Identification of a Novel Homozygous Deletion Region at 6q23.1 in Medulloblastomas Using High-Resolution Array Comparative Genomic Hybridization Analysis

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Abstract

Purpose: The aim of this study is to comprehensively characterize genome copy number aberrations in medulloblastomas using high-resolution array comparative genomic hybridization.

Experimental Design: High-density genomic arrays containing 1,803 BAC clones were used to define recurrent chromosomal regions of gains or losses throughout the whole genome of medulloblastoma. A series of 3 medulloblastoma cell lines and 16 primary tumors were investigated.

Results: The detected consistent chromosomal aberrations included gains of 1q21.3-q23.1 (36.8%), 1q32.1 (47.4%), 2p23.1-p25.3 (52.6%), 7 (57.9%), 9q34.13-q34.3 (47.4%), 17p11.2-q25.3 (89.5%), and 20q13.31-q13.33 (42.1%), as well as losses of 3q26.1 (57.9%), 4q31.23-q32.3 (42.1%), 6q23.1-25.3 (57.9%), 8p22-23.3 (79%), 10q24.32-26.2 (57.9%), and 16q23.2-q24.3 (63.2%). One of the most notable aberrations was a homozygous deletion on chromosome 6q23 in the cell line DAOY, and single copy loss on 30.3% primary tumors. Further analyses defined a 0.887 Mbp minimal region of homozygous deletion at 6q23.1 flanked by markers SHGC-14149 (6q22.33) and SHGC-110551 (6q23.1). Quantitative reverse transcription-PCR analysis showed complete loss of expression of two genes located at 6q23.1, AK091351 (hypothetical protein FLJ34032) and KIAA1913, in the cell line DAOY. mRNA levels of these genes were reduced in cell lines D283 and D384, and in 50% and 70% of primary tumors, respectively.

Conclusion: Current array comparative genomic hybridization analysis generates a comprehensive pattern of chromosomal aberrations in medulloblastomas. This information will lead to a better understanding of medulloblastoma tumorigenesis. The delineated regions of gains or losses will indicate locations of medulloblastoma-associated genes. A 0.887 Mbp homozygous deletion region was newly identified at 6q23.1. Frequent detection of reduced expression of AK091351 and KIAA1913 genes implicates them as suppressors of medulloblastoma tumorigenesis.

Medulloblastoma is one of the most common malignant pediatric tumors of the central nervous system. It accounts for 20% to 30% of pediatric tumors with the peak incidence occurring in children at age of 5 to 10 years old. Effective treatments of this cancer are combinations of surgery, radiation, and chemotherapy. However, recurrence and metastasis are still being encountered and only 50% of the patients survive for 5 years after diagnosis. A better understanding of the molecular basis of this disease is clearly needed to improve its diagnosis and treatment (1). Molecular studies have linked development of medulloblastomas to Sonic hedgehog-Patched signaling pathway and Wnt pathway (2, 3) and overexpression of C-myc was reported to be significantly associated with shortened patient survival times (4). Amplification of N-myc, C-myc, and epidermal growth factor receptor occurs in <10% of this cancer (5–10). Cytogenetic studies have identified gain of chromosome 7 and loss of chromosome 17p together with isochromosome 17q formation as the most frequent chromosomal aberrations, whereas our earlier analyses of loss of heterozygosity have revealed regions of frequent loss on chromosomes 8p, 10q, 11, and 16q. The corresponding minimal deletion regions were 10q25, 11p13-15.1, and 16q24.1-24.3. Detailed fine mapping analysis of chromosome 8 defined a region of homozygous deletion on 8p22-23.1 (11, 12).

Comparative genomic hybridization (CGH) analyses of medulloblastomas have described losses of 8p, 10q, 11q, 16q, and 17p, and gains of 1q, 7, and 17q with infrequent amplifications at 17q. 7q, 8q24, 2p21, 5p15.3, and 11q22.3 (9, 13). Recently, we have reported frequent chromosomal losses of 8p and 17p as well as gain of 17q, 12q, and 7q in 14 medulloblastomas (14). However, CGH analyses reported thus far have mapped aberrations onto metaphase

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chromosomes and so have been limited in resolution to ~10 Mbp (15). More recently, CGH has been carried out by mapping aberrations onto arrayed DNA elements selected to cover the genome at much higher resolution (16, 17). In the present study, we have used array CGH to interrogate medulloblastoma genomes in a series of 16 primary tumors and three medulloblastoma cell lines at 1 Mbp resolution to delineate the minimal regions of genetic aberration. The comprehensive information will indicate minimal regions of genetic aberrations, including microdeletion regions and amplions. This will provide important clues for identification of tumor suppressor genes and oncogenes associated to the development of this cancer.

