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Enhanced Expression of the c-myc Protooncogene in Human Intracranial Meningiomas

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We examined the alteration and expression of c-myc protooncogene in 11 human intracranial meningiomas using Southern blot, Northern blot and immunohistochemical techniques. Southern blot showed neither amplification nor rearrangement but Northern blot and immunohistochemical study revealed enhanced expression of the c-myc gene. Immunohistochemically, c-myc product was found in all of the11 cases and seven of these cases showed an above moderate degree of immunoreaction in semiquantitative analysis. Loss of heterozygosity at IGLC2 locus on chromosome 22 was detected in four of the 8 informative cases. But extent and intensity of immunoreactivity did not correlated with loss of heterozygosity on chromosome 22. These genetic changes may play important roles in the pathogenesis of human intracranial meningioma.

Key Words: *Intracranial meningioma, c-myc protooncogene, Southern blot, Northern blot, Immunohistochemical study, Loss of heterozygosity*

INTRODUCTION

The c-myc protooncogene, the cellular homologue of the transforming sequence of the avian myelocytomatosis virus (Vennstrom et al., 1982), is supposed to play an important role in the normal proliferation and differentiation of cells, including those of the central nervous system (Adamson et al., 1987). The c-myc gene locus is situated on the long arm of chromosome 8 (Hamlyn et al., 1983) and its product is a 62,000 molecular weight protein, c-myc p^{62}, which is dominantly located in the nucleus (Eisenman et al., 1985). This protein is absent in quiescent tissues such as neuron, ova, cardiac myocyte, whereas it is abundant in highly proliferative cells (Jack et al., 1986; Loke et al., 1988). The c-myc gene is frequently involved in genetic alteration, such as the amplification, rearrangement and overexpression, of many cancers (Little et al., 1983; Hamlyn et al., 1983; Matsumura et al., 1990; Riou et al., 1987; Varley et al., 1987; Field et al., 1989, 1990). Deregulation of its expression, usually implying an increased steady-state mRNA level, has often been cited as a possible step in oncogenesis (Reed et al., 1985). With the availability of monoclonal antibodies, immunohistochemical study can be undertaken to determine the subcellular distribution and possible functions of the c-myc protein. Among central nervous system tumors, c-myc amplification and /or overexpression were found in glioblastoma multiforme (Bigner et al., 1988; Engelhard et al., 1989; Helseth et al., 1988; Fujimoto et al., 1989) and medulloblastoma (Raffel et al., 1990; Bigner et al., 1990; Wasson et al., 1990; MacGregor et al., 1990). There are few studies of the c-myc gene in meningiomas and the results are not consistent. Kazumoto et al (1990) reported that the expression of c-myc mRNA was increased in meningiomas. Conversely, Carstens et al (1988) did not find genetic alteration of the c-myc gene. Morevoer, Nakajima et al (1989) also reported that the nuclear immunoreaction was usually absent or only weak in immunohistochemical study of the c-myc gene in meningiomas.

In the present study, we examined the alteration and expression of c-myc gene and c-myc protein in human intracranial meningiomas using Southern blot hybridization, Northern blot hybridization and im-

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munohstochemical techniques. And to find any correlation between expression of c-myc gene and loss of putative suppressor gene of human intracraninal meningioma on chromosome 22q, we performed a restriction fragment length polymorphism (RFLP) analysis to detect loss of heterozygosity on chromosome 22q with IGLC2 probe.

MATERIALS AND METHODS

Tissues

Surgically removed meningioma tissue was obtained from 11 patients. Peripheral blood sample was obtained from the same patient. The specimens were stored at -70°C until DNA and RNA were extracted. The clinical details of the patients from whom the tumors were obtained are summarized in Table 1.

DNS Extraction and Southern Blot Hybridization

The high molecular weight DNA was extracted by the phenol-chloroform method after treatment with sodium dodecyl sulfate and proteinase K. 15 micrograms of DNA was digested with restriction enzyme EcoRI under the conditions recommended by the supplier, size fractionated in 0.8% agarose gel, denaturated, neutralized, transferred to a nylon membrance and then hybridized to 32p labeled c-myc probe. The c-myc probe used was a 0.4 kilobase pair, PstI-PstI fragment of pHSR-1. The probe was labeled with ^{32}P-dCTP by the multiprime DNA labeling system. The filter was then washed and autoradiographed with an intensifying screen at -70°C. We also performed a RFLP study to detect loss of heterozygosity on chromosome 22 with IGLC2 probe.

