Clinical and Cytogenetic Analyses in Uveal Melanoma

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PURPOSE. Uveal melanoma is one of the most frequently occurring primary intraocular malignancies in the Western world. Cytogenetically these tumors are characterized by typical chromosomal losses and gains, such as loss of 1p, 3, and 6q and gain of 6p and 8q. Whereas most studies focus on known aberrations, in this one, cytogenetic changes were characterized and correlated with clinical and histopathologic parameters.

METHODS. Karyotypes of 74 primary uveal melanomas were analyzed with respect to the presence or absence of chromosomal gains and losses. In the analysis, classic clinical and histopathologic parameters were analyzed together with the chromosomal aberrations.

RESULTS. At a median follow-up of 43 months, 34 patients had died or had metastatic disease. Clonal chromosomal abnormalities were present in 59 tumors. The most frequent chromosomal abnormalities involved chromosome 8 (55%); loss of chromosome 3, p-arm (41%) and q-arm (42%); partial loss of chromosome 1, p-arm (24%); and abnormalities in chromosome 6 that resulted in gain of 6p (18%) and/or loss of 6q (28%). Less frequent aberrations were abnormalities in chromosome 16, in particular loss of chromosome 16 q-arm (16%). In the univariate analysis, loss of chromosome 3, largest tumor diameter, gain in 8q, and mixed/epithelioid cell type in the tumor compared with tumors without these chromosomal changes or with a spindle cell type was associated with decreased disease-free survival. When corrected for confounding variables, significance of gain of 8q and cell type was decreased, whereas the significance of loss of chromosome 3p or 3q and largest tumor diameter remained the same.

CONCLUSIONS. Monosomy 3 and largest tumor diameter are the most significant in determining survival of patients with uveal melanoma. Abnormalities in the q-arm of chromosome 16 are relatively common in uveal melanoma, but are not associated with survival or other cytogenetic or histopathologic parameters. (Invest Ophthalmol Vis Sci. 2006;47:3703–3707) DOI:10.1167/iovs.06-0101

Uveal melanoma (UM) is the most common primary intraocular tumor in the Western world, affecting approximately 7 in 1 million people each year. Tumorigenesis and progression of cancer is in general preceded by the occurrence of genetic changes in normal cells.1 In this respect, UM are quite homogeneous, with a few tumor-specific cytogenetic aberrations. Some of these aberrations correlate with the metastatic potential of the tumor, resulting in metastatic disease followed by death. Recurrent aberrations in UM concern loss of 1p, monosomy of chromosome 3, loss of 6q and 8p, and gain of 6p and 8q.

Loss of chromosome 1, p-arm, was observed in metastases,2 and concurrent loss of 1p and 3 is associated with decreased survival.3–4 Furthermore, monosomy 3 is considered to be an early event in UM, and several studies have shown that it is a strong predictor of survival.5–7 Loss of chromosome 3 is frequently associated with amplification of 8q, often seen as isochromosome 8, q-arm.8,9 Recently, Hoglund et al.10 elucidated a common genetic pathway for both uveal and cutaneous melanoma. Monosomy 3 probably occurs as an early event, and loss of 1p, 8p, and gain of 8q as secondary events.

Regions of chromosomal loss are thought to harbor tumor-suppressor genes and regions of gain, oncogenes. Previous cytogenetic analyses have focused in general on the known aberrations. In this study, we performed cytogenetic analysis on short-term cell cultures of fresh tissue from 74 primary UM to characterize all chromosomal changes and correlate these changes with clinical and histopathologic parameters. Significant prognostic parameters for UM, at high-risk for metastases, were identified.

MATERIALS AND METHODS

Patients and Tumor Samples

From March 1992 to April 2003, we collected tumor tissue of patients who underwent enucleation for ciliary body or choroidal melanoma. Informed consent was obtained before enucleation, and the study was performed according to the tenets of the Declaration of Helsinki. Fresh tumor tissue was obtained within 1 hour after enucleation and processed as described before.9 Conventional histopathologic examination was performed on all tumors and the origin of the tumor was confirmed. Cytogenetic studies were also performed on stimulated peripheral blood samples of each patient to exclude the presence of congenital chromosome abnormalities. Follow-up data from time of diagnosis until the end of the study in December 2005 were obtained by reviewing the charts patient’s and contacting their general physicians.

