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ORIGINAL ARTICLE

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Nonrandom gain of chromosome 7 in central neurocytoma: a chromosomal analysis and fluorescence in situ hybridization study

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Abstract Central neurocytoma is a benign, slow-growing neoplasm with favourable prognosis. Biomolecular analysis has failed to demonstrate significant alterations, and no cytogenetic alterations have been reported. In this study we demonstrate chromosome 7 gain in three of nine neurocytomas (33%). Traditional cytogenetic analysis performed in four of the nine cases identified trisomy 7 as the sole chromosomal abnormality in one case. Interphase cytogenetics utilizing fluorescent in situ hybridization (FISH) on cell suspensions from formalin-fixed paraffin-embedded tumour tissue performed in all nine cases detected trisomy 7 in two more cases and tetrasomy in another. Our results suggest that chromosome 7 gain is a feature of neuroectodermal tumorigenesis, possibly conferring growth advantage on the neoplastic cells. FISH on interphase nuclei is a valuable adjunct in the genetic evaluation of rare central nervous system neoplasms with low baseline proliferative activity.

Key words Neurocytoma · Cytogenetics · FISH · Chromosome7

Introduction

Central neurocytomas (CN) are rare intraventricular tumours that occur predominantly in young adults [9]. These neoplasms generally arise in the lateral or III ventricle near the foramen of Monro and are generally slow-growing and benign. The neuronal nature of neurocytomas is apparent from the ultrastructural findings of synapses, neuritic processes and neurosecretory vesicles, and from immunohistochemical reactivity for synaptophysin. Biomolecular analysis of a few cases has failed to demonstrate significant alterations [18, 22]. Similarly, as far as we know, no cytogenetic alterations have been reported.

In this study we performed a traditional cytogenetic analysis in four cases. The discovery of trisomy of chromosome 7 as single chromosomal abnormality in one case, prompted us to an interphase cytogenetic study using fluorescent in situ hybridization (FISH) on cell suspensions prepared from paraffin-embedded tumour tissue in these four cases and in five more in which conventional cytogenetic information was lacking.

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Materials and methods

Nine cases of CN were examined. All cases had an intraventricular location. The age of the patients ranged from 20 to 35 years; four were male and five female (Table 1).

In all cases multiple paraffin blocks were available. Paraffin sections were stained with haematoxylin and eosin (H&E). Immu-

Table 1 Clinical features, cytogenetic and FISH results of patients with central neurocytoma (*LLV* left lateral ventricle, *RLV* right lateral ventricle, *3rd V* III ventricle, *MF* Monro's foramen, *NP* not performed)

Case no.	Age\sex	Site	Karyotype	FISH Chr.7
1	34\M	LLV	47, XY, +7	Trisomic
2	20\M	RLV+MF	46, XX	Disomic
3	27\M	LLV	46, XY	Disomic
4	22\F	RLV	46, XY	Disomic
5	25\F	LLV	NP	Disomic
6	22\F	RLV	NP	Disomic
7	29\F	LLV	NP	Tetrasomic
8	29\F	LLV	NP	Trisomic
9	35\M	RLV+3rd V	NP	Disomic

