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Molecular Analysis of a Myxoid Chondrosarcoma with Rearrangements of Chromosomes 10 and 22

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ABSTRACT: Myxoid chondrosarcoma is a rare tumor of adulthood. An associated nonrandom reciprocal translocation between chromosome 9 and 22 was previously reported in this tumor. We performed cytogenetic and molecular genetic analysis of a myxoid chondrosarcoma derived from the sphenoid bone. Using restriction fragment length polymorphism (RFLP) analysis, we demonstrated rearrangement and a possible allele loss close to the chromosome 22 breakpoint. In addition, structural rearrangement in the chromosome 10q21.1 region and an allele loss in the chromosome 10q21-q23 region were also detected. In tumor DNA an additional hybridization fragment was detected by pAS-1 probe, which recognizes multiple pseudogenes of argininosuccinate synthetase dispersed in various chromosomes. We were unable to detect chromosomal abnormalities with two additional chromosome 9q probes. This study suggests that multiple gene rearrangements occurred in the myxoid chondrosarcoma and the significance of this is discussed.

INTRODUCTION

Identification of the specific cytogenetic abnormalities in cancers is important in disease diagnosis, defining tumor subtypes as well as providing the clue to the location of the genes involved in formation of the tumors. Nonrandom structural chromosome abnormalities have been identified in various soft tissue sarcomas [1]. Reciprocal translocation between chromosome 9 and 22 has been reported in two rare myxoid chondrosarcomas [2, 3]. Recently, molecular analysis was used to study the genotype of solid tumors. The molecular genetic study can be performed directly on the tumor tissue and avoids the possible secondary change occurring in *in vitro* propagation. The method can detect small chromosome deletions and rearrangements beyond the resolution of cytogenetic study. We performed molecular analysis of a sphenoid myxoid chondrosarcoma. In this tumor, rearrangement and a possible allele loss were detected in the chromosome 22q11-q12 region with the DNA probes and were consistent with the previous cytogenetic observation. We also demonstrated rearrangement and allele loss on chromosome 10.

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CASE REPORT

A 27-year-old white woman was admitted in March 1987 with the chief complaint of right-sided vision and hearing loss and right facial numbness. Computerized tomography and magnetic resonance imaging scans of the head showed the presence of a large mass at the right frontotemporal region. A large encapsulated tumor mass, very mucoid and with focal calcification, was surgically removed. The major histologic constituent was a mildly to moderately cellular neoplasm composed of small to medium-sized polyhedral cells with bright pink cytoplasm and small, rounded, single or multiple nucleolated nuclei (Fig. 1). The pathologic diagnosis was chondrosarcoma of the right sphenoid ring. Two years after operation the patient is alive without recurrence.

MATERIALS AND METHODS

Isolation of DNA and RFLP Analysis

Tumor that had been frozen on dry ice at the time of removal and stored at -70°C was used for DNA isolation. The frozen tumor was pulverized in liquid nitrogen and then suspended in a lysis buffer containing 20 mM Tris-HCl, 1 mM EDTA, 1% sodium dodecylsulfate (SDS) and 100 $\mu\text{g}/\text{ml}$ proteinase K and incubated at 37°C up to 18 hours. DNA was then extracted with phenol-chloroform-isoamyl alcohol (CHISM) and precipitated with 0.4 M ammonium-acetate and cold ethanol. The DNA pellets were dissolved in Tris-EDTA buffer. The patient's lymphocyte DNA was used as a control. Mononuclear cells isolated from the peripheral blood were resuspended in 10 mM Tris and then lysed with lysis buffer and extracted as above.

Ten micrograms of the control and the tumor DNA were digested to completion with the appropriate restriction enzymes, fractionated by 0.8% agarose gel electrophoresis, and transferred to Gene Screen plus nylon membrane according to the manufacturer's protocol. Prehybridization and hybridization were performed in a solution containing 1% SDS, 1 M NaCl, 10% dextran sulfate, 0.05 M Tris pH 7.5, and 0.5 mg/ml denatured herring sperm DNA at 65°C with shaking. The probe was radiolabeled with ^{32}P -ATP by random oligonucleotide primer procedure [4]. Filters were washed twice in $2 \times \text{SSC}/0.1\% \text{ SDS}$ at room temperature, once in $1 \times \text{SSC}/0.1\% \text{ SDS}$ for 15 minutes, and once in $0.1 \times \text{SSC}/0.1\% \text{ SDS}$ at 60°C for 30 minutes. Autoradiography was performed using Kodak XAR-5 film with intensifying screen at -70°C overnight or for several days depending on the particular probe used.