Materials and Methods

**Tumor samples and patients.** For array CGH study, three medulloblastoma cell lines, DAOY, D283, and D384, were obtained from American Type Culture Collection (Manassas, VA). The cells were cultured in α-MEM (Invitrogen, Carlsbad, CA) supplemented with 10% to 20% fetal bovine serum and 2 mmol/L L-glutamine. All of the medulloblastoma samples (cases MB1 to MB16) were obtained from our frozen tumor bank (Anatomical and Cellular Pathology, Chinese University of Hong Kong). Each tumor was histopathologically classified according to the current WHO criteria (18). Cases were considered suitable for the study when >80% tumor cell content was confirmed histologically. High molecular weight DNA was isolated from 16 tumor samples using conventional proteinase K digestion and phenol/chloroform extraction method. Normal reference DNA was considered suitable for the study when >80% tumor cell content was confirmed histologically.

**Array comparative genomic hybridization analysis.** The BAC arrays used in the current study were obtained from Prof. J.W. Gray. Each microarray contained 1,803 clones distributed throughout the human genome. Array CGH analysis was done as described previously. Briefly, tumor DNAs and reference DNAs were labeled by random priming (Invitrogen) with Cy3-dUTP and Cy5-dUTP (Amersham Pharmacia, Piscataway, NJ), respectively. Unincorporated fluorescent nucleotides were removed using Sephadex G-50 spin columns (Amersham Pharmacia). Five hundred nanograms of each of the labeled tumor and reference DNAs were cohybridized to microarrays in the presence of 100 μg of tRNA and 50 μg of human Cot-1 DNA (Invitrogen) to suppress hybridization of labeled probe to repeat sequences. This probe mixtures were ethanol precipitated and resuspended in 60 μL of a hybridization solution containing 50% formamide, 10% dextran sulfate, 4% SDS in 2× SSC, and applied to microarray templates. After hybridization for 2 days, posthybridization wash with 50% formamide/2× SSC was done to remove unhybridized probes. Sixteen-bit fluorescence single-color intensity images of Cy-3 and Cy-5 microarray images were captured using GenePix Pro 4.0 (Axon Instruments, Inc., Foster City, CA). The image data was analyzed by Spot and Sproc software as described previously (19, 20). The software automatically identified each spot for analysis of the set of Cy3/Cy5 ratios on all targets. This Cy3/Cy5 ratio of a target indicates the degree of gain or loss of copy number.

**Fluorescence in situ hybridization.** FISH analyses were done to confirm high-level amplification and homozygous deletion. For verification of 7q35 amplification in three formalin-fixed and paraffin-embedded sections of primary medulloblastoma, BAC clone RP4-545C24 (7q35) was used as a test probe and a commercially available chromosome 7 α-satellite as a reference probe. For homozygous deletion of 6q23.1, dual-color FISH analysis were conducted on both the three medulloblastoma cell lines and 17 formalin-fixed and paraffin-embedded sections using the BAC clone RP11-7306 as a test probe and a commercially available chromosome 6 α-satellite as a reference probe. FISH analysis was done as described in our previous studies (21). High molecular weight DNA was isolated from the BAC clones and labeled with spectrum Green-dUTP.
Homologous Deletion of 6q23.1 in Medulloblastoma

(Vysis, Downers Grove, IL) by nick translation. Chromosomal location of the clones had been validated by previous FISH analysis of metaphase spreads prepared from peripheral blood of normal healthy individuals. The reference probes are digoxigenin-labeled centromere probes (Appligene oncor), which detected with antidigoxigenin rhodamine (Sigma Chemical, Co., St. Louis, MO). FISH probe was prepared by combining the labeled test and reference probes and applied to pretreated paraffin section slides. After overnight hybridization, washing, detection, and counterstaining with 4',6-diamidino-2-phenylindole for imaging, cells were examined with a Zeiss fluorescence microscope (Iena, Germany), which connected with a triple band pass filter set. In each case, at least 100 cells were analyzed. Amplifications were scored when ratios of test to reference clones were >2. Samples with signal ratios of test to reference probes <0.8 were counted as deletion cases.