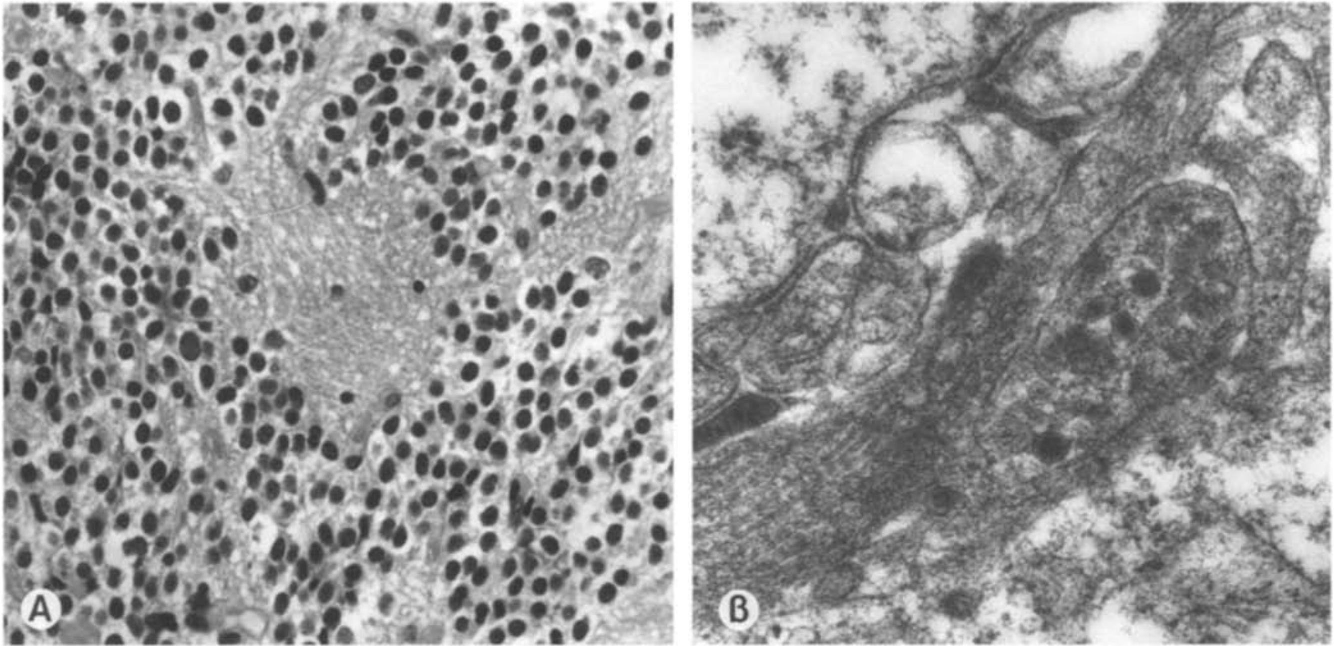

Fig. 1 **A** Uniform population of small round cells with clear cytoplasms (case 1). **B** Cytoplasmic processes containing microtubules, dense core granules and synaptic vesicles

nohistochemistry was carried out with the ABC method (ABC Kit by Vector) using diaminobenziydine (DAB) as substrate. The following primary monoclonal antibodies (mab) were used: anti-synaptophysin (SY38; Progen, Heidelberg, Germany) 1:300; anti-glial fibrillary protein (GFAP; Dako, Copenhagen, Denmark) 1:200; anti-neurofilament (NF; triplet proteins; Ortho Diagnostic Systems, Milan, Italy 1:500. For controls the primary antibody was omitted.

Fresh tumour specimens were obtained from all cases and used for electron microscopy. They were fixed in 2% cold glutaraldehyde in cacodilate buffer, postfixed in osmium tetroxide 1%, dehydrated and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by transmission electron microscopy.

Standard cytogenetic evaluation was performed in four cases (1–4). Fresh sample of neoplastic tissue was minced with scissors on a glass petri dish and resuspended in culture medium RPMI 1640 supplemented with 20% fetal calf serum, L-glutamine and penicillin-streptomycin (100 IU/ml). The cell suspension was placed in flasks and incubated in 5% CO_2 at 37°C. Outgrowth of cells from tissue fragments was observed daily by phase-contrast microscope for attachment and beginning mitotic activity. Culture media were changed twice a week and after 20–30 days the growing cells occupied about 70–80% of the area of the bottom surface of the flasks. The cells were washed with culture medium, dispersed with trypsin, resuspended in medium and centrifuged three

