

Genetic instability in nerve sheath cell tumors

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ABSTRACT

After *in vitro* culture, we analyzed cytogenetically four acoustic nerve neurinomas, one intraspinal neurinoma and one neurofibroma obtained from unrelated patients. Monosomy of chromosomes 22 and 16 was an abnormality common to all cases, followed in frequency by loss of chromosomes 18 (three cases) and chromosomes 8, 17 and 19 (two cases). Trisomy of chromosome 20 was also detected in two cases. Structural rearrangements were detected at low frequencies, with del(10)(p12) being present in two cases. In addition, we observed cell subpopulations showing a certain degree of genetic instability, reflected by the presence of polyploid cells with inconsistent abnormalities, endoreduplications and telomeric associations resulting in dicentric chromosomes. It is probable that these cytogenetic abnormalities represent some kind of evolutionary advantage for the *in vitro* progression of nerve sheath tumors.

INTRODUCTION

Neurinomas (or schwannomas) are tumors originating from cells of the nerve sheath, are typically benign, encapsulated, and or intracranial intraspinal or peripheral location. Within the cranium the commonest site of origin is the vestibular portion of the acoustic nerve. When bilateral, they are usually associated with bilateral acoustic neurofibromatosis (BANF) or neurofibromatosis type 2 (NF-2), a disorder inherited as an autosomal dominant trait (Rowlands, 1986; Muir, 1987). Neurofibromas are thought to arise from Schwann cells, although they differ from neurinomas in that they tend not to be encapsulated and have a much softer consistency. These lesions may be locally

infiltrative and multiple neurofibromas are a feature of von Recklinghausen's disease or neurofibromatosis type 1 (NF-1). In this condition, neurofibromas may occur in virtually all sites of the body and be associated with virtually any peripheral or intraspinal nerve (Yang *et al.*, 1989).

Approximately 90 neurinomas have been analyzed cytogenetically with chromosome banding (Rey *et al.*, 1987a; Couturier *et al.*, 1990; Webb and Griffin, 1991; Stenman *et al.*, 1991; Bello *et al.*, 1993). Monosomy of chromosome 22 is consistently reported as the only alteration, suggesting its specific involvement in the genesis of acoustic nerve neurinomas, of spinal and peripheral neurinomas, and of neurofibromas (Krone and Hogemann, 1986). Complex karyotypes showing other chromosome abnormalities in addition to monosomy 22 have been described in a few cases (Stenman *et al.*, 1991; Bello *et al.*, 1993). Recently, Bello *et al.* (1993) suggested the existence of different cytogenetic subgroups in neurinomas.

We report here the cytogenetic analysis of six sporadic primary tumors of nerve sheath cells: four acoustic nerve neurinomas, one intraspinal neurinoma and one neurofibroma. Clonal chromosome abnormalities were detected in all cases.

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MATERIAL AND METHODS

Four acoustic nerve neurinomas (cases 1-4), one intraspinal neurinoma (case 5) and one neurofibroma (case 6) were obtained from unrelated patients, five females and one male, ranging in age from 31 to 50 years. The tumors were classified according to the World Health Organization (WHO) brain tumor classification (Zülch, 1979). All cases represented primary sporadic neoplasias and the patients did not receive presurgical radio- or chemotherapy.

Cytogenetic study

Fresh tumor samples collected under sterile conditions were processed promptly. The fragments were first sectioned and then dissociated enzymatically with a 0.4% type IV collagenase solution, and transferred to flasks containing HAM F-10 medium (SIGMA), supplemented with 20% fetal calf serum, vitamins and antibiotics. Culture time was adjusted individually for each flask, depending on mitotic activity. For cytogenetic analysis, cells in the exponential growth phase were first treated with 0.0016% colchicine for at least six hours. Hypotonic 0.075 KCl was used for approximately 30 minutes, and methanol: acetic acid (3:1) was used as a fixative.

Chromosome studies were done only on primary cultures. Mean time for culture was 26 days (range 18-45 days). Slides were subjected to standard Giemsa staining, GTG banding (Scheres, 1972) and CBG banding (Sumner, 1972). At least five cells were analyzed after CBG banding to determine the occurrence of heterochromatin polymorphisms. The International Standard for Human Cytogenetic Nomenclature (ISCN, 1991) was used for karyotypic description.

RESULTS

The four acoustic nerve neurinomas (cases 1-4) had a modal chromosome number equal to 46 (ranging from 25 to 70% of the cells in the different tumors), for a total of 100 cells analyzed. Cells with hypodiploid up to near tetraploid chromosome numbers were also commonly observed. In cases 5 and 6 (intraspinal neurinoma and neurofibroma, respectively), the modal number observed was 45 chromosomes, with variations in the peridiploid range (Figure 1).

Normal karyotypes were observed in five cases (1-4,6). Nonclonal numerical and structural chromosome abnormalities were considered only when at least another case presented the same alteration in clonal form. Monosomy of chromosome 22 was the most frequent abnormality (clonal in cases 2-5 and nonclonal in cases 1 and 6), followed in frequency by the loss of chromosomes 16 (clonal in cases 2-6), 8 and 17 (clonal in cases 2 and 3), 18 (clonal in cases 2 and 3 and nonclonal in case 1) and 19 (clonal in cases 2 and 5). Trisomy of chromosome 20 was observed in cases 2 and 3 (Table I). Structural rearrangements were detected at low frequency. Among them was del(10)(p12), found in two cells in case 2 and in one cell in case 4 (Figure 2). In addition, del(10)(q24) was detected in a sporadic cell of case 3.

