Oncogene Amplification in Pediatric Brain Tumors


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ABSTRACT

Despite a considerable amount of information concerning chromosomal and molecular abnormalities found in gliomas in adults, relatively little is known regarding these abnormalities in pediatric brain tumors. We have analyzed DNA from 37 primary brain tumors and 4 tumor-derived cell lines for oncogene amplification. Probes utilized represent 11 known oncogenes (erbB1, gli, neu, myc, L-myc, N-myc, H-ras, K-ras, N-ras, sis, and src). Of 20 primary medulloblastomas studied, only one tumor was found to have erbB1 amplification. In contrast, of the 4 medulloblastoma cell lines studied, 1 had c-myc amplification, 1 had erbB1 amplification, and 1 had amplification of N-myc. Twelve glial brain tumors were analyzed, and only 1 case with amplification of the erbB1 oncogene was found. Other tumors studied include 1 meningioma, 2 ependymomas, 1 anaplastic ependymoma, and 1 cerebral primitive neuroectodermal tumor, none of which had oncogene amplification. These results suggest that oncogene amplification is relatively uncommon in primary medulloblastomas, but the frequency and diversity of oncogene amplification is greater in tumors that can be established as cell lines. The lower frequency of erbB1 amplification in glial brain tumors in children compared to adults is consistent with the generally lower grade of glial tumor histology seen in pediatric patients. However, the case with amplification of the erbB1 oncogene represented 1 of 2 cases of glioblastoma multiforme we studied, which suggests that pediatric glioblastoma multiforme may have a similar frequency of erbB1 oncogene amplification to glioblastomas seen in adults. Our results suggest that oncogene amplification is a relatively uncommon mechanism of oncogene activation in pediatric brain tumors, and they provide molecular evidence for heterogeneity in tumors classified as medulloblastomas.

INTRODUCTION

Brain tumors are the most common solid tumors in children, accounting for 20% of all malignant diseases in children under 15 years old. Understanding of the biology and treatment of this diverse group of tumors has not kept pace with the considerable advances made over the past 25 years in the treatment of other forms of childhood cancer. This situation has improved somewhat in the past several years with the appearance of several reports detailing some of the cytogenetic and molecular biological characteristics of pediatric brain tumors.

The majority of cytogenetic and molecular studies of brain tumors published thus far involve glial tumors in adults, especially glioblastoma multiforme, whereas the few studies in children generally involve medulloblastomas. In contrast to gliomas, numerical gains or losses of whole chromosomes are relatively infrequent in medulloblastomas (1). However, structural abnormalities are common and include i(17q), t(8;11), 22p+, 11q-, as well as other nonreciprocal translocations and deletions (1-4). Double minutes have also been found in medulloblastomas, both in primary tumors and cell lines (1, 5), providing evidence for gene amplification in these tumors.

Molecular analysis of medulloblastoma has shown amplification of c-myc in a medulloblastoma cell line as well as in some of 3 dm3 bearing tissues (5, 6). N-myc amplification has also been demonstrated in 2 of 20 cerebellar PNETs with neuronal differentiation (7), and a 10-fold increase in N-myc expression has been demonstrated in a single medulloblastoma sample (8).

In order to extend these observations to a large number of pediatric brain tumors, we have undertaken a survey of oncogene amplification in 37 primary tumors and 4 tumor-derived cell lines. Our results indicate that oncogene amplification is a relatively uncommon event in pediatric brain tumors of all histological types, suggesting that mechanisms of oncogene activation other than amplification are utilized in this heterogeneous population of tumors, and that these mechanisms vary even within relatively homogeneous tumor types, such as the medulloblastomas.

MATERIALS AND METHODS

Brain tumor tissue was obtained from the primary tumors of 36 patients undergoing initial diagnostic surgery and from 1 patient, previously treated with radiation therapy, undergoing repeat surgery. The patients ranged in age from 2 months to 17 years at the time of diagnosis. Cases were collected at the referring institutions from 1979 to 1986 and were selected only on the basis of availability of sufficient tissue for study. The samples were frozen at the referring institution, sent on dry ice to Washington University, and were kept frozen until the time of analysis. Samples were obtained from the University of Texas Health Science Center at San Antonio, St. Jude Children's Research Hospital, Duke University, and Washington University. Cell lines used were obtained from Duke University [D283 Med (10), DAOY (11), D341 Med (6)] and St. Jude Children's Research Hospital (MTS), and were propagated in improved minimal essential zinc option medium supplemented with 20% fetal calf serum. Cell line MTS, which has not been previously described, was derived from tumor tissue obtained at the initial diagnostic surgery of a 26-month-old black male who expired 3 months after diagnosis. The primary tumor was cerebellar in location and was described histologically as a medulloblastoma containing focal areas appearing unusually epithelial and pleomorphic. The initial karyotype demonstrated both dms and homogeneously staining regions.

