MORAN FOUNDATION AWARD PROGRESS REPORT

Molecular Genomic Profiling of Medulloblastoma

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Based on the working hypothesis that multiple, non-random genomic alterations occur in medulloblastomas and other PNETs and account for the neoplastic transformation of these tumors, the principal objective of this study was to identify multiple specific genetic alterations in medulloblastomas and related supratentorial primitive neuroectodermal tumors (PNETs).

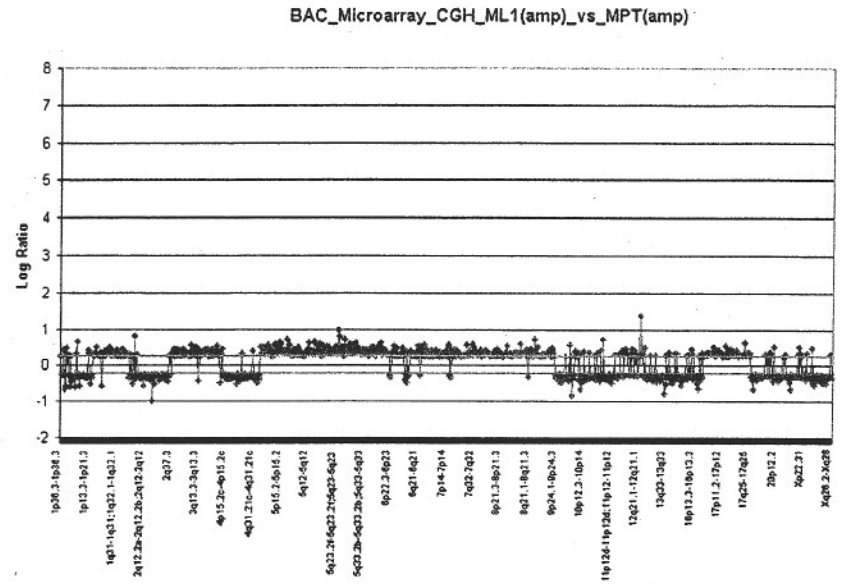
The specific aims were:

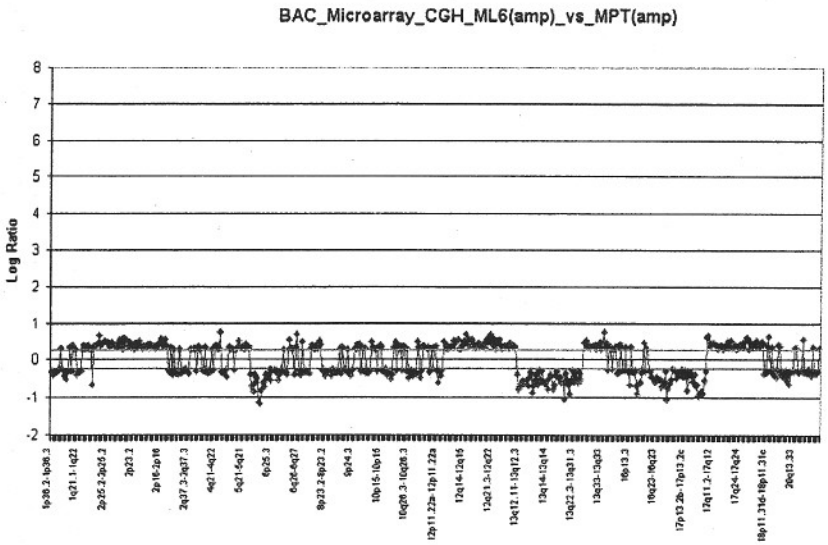
1. To identify multiple sites of genomic alteration which may indicate putative tumor suppressor gene or oncogene loci in medulloblastomas and related PNETs.
2. To identify whether such altered loci are associated with medulloblastomas in general or specific for certain subsets of medulloblastomas with the possibility that the finding of subset specificity may have implications for differing tumor biology or clinical outcome