Genetic instability reflected by the presence of endoreduplications, polyploidies, telomeric associations resulting in dicentric chromosomes, and chromosome breaks were observed at a low frequency, or in sporadic cells in all cases (Figure 3).

After CBG banding, heterochromatin polymorphisms were observed in all cases. Pericentric inversion of chromosome 9 was an alteration shared by five cases (Table II). In case 4 it was also possible to perform chromosomal analysis of normal cells by peripheral blood lymphocyte culture. Conventional analysis and analysis after GTG banding revealed a normal 46,XX karyotype. Analysis after CBG banding revealed partial pericentric inversion of chromosome 9 but not polymorphism of chromosome 1 (1qh+), which was also present in tumor cells (Figure 4).

DISCUSSION

In cytogenetic studies of nerve sheath tumors there is a predominance of normal karyotypes, and karyotypically abnormal stem or side lines are detected in approximately 30% of cases, with a predominance of numerical chromosome alterations. Mark (1972) provided data on non-banded neurinomas which suggested that the loss of a G group chromosome is characteristic of this neoplasm. Hypodiploid cells with losses of chromosome 22 are consistently reported (Rey *et al.*, 1987a; Couturier *et al.*, 1990; Webb and Griffin, 1991; Stenman *et al.*, 1991; Bello *et al.*, 1993). In a previous report we described two neurinomas with a monosomy 22; normal diploid numbers were found in two others acoustic neurinomas, which also displayed a small polyploid population and structural rearrangements (Rogatto and Casartelli, 1989). Recently, other

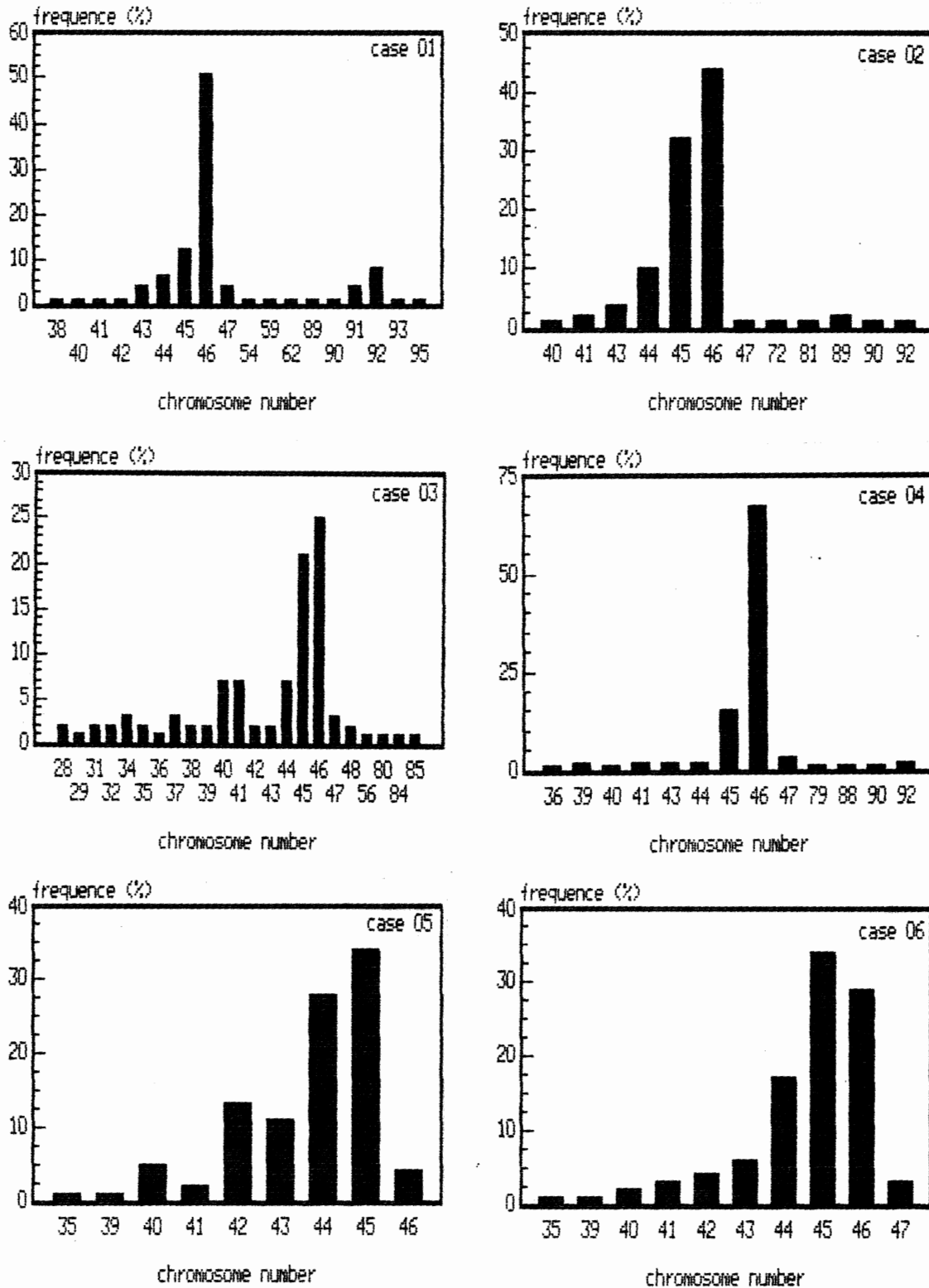


Figure 1 - Frequency distribution of chromosome numbers per cell in the acoustic nerve neurinomas (cases 1-4), intraspinal neurinoma (case 5), and neurofibroma (case 6).