The histology of most tumors was determined by two of us (S. H., P. C. B.) and was compared to the original pathology reports. The classification scheme used was as described by Russell and Rubinstein (12). For this report, PNETs of the cerebellum in children and adolescents were called medulloblastomas. Supratentorial tumors histologically resembling medulloblastomas were called cerebral PNETs.

DNA was prepared from about 100 mg (wet weight) of each tumor.
and quantitated by fluorometric assay as previously described (13). Samples (5 μg) of DNA from each tumor or cell line were digested to completion with EcoRI, separated by electrophoresis on 0.8% agarose gels, and transferred to nitrocellulose or nylon filters by a modification of the method of Southern (13). Positive controls utilized include the neuroblastoma cell line NGP, which has 150-fold amplification of N-myc (14, 15), the epidermoid carcinoma cell line A431, which has 40-fold amplification of erbB1 (16), the breast carcinoma cell line MCF-7, which has 10-fold amplification of N-ras (17), and the colon carcinoma cell line COLO-320, which has 50-fold amplification and rearrangement of c-myc (18). Filters were baked and hybridized with 32P-labeled probes representing 10 known oncogenes (c-erbB1, neu, c-myc, L-myc, N-myc, H-ras, K-ras, N-ras, sis, and src) and were washed under high-stringency conditions. The rationale for choosing these 10 oncogenes was the existence, at the time the study was initiated, of published data suggesting the involvement of these oncogenes in tumors of either the central or peripheral nervous systems. The following individuals generously provided DNA probes for these studies: J. M. Bishop, human N-myc probe pNb-1 (14); J. D. Minna, human L-myc probe pLMyc 10 (19); R. A. Weinberg, human neu probe; J. M. Trent, human erbB1 probe pE7 (20). Other probes were obtained from the American Type Culture Collection. Filters were exposed for 18–24 h at −70°C with an intensifying screen. The gene copy number was determined by serial dilution and laser densitometry. Filters were also stripped and rehybridized with other oncogene probes so that the densitometric confirmation of the single copy nature of these other oncogenes would represent an internal control to confirm the loading of equal amounts of DNA in each lane (data not shown).

A probe for human gli was also tested. The gli probe pKK380 was generously provided by B. Vogelstein and is derived from an amplified, expressed gene in an adult glioma that is a zinc finger protein with homology to the Kruppel family of genes in Drosophila (21–23). Although there is no direct evidence that gli actually functions as an oncogene in transfection assays, it is also amplified and overexpressed in a subset of childhood sarcomas (24), and in some glial tumors (21).

RESULTS

Medulloblastomas. Analysis of DNA samples from 20 primary tumors and 4 cell lines was undertaken (Table 1). Of the primary medulloblastomas, one was found to have about 10-fold amplification of the erbB1 oncogene (Fig. 1A). In addition, the cell line DA0Y was found to have about 5-fold amplification of erbB1 (Fig. 1A). The medulloblastoma cell line MTS had about 150-fold amplification of the N-myc oncogene (Fig. 1B). As previously reported (6), cell line D341 Med had about 20-fold amplification of c-myc (Fig. 1C). All 3 of the cell lines with amplification of an oncogene had been shown to have dms on cytogenetic analysis prior to the molecular studies (S. H. B., E. C. D.).

Glial Tumors. Analysis of DNA samples from 12 primary tumors was undertaken. These included 1 oligodendroglioma, 2 xanthoastrocytomas, 2 cerebellar astrocytomas, 3 astrocytomas, 2 anaplastic astrocytomas, and 2 glioblastoma multiforme (Table 1). One primary tumor, a glioblastoma multiforme, was found to have amplification of erbB1 (Fig. 1A).

Other Tumors. Other tumors tested include 1 meningioma, 2 ependymomas, 1 anaplastic ependymoma, and 1 cerebral PNET (Table 1). None were found to have amplification of any of the oncogenes tested.

DISCUSSION

We have assayed 37 pediatric brain tumors and 4 cell lines derived from pediatric brain tumors for amplification of a panel of 11 oncogenes. We detected amplification in only 2 of 37 tumor samples and in 3 of 4 cell lines. We thus conclude that oncogene amplification, at least of the 11 oncogenes tested, is a relatively uncommon event in pediatric brain tumors. On the other hand, there appears to be selection in vitro for brain tumors with preexisting oncogene amplification, based on our finding of oncogene amplification in 3 of the 4 cell lines studied. A more detailed examination of this question will be published elsewhere (25).

