Human medulloblastoma samples

Tumor specimens were obtained in accordance with the Research Ethics Board at the Hospital for Sick Children (Toronto, Canada). 201 primary medulloblastomas were obtained as surgically-resected, fresh-frozen samples. Tumor specimens were acquired from the Cooperative Human Tissue Network (CHTN; Columbus Ohio, USA), the Brain Tumor Tissue Bank (London, Ontario, Canada). SNP array and RT-PCR were carried out as described, below.

SNP Array Data Processing

Affymetrix CEL files were extracted using the Affymetrix Data Transfer Tool (Version1.1.0). For SNP genotyping, the BRLMM Analysis Tool (Version 1.0) was employed for individual array platforms using default parameters. Copy number analysis was performed in CNAG 2.0 (1), with non-self analysis performed automatically using 100 normal control samples as a reference with a maximum of 10 reference samples of the same gender employed per analysis. Inferred copy number changes were predicted using Hidden Markov Model (HMM) with default parameters.

To identify homozygous deletions, the following criteria were used: - $\geq 3$ contiguous SNPs; - size range: 1Kb-10Mb; - mean CNAG HMM copy number $\leq 0$. To identify amplifications, the following criteria were used: - $\geq 5$ contiguous SNPs; - size range: 10Kb-10Mb; - mean CNAG HMM copy number $\geq 5$. Recurrent, focal single copy losses were reported using the following criteria: - $\geq 3$ samples with overlapping interstitial loss (CNAG HMM copy number = 1); - size range of individual losses: 10Kb-5Mb.

RT-PCR analysis
For RT-PCR analysis, total RNA from primary medulloblastomas was extracted using Trizol (Invitrogen). Taqman microRNA assays (Applied Biosystems) were used to quantify mature miRNA expression using StepOnePlus and ABI Prism 7900HT Sequence Detection Systems (Applied Biosystems). Statistical significance was determined using two-tailed Student’s T-test in Excel. Total RNA from two independent pooled wild-type and TSC2-RGΔ PN4 CGNPs were extracted using Trizol (Invitrogen). 1 ug total RNA was used to generate cDNA and PCR product using Superscript One-Step RT-PCR kit (Invitrogen). Primers were used as follows: TSC2-RGΔ 5’(GTCCAGGAGAGACTCAGGTGCCAGT) and TSC2-RGΔ 3’ (CTGTAAGGTCTGCAAAGTCCAGGAGAA); β-actin 5’ (CACAGCTACAAAGAGCGGCTCCACC) and β-actin 3’ (CACTGCATTCTAGTTGTGGTTTGTCC).

Expression analysis of TSC2 and CDKN1B in human medulloblastoma

Gene expression profiling of normal human cerebellum (fetal and adult) and primary human medulloblastomas was performed using Affymetrix exon arrays as described previously (2).

References