In the original grant, a combination of cDNA and BAC array based analyses were proposed. Due to the high cost of the BAC arrays and to ensure that the data generated from these studies are meaningful enough to form the basis for the submission of a grant proposal to other funding agencies such as the NIH, the initial analysis focused on the use of BAC arrays only. In pursuance of these objectives, a total of nine (9) medulloblastoma tumors were subjected to micro-array comparative genomic hybridization. The BAC array contained 2500 chromosome specific BACs (Spectral Genomics). All the human BAC clones had been end-sequenced and mapped by fluorescent *in situ* hybridization (FISH). They represent second generation human BACs, which are well distributed across the whole genome and provide a resolution of approximately 1 Mb. We optimized the hybridization protocol. Images were quantified using the Scan Array express software (Perkin Elmer). The fluorescence intensities for Cy3 and Cy5 for each of the hybridization spots were normalized using the Lowess's method and following which the ratio of the intensities of test DNA to reference DNA for each spot was determined. A duplicate dye swap experiment was done to compensate for the non-linearity of intensities between the two dyes. Gains or losses were called when ratio of normalized intensities were greater than two standard deviations above or below 1, respectively.

Results

BAC array CGH was done on 9 of 21 tumors previously characterized by chromosomal CGH. Fig. 1a-d shows the copy-number profile for all nine tumors. The BAC array CGH was able to detect smaller regions of chromosomal gain or loss when compared with chromosomal CGH. As revealed by the BAC array analysis, the regions of gain detected by chromosomal CGH represented regions of high to medium copy number gains. The BAC array CGH was therefore, more sensitive in detecting genomic alterations. Although multiple genetic alterations were identified, a high frequency of genomic copy-number gain was observed to involve chromosomes 2p (6/9) and 17q 6/9) respectively. For example, ML-13 showed a high chromosome copy-number gain (42 – 54 fold) on 2p24 (Fig. 1b). This represents the chromosomal locus for *N-myc* a gene previously implicated in the biology of a subset of medulloblastomas. Interphase *in situ* hybridization confirmed the presence of *N-myc* amplification in this tumor (Fig. 2).





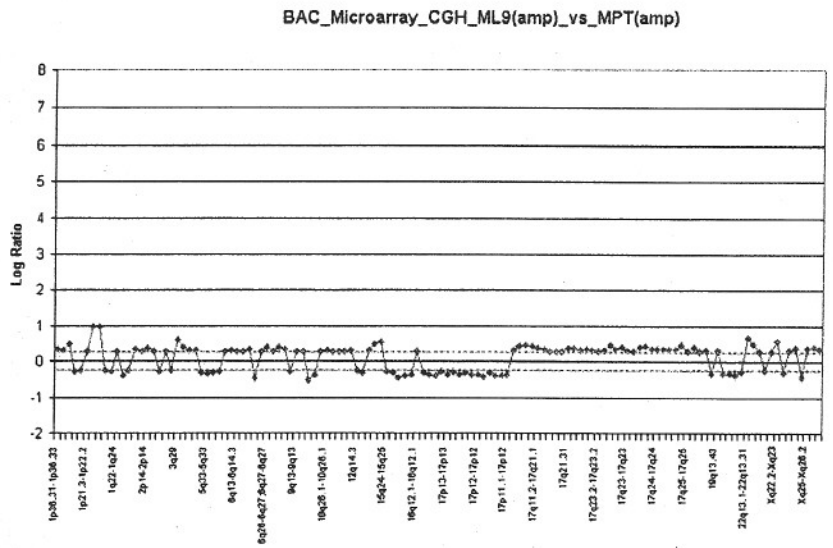


Fig 1a: Chromosome copy-number profiles for medulloblastoma tumors ML-1, ML-6 and ML-9