**PCR.** Thirty pairs of primers for sequence-tagged sites spanning the 6q22-23 region were used to fine map the boundaries of homologous deletion regions at 6q23.1. All of the primer sequences were retrieved from UniSTS at the National Center for Biotechnology Information. Detailed information on the primers was shown on Fig. 1. PCR reactions were conducted in a total volume of 25 μL containing 1× PCR buffer (Perkin-Elmer/Cetus), 2.5 mmol/L MgCl₂, (Perkin-Elmer/Cetus, Norwalk, CT), 62.5 μmol/L of each of deoxyribonucleotide triphosphates, and 0.25 pmol each of the left and right primers (GeneAmp, PE 9700). The reaction mixes were first heat denatured for 10 minutes at 95°C, followed by 40 amplification cycles, and another 7 minutes of extension at 72°C. Each PCR cycle consisted of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. The resulting PCR products were mixed with 5 μL of loading buffer and resolved by electrophoresis through 2% PAGE gels, stained with ethidium bromide, and visualized under UV illumination.

**Quantitative reverse transcription-PCR analysis.** According to University of California Santa Cruz Genome Browser on Human (July 2003 Freeze), the 0.887 Mbp homologous deletion region covered three candidate genes. These are BC060845 (LMBT3 protein), AK091351 (Hypothetical protein FLJ34032), and KIAA1913. RNA transcript levels of these three genes were measured in medulloblastoma cell lines (DAOY, D283, and D384) and 10 primary tumors. Total RNAs for these samples and from normal cerebellum (Life Technologies, Inc., Rockville, MD) were extracted with TRizol (Invitrogen). mRNA copy numbers of the three genes and a housekeeping gene (β-actin) were determined by real-time quantitative reverse transcription-PCR using SYBR Green PCR Master Mix (Applied Biosystem) and an iCycler instrument (Bio-Rad, Richmond, CA). β-actin levels were used to normalize the investigated samples. Gene-specific primers were used to assay the quantity. The designed primer pairs were:

**BC060845**, forward: 5'-ATTACCCCTGAGGCTGTGTT-3'; reverse: 5'-ACAGTTGGCAGCAGCTGG-3'.

**AK091351**, forward: 5'-CACAAGACTACATGGGACAA-3'; reverse: 5'-GGGTACAGTGAGCCTTCA-3'.

**KIAA1913**, forward: 5'-GGCTTTATCCCCATCTGGT-3'; reverse: 5'-GAATGCAATGATGCTGCTCAA-3'.

**β-actin**, forward: 5'-TGAGCGCGCCTACAGCTT-3'; reverse: 5'-TCCCTAATGGTACGCGCAATT-3'.

The PCR thermal profile consisted of UNG incubation at 50°C for 2 minutes, Ampli-Taq Gold activation at 95°C for 10 minutes followed by 50 cycles of denaturation at 95°C for 15 seconds, and annealing/extension at 63°C for 1 minute. The cycle number (C value) was recorded at which the increase in fluorescent signal associated with an exponential growth of PCR products was firstly detected by the laser detector. The results of the n-fold difference in the expression level of investigated genes relative to the β-actin gene were calculated using the formula, n = 2⁻ⁿc, sample. The ΔC value of each sample was determined by subtracting the average C value of target gene from the average C value of the β-actin gene. On the other hand, the n value of each sample was subsequently normalized, with the mean n value in the normal cerebellum normalized to 1.