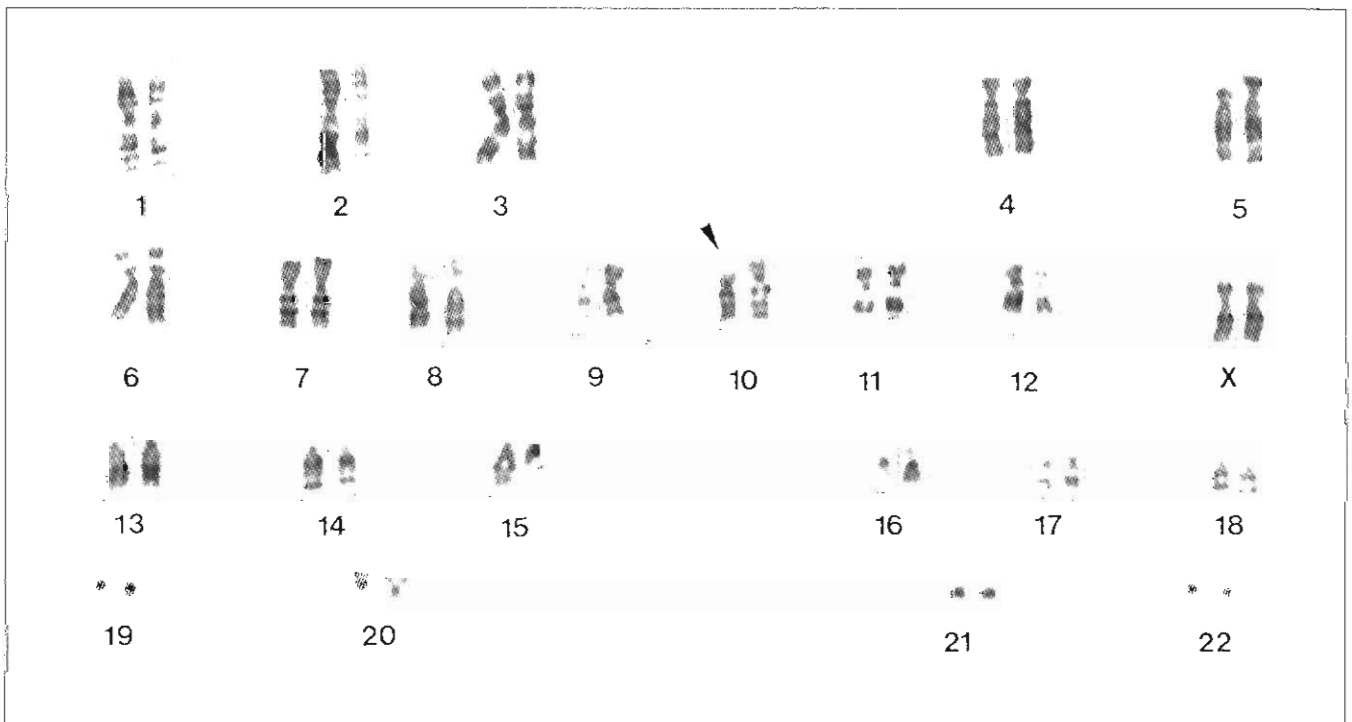
research groups have provided additional cytogenetic data. Normal karyotypes or monosomy 22 as the sole abnormality are seen to be the most frequent cytogenetic characteristics of the neurinomas (Rey et

al., 1987a; Couturier *et al.*, 1990; Webb and Griffin, 1991; Stenman *et al.*, 1991; Bello *et al.*, 1993).

The high frequency of cells with normal karyotypes in acoustic nerve neurinomas, especially

Table I - Summary of cytogenetic analysis after GTG banding. Nonclonal chromosome abnormalities were considered only when at least another case presented the same alteration in clonal form.

Case no.	Age/sex	Histopathological diagnosis	Days <i>in vitro</i>	Normal/abnormal Cells	Karyotype
1	31/F	acoustic neurinoma	34	10/7	44-46,XX,-18[1],-21[2],-22[1]
2	41/F	acoustic neurinoma	20	20/4 /16 /11 /2	39-48,XX, add(2)(p25)[2],-8[3]. del(10)(p12)[2],-16[3],-17[3],-18[6],-22[4]/ 60-74,XXX,-X[3],+1[2],+2[3],+5[2],+7[2], +10[2],+11[2],+16[2],+17[2],-19[3],-20[3],+22[2]/ 86-97,XXXX,-X[3],+8[3],-17[4],-21[3],-22[3]/ 92,XXXX
3	44/F	acoustic neurinoma	45	3/2 /27	46,XX,-X,+20/ 24-47,XX,-X[3],del(1)(p34.2)[2],-3[7],-4[6], -5[4],-6[3],-8[8],-9[6],-10[3],-11[4],-12[7], +der(13;15)(q10;q10)[2],-13[7],-15[6],-16[4], -17[7],-18[9],-20[5],-21[4],-22[5],+mar1[2],+mar2[3]
4	50/F	acoustic neurinoma	18	13/5 /16	46,XX,del(5)(q31)/ 40-46,XX,del(5)(q31)[3],del(10)(p12)[1],del(14)(q11.2q21)[2], -16[3],+20[2],-22[5]
5	50/F	intraspinal neurinoma	27	0/10 /12	45,XX,-22/ 40-46,XX,del(12)(p13)[3],-16[3],-19[3],-21[3],-22[11]
6	44/M	neurofibroma	30	4/2 /4 /5	45,XY,-22/ 46,XY,del(2)(q33)/ 45,XY,del(2)(q33)[2],-16[4]