RFLP probes used in this study included D22S1 (Dr. Y. Nakamura); D22S9 and D10S5, (Dr. B. N. White); *c-sis*, HoxII, D10S1, D10S4 and NGF-B (American Tissue Type Collection); pAS-1 (Dr. A. L. Beaudet), and Aldo-B (Dr. F. Salvatore).

Determination of Allelic Dosage

BglII blot hybridized with the D22S1 probe was stripped in 0.4N NaOH at 65°C for 30 minutes followed by $0.1 \times \text{SSC}$, 0.1% SDS, and 0.2 M Tris-HCl pH 7.5 at 65°C for 30 minutes and was rehybridized with NGF-B. TaqI blot was hybridized with D22S9, stripped in the same fashion and rehybridized with the D10S4 and D10S5 probes. HindIII blot was used for both pAS-1 and D10S5 probes. The stripped blot was checked with an autoradiogram before reuse. The intensity of the hybridization signals were determined by laser densitometry (LKB ultrascan XL), and peak areas corresponding to each hybridization signal were calculated by electronic integration.

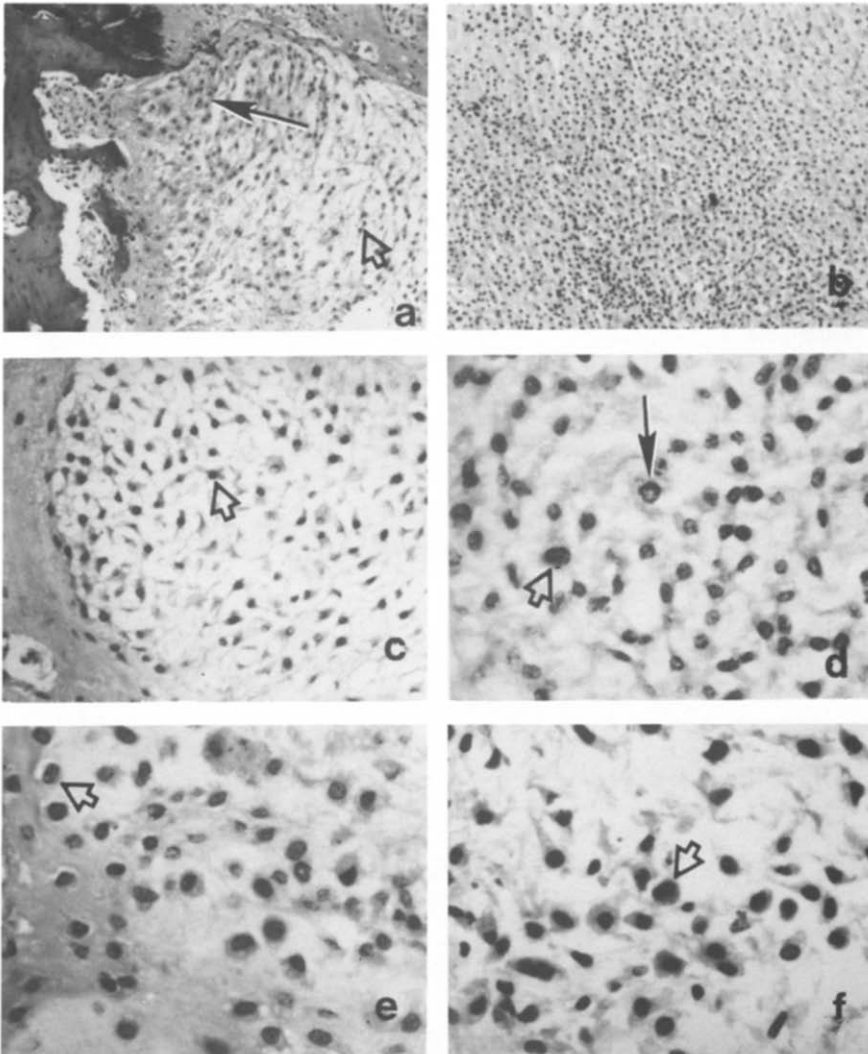