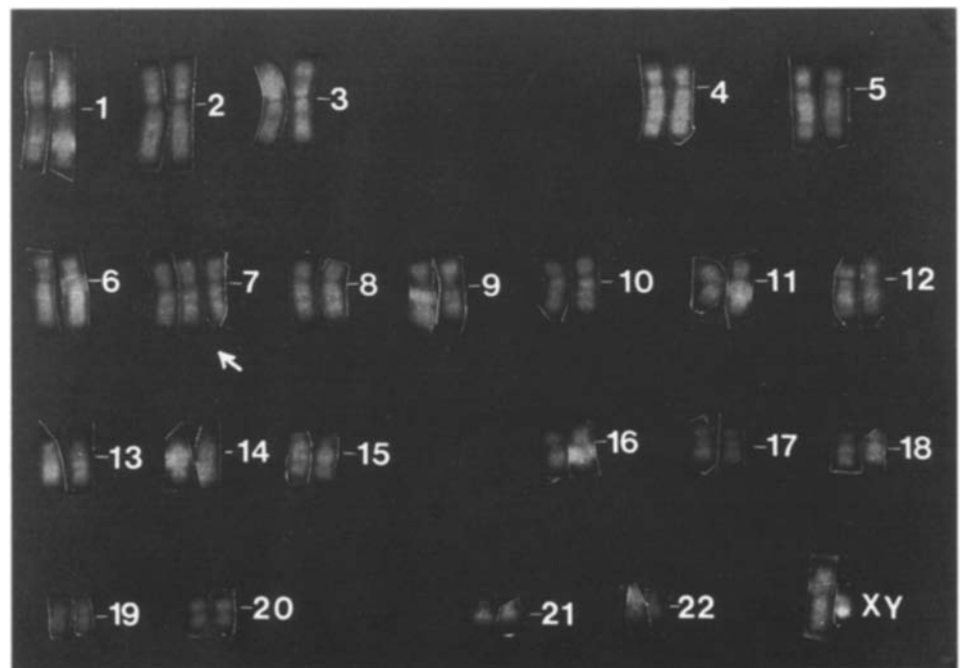

Fig. 2 Case 1: karyotype showing trisomy 7 (*arrow*) as single abnormality

times. The pellet was incubated in 2 ml of RPMI 1640 on glass chamber slides for 24 h. Then cells from each dish were harvested for treatment with Colcemid (10mg/ml) for 3 h. Coverslips were exposed to hypotonic solution (KCl × 12 min) and gradual admixture of fixative (3:1 methanol: glacial acetic acid) with three changes. Slides were air-dried and banded by a conventional banding method. Quinacrine fluorescein Q (QFQ) banding was used to evaluate the centromeric heterochromatin. Karyotype was determined by arranging all photographed metaphases that were technically satisfactory according to the International System for Human Cytogenetic Nomenclature (ISCN) [12].

For FISH, areas composed exclusively of neoplastic tissue and free of haemorrage were selected from the H&E-stained sections of each case. The sections were then matched to the paraffin blocks, and the selected region was removed with a scalpel blade, deparaffinized, dehydrated and minced by scalpel blade in a petri dish. Pieces were digested with 0.5% pepsin solution at 37°C for 30 s and the suspension obtained was filtered (filter 80 μm diameter) and fixed routinely with a by methanol/acetic acid mixture (3:1).

Two DNA probes were used: clone p7tet (24) and clone pMR9A [19], which specifically hybridize to the centromeric region of chromosomes 7 and 9, respectively. These two centromere-specific probes detect the cognate chromosome in metaphases as well as in interphase cells. The DNA probes were labelled with biotin-11-dUTP by nick translation [16].

FISH was performed on nuclei according to the protocol of Hopman et al. [11], modified as follows: slides containing nuclei were incubated in 0.2 N HCl solution for 10 s at room temperature and subsequently treated by Proteinase K (20 μg/ml) for 1 h at 37°C. After washing, RNase treatment (100 μg/ml) was applied to each specimen for 30' at 37°C. The slides were extensively washed in 1 × PBS and then dehydrated. Seventy ng of each DNA-labelled probe was precipitated with 9 μg of salmon sperm DNA and resuspended in 10 μl of 50% formamide/2 × SSC/10% dextran sulfate. The hybridization mixture was applied to the specimen, which was covered with a coverslip and sealed with rubber cement. Simultaneous denaturation of probe and nuclei was done at 86°C × 12–15 s. Slides were then incubated in a moist chamber overnight at 37°C. Posthybridization washing was at 42°C in 2 × SSC-50% formamide (3 × 5 min), followed by three washes in 0.7 × SSC at 60°C. The biotinylated probes were detected using FITC-conjugated avidin DCS (5 μg/ml) (Vector Laboratories). Total DNA was counterstained with DAPI and/or with propidium iodide (PI). Colour images were collected using a computer-controlled Zeiss Axioscope epifluorescence microscope equipped with a cooled charge-coupled device (CCD) camera (Photometrics). Fluorescein DAPI and PI fluorescences were recorded separately as grey scale images and then pseudocoloured and merged using a software program developed by T. Rand in D.C. Ward's laboratory (Yale University, USA).

