Transformation of Cell Type in Uveal Melanomas
A Quantitative Histologic Analysis
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Objective: To describe the cytologic transformation and tumor progression in a series of uveal melanomas.

Methods: Fifteen cases of uveal melanoma, treated by primary transscleral local resection without primary adjuvant treatment, needed enucleation because of local tumor recurrence. Cytologic and cell morphometric features of the primary tumor and the intraocular recurrence were compared, with evaluation of the amounts of intermediate cells, epithelioid cells, mitotic figures, and nucleolar area.

Results: The cases were categorized into 2 groups, according to their cytologic characteristics. In the first group (5 cases), there was no cytopathological transformation in the recurrent tumor. The nucleolar area was increased in only 1 case. In the second group (10 cases), the recurrent tumors showed transformation into a more epithelioid cell type. In all but 1 case there was an increase in epithelioid cells in the tumor recurrence. The nucleolar area was increased significantly in all cases. The mean local recurrence interval in all cases was 15.3 months, with no difference between the groups. Death from metastases occurred in 7 cases in which the nucleolar area was 4.2 µm² in the primary tumor.

Conclusions: These findings demonstrate that, in an individual tumor, the cytologic phenotype can change considerably even after a relatively short time, resulting in an increase in tumor-related mortality.

Clinical Relevance: Studies on the natural course of uveal melanoma have been very limited and based purely on observations on the progression of melanomas in terms of size and alteration of various clinical characteristics.


The understanding of the natural course of uveal melanomas is limited and has been based mainly on clinical observations either at a very early stage of the disease or in patients who have refused therapy. It has been proposed that uveal melanomas have variable growth rates, which are usually slow in the early stages of the disease and more rapid in the later stages. It is generally accepted that uveal melanomas arise from pre-existing benign melanocytic lesions such as nevi, but it is still impossible to establish with certainty which lesions are harmless and which will undergo a malignant transformation during their evolution. Furthermore, it is difficult to estimate when malignant transformation occurs.

Sequential histopathological examination of uveal melanomas has not previously been performed, leaving unanswered the question of whether the cytologic characteristics of a melanoma can change over time. Tumor growth has been studied in vivo by measuring changes in tumor dimension, but a cytopathological and morphometric study of the histologic features of an intraocular melanoma at 2 stages in its evolution has not yet been performed.

Each case represents a biological entity and therefore should be treated according to its individual characteristics. Treatment modalities against uveal melanomas range from invasive, eg, enucleation, transscleral resection, and recently enucleosurgery, to conservative approaches, such as brachytherapy (ruthenium, iodine, etc), teletherapy (proton beam, helium ion, stereotactic irradiation, etc), transpupillary thermotherapy, and others. To date there is no evidence that any treatment modality is superior in terms of protecting from death from metastases, and that treatment should be dependent on the progression and stage of the disease. Therefore, a better knowledge of the natural course of uveal melanomas may improve our management of them.
PATIENTS AND METHODS

Between 1970 and 1989, 313 cases of uveal melanoma were treated in the Tennent Institute in Glasgow by transscleral local resection. Fifteen of these patients developed local intraocular recurrence and were treated by enucleation. None of these patients received primarily any additional adjuvant treatment during this period. The follow-up information was obtained from hospital records and by correspondence with referring centers.

PRIMARY TRANSSCLERAL RESECTION SPECIMENS

After 4% glutaraldehyde fixation and measurement of dimensions, each primary tumor resection specimen was cut in the long axis and additional blocks were taken from the edges of the tumor to assess clearance. At least 5 blocks were examined per specimen, and in most, between 6 and 8 blocks were processed for paraffin embedding.

ENUCLEATION SPECIMENS

After fixation, the globe was cut in a plane to include the pupil, the optic nerve, and the surgical coloboma, with the bulk of the recurrent tumor in a single block. If there were multifocal tumor nodules, appropriate additional blocks were taken. In all cases, multiple sections were obtained from the paraffin-embedded blocks and were stained routinely with hematoxylin-eosin, periodic acid-Schiff, Prussian blue, Loyez, and Bodian stains. If the tumor was heavily pigmented, bleached preparations were examined.

Transscleral local resection has been practiced in Glasgow, Scotland, in a large number of patients with melanoma since the early 1970s.2 Fifteen of these patients, who had not received any additional adjuvant treatment and developed local recurrence, were treated by enucleation. Comparison of the histologic characteristics of the tumor in the primary excision specimen with those of the recurrent tumor in the enucleated eye allowed us to investigate changes in the cytologic features of the individual tumors, giving an insight in the natural progression of the disease. Since the time intervals between primary and secondary surgery were known, we had the opportunity to determine the rapidity with which an individual melanoma could undergo transformation from a more benign to a more malignant phase.