**Figure 2** - Karyotype of a cell from case 4 (acoustic nerve neurinoma) showing del(10)(p12) (arrow).

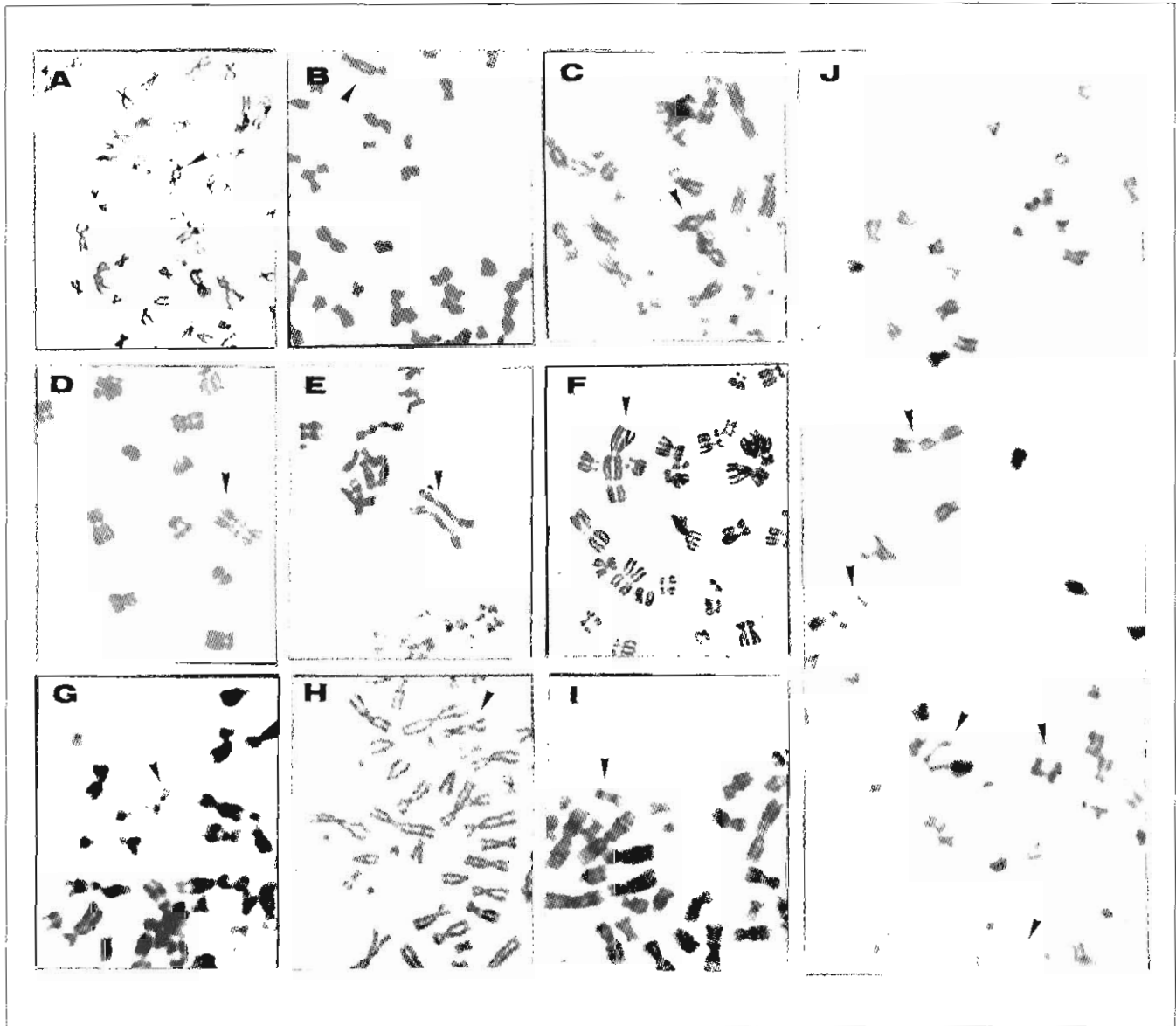


Figure 3 - **A**, Partial metaphases showing a CBG-banded polyploid cell with a dicentric chromosome (case 1); **B**, polyploid cell with the presence of a dicentric (case 1); **C**, dicentric chromosome (case 4); **D**, **E**, and **F**, endoreduplicated cells showing telomeric associations resulting in dicentrics (**D**, case 4; **E** and **F**, case 5); **G**, **H**, and **I**, dicentric chromosomes observed in cases 2 and 4, **J**, polyploid cell showing chromatid separation in some chromosomes and fragments (case 5).

Table II - Chromosome heteromorphisms observed after CBG banding.

