Original article

Genetic analysis in retinoblastoma and peripheral blood correlation

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ABSTRACT

Objective: To determine the importance of intratumoral genetic analysis in the diagnosis of germ-line mutations in patients with retinoblastoma. To underline the importance of performing these genetic tests in every case of retinoblastoma.

Method: Intratumoral genetic analysis of RB1 mutation was performed on 17 enucleated eyes that were non-responsive to conservative treatment. Patients had no family history of retinoblastoma, and lesions were always single. The identified mutations were then also studied in peripheral blood analysis.

Results: There were 12 (70.6%) cases with positive results in intratumoral analysis. In 8 cases (47.1%) mutation of both RB1 alleles were detected, and in 4 (23.5%) cases only one allele was found mutated. In 5 patients (29.4%) no mutation was identified. In the first hit, mutations comprised 7 frameshift or nonsense and 2 splice, whereas in the second hit, one splice, mutation, 2 nonsense and 8 loss of heterozygosity were identified. Among 6 patients where intratumoral analysis detected a single mutation associated with a loss of heterozygosity, the peripheral blood analysis was able to detect the same mutation in 3 cases (50%).

Conclusions: Intratumoral genetic analysis of sporadic retinoblastoma can detect germ-line mutations. These patients are at higher risk of bilateralization and development of second tumors or trilateral retinoblastoma. Genetic screening is recommended in every patient diagnosed with retinoblastoma.

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Análisis genético intratumoral en retinoblastoma

RESUMEN

Objetivo: Determinar la importancia del análisis genético en tejido tumoral para identificar la afectación de la línea germinal. Demostrar la importancia de realizar el test genético en todos los casos de retinoblastoma.

Método: Se realiza análisis genético intratumoral para el gen RB1 en 17 globos enucleados sin respuesta al tratamiento conservador, con lesiones únicas y unilaterales en pacientes sin antecedentes familiares de retinoblastoma. Posteriormente, se comprueba la presencia de las mutaciones identificadas intratumoralmente en sangre periférica.

Resultado: De un total de 17 casos con estudio intratumoral, con resultados positivos en 12 (70,6%), se ha identificado la mutación de los 2 alelos del gen RB1 en 8 (47,1%), en 4 (23,5%) se ha identificado la mutación de un solo alelo y en 5 (29,4%) no se ha podido identificar ninguna de las alteraciones genéticas. El tipo de alteraciones genéticas identificadas en el primer hit han sido 7 mutaciones del tipo frameshift o nonsense y 2 del tipo splice. En el segundo hit se ha identificado una mutación de tipo splice, 2 tipo nonsense y 8 pérdidas de heterocigósidad. Se ha identificado en sangre periférica la mutación puntal de 3 de los pacientes en los que se había identificado en tejido intratumoral una mutación puntual y una pérdida de heterocigósidad.

Conclusiones: El análisis genético intratumoral en casos clínicamente esporádicos ha resultado eficaz para demostrar la existencia de la afectación de la línea germinal, con riesgo de bilateralización o desarrollo de retinoblastoma trilateral o segundas neoplasias. El screening genético se debe realizar en todos los pacientes con retinoblastoma.

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Introduction

Retinoblastoma may appear sporadically without previous familial history or as a family-related tumor. Between 5% and 10% of cases exhibit familial antecedents and 90-95% are sporadic. The RB1 gene behaves as an anti-oncogene, i.e., both copies must be altered for the disease to manifest. If one of the mutations appears in the germinal line, that is in one of the first cell divisions after fertilization, the subject becomes susceptible to developing retinoblastoma and passing it on to descendants. The second mutation would take place only in retinoblasts.2

There is a period of higher susceptibility for the second mutation to occur between gestation week 12 and 5 years. According to the Knudson theory, this would be the period where the second hit takes place, thus predisposing the subject to developing the tumor.3

There are many cases without familial antecedents in which one of the RB1 gene mutations takes place in the germinal line. These are cases without familial antecedents although with potential inheritance, i.e., de novo mutations which will become familial cases because they could be inherited by descendants.

The involvement of the germinal line explains why patients with family antecedents exhibit bilateral and multiple tumors in the same eye with greater frequency, as well as a susceptibility to the development of second neoplasias.4 The Rb protein, which is encoded by the RB1 gene, plays a crucial role in controlling the cell cycle and intervenes in the appearance and development of many more tumors in addition to retinoblastoma.

Material and method

Seventeen cases of patients with unilateral tumors without familial retinoblastoma history who had to be enucleated due to poor local control of the disease. After enucleation, the fresh tumor sample was sent to the genetics department for processing. Both copies of the RB1 gene were analyzed, establishing whenever possible the genetic mutation that took place. Subsequently, the existence of the identified intratumor alterations in the peripheral blood was verified.