These results are in contrast to the experience in adult gliomas in which oncogene amplification is reported in 40–50% of glioblastoma multiforme primary tumors. Bigner et al. (26) have reported results from 33 malignant gliomas, 15 of which demonstrated amplification of the epidermal growth factor receptor gene, erbB1. They correlated erbB1 amplification with the presence of dms in the majority of cases, reporting that 81% of the tumors with amplification contained double minutes. Wong et al. (27) have also reported that a large number of adults with malignant gliomas have amplification and increased expression of the epidermal growth factor receptor gene. Of the 63 patients in this report, 24 had amplification of the epidermal growth factor receptor gene, 2 had amplification of N-myc, and 1 had amplification of gli. Increased expression of the corresponding mRNA was found in cases with amplification of the oncogene. [Some of the patients studied by Bigner et al. (26) were included in the larger study by Wong et al. (27).]
Oncogenes reported by other authors to be amplified in individual malignant gliomas in adults are N-myc and gli (21, 26, 28). A single case report has also appeared describing c-myc rearrangement, amplification, and expression in a childhood glioblastoma multiforme (29).

An analogous situation has been reported in human neuroblastomas (13, 30). N-myc amplification occurred in one-third of neuroblastomas from untreated patients, and was strongly associated with advanced stages of disease and a poor prognosis. An additional correlation was found between histology and N-myc amplification or expression. N-myc amplification was associated with more immature histological types (31), and, within a given tumor, the most differentiated tumor cells had the lowest levels of N-myc expression (32).

Based on our data, pediatric brain tumors are unlike adult gliomas and neuroblastomas in terms of the frequency and specificity of oncogene amplification. The most obvious explanation for this is the heterogeneity of the tumor population studied. Neuroblastomas represent a spectrum of differentiation from immature neuroblasts to mature ganglion cells and only amplification of N-myc has been found in the most immature tumors. Gliomas also demonstrate considerable histological heterogeneity but only erbB1 amplification has been consistently demonstrated in the more malignant glioblastoma multiforme. Medulloblastomas, which comprise the majority of the tumors studied, are also a heterogeneous population, consisting of primitive neural elements, glial elements, or both (12). Despite the heterogeneity of oncogenes amplified, the overall prevalence is low but consistent with the frequency with which dms are deleted by cytogenetic analysis.

The lower frequency of erbB1 amplification in gliomas from children may be due to the generally lower grades of malignancy seen in these tumors in terms of both histology and clinical behavior. Indeed, of the two glioblastomas multiforme we studied, one had erbB1 amplification, a proportion similar to that found in series of adult glioblastoma multiforme (26, 27). An alternative explanation is that tumorigenesis of glial neoplasms in children is fundamentally different than in adults. Although there were no examples of oncogene amplification in the other histological types studied, only a small number was included in our series. Since meningiomas are generally not aggressive neoplasms, and dms have not been reported in this tumor, our results are not surprising. No specific cytogenetic abnormality has been identified in ependymomas to date, but dms are rarely if ever seen.

An additional type of molecular analysis that has yielded important information is the assessment of restriction fragment length polymorphisms and LOH. Allelic loss for all or part of chromosome 10 sequences has been reported with the most malignant histological type, glioblastoma multiforme, while nonrandom abnormalities involving chromosome 17 can be detected in lower grades of malignant gliomas as well (33–36). Chromosome 22 monosity, deletion, or LOH has been shown to be characteristic of meningiomas (37–40). To date, LOH for one or more specific chromosomes has not been reported in medulloblastomas, although trisomy for 17q is a common finding and is sometimes associated with monosity for 17p.

Our data suggest that amplification is not the principle mechanism of oncogene activation in pediatric brain tumors. Other possible mechanisms that must be considered include increased or unregulated transcription of oncogene message, increased mRNA or protein half-life, or structural alteration of the gene and protein product leading to altered or enhanced activity. Published studies addressing these alternative pathways of oncogene activation have been infrequent in medulloblastomas, although increased expression of the c-myc and N-myc genes has been detected at the RNA level (8, 41). Alternatively, there may be additional genes that are either activated, as described above, or inactivated by deletion or mutation as seen in glioblastoma (34, 36), meningioma (37–40), neuroblastoma (42–44), and many other tumors. A comprehensive cytogenetic and molecular analysis of pediatric brain tumors and correlation with histological and clinical variables will be necessary to understand mechanisms of tumorigenesis and progression in these tumors, particularly in the subset of tumors presently classified as medulloblastomas.

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