**Results**

**Array comparative genomic hybridization analysis.** A series of normal versus normal hybridizations were done to define the normal variations of the test to reference (Cy3/Cy5) intensity ratio for each target clone (19, 20). Hybridizations were normalized so that the overall ratio of green to red signals was centered at 1. With reference to previous array CGH studies, thresholds for copy number gain and loss were log₂ ratio 0.2 and −0.2, respectively (19, 20, 22). The array CGH analysis results for the 19 medulloblastoma samples and representative array CGH profiles are illustrated in Fig. 2. The major findings of this study are illustrated in Table 2. Chromosomal gains were more frequently detected than losses. Genomic occurrence in >7 of 19 cases were found at chromosome 1q21.3-q23.1 (36.8%), 1q32.1 (47.4%), 2p23.1-p25.3 (52.6%), 7 (57.9%), 9q34.13-q34.3 (47.4%), 17p11.2-q25.3 (89.5%), and 20q13.1-q13.33 (42.1%). Lengths of the corresponding small regions of gain were 2.49 Mbp at 1q21.3-q23.1, 2.61 Mbp at 1q32.1, 30.4 Mbp at 2p23.1-p25.3, 5.3 Mbp at 9q34.13-q34.3, and 6.51 Mbp at 20q13.31-q13.33. The gains at 9q34.13-q34.3 and 20q13.31-q13.33 have not been reported in previous CGH analyses. Regions of chromosomal losses were detected on 3q26.1 (57.9%), 4q31.23-q32.3 (42.1%), 6q21.1-25.3 (57.9%), 8p22-23.3 (79%), 10q24.32-26.2 (57.9%), and 16q23.2-q24.3 (63.2%). Lengths of the defined minimal deletion regions were 4.79 Mbp at 3q26.1, 18.76 Mbp at 4q31.23-q32.3, 9.46 Mbp at 6q21.1-25.3, 11.74 Mbp at 8p22-23.3, 23.6 Mbp at 10q24.32-26.2, and 9.18 Mbp at 16q23.2-q24.3. The loss of 4q31.23-q24.3 has not been reported. The frequencies of gains at 1q, 2p, and 17q and losses of 3q, 6q, 8p, 10q, and 16q in our study were higher than that previously reported (Table 2). Nonrandom (detected in more than two cases) apparent homologous deletions and amplifications were scored when log₂ ratio was <−1 (loss of two copies) or >1 (gain of two copies), respectively. As shown in Table 3, amplifications were found on chromosomes 2, 7q, 11p, and 15q, whereas nonrandom homologous deletions were detected on chromosomes 1p, 5q, 6p, 6q, 7p, 7q, 8q, 9p, 10q, 14q, and 16q.

**Verification of amplification and homologous deletion by fluorescence in situ hybridization analysis.** Array CGH analyses showed amplification in three primary tumors at the point of the genome interrogated by BAC clone RP4-545C24 (7q35). The results were confirmed by FISH analysis of formalin-fixed and paraffin-embedded sections of the samples. As shown in Fig. 3A, two representative cases showed that majority of the cells showed two copies of the chromosome 7 centromere and multiple copies of the regions probed by RP4-545C24.

Homologous deletion of the clone RP11-73O6 at 6q23.1 was one of the significant new findings in the present study. Array CGH analyses indicated that this region may be homologously deleted in the cell line DAOY and single copy losses in 5 of the 16 (31%) primary tumors. This result was further confirmed with FISH using the same BAC clone RP11-73O6 that detected an apparent homologous deletion during