Cytogenetic Analysis

Chromosome preparations were made according to standard procedures and stained with acridine orange or atebrine to obtain R or Q
TABLE 1. Recurrent Changes in Karyotype of Primary Uveal Melanoma

<table>
<thead>
<tr>
<th>Chromosomal Region</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>1p loss</td>
<td>18 (24)</td>
</tr>
<tr>
<td>2p loss</td>
<td>30 (41)</td>
</tr>
<tr>
<td>3q loss</td>
<td>31 (42)</td>
</tr>
<tr>
<td>6q loss</td>
<td>13 (18)</td>
</tr>
<tr>
<td>8p gain</td>
<td>21 (28)</td>
</tr>
<tr>
<td>8p loss</td>
<td>13 (18)</td>
</tr>
<tr>
<td>8q gain</td>
<td>18 (24)</td>
</tr>
<tr>
<td>9q gain</td>
<td>13 (18)</td>
</tr>
<tr>
<td>11q gain</td>
<td>12 (16)</td>
</tr>
<tr>
<td>12q loss</td>
<td>4 (5)</td>
</tr>
<tr>
<td>12p gain</td>
<td>4 (5)</td>
</tr>
<tr>
<td>13q gain</td>
<td>7 (9)</td>
</tr>
<tr>
<td>15q gain</td>
<td>7 (9)</td>
</tr>
<tr>
<td>15p gain</td>
<td>7 (9)</td>
</tr>
<tr>
<td>16p loss</td>
<td>7 (9)</td>
</tr>
<tr>
<td>16q gain</td>
<td>7 (9)</td>
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<tr>
<td>17q gain</td>
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<tr>
<td>18q gain</td>
<td>7 (9)</td>
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<tr>
<td>19p gain</td>
<td>7 (9)</td>
</tr>
<tr>
<td>20p gain</td>
<td>7 (9)</td>
</tr>
<tr>
<td>21p loss</td>
<td>7 (9)</td>
</tr>
<tr>
<td>22q loss</td>
<td>7 (9)</td>
</tr>
</tbody>
</table>

Data are the number of tumors (% of total group).

The different parameters (SPSS, ver. 11.0; SPSS, Chicago, IL). Corresponding probabilities were calculated to identify association between loss and gain.

Follow-up time was 42.8 months (range, 6.4–164.4 months). At the end of follow-up time 31 patients had died of melanoma-related disease, 3 had diagnosed metastases, 9 had died due to other causes, and 31 were still alive without metastases. The median follow-up time was 42.8 months (range, 6.4–164.4 months).

Results

Patients

From March 1992 to April 2003, of the 152 patients available for the study, chromosome analysis was successfully performed in 74. The clinical and histopathologic features of the 74 primary UMs are listed in Supplementary Table S1, available online at http://www.iovs.org/cgi/content/full/47/9/3703/DC1. The median age of the patients at the time of enucleation was 60 years (range, 21–87 years), 29 women and 45 men. One patient was lost to follow-up after 27 months. At the end of follow-up time 31 patients had died of melanoma-related disease, 3 had diagnosed metastases, 9 had died due to other causes, and 31 were still alive without metastases. The median follow-up time was 42.8 months (range, 6.4–164.4 months).

Histopathology

All tumors were confirmed histopathologically as UM. Based on cell type, 16 tumors were classified as epithelioid, 24 as mixed, and 34 as spindle. The mean tumor diameter and thickness were 13.2 mm (range, 6–19 mm) and 8.4 mm (range, 2–22 mm), respectively. Four tumors were located in the ciliary body and 70 were located in the choroid. Of the tumors located in the choroid four showed involvement of the ciliary body.

Cytogenetic Analysis

Seventy-four UMs were analyzed for cytogenetic changes (Supplementary Table S1, http://www.iovs.org/cgi/content/full/47/9/3703/DC1) and classified for gain and loss in all chromosomal regions (Table 1). Clonal chromosomal abnormalities were present in 59 tumors. The most frequent chromosomal abnormality involved chromosome 8, trisomy of chromosome 8 or gain in 8q, most often in the form of an i(8q) (53%). Other abnormalities involved loss of chromosome 3, p-arm (41%) and q-arm (42%), partial loss of 1p (24%), and abnormalities in chromosome 6, resulting in gain of 6p (18%) and/or loss of 6q (28%). Other less-frequent aberrations were abnormalities of chromosome 16, in particular loss of chromosome 16, q-arm (16%; Fig. 1). Other chromosomal aberrations, such as loss of 6p, 9p, 15p, 15q, 21p, and 22p and gain of 2p, 2q, 7q, 9p, and 11q were present but did not reach 10%.

