Original article

Cytogenetic analysis of choroidal melanoma

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ABSTRACT

Purpose: To investigate the presence of known cytogenetic alterations of choroidal melanoma in a series of patients diagnosed and treated in our Ocular Oncology Service. A review of the present literature on this topic is also presented.

Methods: Microsatellite analysis (MSA) studies on loss of heterozygosity (LOH) of chromosome 3, as well as multiplex ligation probe amplification (MLPA) on chromosomes 1, 3, 6 and 8, were performed on enucleation or local resection samples obtained from a total of 27 patients, over a 2-year period.

Results: Twenty patients showed at least one of the cytogenetic alterations looked for. A total of 11 cases were found that showed LOH of chromosome 3 (44%), 8 gains of chromosome 8 (30%), 8 gains of chromosome 6 p (30%), and 7 partial or total losses of chromosome 1 (26%).

Conclusions: This is the first study on the cytogenetics of choroidal melanoma performed in our country.

The results are similar to that published in the literature.

Cytogenetic analysis provides more accurate knowledge on a vital individual prognosis. It may also become a valuable tool for establishing the most adequate follow-up regimes, and the need for adjuvant therapies.

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Análisis citogenético del melanoma de coroides

RESUMEN

Objetivo: Investigar la presencia de las alteraciones citogenéticas conocidas del melanoma de coroides en una serie de pacientes diagnosticados y tratados en nuestra Unidad de Oncología Ocular. También exponemos una revisión de la literatura actual sobre este tema.

Método: Durante dos años se han estudiado muestras procedentes de piezas de enucleación o de resección de melanoma coroideo de un total de 27 pacientes mediante análisis de microsatélites (MSA) para estudio de pérdida de heterocigosidad (LOH) del cromosoma 3 y mediante multiplex-ligation-probe amplification (MLPA) para los cromosomas 1, 3, 6 y 8.

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Introduction

The objective of this study is to research the presence of known uveal melanoma cytogenetic alterations in samples obtained from patients in our Ocular Oncology Unit, comparing the results with those published in current literature.

Approximately 90% of uveal melanoma expresses in the choroids. Despite adequate local treatment, about 50% of patients die due to metastatic disease which will normally involve the liver.3

Estimated survival is based on clinical characteristics, mainly the maximum tumor diameter, height, ciliary body involvement and extracocular extension.2

The anatomic/pathological characteristics leading to poor prognosis are, among others, the presence of epithelioid cells, high index of mitosis and specific vascular patterns.3

Since a correlation was demonstrated in 1996 between the loss of chromosome 3 in tumor cells and demise due to metastasis,6 other negative prognosis cytogenetic markers have been identified, including gain of 8 q, loss of 8 p, loss of 1 p and gain of 6 p.

Subjects, materials and methods

In the past 2 years, choroidal melanoma samples of 27 patients were researched using enucleation parts (derived from primary treatment or secondary treatment due to local relapse after conservative treatments) or from local resection (both from ab externo [trans-scleral] resection or ab interno [endoresection]). Together with tumor samples, peripheral blood samples were obtained to compare cytogenetic analysis of tumor cells with that of blood lymphocytes. Cytogenetic and anatomic/pathologic studies were carried out simultaneously.

The cytogenetic study was performed by microsatellite analysis (MSA) which consisted in using the DNA-polymerase reaction chain (PCR) to detect the loss of 10 polymorphic markers of chromosome 3 and accordingly studying the loss of heterozygosity (LOH) thereof, defined as a substantial loss of one of the chromosome alleles in comparison to a healthy cell of the same individual (commercial markers owned by Kit Human Genome Mapping Kit v2.5 de applied Biosystem Inc., CA, USA).

In addition, the multiplex ligation-dependent probe amplification (MLPA) was utilized for detecting chromosome imbalances in chromosomes 1 (loss of 1 p), 3 (loss of 3 p), 6 (gain of 6 p) and 8 (gain of 8 q and loss of 8 p) (MLPA kit Salsa po27; MRC-Holland, Amsterdam, Holland).

