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>17</td>
<td>Ch</td>
<td>12</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>18</td>
<td>Ch</td>
<td>15</td>
<td>S</td>
<td>Yes</td>
</tr>
<tr>
<td>19</td>
<td>Ch</td>
<td>10</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>20</td>
<td>Ch</td>
<td>16</td>
<td>E</td>
<td>Yes</td>
</tr>
<tr>
<td>21</td>
<td>Ch</td>
<td>14</td>
<td>S</td>
<td>No</td>
</tr>
<tr>
<td>22</td>
<td>Ch</td>
<td>19</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>23</td>
<td>Ch</td>
<td>11</td>
<td>M</td>
<td>No</td>
</tr>
<tr>
<td>24</td>
<td>Ch (CB)</td>
<td>20</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>25</td>
<td>Ch</td>
<td>17</td>
<td>S</td>
<td>No</td>
</tr>
<tr>
<td>26</td>
<td>Ch (CB)</td>
<td>14</td>
<td>M</td>
<td>Yes</td>
</tr>
<tr>
<td>27</td>
<td>Ch</td>
<td>15</td>
<td>M</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Ch, choroid; CB, ciliary body; (CB), ciliary body involvement; S, spindle; E, epithelioid; M, mixed.

* Diameter across the tumor base in millimeters.

**Statistical Analysis**

The Fisher exact test was used to determine statistical correlations between LOH and clinicopathologic parameters, specifically tumor location, largest basal diameter, cell type, and the presence of microvascular loops.

**Loss of Heterozygosity in Uveal Melanoma**

Initial multiplex fluorescence microsatellite assays and analysis on an automatic sequencer were used to detect LOH, using markers close or intragenic to known or putative TSGs on chromosomes 3, 9, 13, and 17. Further analyses of all tumors were undertaken, by using an additional 10 chromosome 3 markers relatively evenly spaced along the p and q arms, to determine whether the LOH detected on chromosome 3 involved discrete regions or was consistent with monosomy 3 and to identify other regions potentially containing melanoma TSGs. All tumors were analyzed also with an additional seven 9p22-p21 markers, to determine whether any LOH detected incorporated CDKN2A (p16) and to identify further tumors with LOH in this region.
Fluorescence microsatellite assays on chromosomes 3, 9, 13, and 17 identified LOH at one or more loci in 17 (65%) of 27 uveal melanomas (Figs. 1 and 2). Imbalance was most frequently detected on chromosome 3, in 11 (52%) of 21 informative tumors using marker D3S1304 (3p25-p26), close to the VHL gene. All informative tumors showing LOH at D3S1304 also showed alteration at D3S1300, intragenic to the FHIT gene at 3p14.2. The uveal melanomas were then analyzed with further chromosome 3 markers. All tumors with chromosome 3 LOH on initial assay subsequently showed LOH at all informative markers (Fig. 3).

In addition, two further tumors with LOH on chromosome 3 were identified. Tumor 16 was non-informative with both of the chromosome 3 markers used in the initial multiplex analysis (Fig. 1) but showed imbalance at all seven informative markers analyzed subsequently. Tumor 10 showed LOH at 3q markers D3S1605, D3S1580, and D3S1311, but retention of the remaining 3p and 3q markers, thus representing LOH restricted to 3q25.1-qter.

The second most common alteration found in this set of uveal melanomas was LOH on chromosome 13. Two 13q markers were assessed by multiplex fluorescence assays: D13S153 within the RB1 gene (13q14.2) and D13S171 (13q12.3-q13), near the BRCA2 gene. The RB1 intragenic marker D13S153 showed LOH in 5 (21%) of 23 informative tumors, with retention of D13S171 in all but one of these tumors (Figs. 1, 2). LOH at D13S171 was detected in only one tumor, together with D13S153. Four (80%) of the five tumors with LOH at the RB1 locus also showed imbalance on chromosome 3.

Allelic imbalance was less frequently detected on chromosomes 9 and 17. Marker D9S157 (9p23-22) showed LOH in 3 (14%) of 21 informative tumors; imbalance was detected at D9S161 (9p21) in one tumor (tumor 14; Fig. 1). These three tumors were then analyzed with seven additional markers, including markers flanking the CDKN2A (p16) gene. All markers in tumor 14 showed imbalance but were retained in the remaining two tumors. Tumors that did not show 9p LOH on initial assessment were also analyzed with these additional markers, but LOH was not detected. Alterations on both chromosomes 9 and 13 were not found in any of the tumors studied. Allelic imbalance at a marker intragenic to the p53 gene was detected in 1 (7%) of 14 informative tumors in the absence of alterations at other loci analyzed.