RNA Extraction and Northern Blot Hybridization

The RNA was isolated by a single extraction with an acid guanidinium thiocyanate-phenol-chloroform mixture (Chomczynski et al., 1987). RNA was electrophoresed on 1.0% agarose gel containing 6.0% formaldehyde, then transferred to a nylon membrane and then hybridized to the same ^{32}P labeled c-myc probe as used in Southern blot hybridization. After exposure to the film, the filter was reprobed with beta actin cDNA probe to determine the amount of RNA in each lane. The c-myc/beta actin expression ratio was compared to that of a normal baboon brain tissue.

Immunohistochemical study

For immunohistochemical study, ABC (avidin-biotin complex) method was used (Vector, USA). Five micrometer-thick section of formalin-fixed and paraffin-embedded tissue was deparaffinized in xylene and treated with 0.3% H_2O_2 for 30 minutes in order to block the endogenous peroxidase activity. After washing with phosphate-buffered saline (PBS), the section was treated with normal horse serum for 20 minutes for blocking of non-specific binding. Primary antibody, mouse monoclonal antibody to myc family protein (CRB, England), was applied overnight at 4°C. After washing, biotinylated secondary antibody was given for 20 minutes, followed by application of ABC for 30

Table 1. Summary of Study

Patien	Age/Sex	Pathology	Immunoreaction		Loss of Hetecozygosity on chromosome 22
			Extent	Intensity	
1.	62/F	Transitional	+ + +	+ + +	−
2.	59/F	Transitional	+ +	+ + +	a/−
3.	47/F	Meningotheliomatous	+ +	+ +	a/b
4.	25/M	Meningotheliomatous	+	+ + +	−/b
5.	46/F	Meningotheliomatous	+	+ + +	a/b
6.	34/F	Meningotheliomatous	+	+ +	−/b
7.	62/F	Meningotheliomatous	+ +	+ +	−
8.	38/F	Meningotheliomatous	+	+ +	a/b
9.	32/F	Meningotheliomatous	+	+ +	a/b
10.	56/F	Meningotheliomatous	+ + +	+ + +	not checked
11.	47/F	Meningotheliomatous	+	+ +	a/−

Extent (+: below 1/3 of field, + +: between 1/3 and 2/3, + + +: above 2/3)
Intensity (+: weak, + +: moderate, + + +: marked)
a/b: maintenence of heterozygosity, −: constitutionally homozygous
a/− or −/b: loss of heterozygosity

minutes. Aminoethylcarbazole and Meyer's hematoxylin were used as chromogen and counterstain. The immunoreaction in the tumor was analysed semiquantitatively according to the extent and intensity.