In summary, both techniques are based on molecular probes prepared for detecting known chromosome regions (Fig. 1).

Statistics

A descriptive analysis of the study variables was carried out together with a study of proportions by means of the Chi square or the Fisher’s test by demand between different variables.

All the statistical tests were considered to be significant with a P value of ≤0.05. The analysis was performed utilizing the statistical application SPSS 12.0 (SPSS, Inc., Chicago, IL, USA).

Results

Twenty-seven cases were studied, with a maximum follow-up period of 2 years from treatment and sample taking. The mean age of patients was 61.5 ± 11.16 years with a similar distribution per gender (48.1% females and 51.9% males). The proportion of right eyes against left eyes was similar, approaching 50% in both cases. In 11 cases (40.7%) tumor samples were derived from primary ocular globe enucleation, in 8 cases (29.6%) from secondary ocular globe enucleation (relapse or complications), and in 8 cases (29.6%) from local tumor resection (5 endo-resections and 3 trans-scleral resections). Taking into account the size of the tumor (T) according to the seventh edition of the AJCC (TNM) classification, it was of T1 for 3 patients (12%), T2 for 7 patients (28%), T3 for 9 patients (36%) and T4 for 6 patients (24%). In 6 patients (22.2%) an additional neoplasia other than melanoma was diagnosed at some point (before, during or after uveal melanoma diagnostic).

In two patients (7.4%) a macroscopic extrascleral extension was evidenced at diagnostic.

In 19 cases (70.3%), some of the alterations that were sought were found (Table 1).

Seven patients exhibited complete or partial losses of chromosome 1 (26% of cases), 12 exhibited LOH of chromosome 3 (44% of cases), 7 exhibited gains in chromosome 6 p (26% of cases) and 8 exhibited gains in chromosome 8 (30% of cases).
Fig. 1 – Graph example showing the LOH search for chromosome 3, comparing the presence of polymorphic regions between blood cells (upper half) and tumor cells (lower half). Reductions can be seen in 3 regions of chromosome 3q in tumor cells (arrows) in comparison with the same chromosome in healthy blood cells.

Table 1 – Study results.

<table>
<thead>
<tr>
<th>Patient</th>
<th>LOH CR 3</th>
<th>MLPA CR 1</th>
<th>MLPA CR 3</th>
<th>MLPA CR 6</th>
<th>MLPA CR 8</th>
<th>Treatment</th>
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<tr>
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<td>N</td>
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<td>27</td>
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<td>N</td>
<td>LOH</td>
<td>N</td>
<td>N</td>
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</tr>
</tbody>
</table>

Braqui: brachytherapy; CR: chromosome; Endo: endoresection; Enuc: enucleation; LOH: loss of heterozygosity; MLPA: multiplex-ligation-probe amplification; N/A: not available; N: normal; Trans-sc: trans-scleral.
Six patients (22.2%) were identified with a single chromosome anomaly, nine (33.3%) with 2 alterations, two patients (7.4%) with 3 alterations and one patient (3.7%) with 4 alterations. The latter was the only patient in the series that in the 2 years of the follow-up period developed metastatic disease.

The combination of chromosome 3 loss and chromosome 8 gain, described as that which involves the worst vital prognostic, was found in 5 patients (18.5%).

On the basis of the treatments, 100% of cases who required primary enucleation and 44% of those in which enucleation was secondary after relapse, followed by radiotherapy, exhibited at least one cytogenetic alteration. The rate of alterations found amongst those who received resection treatment was of 22%. This difference is explained because the larger tumors or those who exhibit growth after radiotherapy are the ones which have the highest probabilities of developing additional poor prognostic factors.