**Relationship between LOH and Clinicopathologic Parameters**

Results of microsatellite assays were correlated with clinical and histopathologic features. Chromosome 3 alterations showed a significant correlation with the presence of epithelioid cells and microvascular loops. LOH on chromosome 3 was detected in none of eight spindle cell tumors compared with 15 (79%) of 19 tumors containing epithelioid cells ($P = 0.0002$) and in 14 (82%) of 17 melanomas with microvascular loops compared with 1 (10%) of 10 melanomas without these microvascular channels ($P = 0.0008$). All four tumors with involvement of the ciliary body showed chromosome 3 alterations compared with 11 (48%) of 23 tumors restricted to the choroid, but this difference was not statistically significant due to the small number of ciliary body melanomas.

**DISCUSSION**

To determine whether LOH at specific tumor suppressor loci is involved in the pathogenesis of uveal melanoma, we undertook microsatellite assays with markers close or intragenic to known or putative TSGs on chromosomes 3, 9, 13, and 17. The analysis of the complete data set indicated LOH in 63% of the melanomas—most commonly found on chromosome 3, followed by chromosome 13q at RB1, chromosome 9p telomeric to CDKN2A (p16), and chromosome 17p at TP53. Chromosome 3 LOH was significantly associated with the presence of epithelioid cells ($P = 0.0002$) and microvascular loops ($P = 0.0008$).

Previous cytogenetic studies of uveal melanoma have identified monosomy chromosome 3 in 50% to 56% of tumors, and this has been associated with a poor prognosis. The implication of monosomy 3 is that multiple genes located on both arms of the chromosome have to be inactivated for uveal melanoma progression. We anticipated that the use of microsatellite assays would allow identification of regional LOH and thus guide localization of potential tumor loci in uveal melanoma. Regional LOH, however, was detected in only one tumor (on the long arm of chromosome 3). The majority of tumors showed LOH at all informative markers, suggesting involvement of an entire chromosome 3 homologue. Genetic alterations other than deletion, for example duplication and amplification, can be detected by microsatellite analysis, but are not readily distinguishable from each other. It is probable, however, that the chromosome 3 LOH detected in the present study was a result of deletion. Major cytogenetic studies of uveal melanoma invariably have reported loss of chromosome 3 (monosomy), and recently a comparison of detection of monosomy 3 in uveal melanoma by microsatellite analysis and comparative genome hybridization showed concordant results, indicating that microsatellite analysis can be used reliably to detect this aberration.

Our findings on chromosome 3 in uveal melanoma are analogous to the alterations found on chromosome 10 in cutaneous melanoma, where monosomy 10 is more common than segmental deletions, and raise the possibility that chromosome 3 is more commonly lost by chromosome segregation than progressive deletion events in uveal melanoma. However, it is possible that multiple deletion events occurred earlier in the development of these tumors.
tumorigenesis, because the samples used in our study were from surgically resected melanomas of relatively large size (small tumors are usually treated conservatively with, for example, radiotherapy). In addition, small deletions may have been missed because of the selection of microsatellites in this study, and further analyses are now under way with a high-density set of chromosome 3 markers.

The presence of epithelioid cells has been reported as a histologic indicator of poor prognosis in many clinicopathologic studies of uveal melanoma.2,25,26 We have shown a correlation between chromosome 3 imbalance and the presence of epithelioid cells (P = 0.0002). However, cytogenetic studies have not found this correlation6 or have reported a weak association only.4,5 These cytogenetic studies also found no significant correlation between epithelioid cells and survival.4–6 The reason for the discrepancy between clinicopathologic and cytogenetic studies regarding tumor cell type and survival is unclear, but may relate to length of patient follow-up (cytogenetic study follow-up in published studies is at present much shorter than follow-up in many clinicopathologic investigations). The difference between our LOH analysis and previous cytogenetic studies may reflect the increased sensitivity of microsatellite assays. However, because the proportion of tumors with chromosome 3 alterations was similar in all reports, it appears that a difference in tumor characteristics or classification between studies may be largely responsible. In the present study of consecutive cases, for example, spindle cell tumors comprised 30% of all the tumors analyzed, whereas in previous studies this tumor type constituted 48%,2 26%,5 and 9%6 of the uveal melanomas investigated.