FISHs on nuclei were examined by two independent investigators. For each probe, and for each sample, 200 intact and non-overlapping nuclei were selected randomly, and the number of bright fluorescent spots per nucleous was counted [21].

In all samples we measured the frequency of nuclei with 1, 2, 3, 4 hybridization spots for each of the two autosomes; we then calculated the mean and SD of the frequencies of 1 and 3 and 4 hybridization spots for chromosome 9, which were used as control values. A conservative approach was applied and, therefore, we considered monosomy, trisomy or tetrasomy of chromosome 7 to be present when the value exceeded the mean + 4 SD observed for the reference chromosome 9. Accordingly, a monosomy was defined when the frequency of single spots was ≥ 11 (i.e., 5.5%), trisomy when the frequency of triple spots was ≥ 7 (3.5%), and tetrasomy when the frequency of four spots was ≥ 6 (3%).

Results

All cases were composed of a uniform population of small round cells with clear cytoplasm embedded in a neuropil-like background (Fig. 1A). Mitoses and necrosis were absent. All cases showed a strong immunoreactivity of the fibrillar areas for synaptophysin. In all cases GFAP positivity was observed only in reactive astrocytes. Immunostaining for NFs was negative. The neuronal nature of neoplastic cells were confirmed by electron microscopy in each case by the presence of processes containing microtubules, dense core granules and synaptic vesicles (Fig. 1B).

Cytogenetic analysis of case 1 showed the following karyotype: 47, XY, +7 (Fig. 2). Analysis of cases 2–4 disclosed normal karyotypes.

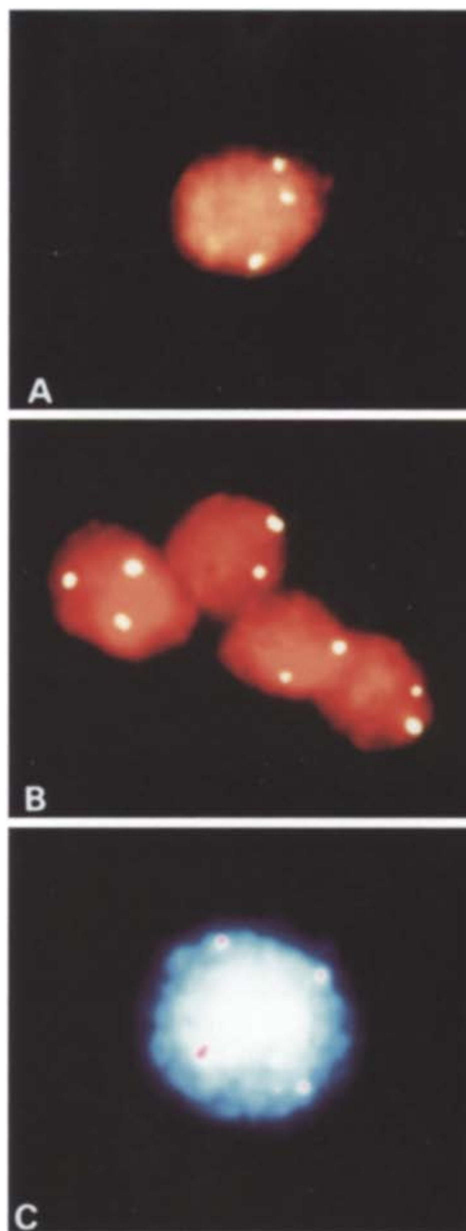

Fig. 3 **A–C** FISH with biotinylated centromeric repeat probe specific for chromosome 7. **A** Trisomy of chromosome 7 (case 1). **B** One nucleus shows trisomy of chromosome 7, whereas three nuclei show disomy of the same chromosome. **C** Tetrasomy of chromosome 7 (case 7)

Table 2 Results of FISH using two probes specific for the centromeres of chromosomes 7 and 9

Probe for chromosome 7 Case no.	Number of spots\nucleus				Probe for chromosome 9 Case no.	Number of spots\nucleus			
	1	2	3	4		1	2	3	4
1	5	180	15	0	1	4	193	3	0
2	6	191	3	0	2	4	193	3	0
3	2	196	2	0	3	nd			
4	0	197	3	0	4	0	197	3	0
5	5	194	1	0	5	3	194	3	1
6	0	199	1	0	6	5	194	1	0
7	0	184	3	13	7	6	190	4	0
8	7	184	9	0	8	4	193	3	4
9	1	197	2	0	9	5	191	4	0

Table 2 summarizes the results of FISH performed on chromosome 7 and 9 centromeres. The single spot frequency was comparable between chromosome 7 and the reference chromosome 9. Trisomy 7 was detected in two of the nine cases, namely case 1 (15/200, 7.5%) and 8 (9/200, 4.5%); tetrasomy 7 (13/200, 6.5%) was observed in case 7 (Fig. 3).

