Genomic imbalances in childhood medulloblastoma by comparative genomic hybridization

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ABSTRACT

We used comparative genomic hybridization (CGH) technique to identify the genetic changes in 12 childhood medulloblastoma samples obtained from archived paraffin-embedded tissues belonging to children (0.7-15 years-old) using double step degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR). CGH analysis showed that the most common genetic alterations were gains of chromosomes 4p15p16, 7p21p22, 9q22q31, 14q31, 17p12, and Xp11 and non-specific losses. In conclusion, the regions of the chromosomes 9q22q31, 14q31, and 17p12 may harbour the novel site for oncogene(s) and/or tumor suppressor gene(s) playing role in transformation and progression of childhood medulloblastoma tumors. [Turk J Cancer 2003;33(4):177-180]

KEY WORDS:

Medulloblastoma, comparative genomic hybridization (CGH), DOP-PCR, genomic imbalances

INTRODUCTION

Medulloblastoma is the most common embryonal central nervous system tumor in children, and accounts for approximately 20% of childhood brain tumors overall. Cytogenetic studies have demonstrated loss of the distal end of chromosome 17p, usually with an i(17q), in 30-50% of cases analysed with conventional cytogenetics (1). Additional findings in small subsets of tumors, either by cytogenetic analysis or by LOH allelotyping include deletions on chromosomes 5q, 6, 9, 10, 11, 16q and 22 (2-6). More recently, the gains of 7 and 17q and the loss of 17p were present in a series of 23 children with primitive neuroectodermal tumors (PNET) (7). Comparative genomic hybridization (CGH) allows a genome-wide screening for unbalanced abnormalities, without the requirement for tumor metaphases (8). Only three studies in medulloblastoma have previously been reported (7,9,10). We studied archived tumor samples obtained from 12 children with medulloblastoma using CGH.

MATERIALS AND METHODS

Twelve archival formalin-fixed, paraffin-embedded medulloblastoma samples from children (age range 0.7-15 years-old) were obtained from Pathology Department, University of Çukurova, in Turkey. Sections (5 μm) were
cut from tumor blocks and stained with hematoxylin and
eosin (H&E) to ensure the histological representation of
the sample and cross detecting of areas of tumor cells.
Based on microscopic evaluation, areas of interest were
identified, scraped with a scalpel, collected in sterile
eppendorf tubes containing 50 µl DOP-PCR reaction
solution in order to amplify the DNA directly.

CGH experiments and the evaluation of the results were
performed as described previously with minor modifications
(11-15). Briefly, tumor DNA and normal male or female
reference DNA (from normal male and female DNA) were
labeled with spectrum green-dUTP and spectrum red-dUTP
(Vysis Co.), respectively by applying DOP-PCR.

Metaphase spreads were prepared from phytohemagglutinin stimulated lymphocytes of healthy male (46,XY)
and female (46,XX) by standard procedures of colcemid
arrest, hypotonic treatment and 3:1 methanol/glacial acetic
acid fixation.

<table>
<thead>
<tr>
<th>No</th>
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<th>CGH results</th>
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*MB: Medulloblastoma
For CGH, 500 ng of tumor DNA, 500 ng of normal DNA and 50 µg unlabeled Cot-1 DNA were co-precipitated and redissolved in 15 µl hybridization buffer. DNA and metaphase spreads were denatured at 70 °C and at 68 °C, respectively.

Hybridization was allowed to proceed for 2 days. Post-hybridization washes were carried out to a stringency of 50% formamide/2xSSC at 45 °C. Imaging processing was carried out using Applied Imaging MacProbe version 5.1 software. Average green:red fluorescence intensity ratio profiles were calculated for each chromosome in 10 metaphases. Defining the gain and loss in DNA sequence copy number in tumors were based on comparison of normal DNAs labeled with two different colors according to previously described protocol (16-18). The decision limits of the green-to-red ratios were <0.85 for the loss of DNA copy number and >1.40 for the gain of DNA copy number. High level increases were distinguished from low level increase by cut-off level of 2.0. Also, for DOP-PCR and CGH, various negative and positive control experiments were carried out by crossing different labeled test and control DNAs and as well as hybridization on chromosomes.

RESULTS

Comparative genomic hybridization analysis of 12 childhood medulloblastoma samples showed different chromosomal rearrangements. Figure 1 shows minimal common regions of all losses and gains detected by CGH. The gains were found on chromosomes 4p15p16, 7p22, 9q22q31, 14q31, 17p12, Xp11. The losses were differently observed on chromosomes such as 2p, 3p, and 18q. Of 12 cases, 6 and 6 cases were males and females, respectively. Male:female ratio was 1:1. Twelve tumor samples showed amplifications rather than deletions (Table 1).

DISCUSSION

CGH has been used for the identification of chromosomal imbalances in a wide variety of solid tumors and hematologic malignancies. This technique circumvents many limitations associated with conventional cytogenetics, such as the difficulty to obtain metaphases in tumor cells or the often poor quality of chromosome spreads associated with the frequent high complexity of chromosomal changes. Moreover, CGH does not require fresh tumor material, and even small pieces of tumor can be analysed. Archived samples can also be used for CGH studies. For all these reasons, CGH is a very powerful technique for the analysis of chromosomal changes in solid tumors. However, some limitations exist that balanced chromosomal rearrangements are not detected and some regions including 1p, 16q, 19, 22, and Y are difficult to analyse. In this study, we detected chromosomal imbalances in 12 medulloblastoma tumors analysed by CGH. Gains were slightly more frequent than losses, and many recurrent chromosomal changes were found in our study. In agreement with published cytogenetic data, chromosome 7 was the most frequently involved (7). The minimal common site 7p21p22 on chromosome 7 harbours the RALA oncogene and this site involves in all cases in this study. Also, the chromosomes 4p16 and Xp11 regions harbour the RAF1P1 and ARAF1 oncogenes, respectively (19). Moreover, the chromosomes 14q31 and 17p12 were identified as novel sites in this tumor series. In conclusion, oncogenes in the minimal common regions and other chromosomal imbalances may play an important role in transformation and progression of medulloblastoma in childhood. If more data about the childhood medulloblastoma can be obtained, their diagnostic criteria will be more reliable.
References