Figure 1 a) Tightly packed tumor cells in close proximity to cancellous bone (arrow). Loosely organized tumor cells show myxoid appearance (open arrow) (Hematoxylin and eosin $\times 88$.) b) Highly cellular region of tumor; tumor cells have small, rounded, and vesicular nuclei. (Hematoxylin and eosin $\times 88$.) c) Myxoid appearance of tumor cells (open arrow) with small to moderate amount of pink cytoplasm. (Hematoxylin and eosin $\times 220$.) d) Higher magnification of tumor cells showing nuclear pleomorphism and hyperchromatism (open arrow) and occasional mitotic figures (arrow). (Hematoxylin and eosin $\times 440$.) e) Higher magnification of the tumor cells showing cartilaginous differentiation (open arrow) and hyperchromatic nuclei of the pleomorphic tumor cells. (Hematoxylin and eosin $\times 440$.) f) Higher magnification of tumor cells shows moderate to marked pleomorphism of nuclei with hyperchromatism (open arrow). (Hematoxylin and eosin $\times 440$.)

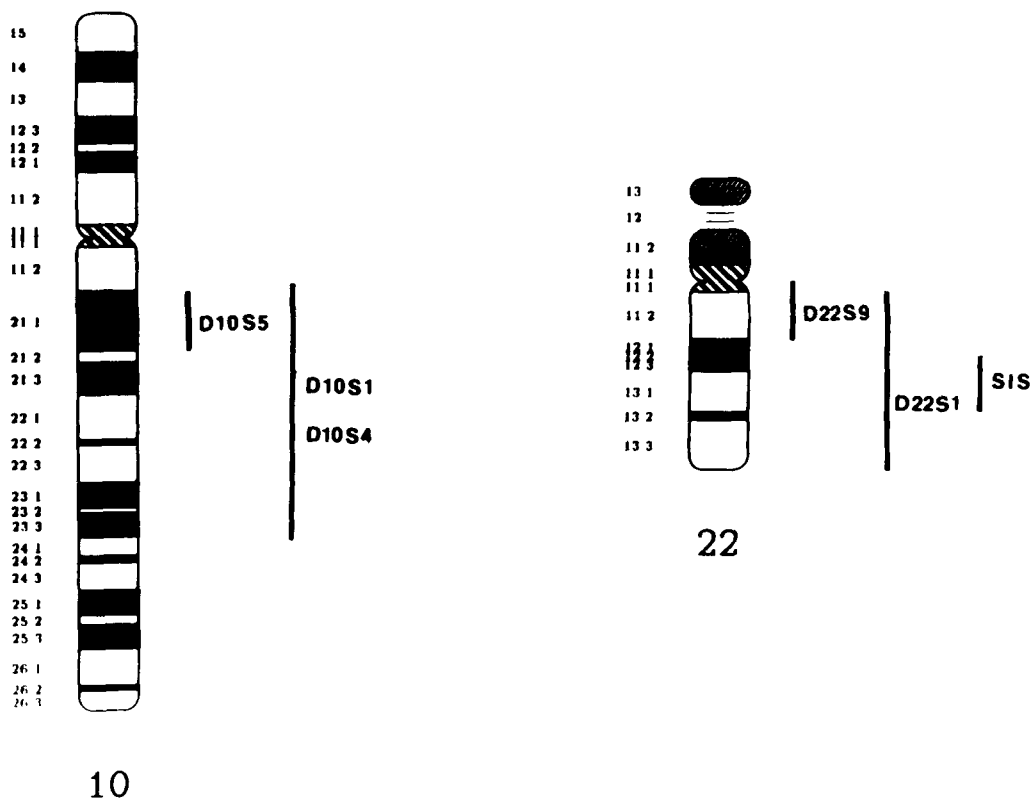


Figure 2 Chromosome location of some of the probes used.