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Fig. 1. Study of homozygous deletion of 6q23.1 in medulloblastoma. A, fine mapping of small deletion region in medulloblastoma cell lines. In cell line DAOY, a 0.887 Mbp novel homozygous deletion was defined at chromosome 6q23.1 and flanked by the sequence-tagged site markers SHGC-13149 and SHGC-110551. B, quantitative reverse transcription-PCR analysis of the three genes covered in the 0.887 Mbp smallest region of homozygous deletion at 6q23.1. mRNA copy numbers of these genes and the housekeeping gene (β-actin) were determined. β-actin levels were used to normalize samples. Expression level of the three genes in normal cerebellum (Normal) is 1. Complete loss of expression of all of the three genes was detected on cell line DAOY. Loss of mRNA expression (with >2-fold reduction of expression) was detected in 10% (BC060845), 50% (AK091351), and 70% (KIAA1913) of 10 medulloblastomas as well as in cell lines D283 and D384.
Fig. 2. Results of array-based CGH analysis. A, summary of copy number profiles of 19 medulloblastoma cases. Clones are ordered from chromosomes 1 to 22 and within each chromosome on the basis of the University of California Santa Cruz mapping position (http://genome.ucsc.edu/). Incidences of gains and losses of the BAC clones were indicated on the top and bottom parts of the figure, respectively. B and C, representative array CGH profiles of two medulloblastomas. Vertical lines, separation of chromosomes. Log2 ratios of the 1,803 clones were plotted on the basis of chromosome position. The clones were ordered from the 1p telomere on the left to the Xq telomere on the right. D, corresponding detail array CGH profile of chromosome 7 amplification in the case of (B). E, corresponding detail array CGH profile of chromosome 6q homozygous deletion in the case of (C).
array CGH. As shown in Fig. 3B, no FISH signals were detected in the cell line DAOY after hybridization with this clone. In a control experiment, FISH with this probe to the cell line D283 showed the two hybridization signals expected on the basis of array CGH. To investigate the significance of this genetic alteration in medulloblastomas, FISH analyses were also conducted on 17 formalin-fixed and paraffin-embedded sections of medulloblastomas. Among these cases, two were analyzed using both array CGH and FISH. Neither showed loss at RP11-73O6. However, FISH analyses revealed

### Table 2. Summary of array CGH analysis of 19 medulloblastoma cases

<table>
<thead>
<tr>
<th>Regions of frequent chromosomal gains</th>
<th>Incidence (%)</th>
<th>Flanking regions, bp (length)</th>
<th>Incidence of previous publications (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1q21.3-q23.1</td>
<td>7 (36.8%)</td>
<td>Chr1: 151,108,268-153,594,838 (2.49 Mbp)</td>
<td>24-33&lt;sup&gt;1&lt;/sup&gt;-&lt;sup&gt;2&lt;/sup&gt;-&lt;sup&gt;4&lt;/sup&gt;-&lt;sup&gt;9&lt;/sup&gt;</td>
</tr>
<tr>
<td>1p32.1</td>
<td>9 (47.4%)</td>
<td>Chr1: 200,720,382-203,329,259 (2.61 Mbp)</td>
<td>24-33&lt;sup&gt;1&lt;/sup&gt;-&lt;sup&gt;2&lt;/sup&gt;-&lt;sup&gt;4&lt;/sup&gt;-&lt;sup&gt;9&lt;/sup&gt;</td>
</tr>
<tr>
<td>2p23.1-p25.3</td>
<td>10 (52.6%)</td>
<td>Chr2: 51,060-30,451,080 (30.40 Mbp)</td>
<td>50&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>11 (57.9%)</td>
<td>Chr7: 1-158,628,139 (158.63 Mbp)</td>
<td>20-60&lt;sup&gt;7&lt;/sup&gt;-&lt;sup&gt;8&lt;/sup&gt;-&lt;sup&gt;10&lt;/sup&gt;-&lt;sup&gt;11&lt;/sup&gt;</td>
</tr>
<tr>
<td>9q34.13-q34.3</td>
<td>9 (47.4%)</td>
<td>Chr9: 131,414,550-136,715,127 (5.30 Mbp)</td>
<td>Nil</td>
</tr>
<tr>
<td>17p11.2-q25.3</td>
<td>17 (89.5%)</td>
<td>Chr17: 21,014,521-77897241 (56.89 Mbp)</td>
<td>48-85&lt;sup&gt;7&lt;/sup&gt;-&lt;sup&gt;11&lt;/sup&gt;-&lt;sup&gt;12&lt;/sup&gt;-&lt;sup&gt;13&lt;/sup&gt;-&lt;sup&gt;14&lt;/sup&gt;-&lt;sup&gt;15&lt;/sup&gt;</td>
</tr>
<tr>
<td>20q13.31-q13.33</td>
<td>8 (42.1%)</td>
<td>Chr20: 55,173,138-61,685,988 (6.51 Mbp)</td>
<td>Nil</td>
</tr>
</tbody>
</table>