Discussion

Central neurocytoma (CN) is a benign, slow-growing neoplasm composed of small round well differentiated neurons. To explain the characteristic intraventricular location, an origin from the subependymal plate of the lateral ventricle has been proposed [9]. Biomolecular analysis of a few cases has not disclosed any amplification of *N-myc* and *c-myc* oncogenes, which are frequently amplified in neoplasms of neuronal origin [22]. Similarly, mutations of the *p53* tumour suppressor gene were not detectable in 12 neurocytomas analysed by single-strand conformation polymorphism [18]. In addition, as far as we know, chromosomal analyses have not been reported. This lack of cytogenetic information might be related to the low proliferation rate of most central neurocytomas, rendering isolation of metaphase plates a relatively harduous task.

In this study we demonstrate chromosome 7 gain in three of nine CN (33%). Traditional cytogenetic analysis of four cases identified trisomy 7 as the sole chromosomal abnormality in only one case (case1). A FISH study on cell suspension from formalin-fixed paraffin-embedded tumour tissue in these four cases and in five more (without traditional cytogenetic analysis) confirmed trisomy 7 in case 1 and detected trisomy 7 in one more case (case 8) and tetrasomy 7 in another (case 7).

Trisomy 7 is a frequent finding in high-grade astrocytoma such as glioblastoma multiforme [4, 5]. In addition, gain of chromosome 7 has been documented by karyotype analysis in primitive neuroectodermal tumours [13] and in benign CNS neoplasms of both glial and neuronal lineage, such as pilocytic astrocytoma [3, 26] and gangliogliomas [2]. Trisomy 7 also occurs in many different extracerebral neoplasms, such as those of lung [14], kidney [7, 25, 27] and bladder [20, 23]. In bladder cancer gain of chromosome 7 may be correlated with aggressive biological behavior [23]. However, various studies based on conventional cytogenetic analysis have shown that gain of chromosome 7 and loss of a sex chromosome are present in various nonneoplastic conditions, suggesting that these aberrations are not solely associated with neoplastic transformation [8, 14, 28]. Similarly, trisomy 7 and loss of sex chromosomes have been reported in short-term cultures of apparently nonneoplastic brain tissue obtained from patients with malignant gliomas [10, 15] and in gliotic tissue [17]. However, studies using FISH analysis on interphase nuclei have not documented extra copies of chromosome 7 in normal and gliotic brain tissue [1, 6, 26]. These discrepancies between conventional and interphase cytogenetic have indicated that short-term cultures of cell populations with low intrinsic mitotic activity, such as normal or gliotic brain tissue, can introduce "in vitro" artefacts into karyotype analysis. Conversely, interphase cytogenetics utilizing FISH reduces the likelihood of these in vitro artefact [6]. These observations support our finding that gain of chromosme 7 is a nonrandom cytogenetic event in CN. In fact, our observation of trisomy 7 as single chromosomal aberration in one case obtained with conventional karyotyping was confirmed in the same case by FISH in cells obtained from selected areas of the paraffin blocks containing only neoplastic tissue. In addition, FISH confirmed gain of chromosome 7 in two other cases. Recently, White et al. [26] used FISH on interphase nuclei to show nonrandom chromosomal gains in pilocytic astrocytomas of childhood. The authors suggest that trisomy 7 and trisomy 8 are relatively common events in the tumorigenesis of these neoplasms. Still several lines of evidence indicate that trisomy 7 may not be a primary marker for neoplasia. However, our results provide further support for the notion that chromosome 7 gain is a feature of neuroectodermal tumorigenesis of both glial and neuronal lineage, possibly conferring growth advantage on the neoplastic cells.

This study confirms that FISH on interphase nuclei, by eliminating the need for short-term cell culture and minimizing cell culture artefacts, is a valuable adjunct in the genetic evaluation of rare central nervous system neoplasms with low baseline proliferative activity.

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