RESULTS

Rearrangement Detected at Locus on Chromosome 22

The tumor and control DNA were compared with three RFLP probes on chromosome 22. D22S1 is an anonymous genomic DNA segment mapped to 22q11-22q13 that gives rise to two RFLP alleles with the *Bgl*III restriction enzyme. D22S9 is an anonymous RFLP probe mapped to 22q11. *c-sis* is a protooncogene (PDGF-B chain) that detects two RFLP alleles with a *Hind*III digestion (Fig. 2). The results are shown in Fig. 3 and summarized in Table 2. The control DNA was homozygous for D22S9 and had a 5.8-kilobase (kb) hybridization signal with *Taq*I digestion; the tumor DNA displayed an extra hybridization signal at 5.2 kb, suggesting that the tumor DNA had a possible small internal deletion or rearrangement of one allele in the region of chromosome 22q11. Analysis of control DNA with the D22S1 probe revealed homozygous hybridization signal at 9.5 kb. A marked decrease in allele signal intensity was observed in the tumor sample, indicating a possible hemizygous deletion of chromosome 22 sequences in the tumor cells. This was supported by rehybridizing the same DNA blot with *NGF-b* probe (mapped to chromosome 1); both tumor and constitutional DNA had hybridization signals that were of equal intensity. Densitometry analysis is shown in Table 1. No loss of heterozygosity was detected with the *c-sis* probe.

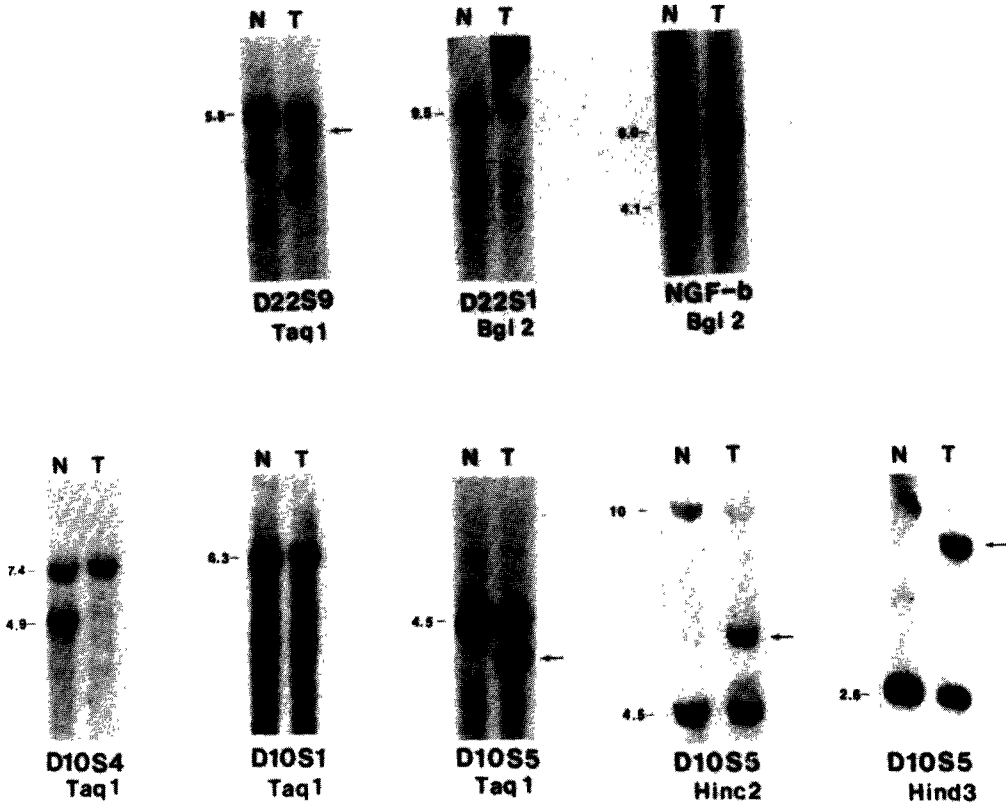


Figure 3 Allele loss and structural changes were detected with various chromosome 22 and 10 probes in a myxoid chondrosarcoma. Probes and restriction enzymes used are indicated beneath each normal (N)/tumor (T) pair. Numbers on left indicate sizes of RFLP alleles; arrows on right indicate aberrant bands.