The following cytologic definitions were used in assessing the various uveal melanoma cell types. Spindle A cells were spindle shaped, with a linear infold in the nucleus. Spindle B cells had round to oval nuclei, prominent nucleoli, and indistinct cell borders (Figure 1A). Small epithelioid or intermediate cells were larger than typical B cells and had round to oval nuclei, prominent nucleoli, abundant cytoplasm, and indistinct cell borders (Figure 2A). Epithelioid cells were larger than intermediate cells but had distinct cell borders and an eosinophilic cytoplasm (Figure 2B and Figure 3B).

The time interval between primary treatment and local recurrence was 15.8 months in the first group and 15.0 months in the second. The 2 groups showed no significant differences with respect to patient age at the time of local resection and the largest tumor diameter of the primary excision specimen (Table 1).

GROUP 1 (NO CHANGE IN PHENOTYPE)

Cytologic Features

In the 5 tumors without apparent change in phenotype, there were no statistical differences in the proportions of intermediate cells, epithelioid cells, and mitotic figures in the primary excision specimen and the recurrence in the enucleated eye (Table 2 and Table 3), al-
though there was some variation between cases. In case 3, although the primary tumor and the recurrence were spindle cell tumors, the recurrence had a higher proportion of intermediate cells. In contrast, in case 4, in which both the primary and recurrent melanomas were of mixed cell type, the recurrent tumor had a lower count of epithelioid cells. There was no difference in the mitotic rate in the primary and the recurrent tumors.

Measurement of Nucleolar Area

In cases 1, 2, and 3 (spindle cell tumors), the nucleolar area in the primary tumors varied between 2.6 and 3.5 µm² and in the corresponding recurrences, between 3.1 and 3.7 µm². Only in case 2 was there a significant increase in nucleolar area in the recurrent tumor. In cases 4 and 5, which were primarily mixed cell tumors, the nucleolar areas were greater (ie, 4.0 and 6.6 µm², respectively), with similar values (3.8 and 5.5 µm², respectively) in the recurrences. In each case, there was no significant difference between the values obtained in the primary and recurrent tumors (Table 4).

GROUP 2 (WITH CHANGE IN PHENOTYPE)

Cytologic Features

Four of the 10 cases with apparent transformation of cell type in the recurrent tumor were primarily composed of spindle cells (Figure 1), and the remaining 6 cases were primarily classified as mixed cell tumors (Figures 2 and 3). All cases showed a marked increase in intermediate and/or epithelioid cell counts in the recurrent tumors (Tables 2 and 3).

On review, we found that all the primary tumors originally classified as of spindle cell type contained vari-
able numbers of intermediate cells. In each of these cases the recurrent tumor contained typical epithelioid cells, which were diffusely scattered within the tumor in cases 8 and 9 and localized only in the extraocular extension in cases 6 and 7. In all but one (case 7), the number of intermediate cells was increased in the recurrence ($P = .002$).

The number of epithelioid cells increased significantly in all but one case (case 7), in which the change was borderline ($P = .06$). The overall $P$ value for the epithelioid cell increase in the second group was .005.

In 7 cases there was a marked increase in the mitotic rate from 0.05 to 0.41 mitoses per high-power field in the recurrent tumors ($P = .004$).

**Measurement of Nucleolar Area**

The nucleolar area in the primary tumors varied between 2.3 and 4.5 $\mu m^2$, with a mean of 3.47 $\mu m^2$, and in the corresponding recurrences between 3.8 and 6.8 $\mu m^2$, with a mean of 5.13 $\mu m^2$. In each case and also overall, the difference was significant (Table 4).

**CORRELATION OF NUCLEOLAR AREA WITH METASTATIC DISEASE IN ALL CASES**

The mean nucleolar area of the primary tumors in patients who did not develop metastatic disease was $3.2 \pm 0.7 \mu m^2$, whereas the mean nucleolar area of the primary tumors in patients who died of metastatic uveal melano-