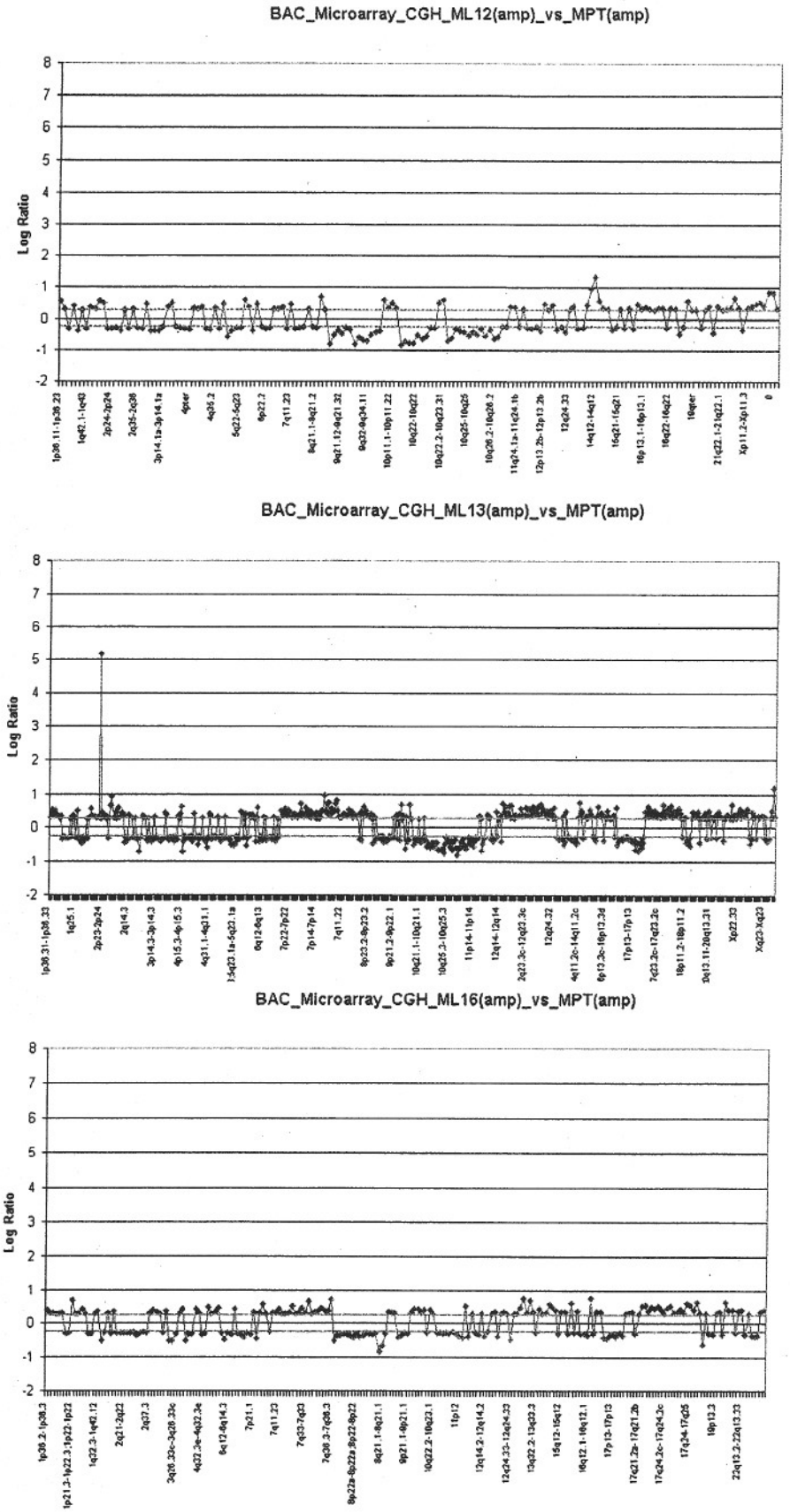


Fig 1b: Chromosome copy-number profiles for medulloblastoma tumors ML-12, ML-13 and ML-16