Loss of Heterozygosity and Rearrangement at Loci on Chromosome 10

The tumor and control DNA were compared with three chromosome 10 RFLP probes: D10S4, D10S5, and D10S1. The chromosomal localization of these probes is shown in Fig. 2. Control DNA was heterozygous at D10S4 locus and yielded 7.4 kb and 4.9 kb bands, whereas tumor DNA retained only a 7.4-kb band. An aberrant band was observed only in the tumor DNA with D10S5 probe using three different enzymes

Table 1 Densitometric analysis of allelic signals

	D22S1	NGF-b	Ratio (D22S1/NGF)
Tumor	0.96	6.06	0.16
Normal	2.30	8.58	0.27

Comparison of the signal responses produced by D22S1 probe and NGF-b (located on chromosome 1) in both tumor DNA and control DNA.

The numbers in this table represent area of peak density of the hybridization signal measured by LKB ultrascan densitometer. The normalized tumor to normal DNA value at the D22S1 locus was close to 1/2 and suggests that the tumor may have a partial deletion at this region

Table 2 Allele loss and structural changes detected with various chromosome 22 and 10 probes in a myxoid chondrosarcoma

Chromosome	Probe	Enzyme	Normal	Tumor
22	D22S9	Taq I	H	A
22	D22S1	Bgl II	H	↓ signal
22	c-sis	Hind III	1,2	1,2
10	D10S4	Taq I	1,2	1
10	D10S5	Taq I	H	A
10	D10S5	Hind III	H	A
10	D10S5	Hinc II	H	A
9	Aldo-B	Pvu-2	H	H
9	abl-3	Taq I	H	H
?	pAS-1	Hind III	H	A
1	NGF-b	Bgl II	1,2	1,2
17	Hox-II	Sac I	H	H

Abbreviations 1, presence of the larger allele restriction fragment and loss of the smaller allele, 1,2, heterozygosity present, A, presence of aberrant restriction fragment other than RFLP alleles, H, homozygous for the probe tested

–TaqI, HindIII, and Hinc2 (Fig. 3). Because both tumor-DNA and control DNA were homozygous at D10S1 locus they were not informative for allele loss. These findings suggest that the tumor has rearrangement occurring at chromosome 10 q21.1 region and an allele loss at chromosome 10q21-10q23 region.

RFLP Analysis of Loci on Other Chromosomes

A breakpoint in the 9q31 and 22q12.2 regions was previously reported in two myxoid chondrosarcomas [1]. We studied tumor and control DNA with chromosome 9q RFLP probes including pAS-1 (cDNA for argininosuccinate synthetase enzyme; sequence showed that RFLP mapped to 9q11-q22) and Aldo B (mapped to 9q21.3 to q32). The tumor and the control DNA were homozygous for Aldo B probe as well as pAS-1 probe. Tumor DNA had an extra 2.4-kb hybridization signal with pAS-1 probe, however, displayed decreased signal intensity with a 2.1-kb band (Fig. 4). Multiple pseudogenes for human argininosuccinate synthetase are dispersed in multiple autosomes, and the X chromosome pAS-1 detects one argininosuccinate synthetase pseudogene mapped in the 9q34 region [5, 6]. Pseudogenes on chromosome 9q11-q22 give HindIII polymorphism 3.9 kb and 2.7 kb. The 2.1-kb band is derived from the chromosome 12 argininosuccinate synthetase pseudogene (mapped to 12p13-qter). Hence the extra 2.4-kb band may be derived from the chromosome 12. The abl oncogene probe (located at 9q34) did not show a difference between tumor and control DNA. Both tumor and control DNA were homozygous for HoxII (chromosome 17) probe and heterozygous for NGF-B probe (chromosome 1).

DISCUSSION

Hinrichs et al. [2] reported a case of myxoid chondrosarcoma of the right shoulder with a nonrandom reciprocal translocation t(9;22)(q22;q11). Turc-Carel et al. [3] subsequently reported another case of extraskeletal myxoid chondrosarcoma from the left distal thigh with a clonal translocation t(9;15;22) (q31;q25;q12.2) and suggested that breakpoints 9q31 and 22q12.2 were associated with extraskeletal myxoid chondrosarcoma. Our case had abnormalities involving the 22q11-q12 region, as demonstrated

