# Gene Aberrations in Childhood Brain Tumors

( childhood brain tumors, c-*myc* and N-*myc* amplification, *p53* deletion, DNA ploidy, karyotype )

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**Abstract. We present the results of the examination of prognostic markers in 40 children suffering from brain tumors. Prognostic markers such as amplification of the N-*myc* and c-*myc*, deletion of the 17p, and DNA ploidy are indispensable factors for the determination of diagnosis. An increased number of c-*myc* gene copies was found in malignant brain tumors, especially embryonal, more often than reported in the literature. N-*myc* amplification occurs in our group seldom, but it seems to be a sign of worse prognosis in glial and embryonal brain tumors. DNA aneuploidy was not found very frequently, but in high-grade tumors only.**

Brain tumors are the most common solid tumors in children. They account for the most frequent cause of death from cancer in childhood (Heideman et al., 1997). As many as 60–70 patients under 18 years of age are newly diagnosed every year in the Czech Republic as having brain tumors (unpublished data). The prognosis of these tumors is still poor. Surgery remains essential, but for the majority of tumors, radiotherapy and/or chemotherapy has been considered as a standard, often successful treatment modality (Heideman et al., 1997). For this reason the determination of prognostic markers in childhood brain tumors is one of the most important goals in neuro-oncology research. The knowledge gained through these studies may provide new approaches for risk-adapted therapy.

Several lines of evidence support the statement that changes in the cellular genome are significant causes of cancer (Heideman et al., 1997). Various types of genetic aberrations are associated with neoplastic development and tumor progression. These aberrations (gene amplifications, deletions, insertions, rearrangements, changes of ploidy, and point mutations) may be, in some tumors, of diagnostic and prognostic value.

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Abbreviations: ATB – antibiotic, EGF – epidermal growth factor, FCS – fetal calf serum, FISH – fluorescence *in situ* hybridization, PDGF – platelet-derived growth factor, PNET – primitive neuroectodermal tumor.

The most frequent chromosomal aberration described in chidhood brain tumors is loss of 17p. This deletion was found not only in primitive neuroectodermal tumors (PNET), where it is, according to some authors, indicative of worse prognosis, but also in astrocytic tumors (Biegel et al., 1997; Orellana et al., 1998; Hill et al., 1999). The cytogenetic analysis of pediatric brain tumors has revealed +7, 9p abnormalities, and −10 in high-grade astrocytic tumors and a normal karyotype in low-grade tumors. PNETs have often +7, −8, i(17)(10q), and −22. Chorioid plexus papillomas showed a normal karyotype, while carcinomas and atypical papilomas are aneuploid (Bhattacharjee et al., 1997). In oligodendrogliomas, which are not frequent in children, deletions of 1p and 19q have been described (Hill et al., 1999). Abnormalities of chromosome 22 play a role in the development of adult brain tumors – neurinomas, meningiomas and gliomas (Rey et al., 1993). Gliomas of adult patients may be divided according to genetic lesions into two subgroups: first with *p53* loss-of-function mutations and platelet-derived growth factor (PDGF) receptor overexpression, and second with epidermal growth factor (EGF) receptor and *MDM2* genes amplifications without *p53* mutation. The prognosis of patients with type 1 gliomas is somewhat more favorable than with type 2 (Stiles, 1998). The amplification of the c-*myc* gene has been described as a sign of worse prognosis in patients with childhood medulloblastoma/PNET (Scheurlen et al., 1998). In high-grade astrocytomas and medulloblastomas, infrequent N-*myc* gene amplification is a sign of the worst prognosis (Tomkinson et al., 1994).

In the majority of tumors, aneuploidy involves a worse prognosis for the patient. Aneuploidy is a manifestation of a large chromosomal instability and mostly with this is connected greater anaplasia. Three pediatric tumors (preB-ALL, neuroblastoma and medulloblastoma) have a better prognosis in an aneuploid form. One may speculate that the reason is a greater sensitivity to cytostatic drugs (Keren et al., 1994). DNA aneuploidy is found more often in high-grade than in low-grade astrocytomas, and DNA ploidy examination may be useful in brain-tumor grading, which may be in some cases difficult (Keren et al., 1994).

From the above mentioned it is evident that knowledge about chromosomal changes in childhood brain tumors, which may be useful for diagnosis or prognosis evaluation, is so far limited.

## Material and Methods

We studied 40 childhood patients aged between one year and 22 years. The series included 28 glial tumors, 10 medulloblastomas/PNETs, one germ cell tumor of pinealis and one primary brain sarcoma. For details see Table 1.

A single-cell suspension was prepared by mechanical disaggregation of tumor tissue and used for fluorescence *in situ* hybridization (FISH) and DNA ploidy examination.