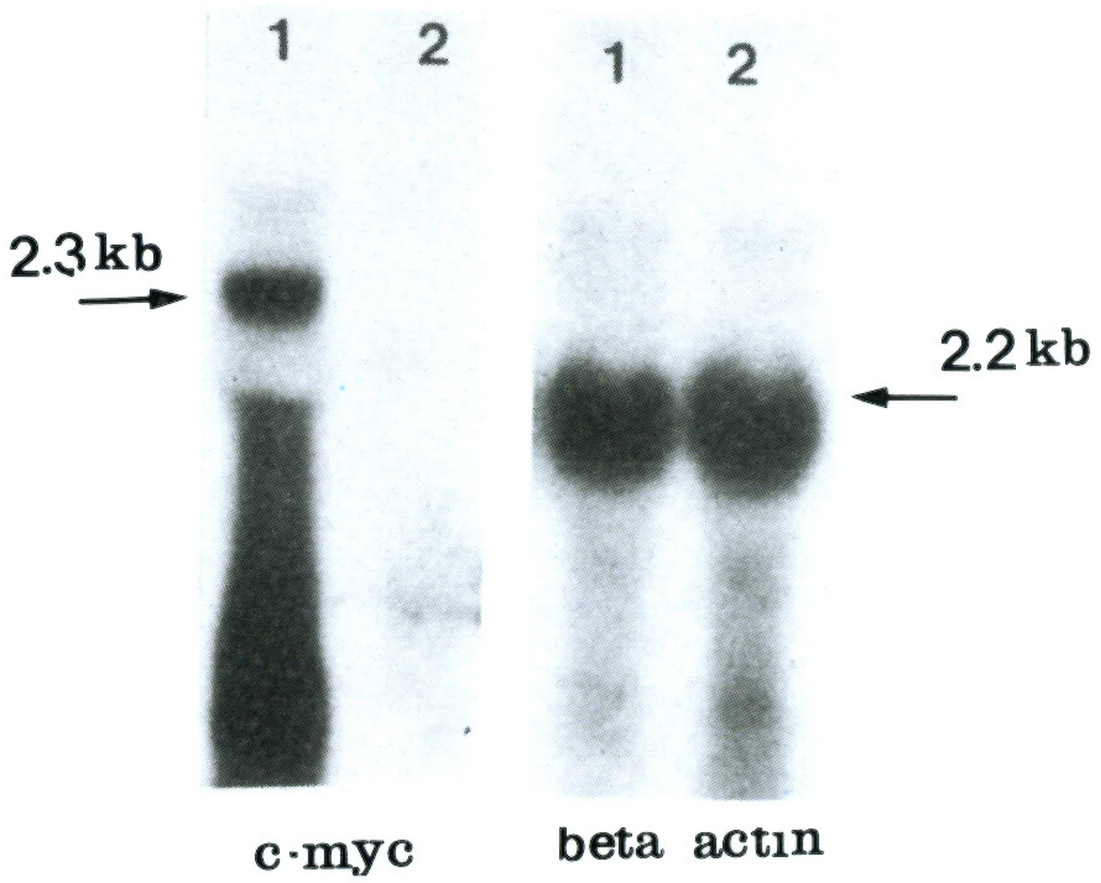

Fig. 1. Northern blot hybridization analysis using c-myc as probe. Case 4 (lane 1) shows the overexpression of c-myc gene when compared with normal baboon brain tissue (lane 2).

RESULTS

Southern Blot Hybridization

We detected a constant band of 12.5 kilobases pair (kbp) EcoRI, c-myc DNA. Southern blot hybridization of meningioma DNAs with the c-myc probe showed neither amplification nor rearrangement of this gene in any sample. Four (50%) of 8 informative cases rev-

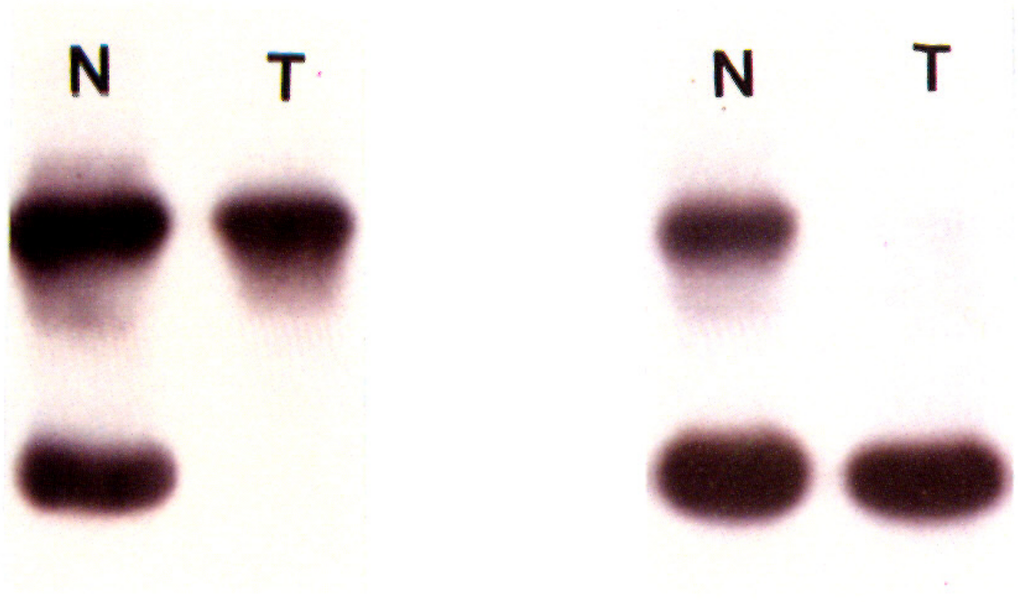

Fig. 3. Loss of heterozygosity at IGLC2 locus on chromosome 22. Normal tissue (N) contains two bands (8 and 18 kilobase) whereas tumor tissue (T) has lost one band.