Case no.	Normal cells	Tumor cells
01	-	partial inv(9)
02	-	partial inv(9), 16qh+
03	-	1qh+
04	partial inv(9)	1qh+, partial inv(9)
05	-	partial inv(9)
06	-	partial inv(9), 16qh+

- analysis not done.

after short periods of *in vitro* culture, have suggested that normal cells may grow more than tumor cells (Webb and Griffin, 1991). Alternately, these tumors may present alterations not detected at the microscope level, as demonstrated by Couturier *et al.* (1990) in three karyotypically normal neurinomas that showed a loss of heterozygosity for loci on chromosome 22. The present case 1 may be included in a subgroup of neurinomas displaying normal karyotypes, as suggested by Bello *et al.* (1993).

Our data show that there may be a correlation between time of *in vitro* culture, variation in number of chromosomes per cell and the presence of different

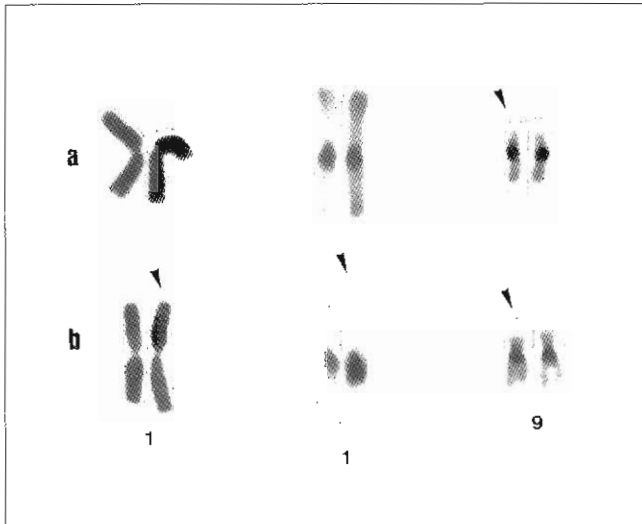


Figure 4 - (a), Chromosomes 1 (standard staining and CBG banding) and 9 (CBG banding), showing partial pericentric inversion in one of the homologues of chromosome 9 (arrow) in peripheral blood lymphocytes. (b), Conventional staining and CBG banding of chromosome pair 1, showing intrapair asymmetry of the 1qh+ type and persistence of partial pericentric inversion of chromosome 9 in tumor cells (case 4).

subpopulations of karyotypically abnormal cells. The greatest variations in chromosome number per cell (from hypodiploid to near tetraploid) were observed in the four acoustic nerve neurinomas, cultured *in vitro* for 20 to 45 days. Similar results have been reported by Stenman *et al.* (1991) in 12 cases of schwannomas that were left *in vitro* six to 34 days. Despite this variation in chromosome number, we were able to detect a recurrent pattern of losses and gains of some chromosomes, in agreement with the chromosome picture already described for this tumor type.

Monosomy of chromosome 22 was detected in all our cases, although it was considered to be non-clonal in two of them (cases 1 and 6). Rey *et al.* (1987a) detected monosomy of chromosome 22 in three tumors and one of them also presented the loss of chromosome 17 and other abnormalities. A study by Webb and Griffin (1991) on 11 acoustic nerve neurinomas only detected one case with a 46,XX/45,XY,-22/44,X,-Y,-22 karyotype, and monosomy 22 was detected in two other tumors as a non-clonal alteration. Monosomy of chromosome 22 has also been detected in cell cultures obtained from peripheral neurofibromas of three patients with neurofibromatosis (Krone and Hogemann, 1986). A recurrent neurofibroma presented t(1;22)(p32;q11) and marker chromosomes (Rey *et al.*, 1987b). In a large sample, Bello *et al.* (1993) reported clonal abnormalities involving chromosome 22 in 23 cases, in 12 of which it was the only chromosome aberration. In 11 samples,

other chromosome alterations were detected in addition to loss of chromosome 22.

Numerical deviations other than those involving chromosome 22 were assessed as clonal and involving the loss of chromosomes 8, 10, 16, 17 and 18 and trisomy 20. The involvement of chromosomes of group E as secondary alterations has been described by several investigators (Mark, 1972; Rey *et al.*, 1987a; Bello *et al.*, 1993). Monosomy 16 was observed in all cases except case 1. Stenman *et al.* (1991) reported t(16;19) as a sporadic abnormality and Bello *et al.* (1993) found monosomy 16 in side lines in two cases. Rey *et al.* (1987a) reported the loss of chromosomes 17 and 18 in separate cases. Bello *et al.* (1993) detected monosomy 17 in seven cases and monosomy 18 in one. Trisomy 20 has been previously observed by Stenman *et al.* (1991) and Bello *et al.* (1993).

Molecular studies indicate an association between neurinomas and the loss of alleles on chromosome 22 both in neurofibromatosis and in sporadic tumors (Seizinger *et al.*, 1986, 1987; Wolff *et al.*, 1992; Rey *et al.*, 1993), suggesting that a suppressor gene may be located on this chromosome. Patients with neurofibromatosis commonly develop tumors of the nervous system, including neurinomas, neurofibromas, meningiomas, and gliomas. The existence of genetically distinct forms of the disease has been suggested: neurofibromatosis type I (NF-1) and neurofibromatosis type II (NF-2). Gene NF1 was mapped at 17q11.2 (Collins *et al.*, 1989) and locus NF-2 might be located at 22q12 (Rouleau *et al.*, 1987; Ahmed *et al.*, 1991). Constitutional deletions in patients with NF-1 have suggested that this disease may originate from a loss of function of gene NF-1, consistent with the hypothesis that this is a tumor suppressor gene (Marshall, 1991). However, analysis of tumor cells from patients with NF-1 has shown loss of heterozygosity at this locus only in a few cases (Skuse *et al.*, 1989; Xu *et al.*, 1992).