### Table 3. Clones with nonrandom high-level amplifications or homozygous deletions in 19 medulloblastoma cases

<table>
<thead>
<tr>
<th>Physical position (bp)</th>
<th>Length (bp)</th>
<th>Chromosomal location</th>
<th>Genes/locus</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP11-547I5</td>
<td>168,506</td>
<td>2p22.2</td>
<td>BX538008, AB037835 (KIAA1414), AK001513, F;K38348, PRKR</td>
<td>3</td>
</tr>
<tr>
<td>RP11-811J9</td>
<td>72,045</td>
<td>7q34</td>
<td>CLCN1, KIAA0773, ZYX, EPHA1</td>
<td>2</td>
</tr>
<tr>
<td>RP4-546C24</td>
<td>155,952</td>
<td>7q35</td>
<td>AF327904, TIM/ARHGEF5, TPK1</td>
<td>3</td>
</tr>
<tr>
<td>RP11-390K5</td>
<td>190,016</td>
<td>11p11.2</td>
<td>MGC4707, ZNF289, PACSIN3, AB107037, AK123492, NRIH3, ACP2, MADD, DDB2</td>
<td>2</td>
</tr>
<tr>
<td>RP11-522G20</td>
<td>180,722</td>
<td>15q21.2</td>
<td>CYP19A1, BC056258</td>
<td>2</td>
</tr>
<tr>
<td>RP11-24C10</td>
<td>56,117</td>
<td>1p35.1</td>
<td>STK22C, FLJ10276, AY358230</td>
<td>2</td>
</tr>
<tr>
<td>RP11-64H16</td>
<td>107,455</td>
<td>6p25.3</td>
<td>IRF4, SEC5L1 (FLJ11026)</td>
<td>2</td>
</tr>
<tr>
<td>RP11-73O6</td>
<td>140,446</td>
<td>12q13.13</td>
<td>BC060845 (L3MBTL3), KIAA1414, SAMD3</td>
<td>2</td>
</tr>
<tr>
<td>RP11-226O1</td>
<td>172,573</td>
<td>11p11.1</td>
<td>AK097033</td>
<td>2</td>
</tr>
<tr>
<td>RP11-697H7</td>
<td>183,846</td>
<td>12q23.1</td>
<td>CD36, SEMA3C</td>
<td>2</td>
</tr>
<tr>
<td>RP11-456A16</td>
<td>194,590</td>
<td>16p11.2</td>
<td>AK093407, BX648731, AF066468</td>
<td>2</td>
</tr>
<tr>
<td>RP11-14912</td>
<td>101,154</td>
<td>15q21.2</td>
<td>MTAP, AFB211119, MTS1/CDK14/CDK2NA1/2, CDK2N2</td>
<td>2</td>
</tr>
<tr>
<td>RP11-145E5</td>
<td>157,533</td>
<td>9p21.3</td>
<td>AFB109294, BC014469, L36444, AFO04819, UI7075, CR5S629</td>
<td>2</td>
</tr>
<tr>
<td>RP11-314P12</td>
<td>75,036</td>
<td>10q11.2</td>
<td>BC021910, U432387, CR541950, BC032221</td>
<td>3</td>
</tr>
<tr>
<td>RP3-449M8</td>
<td>140,425</td>
<td>14q24.3</td>
<td>NUMB, BX248073, BX248775, BX248781</td>
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<tr>
<td>RP11-18C13</td>
<td>151,645</td>
<td>14q32.3</td>
<td>LOC122618, C14ORF79</td>
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<td>RP11-229O3</td>
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<td>16q21</td>
<td>CDH11</td>
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<td>RP11-1B11</td>
<td>161,878</td>
<td>16q23.3</td>
<td>HSPC105, HSD17B2</td>
<td>2</td>
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</table>
five paraffin-embedded cases with loss at RP11-73O6. Figure 3C illustrates detection of copy loss of two medulloblastomas. Two signals of chromosome 6 centromere were scored, whereas none or one signal of RP11-73O6 clone was identified in most of the cells of MB21. In case MB25, signal ratio of test to reference probes was 0.35, which indicate loss of RP11-73O6 clone.