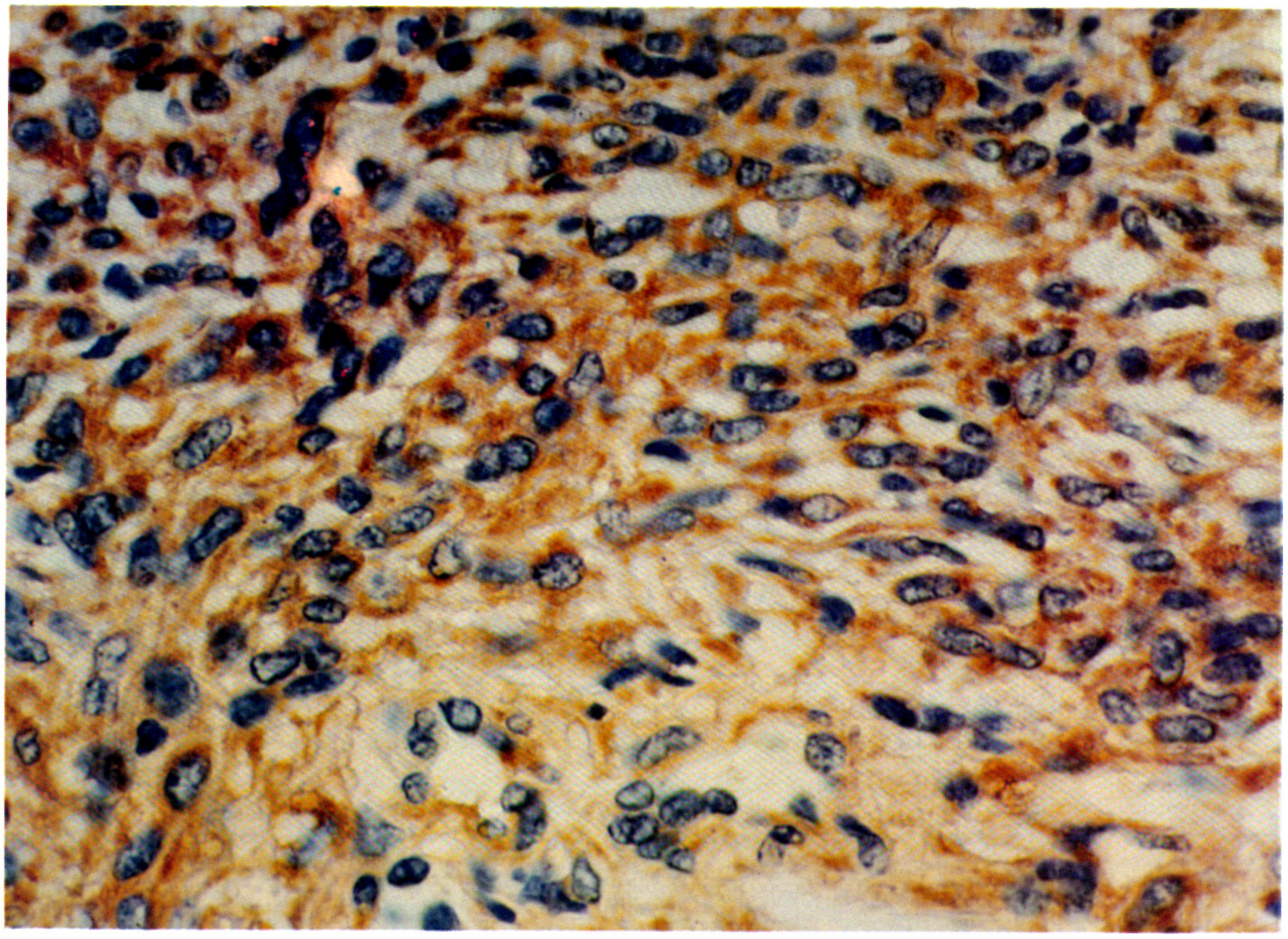

Fig. 2. Immunohistochemical staining of the meningioma with c-myc monoclonal antibody. Immunoreactivity of c-myc monoclonal antibody. Immunoreactivity of c-myc product is observed in the tumor tissue.

ealed loss of heterozygosity at IGLC2 locus on chromosome 22.