**Discussion**

A number of cytogenetic anomalies involving chromosomes 1, 3, 6 and 8 have been identified in the study of uveal melanoma. There is a range of series in the literature with long follow-up periods which have studied the presence of said alterations and related them with patient survival. The objective of this paper is to describe for the first time in our country the said chromosome disorders in a series of patients diagnosed and treated in our facilities.

The most frequently found cytogenetic alteration was monosomy 3 (44% of cases) which numerous papers relate to poor prognosis in uveal melanoma patients.

In a study on 500 cases with samples obtained from fine needle trans-scleral aspiration, it was concluded that the aggregate probability of metastases at 3 years was of 2.6% for disomy 3, of 5.3% for partial monosomy and 24% for complete monosomy.

The authors have found a high proportion of monosomy 3 associated to chromosome 8 gain (18.5%) which is also related to poor vital prognosis. The high proportion of cytogenetic anomalies in the series is explained by the high proportion of large tumors (88% T2, T3 and T4) therein and the fact that this is one of the most relevant poor prognosis clinical factors.

A study carried out by Damato et al. carried out the cytogenetic typification of 452 melanomas with MLPA and correlated the results with the presence of other known choroidal melanoma risk factors and with the probability of death due to metastasis. Their conclusion was that death by metastases amongst these patients occurred predominantly in those whose tumors exhibited loss of chromosome 3 (specific mortality at 10 years, 55%). The frequency increased even more if chromosome 8q gains were also present (specific mortality at 10 years, 71%). In fact, the tumors with the dual findings were practically the only ones that developed metastatic disease. These data are in contrast with zero specific mortality exhibited by patients without alterations in chromosome 3 (0%). In said tumors with both cytogenetic alterations, the epitheloid cell type, vascular loops and high mitotic index were also correlated to poor survival, together with the absence of gain in chromosome 6p (the presence of this alteration would act as a protective factor).

In what concerns analysis techniques, even though fluorescence in situ hybridization (FISH) was utilized initially in many hospitals due to its high sensitivity, microsatellites and MLPA have demonstrated to be an equally reliable technique without the drawbacks of high cost and specific analyzed sentence exhibited by FISH due to the fact of being a technique that studies the average characteristics of all the cells in a sample. For this reason, we began the study of our patients with microsatellites and MLPA, and detected a hyper portion of cytogenetic anomalies in our patients as can be appreciated in this paper.

At present, the clinical usefulness of cytogenetic studies for choroidal melanoma is the subject of controversy. Damato et al. had stated that, considering the high correlation of monosomy 3 and gain in 8 with mortality due to metastasis, the individual vital prognosis obtained by means of cytogenetic analysis is beneficial for the patient because it enables a more lax follow-up in low-risk patients, while high-risk patients receive stricter screening allowing for early diagnostic of metastasis. This should increase the probability of treating them and including them in adjuvant therapy studies.

However, the said paper has been the object of a number of criticisms. It has been said that, when performing a biopsy with fine needle, it is not possible to determine whether the tumor sample has actually been collected or not. In addition, the psychological benefits attributed to the fact that the patient knows about the prognosis have also been questioned. The increased survival that could be achieved with stricter screening in high risk patients could be only a time bias (the fact of diagnosing metastases earlier does not mean that the patient will live longer than if it was identified at a more evolved stage).

At present, in most hospitals choroidal melanoma cytogenetic analysis is carried out only for research purposes. However, it appears that greater knowledge on these genetic alterations will enable knowledge of the mechanisms whereby the disease becomes malignant and the changes in associated genetic expressions, thus paving the way for new diagnostic and therapeutical possibilities. The knowledge derived from these studies and the attention of long series seem to be right now the next step for improving the expectations of these patients.

Due to the low number of patients the authors were not able to find relationships between cytogenetic alterations and other known clinical and histological poor vital prognosis factors.

It yet remains to relate the presence of one or more cytogenetic anomalies with specific survival in our series of patients; however, this requires a higher number of cases.

**Conflicts of interests**

No conflicts of interests has declared by the authors.
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