Mapping of homozygous deletion boundaries of 6q23.1. We analyzed deletions in the medulloblastoma cell lines D283, DAOY, and D384 using 30 primers for sequence-tagged sites at
6q22-23 to refine the boundaries of the minimal region of homozygous deletion. Ten of these markers showed the expected, homozygous deletion in DAOY (Fig. 1A). The extent of the homozygous deletion was ~0.887 Mbp defined by flanking markers SHGC-14149 (6q22.33) and SHGC-110551 (6q23.1). These same markers, 10 sequence-tagged sites, showed no deletions in the cell lines DAOY and D283, confirming the array CGH analyses.

**Examination of expression levels of candidate 6q23.1 tumor suppressor gene(s) by reverse transcription-PCR analysis.** Analysis of 0.887 Mbp region of homozygous deletion using information from the University of California Santa Cruz Genome Browser on Human (http://genome.ucsc.edu; July 2003 Freeze) revealed three candidate genes; BC060845 (L3MBTL3 protein), AK091351 (hypothetical protein FLJ34032), and KIAA1913. We measured expression levels of these three candidate genes in medulloblastoma cell lines and primary tumors using quantitative reverse transcription-PCR analysis with total RNAs of a normal cerebellum as control. None of these genes was expressed in the homozygously deleted cell line DAOY and their expression levels were reduced in cell lines D283 and D384. As shown in Fig. 1B, reduced gene expression levels were also detected in the 10 analyzed primary tumors with the frequencies of 70% in KIAA1913, 50% in AK091351, and 10% in BC060845.

**Discussion**

To understand the tumorigenesis of medulloblastomas, several CGH studies had been conducted to investigate the chromosomal aberrations throughout the whole genome of this cancer. In the current study, we applied a high-throughput approach of high-resolution array CGH analysis to comprehensively delineate the minimal regions of genetic aberrations in medulloblastomas. We have systematically screened a series of 19 medulloblastomas. Several chromosomal aberrations were identified. Most of these chromosomal aberrations were in concordance with the previous findings, but with higher incidences in current study (9, 13, 14, 23–28). Frequent gains of 9q34.13-q34.3 and 20q13.31-q13.33, as well as loss of 4q31.23-q32.3, were newly identified. With a higher-resolution power in detecting chromosomal alterations at 1 Mb, current array CGH results were able to detect smaller regions of gains and losses. Frequent gain of the 5.3 and 6.51 Mbp amplicons on 9q34 and 20q13.3, as well as loss 18.76 Mbp on 4q31-32, might be underestimated in previous CGH analysis due to the resolution limit. Although rarely identified in medulloblastomas, these chromosomal alterations had been reported in other brain tumors. Gain of 9q34 had been identified in pilocytic astrocytomas (29). Chromosome 9q34 locates the common oncogene ABL1 gene, which translocated with BCR gene in leukemia (30). Gain of 20q13.3 was found in both glioblastoma and in our previous CGH analysis of ependymomas (31, 32). Frequent loss of 4q was suggested to be involved in the tumorigenesis of oligodendrogliomas (33).

Epidermal growth factor receptor at 7p and ERBB2 at 17q are two of the oncogenes related with the development of medulloblastoma. ERBB2 amplification is suggested to be a poor prognosis factor (34). However, amplification of these two genes and other oncogenes (such as N-myC and C-myC) was infrequent and detected in <10% of medulloblastomas (5–10).

Hence, the need of searching target oncogenes involved in the tumorigenesis of this cancer remains. In current array CGH analysis, chromosome 7q was detected to have both high incidence of gains and amplifications in 52.6% of the cases. High-level amplification was found at two BAC clones RP11-81119 (7q34) and RP4-545C24 (7q35) on two and three of the primary tumors, respectively (Table 3). These results were confirmed by FISH analysis. Besides chromosome 17, gain of chromosome 7q is the second most commonly reported cytogenetic abnormality in medulloblastomas. The reported incidence of gain of chromosome 7 ranged from 20% to 60% (24, 26–28). Oncogene(s) located on this chromosome may play a critical role in the development of medulloblastomas. However, previous studies did not fine map the minimal region of aberration and no known medulloblastoma-related oncogenes had been identified on this region. Our present array CGH template indicated a 1.04 Mb amplification core at the two BAC clones, RP11-81119 and RP4-545C24. It was flanked by the clones RP11-55613 and RP11-511P7, which were 7.23 Mb apart. According to University of California Santa Cruz Human Genome Browser, this region covered several oncogenes, such as CLCN1, ZYX, EPHA1 TIM/ARHGEF5, TPK1, etc. Target medulloblastoma-associated oncogene(s) residing on this region may be associated with the tumorigenesis of this cancer.

