A case of aggressive medulloblastoma with multiple recurrent chromosomal alterations

Medulloblastomas (MB) arise in the posterior fossa region of the brain and represent 20–25% of central nervous systems (CNS) tumors in childhood, occurring predominantly in the first decade of life [1–3]. The World Health Organization (WHO) defined MB as a homogeneous grade IV neoplasm, even though a broad histopathologic spectrum was demonstrated recently [4].

The prognosis of individual MB patients depends on age, extent of surgical resection, and presence of metastases. These clinical data, however, are not always sufficient for proper classification and therapy planning. Genetic and cytogenetic analysis of MB could provide additional prognostic information because the biological behavior of a tumor is primarily determined by its genomic alterations [5].

From a cytogenetic point of view, MB sometimes have complex karyotypes that exhibit many structural and numerical alterations [6,7]. Some chromosomes, such as chromosome 1, are not coincidentally involved more frequently in a wide variety of alterations than others [5,8,9]. Additionally, amplifications involving some protooncogenes of the MYC gene family (MYCN or MYC) are regularly observed in MB. Preliminary studies suggest that MYC [10] and MYCN amplification [11] and/or overexpression [12,13] may result in more aggressive tumors [8]. The presence of double-minute (dmin) amplifications in some CNS tumors is often associated with these genes [14].

The introduction of molecular cytogenetic techniques has, in general, provided the possibility for a more accurate characterization of cytogenetic changes present in solid tumors. Techniques such as fluorescence in situ hybridization (FISH) [15] and especially Multiplex-FISH (M-FISH) [16] are powerful tools to investigate abnormalities in tumor samples. FISH studies as well as cytogenetic prognostic markers, however, are still limited in MB [5]. Hence, the aim of this study is to analyze a case of medulloblastoma with a complex karyotype by means of M-FISH and locus-specific FISH for MYCN, TP53, and the centromeric region of chromosome 17 (CEP17) to identify recurrent chromosomal rearrangements and possible alterations in the number of copies of these important genes.

A 7-year-old female child was admitted to Ophir Loyola hospital with headache, vomiting, and deambulation problems. She was diagnosed with MB, which was histopathologically defined with classic MB (ICD-O code 9470/3, World Health Organization grade IV). The tumor was localized on the midline of the posterior fossa region, measuring 5 × 2.5 × 1.5 cm. Clinical data confirmed an aggressive tumor. The sample was obtained from the resection surgery at Hospital Ophir Loyola (Belém-PA-Brazil) in October 2005. The patient developed a postoperative pneumothorax and died at the hospital 2 months after the surgery, due to respiratory failure.

Chromosome analysis using Giemsa staining in metaphase plates, obtained from a fresh tumor sample dissociated and incubated in Dulbecco’s modified Eagle medium with colcemid for 2 hours, revealed a modal chromosome number of 74, ranging from 44 to 84 chromosomes per cell. An average of 8 dmin (ranging from 1 to more than 40) per cell was observed in approximately 45% of the cell population.

M-FISH confirmed numerical abnormalities involving every chromosome pair and some structural rearrangements. The composite karyotype was: 44~84,XXX,der(1)t(1;8)(q?;?)x3,+2,+3,–4,–5,+6,–7,–8,–9,–10,–12,–16,+18, +19,der(19)t(19;19)(?:?),+22,dmin[cp15]. Chromosome aberrations found in individual cells and clonal alterations are listed in Table 1.

The der(1)t(1;8)(q?;?) (Figs. 1A and 1B) was found in all the cells analyzed by M-FISH, corroborating the findings of others authors who demonstrated that chromosome 1 is frequently involved in structural alterations in MB with breakpoints scattered over both the short and the long arm [5,6,9,17,18]. Cohen et al. [5] demonstrated that chromosome 1 is the most frequently involved in structural aberrations. Moreover, they found translocations involving chromosomes 1 and 8 in two out of five cases. This might support a special significance of chromosome 1 imbalances and instability in MB.

Generally, amplification of the MYCN region is frequently found in CGH studies of MB patients [19], and the presence of dmin is a hallmark of this gene amplification in these tumors [9]. Contrary to expectations, the MYCN gene was not involved in amplifications or formation of dmin in this case. Although the whole chromosome 2 probe hybridized onto dmin, hybridization signals of the MYCN locus-specific probe were found only on the normal chromosome 2 (Figs. 1C and 1D). Further experiments must be performed to characterize and clarify the origin of these dmin.
The application of a dual-color experiment of the centromere 17-specific probe plus the TP53-specific probe showed the following: (1) 49.5% had four copies of this chromosome, (2) 37% were cells with three copies, and (3) the remaining 13.5% comprised cells with more than four or with less than three copies. The TP53 locus was found deleted in at least one copy of chromosome 17 in 59.5% of the cells. Although the most frequently recurrent chromosomal alteration in MB is i(17q), which occurs in approximately 40% of cases [20–22], we detected it in only one spread metaphase by M-FISH as a nonclonal aberration. It is likely here, however, that this specific tumor achieved the “desired effect” of this alteration directly by loss of the TP53 gene and the presence of additional copies of chromosome 17 in the main clone.

In conclusion, the der(1)t(1;8)(q?:?) seems to have an important role in the progression of this MB, and can be associated with its poor prognosis. Further studies should focus on the identification of genes that could be affected by this rearrangement, as well as on their role in the genesis and evolution of this malignant type of tumor. The high frequency of this alteration in this sample indicates its early origin and importance in the development of this tumor. The analysis of a higher number of samples could also clarify the prognostic value of this rearrangement, as well as the sequence of their appearance during the MB development. On the other hand, the deletion of the TP53 locus-specific region can produce an effect similar to the i(17q), which is usually associated to this type of tumor.

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Table 1

<table>
<thead>
<tr>
<th>Chromosomes</th>
<th>Structural chromosome aberrations</th>
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<tr>
<td>1</td>
<td>Clonal der(1)t(1;8)(q?:?) [15/15]</td>
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<tr>
<td>4</td>
<td>Single t(4;6)(?:?) [1/15]</td>
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<tr>
<td>16</td>
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<tr>
<td>17</td>
<td>Single i(17)(q10) [1/17]</td>
</tr>
<tr>
<td>19</td>
<td>Clonal der(19)t(19;19)(?:?) [8/15]</td>
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* Loss of TP53 was found by FISH in 59.5% of cells.

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Fig. 1. Results of molecular cytogenetics techniques (A) Representative metaphase spread and (B) the pseudocolour scheme after M-FISH treatment and analysis showing one copy of the translocated chromosome 1;8 (arrows). (C) Representative result after FISH applying chromosome 2 and (D) specific MYCN probes in metaphase and interphase. These images exhibit three chromosome 2 and three stained dmin (arrows) (C), and only three signals for MYCN probe (2p24) in both spread metaphase and interphase nuclei (D).
References