Clonal structural rearrangements including deletions, translocations and markers involving a variety of chromosomes were observed by us. All cases with these deviations also had monosomy 22. Neurinomas displaying similar characteristics have been described by other groups (Stenman *et al.*, 1991; Bello *et al.*, 1993). Among the structural alterations detected, we point out the involvement of chromosome 10, although at low frequencies, in the deletions of cases 2, 3 and 4. Loss of chromosome 10 regions is common in advanced stages of the progression of astrocytomas (James *et al.*, 1988; Cavenee, 1992; Rasheed *et al.*, 1992; Fults and Pedone, 1993). Recently, loss of heterozygosity for loci on chromosome 10 has

been reported for meningiomas (Rempel *et al.*, 1993), tumors of the nervous system that, like tumors of the nerve sheath, also show morphological heterogeneity. The loss of alleles in chromosome 10 was associated with morphological signs of malignancy in these meningiomas. The loss of chromosome 10 material (especially 10pter) detected in our cases may not be a random event, and its significance should be better investigated at the molecular level.

The role of chromosome alterations in addition to monosomy 22 and the presence of complex karyotypes in some cases of nerve sheath tumors is unknown. Within this context are the subpopulations of polyploid cells detected in the neurinomas studied here. Abnormal mitoses, including endoreduplications (Levan and Hauschka, 1953) are characteristic events of tumor cells and were occasionally detected during our analysis. Another type of aberration was total or partial mitotic spindle failure. Atkin and Baker (1992) have called attention to the occurrence of this phenomenon in human cancers. In most cases, mitotic abnormalities depend on faulty alignment of chromosomes in the metaphase or anaphase spindle. This results in loss, nondisjunction or misdivision of chromosomes. Such abnormal alignment is rare in untreated normal cells, but common in cancer.

Even though they are characteristics shared by tumor cells, these two events were recorded in cells *in vitro*, and extrapolation to cell behavior *in vivo* would be difficult. These changes in mitosis may confer a certain degree of genetic instability on tumor cells, and lead to new abnormalities such as segregation errors, breaks and telomeric associations, resulting in dicentric chromosomes. In hypodiploid meningiomas, the presence of chromosome rearrangements, breaks and telomeric associations has been commonly reported (Zang, 1982; Vagner-Capodano *et al.*, 1992). An increased dicentric frequency in cultured meningiomas, showing karyotypically more complex stem lines, may reflect chromosome instability, possibly leading to the occurrence of other rearrangements, and possibly related to evolutionary advantages for *in vitro* (Rey *et al.*, 1988) and *in vivo* (Casartelli *et al.*, 1989) progression in these tumors.

Genetic instability is important in the carcinogenesis process (tumor initiation and progression), since it creates different variant clones, permitting the evolution of tumor cells and the acquisition of malignant and/or metastatic properties. In fact, inherent genetic instability is one characteristic separating metastatic and many malignant tumors from benign tumors (Volpe, 1988). The findings reported for these cases of nerve sheath tumors, which are considered to be benign

tumors, show cell subpopulations with a degree of karyotype complexity compatible with that expected for malignant tumors. It is possible that this complex picture is independent of the potential for malignancy. Thus, some benign tumors may contain karyotypically unstable cells, but in general malignant tumors may be much more genetically unstable.

Intraindividual variations in C-banding patterns between normal and tumor cells may represent a greater genetic instability which, in turn, may be related to predisposition or even to the carcinogenesis process itself. In the present study, it was possible to compare the C-banding patterns of normal and tumor cells from a patient with an acoustic nerve neurinoma (case 4) which revealed polymorphism of the 1qh+ type in tumor cells, and which was not observed in peripheral blood lymphocytes. This fact suggests that this abnormality was acquired, possibly by a process of unequal mitotic crossing-over (Doneda *et al.*, 1987) that led to 1qh amplification. An increased incidence of pericentric inversions, involving the heterochromatic regions of chromosomes 1 and 9, was detected in patients with solid tumors (Shabtai *et al.*, 1985; Suciú, 1986), including tumors of the nervous system (Rey *et al.*, 1987c; Rogatto and Casartelli, 1988; Rogatto *et al.*, 1993). The intrapair asymmetry in the heterochromatic regions of chromosome 1 has been associated with certain types of cancers, and it has been suggested that individuals showing such variations may be more susceptible to the development of tumors (Doneda *et al.*, 1987; Kopf *et al.*, 1990).

In conclusion, the consistent involvement of chromosome 22 in nerve sheath tumors at different locations and with different forms of presentation suggests that this is a critical event in the origin of these neoplasias, indicating a common mechanism of development or progression for these tumors. Our data, taken together with those previously reported in the literature, show the loss of chromosomes 16, 17 and 18, the gain of chromosome 20, as well as the involvement of chromosome 10 in deletions as recurrent alterations. These play a pathogenetic role, and should be investigated further. Additionally, the genetic instability detected on the basis of different alterations needs to be evaluated in order to establish whether it is an intrinsic characteristic of nerve sheath cells, or if it reflects the *in vitro* evolution of these cells.