### *FISH*

The FISH protocol used was a modification of the procedure reported by Sharpio et al., 1993, and Eckschlager and McClain, 1996. Several drops of fixed (methanol : acetic acid) cell suspension were placed on precleaned slides, which were air dried. Cells were denatured with 70% formamide solution for 2 min at 72°C, dehydrated with ethanol and dried. A digoxigenin- or biotin-labeled probe in a hybridization solution (Oncor, Gaithersburg, MD) was applied to each slide. The slides were incubated overnight at 37°C, then washed in a buffered detergent solution. The hybridized probe was visualized with a FITC-labeled anti-digoxigenin antibody or labeled avidin (Oncor, Gaithersburg, MD). Cells were counterstained by an antifade solution with propidium iodide.

Used DNA probes: c-*myc* 8q24 Cat No. P5117, N-*myc* 2p23-24 Cat No. P5115, distal 17p (17p13-pter) Cat No. P5433, and *p53* (17p13.1) Cat No. P5106.

### *DNA analysis*

The cell suspension ($5–10 \times 10^9$/l) was permeabilized and stained with propidium iodide using DNA Prep Reagent Kit (Coulter Immunology, Hialeah, FL) according to the producer's protocol. Twenty to 120 min after completing the staining procedure the samples were measured with an EPICS Profile II flow cytometer (Coulter Immunology, Hialeah, FL) and data were analyzed by Multicycle software (Phoenix Flow Software Products, San Diego, CA) (Eckschlager et al., 1996).

### *Cytogenetic analysis*

The biopsy specimens received within 24 h, collected in a transport sterile centrifuge tube with RPMI 1640 medium (Gibco, Gaithersburg, MD), were transferred into a Petri dish and mechanically disaggregated, transferred into T25 flasks with RPMI 1640 + 15% fetal calf serum (FCS) (Sigma, St. Louis, MO) and antibiotics (ATB's) (gentamicin) and were cultured in a humidified atmosphere at 37°C and 5% $CO_2$. The cultures were monitored daily and harvested between 5 and 14 days by standard cytogenetic techniques using a hypotonic solution of 1 : 3 0.075 M KCl/0.8% sodium citrate and 3 : 1 methanol/acetic acid solution according to the growth activity after overnight exposure to Colcemid (Gibco, Gaithersburg, MD) at a final concentration of 0.01 µg/ml.

Slides were made according to conventional techniques in a humidified atmosphere to assist metaphase spreading. After an aging period the slides were trypsin-Giemsa banded and twenty metaphases were analyzed whenever possible.

## Results

We observed six parameters: amplification of the N-*myc* and c-*myc*, deletion of the 17p or *p53*, DNA ploidy and karyotype. The possibility of examination of all those parameters was often limited by a small tissue sample. Our data are summarized in Table 1.

The most frequent aberration was c-*myc* amplification, which was found in 22 cases (55%), more frequently in medulloblastomas/PNETs (80%). In 30 to 60% of cells there were five or more (usually five to ten) copies of this gene. Deletion of 17p and N-*myc* amplification were detected less frequently, in 16.6% and 10.5% of cases, respectively. Deletion of the *p53* gene was detected in only two cases of medulloblastoma (20% of all tumors, 40% of medulloblastomas).

DNA aneuploidy was seen in four cases, two high-grade glial tumors, one medulloblastoma, and one brain sarcoma. It was also observed in one case of malignant ependymoma (ependymoblastoma), which was examined twice: the first DNA diploid sample was examined at the time of onset of the disease and the second DNA aneuploid sample at the time of recurrence.

In two cases a karyotype bearing an i(17q)(q10) was detected; both were medulloblastomas. Aneuploidy was ascertained by cytogenetics in four cases (two low-grade gliomas, one low-grade with high-grade foci, and one brain sarcoma). Other karyotypic changes were not specific except for one case of high-grade astrocytoma with +7. A normal karyotype was found in both high-grade and low-grade glial tumors and also in embryonal tumors. In a case of fibrillar and gemistocytic astrocytoma with foci of anaplastic astrocytoma, cytogenetic examination showed three clones: one with a normal karyotype, one hypodiploid (33–35 chromosomes) and one hyperdiploid 52–53 (XX, + iso (1q), +2, +5, +7, +12, +17) (see Table 1, case 33).

## Discussion

Relatively often, we found c-*myc* amplification, especially in embryonal tumors (80% of medulloblastoma/PNET). Amplification of this gene, which is in embryonal brain tumors a sign of worse prognosis, was described in 40–50% cases (Scheurlen et al., 1998). A possible explanation for a higher incidence of c-*myc* amplification in embryonal brain tumors in our group may be a tardy diagnosis in the examined patients. Bigner et al. explain a higher occurrence of c-*myc* amplification