Frequent detection of homozygous deletion on chromosome 6q23 was one of the most striking finding in the present study. Homozygous deletion of this region was found on cell line DAOY. Furthermore, copy loss was detected in 30% of primary tumors. This result was further confirmed with FISH using the same BAC clone, RP11-73O6, which is located at 6q23.1. Loss of chromosome 6q had been reported by Michiels et al. (35) and Thomas et al. (36) in 33.3% and 26.1% of medulloblastomas. Both studies investigated the whole long arm of chromosome 6 and no fine mapping analysis was done. In the present study, a 0.887 Mb smallest region of homozygous deletion was defined at 6q23.1. For deletion of 6q23.1 in primary cases, 5 of 16 cases and 5 of 17 cases were detected to have loss of RP11-73O6 clone in array CGH and FISH analysis, respectively. Taken together, 10 of 33 (30.3%) of the cases were detected to have chromosomal aberrations at 6q23.1. As array CGH cannot indicate presence of translocations, there may be underestimation of such genetic aberrations. Chromosome 6q23.1 might be a consistent fragile site in medulloblastoma. Tumor suppressor gene(s) located at this novel 6q23.1 homozygous deletion region may be inactivated by the mechanisms of chromosomal deletion and inactivation by translocation. Inactivation of tumor suppressor genes by deletion and translocation were commonly reported, such as in the tumor suppressor genes of RB1, WT1, and FHIT genes (37, 38). Deletion of chromosome 6q23 was also common to diverse tumor types, such as pancreatic cancer, hepatocellular carcinomas, gastric cancer, ovarian cancer, leukemia, etc. (39–43). Chromosome 6q23 is a common break point and frequently involved in translocations of lymphomas. This fragile site was frequently translocated with several other chromosomes, such as chromosomes 2, X, and 12 (44, 45).

Furthermore, homozygous deletion of 6q23-24 has been reported in a study of prostate cancer (46). Previous studies had reported the importance of detection of homozygous deletion in medulloblastomas, such as studies of p15 and p16.
genes, DMBT1 genes, and chromosomal regions 17p13.1 and 8p22-23.1 (12, 47, 49, 50). These results showed that 6q23 is a critical tumor suppressor locus and harbors tumor suppressor genes that are involved in the development of human cancers.

To identify target medulloblastoma-associated tumor suppressor genes located at 6q23.1, expression levels of the genes (BC060845, AK091351, and KIAA1913) covered in this novel homozygous deletion region were investigated. Our results illustrated complete loss of the transcripts of these three genes on cell line DAOY, which has the 0.878 Mbp homozygous deletion region. Expression of these genes was detected in normal cerebellum, but reduced in cell lines D283 and D384. In primary tumors, reduced expression levels were detected on all of these three genes, with higher incidences in AK091351 (50%) and KIAA1913 (70%). Hence, these two genes may be candidate medulloblastoma-associated tumor suppressor genes at 6q23.1. Their tumor suppressor properties will be determined. This information will lead to a better understanding of medulloblastoma tumorigenesis and used as new targets for therapeutic intervention and drug discovery of this cancer.

In conclusion, current high-resolution array CGH analysis generates a comprehensive pattern of chromosomal aberrations involved in medulloblastomas. The findings of our study will be used for identification of medulloblastoma-associated genes and will add to the understanding of this disease. This will be achieved by identifying and studying the roles of medulloblastoma-related genes to elucidate the genetic basis of this cancer and their impact on tumor development. A 0.887 Mbp homozygous deletion region was newly identified at 6q23.1. The AK091351 and KIAA1913 genes may be target medulloblastoma-related tumor suppressor genes at 6q23.1. They may be used as potential molecular targets for diagnosis of medulloblastomas, as well as targets for development of alternative therapeutic options to improve the treatment of this malignant pediatric cancer.

References


Identification of a Novel Homozygous Deletion Region at 6q23.1 in Medulloblastomas Using High-Resolution Array Comparative Genomic Hybridization Analysis

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