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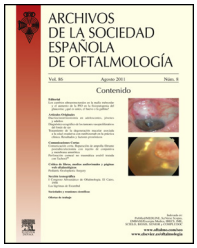

Original article

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 also may become a valuable tool for establishing the most adequate follow-up regimes, and the need for adjuvant therapies.

Análisis citogenético del melanoma de coroides

Palabras clave:
Melanoma
Uveal
Citogenético
Multiplex-ligation-prove amplification
Análisis de microsatélites
Pérdida de heterocigosidad

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-prove amplification (MLPA) para los cromosomas 1, 3, 6 y 8.

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Resultados: Entre los casos estudiados, 20 mostraron como mínimo una de las alteraciones citogenéticas que se buscaban, 11 LOH del cromosoma 3 (44%), 8 ganancias del cromosoma 6 p (30%), 8 ganancias en cromosoma 8 (30%) y 7 pérdidas totales o parciales del cromosoma 1 (26%).

Conclusiones: Este es el primer estudio citogenético del melanoma de úvea en nuestro país.

La presencia y preponderancia de las distintas alteraciones citogenéticas se corresponden con las de las series publicadas en la literatura.

El análisis citogenético nos permite conocer mejor el pronóstico vital individualizado. También puede resultar una herramienta valiosa para establecer el protocolo de seguimiento más adecuado y la necesidad de tratamientos adyuvantes en estos pacientes.

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.[1]

Estimated survival is based on clinical characteristics, mainly the maximum tumor diameter, height, ciliary body involvement and extraocular 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,[4] 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-Holand, 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 seeked 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).