Neovascularization is essential for tumor growth and the establishment of metastatic disease.27 The presence of so-called microvascular loops (identified by PAS staining) is an

![Image of microsatellite markers on chromosomes 3, 9, and 13 in uveal melanoma specimen 26 after PCR amplification in multiplex reactions and analysis on an automatic sequencer to determine LOH.](image)

**FIGURE 2.** Examples of informative microsatellite markers on chromosomes 3, 9, and 13 in uveal melanoma specimen 26 after PCR amplification in multiplex reactions and analysis on an automatic sequencer to determine LOH. Electropherogram traces for the normal (N) and tumor (T) genotypes are shown (the two peaks representing the two alleles). An allelic ratio cut-off level of 0.77 (25% reduction of one allele intensity) was used to score LOH. Markers were chosen close or intragenic to known or putative TSGs. Chromosome 3 marker D3S1304 (3p26-25, VHL), showed LOH (arrow, virtual absence of one tumor allele) whereas the chromosome 9 marker D9S161 (9p21.3, CDKN2A/p16) was retained. On chromosome 13, the RB1 intragenic marker (D13S153, 13q14.2) showed LOH (arrow, if 0.35/65% reduction in the peak area of one tumor allele), but a marker centromeric to RB1, near BRCA2 (D13S171; 13q12.3-13) showed retention of heterozygosity.

![Image of microsatellite assays on chromosome 3 in uveal melanoma.](image)

**FIGURE 3.** Representative results of further microsatellite assays on chromosome 3 in uveal melanoma. Amplification products from six patients with chromosome 3 LOH, by using six different microsatellite markers visualized on silver-stained polyacrylamide gels. Comparison of the normal (lane N) and tumor (lane T) alleles for each case shows a markedly reduced intensity of one allele in each tumor (LOH, arrows).
indicator of poor prognosis in uveal melanoma. The presence of PAS-positive loops and imbalance on chromosome 3, which is also associated with aggressive biological behavior of uveal melanoma. Currently, there is a great deal of interest in the formation of microvascular channels in uveal melanoma, because it has been suggested that these structures can be formed by highly invasive tumor cells with a gene expression pattern characteristic of endothelial cells. Whether the correlation between PAS-positive loops and LOH on chromosome 3 reflects incidental coexistence in aggressive uveal melanoma or whether genes on chromosome 3 influence the formation of these structures remains to be determined.

The RB1 gene on chromosome 13q14.2 is the prototypical TSG, classically inactivated in retinoblastoma, the most common intraocular tumor in childhood. In this study of uveal melanoma, LOH was detected at an RB1 intragenic marker in 21% of tumors, raising the possibility of disruption of the pRB function in these structures.

In this study of uveal melanoma, LOH was detected at an RB1 intragenic marker in 21% of tumors, raising the possibility of disruption of the pRB function in these structures.

The RB1 gene is not the primary target on chromosome 13q, but that a locus closely linked to, but distinct from, RB1 may be involved. The BRCA2 TSG is centromeric to RB1, and the incidence of uveal melanoma appears to be increased in families with BRCA2-related breast cancer. Germ-line alterations in BRCA2, however, have been reported in only a small proportion of patients with ocular melanoma who have a family history of breast or ovarian cancer or ocular melanoma, suggesting that there may be additional loci that contribute to the association between these familial cancers.

In the present study, a marker near BRCA2 was retained in all but one of the tumors with RB1 LOH, suggesting that BRCA2 is not the primary target in uveal melanomas with chromosome 13q imbalance. It is of interest that Brantley and Harbour recently proposed inactivation of pRB by an alternative mechanism in uveal melanoma, through phosphorylation of specific residues in the COOH-terminal region.

An increased risk of uveal melanoma has been reported in patients with cutaneous melanoma and atypical mole syndrome. Linkage analysis has implicated chromosome 9p21 in familial cutaneous melanoma and mutation of the CDKN2A (p16INK4a) TSG has been identified in some, but not all, 9p21-linked families. There is increasing evidence for an additional TSG other than CDKN2A. An increased risk of uveal melanoma has been reported in patients with cutaneous melanoma and atypical mole syndrome. Linkage analysis has implicated chromosome 9p21 in familial cutaneous melanoma and mutation of the CDKN2A (p16INK4a) TSG has been identified in some, but not all, 9p21-linked families. There is increasing evidence for an additional TSG other than CDKN2A.

The pattern described by Merbs and Sidransky was apparent retention of heterozygosity at CDKN2A flanked by markers showing LOH, interpreted as homozygous deletion of CDKN2A. However, in our study an additional pattern was detected, with LOH present only at marker D9S157, 9 centromorgans telomeric to CDKN2A, results similar to those reported previously in a study of sporadic cutaneous and uveal melanomas. These findings support the concept that a tumor suppressor locus other than CDKN2A is present on chromosome 9p23-21, and thus fine mapping of this region is required in uveal melanoma.

In conclusion, we have shown that chromosome 3 LOH correlates with the presence of microvascular loops and epithelioid cells, two of the recognized histologic indicators of poor prognosis in uveal melanoma. Furthermore, our data support a role for RB1 inactivation in the pathogenesis of uveal melanoma, but also raise the possibility of the involvement of additional loci close to RB1 and CDKN2A.

References