Statistical Analysis

Univariate analysis was performed for all clinical, histopathologic, and cytogenetic parameters (Table 2, Fig. 2). Univariate analysis of the single prognostic factors showed significant lower DFS in patients with loss of chromosome 3, largest tumor diameter (LTD), gain of 8q, and a mixed/epithelioid cell type in the tumor compared with tumors without these chromosomal changes or of a spindle cell type. Other potential prognostic factors such as gender, age at time of diagnosis, and tumor location (i.e., involvement of ciliary body) did not reach significance. Also chromosomal changes such as loss of chromosome 1p, gain of 6p, and loss of 6q were not significantly associated with DFS. To examine the possibility that other clinical, histopathologic, or chromosomal variations may affect the prognosis, we performed Cox proportional hazards analysis for each confounding variable (Table 2). Parameters presented in the columns are the investigated prognostic parameters; in the rows the same parameters resemble the confounders with a possible modifying effect. Significance of

FIGURE 1. Karyotype of tumor EOM 63. This tumor showed chromosomal changes caused by UM: −3, i(6)(p), i(8)q (multiple copies), and del(16)(q21).
loss of 3p and 3q did not alter after correcting for the possible confounders. A similar pattern was observed for LTD and cell type. Odds ratios were calculated to identify association between the different parameters (Table 3). Associations were shown for loss of chromosome 3 with gain of 8q, loss of 8p, vascular patterns and LTD (>12 mm), and a weak association with mixed-epithelioid cell type. Presence of vascular patterns and LTD (>12 mm) showed also association with gain of 8q. Associations were also present for loss of 1p with loss of 16q and loss of 3p, and weak association with cell type, vascular patterns LTD, 3q loss, and 8q gain. Loss of 6q was weakly associated with gain of 8q. Loss of 16q was weakly associated with gain of 8p.

**DISCUSSION**

By means of karyotyping we analyzed chromosomal aberrations in UM. Previous reports have revealed that abnormalities in chromosome 1, 3, and 8 occur in a nonrandom fashion in UM. Some of these tumor-specific aberrations have not yet been identified. Because UM cells are derived from neuroectodermal tissue this might be of potential interest. In many reports outcome was correlated with tumor location.

In these tumors, candidate genes have not yet been identified. Because UM cells are derived from neuroectodermal tissue this might be of potential interest. In many reports outcome was correlated with tumor location. However, in our series, we cannot conclude the same for isochromosome 6, p-arm. In addition, gain of 8q may also support this hypothesis, because the odds ratios for gain of 8q or loss of 8p were higher than the combination of loss of 3p or 3q and gain of 8p. However, in our series, we cannot conclude the same for isochromosome 6, p-arm. In addition, gain of 8q was significantly associated with survival in the univariate analysis (Table 2), but when corrected for confounding variables, such as vascular pattern, cell type, LTD, and 3p or 3q loss, significance was absent, implying that gain of 8q occurs together with at least one of those other variables. On the contrary, when this same procedure was followed for 3p or 3q loss we observed that the significance remained. In Table 3 the odds ratios were shown for different chromosomal parameters. If significance was absent, implying that gain of 8q occurs together with at least one of those other variables. On the contrary, when this same procedure was followed for 3p or 3q loss we observed that the significance remained.
FIGURE 2. Kaplan-Meier survival curves for clinical, histopathologic, and chromosomal aberrations.
patient samples from relatively large tumors that were treated by enucleation. Considering monosomy 3 as an early event, it is likely that it would be observed in even the smallest amount of tissue despite the heterogeneity of UM. Though, there are no studies to date that confirm the uniform distribution of cytogenetic abnormalities in UM, and it is at least theoretically possible that small amounts of tissue (e.g., used for karyotyping, FISH, and CGH) do not contain the cytogenetic markers of interest.

Acknowledgments

The authors thank Anne Hagemeijer, Rosalyn Slater, and Ellen van Drunen for performing most of the cytogenetic analyses during the early years of the study.

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3. Kilic E, Naus NC, van Gils W, et al. Detection of genetic abnormalities in UM, and it is at least theoretically possible that small amounts of tissue (e.g., used for karyotyping, FISH, and CGH) do not contain the cytogenetic markers of interest.

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References