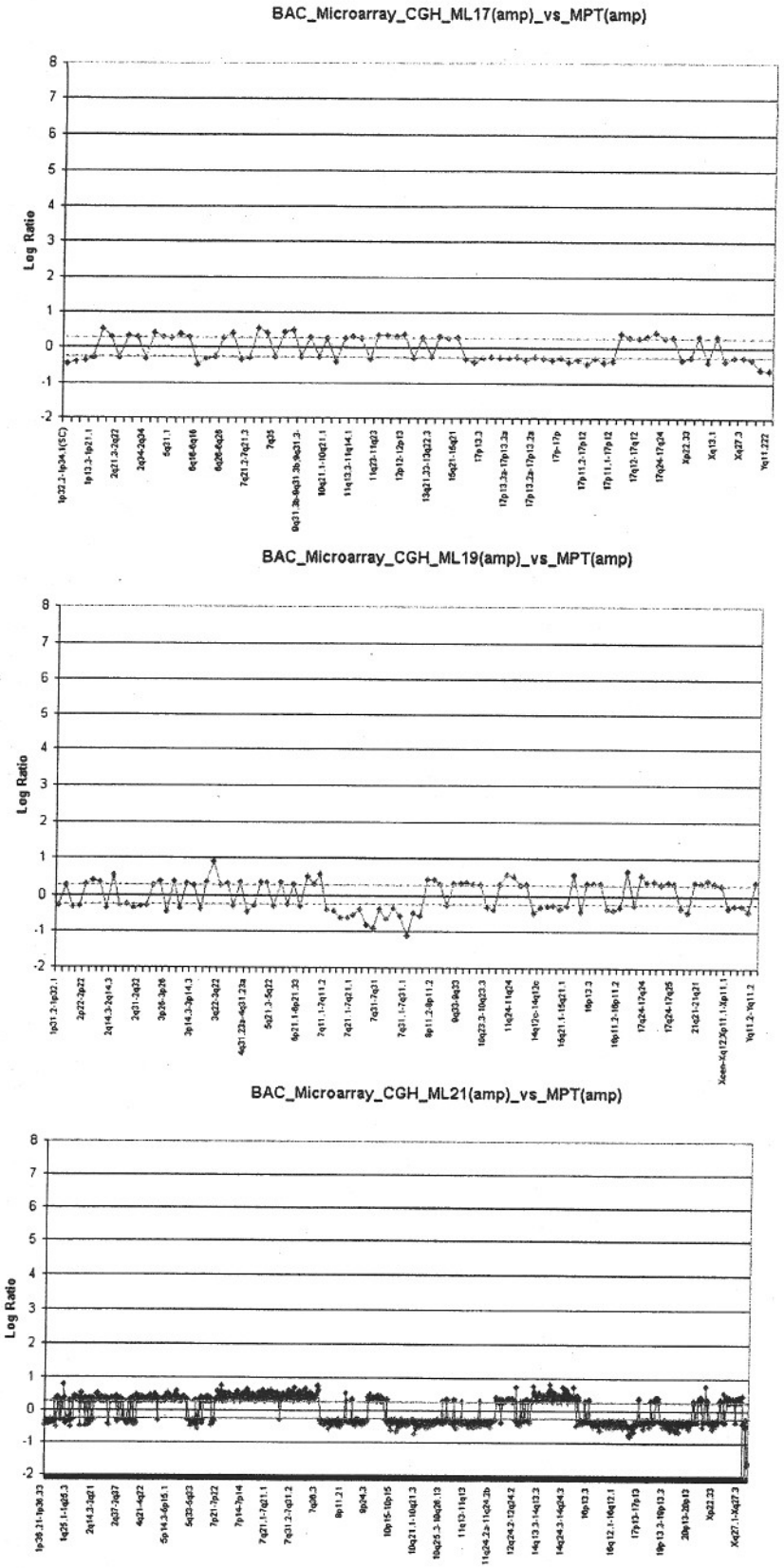


Fig 1c: Chromosome copy-number profiles for medulloblastoma tumors ML-17, ML-19 and ML-21

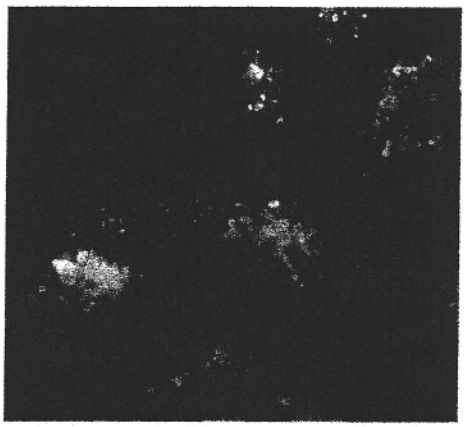


Fig. 2: Interphase *in situ* hybridization showing *N-myc* amplification in ML-13. Note the corresponding chromosome copy-number gain for 2p24 in Fig. 1b

The copy-number gain on 2p frequently involves a variably sized amplicon spanning 2p22 – 2p25.3. Notable among other genes located in this amplicon is GRP3 (Ras guanine releasing protein 3 / guanine nucleotide exchange factor for Rap1); a guanine exchange factor implicated in the activation of both *ras* and *rap1*. Identification of gene copy-number gain or amplification of this gene in medulloblastoma is novel and may partially account for the activation of the RAS/MAPK pathway; a pathway implicated in the biology of medulloblastoma.

Publication Status: No publication has been submitted as at date. However, data will be part of paper in preparation titled: Genomic alterations in medulloblastoma – analysis by conventional and array based comparative genomic hybridization. These preliminary observations will be part of the data for an NIH / NCI R01 grant proposal.

Status of Project: Complete