Supplementary Material

Supplementary Materials & Methods

Medulloblastoma Tumour Specimens. Tumour 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. Tumour specimens were acquired from the Cooperative Human Tissue Network (CHTN; Columbus Ohio, USA) and the Brain Tumour Tissue Bank (BTTB; London, Ontario, Canada).

100K and 500K GeneChip Mapping Arrays. MB samples were processed and hybridized to Affymetrix Genechip Human Mapping 100K and 500K Array sets at The Centre for Applied Genomics (TCAG) at the Hospital for Sick Children (Toronto, Canada). SNP arrays were processed and the data analyzed for copy number changes as described (9).

MicroRNA Arrays. MicroRNA expression profiling of primary human medulloblastomas was performed on Trizol-extracted (Invitrogen) total RNA using The Ohio State University Comprehensive Cancer Center Version 3.0 microRNA microarray. The resulting raw data was quantile normalized and thresholded to a value of 4.5 (log2) as described previously (18). Using only probes designed against known human mature microRNAs, statistical analyses were performed using BRB-ArrayTools developed by Dr. Richard Simon and BRB-ArrayTools Development Team . Reported fold-changes reflect ratios of geometric means and p-values for informative probes were calculated using the class comparison and class prediction tools.

Murine microRNA profiling was carried out using total RNA isolated with the mirVana extraction kit (Ambion) and hybridized to LC Sciences (Houston, TX) miRNA microarrays. These arrays detect miRNA transcripts corresponding to 599 mature miRNAs contained in the Sanger miRBase Release MiRMouse 11.0. Raw data were median normalized across arrays and miRNAs whose expression displayed greater than two-fold differences across triplicate samples when comparing vehicle-treated to Shh-treated samples were validated as described below.

Affymetrix exon arrays. MB samples were processed and hybridized to Affymetrix Genechip Human Exon 1.0 ST Arrays TCAG as directed by the supplier. CEL files were imported into Affymetrix Expression Console (Version 1.1) and gene level analysis (CORE content) was performed. Arrays were quantile normalized (sketch) and summarized using PLIER with PM-GCBG background correction. MB samples were classified into molecular subgroups using TM4 Microarray Software Suite (MeV) by unsupervised hierarchical clustering using Pearson Correlation and bootstrapping analysis of 1300 SD genes. Clustering trees showed at least 97% statistical support for the segregation of samples into 4 unique subgroups. Significant genes representative of the individual subgroups were identified using T-tests with standard Bonferroni correction.

Taqman analysis. Samples were analyzed using StepOnePlus and ABI Prism 7900HT Sequence Detection Systems (Applied Biosystems). Statistical significance of qRT-PCR data was determined using two-sample Wilcoxon and Student's T-tests.

Retrovirus construction and primary culture experiment quantification. The miR-17-19b cassette (gift of Jason Huse and Eric Holland, Memorial Sloan-Kettering Cancer Center) was cloned into the retroviral vector pWzl-ires-GFP. Infection of CGNPs and

subsequent immunostaining were carried out as previously described (7). TIFF images of 4 random fields were taken for each experimental group using the 20X objective and quantified using Volocity.

Supplementary Figures & Tables

Supplementary Figure 1. Unsupervised hierarchical clustering of 90 MBs identifies four unique subgroups. A. Heatmap showing expression profile of 1300 genes used for clustering. **B.** Support tree result for the clustering shown in A. Numbers indicate the statistical support for the nodes of the trees (%), based on resampling of the data by bootstrapping genes.

Supplementary Figure 2. miR-17/92 up-regulation in specific MB subgroups. Boxplots showing expression data, as determined by miRNA array, for components of the miR-17/92 polycistron in molecular subgroups of MB. Expression of miR-17/92 is consistently highest in the SHH subgroup (red), followed by Group C (yellow) and WNT (blue) subgroups of tumours. Group D (green) tumours are characterized by the lowest expression of the miRNA cluster. Expression values represent $\log_2$-transformed signal intensities from the normalized array data.

Supplementary Figure 3. MYC family expression in specific MB subgroups. Boxplots showing expression data, as determined by exon array, for *MYCN* (left panel) and *MYC* (right panel) in molecular subgroups of MB. Expression values represent $\log_2$-transformed signal intensities from the normalized array data.

Supplementary Table 1. Statistical comparison of miRNA expression profiles in MB versus normal CB samples. Table lists comparison data for 548 miRNAs profiled on the miRNA arrays. Significance of differential expression between MB and normal CB

was determined using the class prediction tool in BRB-ArrayTools.

Supplementary Table 2. Statistical comparison of MB subgroups based on mRNA expression profiles. Table shows upregulated genes in each of the identified MB subgroups compared to the other three molecular subgroups as determined by T-Test statistics. Overlap between genes identified in this study with those reported in a recent study is also included (10).

Supplementary Table 3. Statistical comparison of differential miRNA expression in high MYCN/MYC and low MYCN/MYC expressing tumours. Shown are the results for comparison data of 548 miRNAs examined on the miRNA arrays. Significance of differential expression between high MYCN/MYC and low MYCN/MYC expressing tumours was determined using the class prediction tool in BRB-ArrayTools.

Supplementary Table 4. Representative miRNAs identified in MB subgroups. Table lists significant miRNAs representative of the four MB subgroups identified by clustering of mRNA expression array data. Significant miRNAs were identified using the class comparison tool in BRB-ArrayTools.

Supplementary Table 5. Induction/suppression of miRNAs in Shh-treated CGNPs. Table lists miRNAs exhibiting differential expression in CGNPs following Shh treatment. Fold-change between Shh- and vehicle-treated cells is summarized as the mean fold-change observed for probeset pairs that showed differential expression for a given miRNA.