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RESUMO

Analisamos citogeneticamente após cultivo *in vitro* quatro neurinomas de acústico, um neurinoma intraespinhal e um neurofibroma obtidos de pacientes não relacionados. As monossomias dos cromossomos 22 e 16 foram anormalidades comuns a todos os casos, seguida em frequência pela perda dos cromossomos 18 (três casos) e 8, 17 e 19 (dois casos). A trissomia do cromossomo 20 também foi encontrada em dois casos. Rearranjos estruturais foram verificados em baixas frequências, destacando-se a del(10)(p12) presente em dois casos. Adicionalmente, foram observadas subpopulações celulares mostrando um certo grau de instabilidade genética refletida pela presença de células poliplóides com anormalidades inconsistentes, endorreduplicações e associações teloméricas resultando em cromossomos dicêntricos. É provável que estas anormalidades citogenéticas representem alguma vantagem evolutiva para a progressão *in vitro* dos tumores da bainha neural.

REFERENCES

- Ahmed, F.B., Maher, E.R., Affara, N.A., Bently, E., Xuereb, J.H., Rouleau, G.A., Hardy, D., David, M. and Ferguson-Smith, M.A.** (1991). Deletion mapping in acoustic neuroma. *Human Gene Mapping* 11 (1991). *Cytogenet. Cell Genet.* 58: 2044.
- Atkin, N.B. and Baker, M.C.** (1992). Mitotic spindle failure in human cancer. *Cancer Genet. Cytogenet.* 62: 106-107.
- Bello, M.J., Campos, J.M., Kusak, M.E., Vaquero, J., Sarasa, J.L., Pestana, A. and Rey, J.A.** (1993). Clonal chromosome aberrations in neurinomas. *Genes, Chrom. & Cancer* 6: 206-211.
- Casartelli, C., Rogatto, S.R. and Barbieri Neto, J.** (1989). Karyotypic evolution of human meningioma - Progression through malignancy. *Cancer Genet. Cytogenet.* 40: 33-45.
- Cavenee, W.K.** (1992). Accumulation of genetics defects during astrocytoma progression. *Cancer* 70: 1788-1793.
- Collins, F.S., Ponder, B.A., Seizinger, B.R. and Epstein, C.J.** (1989). Editorial: The von Recklinghausen neurofibromatosis region on chromosome 17 - genetic and physical maps come in focus. *Am. J. Hum. Genet.* 44: 1-5.
- Couturier, J., Delattre, O., Kujas, M., Philippon, J., Peter, M., Rouleau, G., Aurias, A. and Thomas, G.** (1990). Assessment of chromosome 22 anomalies in neurinomas by combined karyotype and RFLP analysis. *Cancer Genet. Cytogenet.* 45: 55-62.
- Doneda, L., Conti, A.F., Gualandri, V. and Larizza, L.** (1987). Mosaicism in the C-banded region of chromosome 1 in cancer families. *Cancer Genet. Cytogenet.* 27: 261-268.
- Fulfs, D. and Pedone, C.** (1993). Deletion mapping of the long arm of chromosome 10 in glioblastoma multiforme. *Genes, Chrom. & Cancer* 7: 173-177.
- ISCN** (1991). Guidelines for Cancer Cytogenetics, Supplement to An International System for Human Cytogenetic Nomenclature (Mitelman, F., ed.). S. Karger, Basel 1991, pp. 54.
- James, C.D., Carlbom, E., Dumanski, J.P., Hansen, M., Nordenskjold, M., Collins, V.P. and Cavenee, W.K.** (1988). Clonal genomic alterations in glioma malignancy stages. *Cancer Res.* 48: 5546-5551.
- Kopf, I., Strid, K.G., Islam, M.O., Granberg, S., Friberg, L.G., Levan, G. and Carstensen, J.** (1990). Heterochromatin variants in 109 ovarian cancer patients and 192 healthy subjects. *Hereditas* 111: 7-16.
- Krone, W. and Hogemann, I.** (1986). Cell culture studies on neurofibromatosis (von Recklinghausen). *Human Genet.* 74: 453-455.
- Levan, A. and Hauschka, T.S.** (1953). Endomitotic reduplication mechanism in ascites tumors of the mouse. *J. Natl. Cancer Inst.* 14: 1-46.
- Mark, J.** (1972). The chromosomal findings in seven human neurinomas and one neurosarcoma. *Acta. Path. Microbiol. Scand.* (Section A) 80: 61-70.
- Marshall, C.J.** (1991). Tumor suppressor genes. *Cell.* 64: 313-326.
- Muir, R.** (1987). *Muir's Textbook of Pathology.* 12nd edn. J.R. Anderson, London, pp. 21.64-21.65.
- Rasheed, B.K.A., Fuller, G.N., Friedman, A.L., Bigner, D.D. and Bigner, S.H.** (1992). Loss of heterozygosity for 10q loci in human gliomas. *Genes, Chrom. & Cancer* 5: 75-82.
- Rempel, S.A., Schwechheimer, K., Davis, R.L., Cavenee, W.K. and Rosenblum, M.L.** (1993). Loss of heterozygosity for loci on chromosome 10 is associated with morphologically malignant progression. *Cancer Res.* 53: 2386-2393.
- Rey, J.A., Bello, M.J., Campos, J.M., Kusak, M.E. and Moreno, S.** (1987a). Cytogenetic analysis in human neurinomas. *Cancer Genet. Cytogenet.* 28: 187-188.
- Rey, J.A., Bello, M.J., Campos, J.M., Kusak, M.A., Valcarcel, E. and Benitez, J.** (1987b). C-band pattern in patients with nervous system tumors. *Cancer Genet. Cytogenet.* 27: 185-190.
- Rey, J.A., Bello, M.J., Campos, J.M., Benitez, J., Sarasa, J.L., Boixados, J.R. and Sanchez Cascos, A.** (1987c). Cytogenetic clones in a recurrent neurofibroma. *Cancer Genet. Cytogenet.* 26: 157-163.
- Rey, J.A., Bello, M.J., Campos, J.M. and Kusak, E.** (1988). Incidence and origin of dicentric chromosomes in cultured meningiomas. *Cancer Genet. Cytogenet.* 35: 55-60.
- Rey, J.A., Bello, M.J., Campos, J.M., Vaquero, J., Kusak, M.H., Sarasa, J.L. and Pestaña, A.** (1993). Abnormalities of chromosome 22 in human brain tumors determined by combined cytogenetic and molecular genetic approaches. *Cancer Genet. Cytogenet.* 66: 1-10.
- Rogatto, S.R. and Casartelli, C.** (1988). Cytogenetic studies of human meningiomas. *Rev. Bras. Genet.* 11: 729-744.
- Rogatto, S.R. and Casartelli, C.** (1989). Cytogenetic study of human neurinomas. *Cancer Genet. Cytogenet.* (abstr.) 41: 278.
- Rogatto, S.R., Rainho, C.A., Casartelli, C. and Barbieri Neto, J.** (1993). Chromosomes in the genesis and progression of ependymomas. *Cancer Genet. Cytogenet.* 69: 146-152.
- Rouleau, G.A., Wertelecki, W., Haines, J.L., Hobbs, W.J., Trofatter, J.A., Seizinger, B.R., Martuza, R.L., Superneau, D.W., Conneally, P.M. and Gusella, J.F.** (1987). Genetic linkage of bilateral acoustic neurofibromatosis to a DNA marker on chromosome 22. *Nature* 329: 246-248.
- Rowlands Jr., D.R.** (1986). *Human Pathology. An Introduction to the Study of Disease.* MacMillan Publishing Co., New York, pp. 582.