Northern Blot Hybridization

Tumor samples were collected and frozen at -70°C for 3 months to one year before isolation of RNAs, so we couldn't obtain high quality RNA in all cases. Six of 11 cases showed relatively discrete bands on the ethidium bromide staining of the gel before transfer. And we could find a definite overexpression of c-myc gene in patient 4, who had multiple meingiomas and bilateral acoustic neurinomas.

Immunohistochemical study

The immunoreactivity of the c-myc product within the tumor was found in all of the 11 cases, and seven of these cases showed an above moderate degree of immunoreaction in semiquantitative analysis. C-myc product was localized mainly in the cytoplasm and sometimes in the nucleus of the tumor cells and stromal cells. Extent and intensity of immunoreactivity did not correlate with loss of heterozygosity on chromosome 22.

DISCUSSION

Amplification and overexpression of the c-myc gene have been found in a variety of human malignancies, including lymphomas, leukemia, adenocarcinomas of the colon, lung cancers, hepatocellular carcinomas, breast, cancer mouth cancer, pharynx cancer, glioblastoma multiforme and medulloblastoma (Field et al., 1990; Bigner et al., 1988; Raffel et al., 1990). Several authors have reported that c-myc overexpression or amplification correlated with prognosis or recurrence in breast cancer (Varley et al., 1987) and head and neck cancers (Field et al., 1989).

Meningioma is one of the most common tumors of the human central nervous system. In most cases, they are sporadic, solitary and benign, but there is evidence suggesting genetic predispositions (Russel et al., 1989). Recently a putative tumor suppressor gene is believed to be associated with the chromosomal deletion in meningioma and the menignioma locus has been shown to be located distal to the myoglobin locus within 22q 12.3-qter (Dumanski et al., 1990).

Amplification of c-myc gene has not been reported in meingioma, but Tanaka et al (1989) reported amplification of this gene in two of seven different malignant meningiomas and in one malignant meningioma cell line. Kazumoto et al (1990) reported that overexpression of this gene was found in 63%. But Carsten et al (1988) could not detect any evidence of overexpression.

In the present study, we could not detect amplification and rearrangement of the c-myc gene with Southern blot, but could find loss of heterozygosity on chromosome 22q in four (50%) of 8 informative cases as in previous reports. Over-expression of the c-myc gene was seen in one case compared with normal brain tissue in Northern blot hybridization, inspite of degradation of RNA. And it was interesting that Patient 4, whose meningioma tissue showed not only overexpression of the c-myc but also loss of chromosome 22, was diagnosed with multiple meningiomas and bliateral acoustic neurofibromatosis.

Seizinger et al (1987) reported that there is a common pathogenic mechanism in meningioma and acoustic neurofibroma, like loss of heterozygosity on chromosome 22q. So we can expect enhanced expression of the c-myc gene in acoustic neurinoma as well as meningioma. Unfortunately acoustic neuroma tissue was not available for this patient.

Immunohistochemically, we detected c-myc products in all of the 11 cases, and seven of the 11 cases showed an above moderate degree of immunoreaction in semiquantitative analysis. The demonstration of the c-myc protein in the present study further confirms that the gene is expressed in meningiomas. Immunohistochemistry may be a more sensitive technique for detecting c-myc expression if intact mRNA can not be obtained. The c-myc protein is thought to be a DNA-associated nuclear protein.

However, immunohistochemical studies on normal and tumor tissues have shown conflicting findings on its subcellular distribution. Loke et al (1988) reported that this discrepancy may be due to the effects of formalin fixation. When tumor tissue was studied after formalin fixation and paraffin embedding, a loss of nuclear staining was observed concurrent with the appearance of the c-myc protein immunoreactivity in the cytoplasm.

The extent and intensity of immunoreactivity did not correlate with loss of heterozygosity on chromosome 22.

In conclusion, we could detect increased expression of the c-myc gene without amplification and loss of heteozygosity on chromosome 22. These genetic changes may play important roles in the pathogenesis of intracraninal meningiomas.

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