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**Table 1. Clinical Data**

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Largest Tumor Diameter, mm</th>
<th>Cell Type (Primary)</th>
<th>Recurrence Interval, mo</th>
<th>Follow-up, mo</th>
<th>Status*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/M/38</td>
<td>15</td>
<td>Spindle</td>
<td>17</td>
<td>44</td>
<td>M</td>
</tr>
<tr>
<td>2/M/49</td>
<td>17</td>
<td>Spindle</td>
<td>27</td>
<td>40</td>
<td>A + W</td>
</tr>
<tr>
<td>3/M/61</td>
<td>14</td>
<td>Spindle</td>
<td>3</td>
<td>53</td>
<td>M</td>
</tr>
<tr>
<td>4/M/72</td>
<td>10</td>
<td>Mixed</td>
<td>4</td>
<td>36</td>
<td>A + W</td>
</tr>
<tr>
<td>5/M/57</td>
<td>14</td>
<td>Mixed</td>
<td>24</td>
<td>81</td>
<td>M</td>
</tr>
<tr>
<td><em><em>Mean ± SD</em>/...55.4 ± 12.8</em>*</td>
<td><strong>14.0 ± 2.5</strong></td>
<td></td>
<td><strong>15.8 ± 12.0</strong></td>
<td><strong>50.8 ± 18.0</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/M/68</td>
<td>10</td>
<td>Spindle</td>
<td>25</td>
<td>70</td>
<td>A + W</td>
</tr>
<tr>
<td>7/M/36</td>
<td>11</td>
<td>Spindle</td>
<td>29</td>
<td>97</td>
<td>A + W</td>
</tr>
<tr>
<td>8/M/63</td>
<td>16</td>
<td>Spindle</td>
<td>7</td>
<td>31</td>
<td>M</td>
</tr>
<tr>
<td>9/M/53</td>
<td>20</td>
<td>Spindle</td>
<td>19</td>
<td>30</td>
<td>M</td>
</tr>
<tr>
<td>10/M/57</td>
<td>15</td>
<td>Mixed</td>
<td>11</td>
<td>74</td>
<td>A + W</td>
</tr>
<tr>
<td>11/M/62</td>
<td>12</td>
<td>Mixed</td>
<td>14</td>
<td>39</td>
<td>M</td>
</tr>
<tr>
<td>12/M/58</td>
<td>22</td>
<td>Mixed</td>
<td>11</td>
<td>23</td>
<td>M</td>
</tr>
<tr>
<td>13/F/70</td>
<td>23</td>
<td>Mixed</td>
<td>10</td>
<td>11</td>
<td>A + W</td>
</tr>
<tr>
<td>14/M/72</td>
<td>20</td>
<td>Mixed</td>
<td>6</td>
<td>7</td>
<td>A + W</td>
</tr>
<tr>
<td>15/F/83</td>
<td>12</td>
<td>Mixed</td>
<td>18</td>
<td>33</td>
<td>M</td>
</tr>
<tr>
<td><em><em>Mean ± SD</em>/...60.2 ± 10.4</em>*</td>
<td><strong>16.1 ± 4.8</strong></td>
<td></td>
<td><strong>15.0 ± 7.6</strong></td>
<td><strong>41.5 ± 29.3</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Combined</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em><em>Mean ± SD</em>/...58.6 ± 11.0</em>*</td>
<td><strong>15.4 ± 4.2</strong></td>
<td></td>
<td><strong>15.3 ± 8.9</strong></td>
<td><strong>44.6 ± 25.8</strong></td>
<td></td>
</tr>
</tbody>
</table>

*M indicates metastatic death from uveal melanoma; A + W, alive and well; and ellipses, not applicable.*
This report is concerned with the histologic variations that can occur during the evolution of uveal melanomas. Our sample included 15 recurrent intraocular uveal melanomas treated by enucleation between 3 and 29 months after local resection. The cytologic features (number of intermediate and epithelioid cells, mitotic rate, and nucleolar size) of the primary and recurrent tumors were compared to provide an insight into the cytologic behavior of these tumors. The subjective assessment of intermediate and epithelioid cells is strengthened by the computer-assisted objective measurements of nucleolar area (Table 4).

The major finding is that most of the tumors (10 cases) in this series showed change in tumor cell morphologic characteristics toward a more malignant phenotype.
notype within the time intervals studied (15.0±7.6 months), but in a substantial number (5 cases), no such transformation could be demonstrated either qualitatively or quantitatively in a similar time interval (15.8±12.0 months). To allow a better comparison in a relatively heterogeneous group, the cases were divided into 2 subgroups according to the cytologic characteristics in the recurrent tumor. The tumors that did not expose change in morphologic phenotype constitute group 1, whereas the tumors that showed cellular transformation in the recurrence constitute group 2 (Tables 2 and 4).

Most uveal melanomas have a predominant cell type, but there is usually some degree of pleomorphism. In most cases, a subpopulation of cells of different type is diffusely distributed throughout the tumor. In other cases, distinct areas of intermediate or epithelioid cells are observed. Several groups have tried to overcome the problem of heterogeneity by random but adequate sampling, and valid prognostication has been achieved.4,5 We therefore considered it justifiable to adopt a similar approach and made counts on 40 high-power fields distributed in a representative manner. Our repeatability was between 10% and 20%, which was acceptable in relation to the orders of magnitude that were demonstrated by comparison of counts made on the primary and secondary specimens. The high SDs of the cell and mitotic counts indicate the lack of homogeneity in different areas within an individual tumor. We found it useful to expand the Callender classification to include a subgroup of small epithelioid or intermediate cells as suggested by McLean et al.3 Intermediate cells hold a position between “classic” spindle B and epithelioid cells and might represent a transition phase.8 The inclusion of intermediate cells in the present analysis improved the assessment of the cell subsets and the comparison between the primary and the recurrent tumor in each case. Thus, we were able to establish conclusively that, in group 2, there was a significant increase in the numbers of intermediate cells in all but one tumor (case 7) and also an increase in the number of epithelioid cells in all but one of the recurrent tumors (case 14). Another striking feature was the increased mitotic activity in the majority of cases that exposed a change in their cytologic phenotype (7 of 10 cases).