- Scheres, V.M.J.C.** (1972). Identification of two Robertsonian translocations with a Giemsa banding technique. *Human Genet.* 15: 253-256.
- Seizinger, B.R., Martuza, R.L. and Gusela, J.F.** (1986). Loss of genes on chromosome 22 in tumorigenesis of human acoustic neuroma. *Nature* 322: 644-647.
- Seizinger, B.R., Rouleau, G., Ozelius, L.J., Lane, A.H., Hyslop, P.S.G., Huson, S., Gusela, J.F. and Martuza, R.L.** (1987). Common pathogenetic mechanism for three types in bilateral acoustic neurofibromatosis. *Science* 236: 317-319.
- Shabtai, F., Antebi, E., Klar, D., Kimchi, D., Hart, J. and Halbrechi, I.** (1985). Cytogenetic study of patients with carcinoma of colon and rectum: particular C band variants as possible markers for cancer proneness. *Cancer Genet. Cytogenet.* 14: 235-245.
- Skuse, G.R., Rosciolet, B.A. and Rowley, P.T.** (1989). Molecular genetic analysis of tumors in von Recklinghausen neurofibromatosis: loss of heterozygosity for chromosome 17. *Genes, Chrom. & Cancer* 1: 36-41.
- Stenman, G., Kindblom, L.G., Johansson, M. and Angervall, L.** (1991). Clonal chromosome abnormalities and *in vitro* growth characteristics of classical and cellular schwannomas. *Cancer Genet. Cytogenet.* 57: 121-131.
- Suciu, S.** (1986). Constitutive heterochromatin studies in patients with solid tumors. *J. Cancer Res. Clin. Oncol.* 111: 291-294.
- Sumner, A.T.** (1972). A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* 75: 304-306.
- Vagner-Capodano, A.M., Grisoli, F., Gambarelli, D., Figarella, D. and Pellissier, J.L.** (1992). Telomeric associations of chromosomes in human meningiomas. *Ann. Génét.* 35: 69-74.
- Volpe, J.P.G.** (1988). Genetic instability of cancer. Why a metastatic tumor is unstable and a benign tumor is stable? *Cancer Genet. Cytogenet.* 34: 125-134.
- Webb, H.D. and Griffin, C.A.** (1991). Cytogenetic study of acoustic neuroma. *Cancer Genet. Cytogenet.* 56: 83-84.
- Wolff, R.K., Frazer, K.A., Jackler, R.K., Lanser, M.I., Patts, L.H. and Cox, D.R.** (1992). Analysis of chromosome 22 deletions in neurofibromatosis type-2 related tumors. *Am. J. Hum. Genet.* 51: 478-485.
- Xu, W., Mulligan, L.M., Ponder, M.A., Liu, L., Smith, B.A., Matheu, C.G.P. and Ponder, B.A.J.** (1992). Loss of NF1 alleles in pheochromocytomas from patients with type I neurofibromatosis. *Genes, Chrom. & Cancer* 4: 337-342.
- Yang, C.J., Rosenberg, S.A., Glatstein, E.J. and Antman, K.H.** (1989). Sarcomas of the soft tissues. In: *Cancer, Principles & Practice of Oncology* (De Vita Jr., V.T., Hellman, S. and Rosenberg, S.A., 3rd edn.). L.B Lippincott Co., Philadelphia, pp. 1355.
- Zang, K.D.** (1982). Cytological and cytogenetical studies on human meningiomas. *Cancer Genet. Cytogenet.* 6: 249-274.
- Zülch, K.J.** (1979). Histological typing of tumours of the central nervous system. Geneva: World Health Organization (WHO).

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