Results

Positive results were found in 12 of the 17 studied cases (70.6%). The mutation of both alleles was identified in 8 cases (47.1%), while the mutation of a single allele was identified in 4 cases (23.5%) and in 5 cases (29.4%) it was not possible to identify any genetic alterations in the RB1 gene (Table 1).

In what concerns the type of identified genetic alteration, in the first hit 7 frameshift or nonsense mutations and 2 splice mutations were identified. In the second hit 1 splice mutation and 2 nonsense mutations were identified together with 8 heterozygosity losses.

The combination of the identified mutation type is illustrated in Table 2.
Subsequently, a genetic analysis was performed on peripheral blood, confirming one of the intratumor alterations in 3 cases, all of which had a mutation and a loss of intratumor heterozygosity. For the 2 cases in which 2 specific mutations were found, none exhibited any alteration in peripheral blood, which means that they were either actual sporadic or could be mosaics in which the mutation was not evidenced. In what concerns the other 9 patients in which only one alteration of intratumor alleles was found (or none at all), the results of the analysis did not provide information.

In the cases exhibiting the mutation together with heterozygosity loss in peripheral blood, the specific mutation was identified in the germline line. In half of these patients the identified mutation was verified in the intratumor tissue in peripheral blood.

The matches between the identified genetic lesions intratumor and in peripheral blood are detailed in Table 3. The differences are not statistically significant (Fisher’s exact statistics p = 0.115) probably due to the sample size.

### Discussion

In recent years, daily ophthalmological practice has included a new protocol for diagnosing patients with unilateral sporadic retinoblastoma. When these patients are enucleated, live tumor tissue samples are taken for processing and analyzing their genetic makeup.

By definition, tumor tissue comprises alterations in the 2 RB1 gene alleles. When these 2 mutations are identified, both are searched in peripheral blood to identify patients with terminal line mutations who, for example, exhibit a disease with low penetration.\(^\text{1-12}\)

By applying said techniques in the present series, it was possible to identify both mutations in 8 of 17 patients, i.e., 47% of cases, and one mutation in 23.5% of cases. In other words, 47% of patients with sporadic unilateral tumors can be informed with certainty whether they are carriers of the germline line mutation, with the consequences this involves. These patients, who otherwise would have been considered to be sporadic, now know that they could exhibit bilateral tumors, second malign neoplasias and even trilateral retinoblastoma, in addition to knowing that they could pass on the disease to their descendants, thus making genetic counseling essential. When the mutation is identified in the germline line, the patient approach is very different.

In patients exhibiting the mutation in one of the alleles together with heterozygosity loss in the other in peripheral blood, the specific mutation is identified. Heterozygosity loss could have taken place only in the retinoblast.

The above demonstrates the necessity of a genetic study in cases with unilateral expression.\(^\text{13-16}\) If the mutation is identified in the germline line of cases in which the disease expresses unilaterally, the therapeutic protocol is more conservative due to the quantifiable risk of late bilateralization. The objective is to preserve both ocular globes to the fullest possible extent.

With improvements in genetic analysis techniques and the appearance of new technologies, genetic study efficiency has improved and there is an increasing number of cases in which the predisposing mutation is identified due to the existence of a huge genetic variety in RBI gene alterations.

### Conflict of interests

No conflict of interests was declared by the authors.

### References


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**Table 1** - Distribution of cases based on the identification of intratumor genetic alterations.

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Done-not identified</td>
<td>5</td>
<td>29.4</td>
</tr>
<tr>
<td>1 hit identified</td>
<td>4</td>
<td>23.5</td>
</tr>
<tr>
<td>2 hits</td>
<td>8</td>
<td>47.1</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100.0</td>
</tr>
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</table>

**Table 2** - Genetic mutation combination type identified in intratumor tissue.

<table>
<thead>
<tr>
<th>Type</th>
<th>Frequency</th>
<th>%</th>
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<tbody>
<tr>
<td>Mut-mut</td>
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<td>11.8</td>
</tr>
<tr>
<td>Mut-LOH</td>
<td>6</td>
<td>35.3</td>
</tr>
<tr>
<td>1 hit</td>
<td>4</td>
<td>23.5</td>
</tr>
<tr>
<td>Not identif.</td>
<td>5</td>
<td>29.4</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100.0</td>
</tr>
</tbody>
</table>

LOH: loss of heterozygosity; Mut: mutation.

**Table 3** - Match between genetic alterations identified in tumor tissue and peripheral blood.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Mut-mut</th>
<th>Mut-LOH</th>
<th>1 hit</th>
<th>Not identif.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identif. in peripheral blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>14</td>
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</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>17</td>
</tr>
</tbody>
</table>

Fisher’s statistic p = 0.115.