With regard to the measurement of nucleolar area, we found it helpful to validate our analysis of cell type transformation with the objective measurement of nucleolar area. The mean of the largest nucleoli and the SD of nucleolar area are known to be highly predictive factors for metastatic disease in uveal melanomas.9,10 In our series we found a highly significant increase in nucleolar area in all cases in group 2 (P=.008). In group 1, only 1 tumor had an increased nucleolar area (case 2). It was reassuring to find that our values were almost identical to those provided in the original report by Gamel and McLean series was 4.6±1.8 µm², whereas in the present group of primary excision specimens, the corresponding value was 4.2±1.3 µm² (cases 1, 3, 5, 8, 9, 11, 12, and 15). The use of the inverse of the SD of the nucleolar area has been recommended as a convenient variable when 200 measurements are made on each tumor.6,9 However, our data were inconclusive when this analysis was used with only 50 measurements on each tumor, because the SD was too wide.

Although the present sequential study of transformation of tumor cell type does not meet the criteria for a study of natural course because of the surgical intervention, we feel it justified to consider our results representative of the biologic behavior of the individual tumors, since the patients did not receive any additional adjuvant therapy such as radiation or laser treatment. It is not known whether the tumors would have exhibited the same cytologic features if no treatment at all had been applied.

One of the important issues when biological behavior is studied is the time window in which changes occur. With the hypothesis that there is a continuous spectrum of cellular transformation from spindle to intermediate, to epithelioid cells, one would expect to observe more cytologic changes after a longer time interval. When the recurrence intervals are compared between the 2 subgroups in the present series, no marked differences were observed (Table 1), suggesting that there must be some trigger mechanism for cellular transformation that was present in some tumors (group 2) but not in others (group 1). It could be possible that genetic alterations in tumor cells, as have been described,11 give rise to faster-growing, more malignant cell subpopulations, resulting in the growth of tumors with higher mortality rates.14 A positive correlation between cyclin D1 cell positivity and tumor cell type has been observed in uveal melanoma,13 suggesting that alterations of the oncogene–tumor suppressor homeostasis must be implied in the mechanisms of genetic and hence cellular transformation.

Cytologic transformation in uveal melanomas has been demonstrated in vitro: cultivated, uveal melanoma cell lines have been shown to convert their cell type from spindle to epithelioid and vice versa.16,17 In vivo transformation of cell type in uveal melanoma has until now been described only in iris melanomas.18 This series is unique insofar as a histologic comparison has been made between an intraocular primary melanoma and an intraocular recurrence, thereby providing “snapshots” that illustrate variations in the evolution of uveal melanomas. The findings are open to variable interpretation. First, they could represent dedifferentiation of spindle cells into intermediate and, subsequently, to epithelioid cells. Alternatively, the intermediate and epithelioid cells, having a higher mitotic rate, may have overgrown the spindle cells in the population, giving the impression of cytologic transformation.19 Cases 6 to 9 are of special importance in the interpretation of the findings in the present study. In these cases, the primary tumor did not have any epithelioid cells, whereas the recurrent tumors contained 0.40 to 3.53 epithelioid cells per high-power field.
Unless a sampling error occurred (the chance of which was minimized as much as possible through multiple random sampling in the entire tumor area), the findings in cases 6 to 9 suggest that the presence of epithelioid cells in the recurrence occurred through transformation of spindle or intermediate cells and not because these faster-growing cells simply overran the tumor, since they were not present in the primary tumor. On a priori grounds, one would assume that the change in cytologic phenotype would be related to the histologic features of the primary tumor. Our results demonstrate, however, that it is impossible to predict purely on histologic grounds which tumors will eventually change their phenotype into a more malignant type.

The implication of the study is that there is intratumor and intertumor heterogeneity in uveal melanomas, and this must be taken into consideration when hypothetical models of tumor growth are being constructed. Apple and Blodi have suggested on clinical and pathological grounds that there is a biphasic growth pattern in uveal melanomas. In vivo observations have also suggested that there is first a slow-growth or premalignant phase, and second a rapid-growth phase. However, a number of authors have assumed that growth rate is constant for an individual tumor. Our results demonstrate, however, that it is impossible to predict purely on histologic grounds which tumors will eventually change their phenotype into a more malignant type.

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REFERENCES