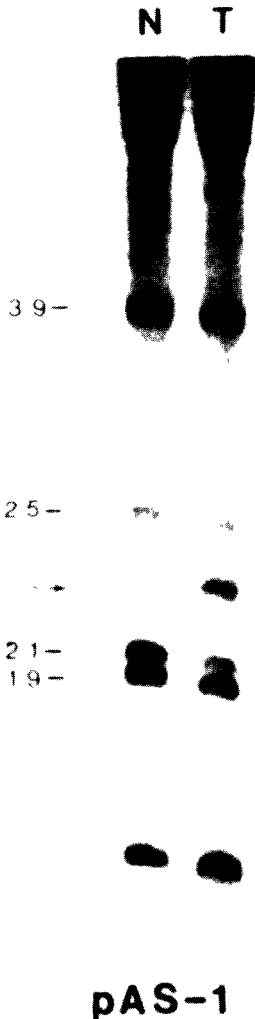


Figure 4 Comparison of hybridization patterns of HindIII-digested DNA from normal and tumor tissue with pAS-1 probe. Tumor DNA exhibited an extra 2.4-kb band. The pAS-1 probe recognized one expressed gene and 14 pseudogenes of argininosuccinate synthetase dispersed on 11 different chromosomes. The 3.9-kb and 1.4-kb bands were derived from argininosuccinate synthetase pseudogenes on chromosome 9; the 2.5-kb band was derived from chromosome 7, and the 1.9-kb band was derived from chromosome 5. The 2.1-kb band derived from chromosome 12 was significantly weaker in tumor DNA.

by RFLP analysis of the tumor and the constitutional DNA. Two different chromosome 10 probes have detected abnormalities in the tumor DNA, one showing allele loss in 10q21-q23 region and the other showing a possible internal deletion or rearrangement at 10q21.1 region. Three chromosome 9q probes were used to detect molecular evidence of rearrangement; no differences were observed with the aldolase B probe (mapped to 9q21-32) and abl probe (9q34). pAS-1 probe revealed an aberrant band, however, owing to dispersed chromosome location of multiple argininosuccinate synthetase pseudogene, this aberrant band may not be derived from the chromosome 9. In addition a decreased hybridization signal of chromosome 12 argininosuccinate synthetase pseudogene derived sequence was observed. Because the rearrangement may not be detected by standard Southern analysis, the negative data on chromosome 9 do not exclude the possible involvement of chromosome 9 in myxoid chondrosarcoma. No abnormality was detected with probes from chromosome 1 and 17.

Loss of heterozygosity or hemizygous deletion at a specific chromosome locus often indicates that chromosomal deletions have occurred; such deletions may also be accompanied by chromosomal rearrangement. In certain tumors, deletions in a particular gene region led to identification of a recessive cancer gene or tumor suppres-

tor gene [7]. The first characterized tumor suppressor gene-retinoblastoma gene (Rb) [8] is deleted or mutated not only in retinoblastomas but also in osteogenic sarcomas, small cell lung carcinomas, breast cancers, and bladder cancers [9–12]. DNA rearrangement can move a cellular protooncogene adjacent to another oncogene or into an active transcription unit and result in increased production or production of an altered gene product involved in cell growth. This has been best illustrated in chronic myelogenous leukemia (CML) and Burkitt's lymphoma [13, 14].

The present study demonstrates allele loss, rearrangement, or a small deletion involving chromosomes 10 and 22 occurring in a rare sporadic myxoid chondrosarcoma, a tumor of mesenchymal origin. Cytogenetic and molecular genetic abnormalities involving chromosome 22q11-q22 have been reported in various neuroectodermal tumors (eg, meningioma, Ewing's sarcoma, peripheral neuroepithelioma, acoustic neuroma, and astrocytoma [15–19]). Loss of chromosome 10 is also commonly found in high-grade gliomas and meningiomas [20, 21] implicating a possible common molecular mechanism involved in the evolution of these neoplasms. The marker D10S5 showing rearrangement in this tumor is linked to multiple endocrine neoplasia (MEN) 2A locus with a maximum lod score of 3.58 at a recombination frequency of 0.19 [22]. Patients with MEN 2 are predisposed to develop medullary thyroid cancer, pheochromocytoma, and parathyroid adenoma. These findings suggest the presence of genes closed to D10S5 locus and chromosome 22q11-q22 region involved in the tumorigenesis. To determine whether chromosome 10 and 22 abnormalities are specific for myxoid chondrosarcoma, more tumors need to be studied with similar probes. Detailed mapping of the chromosome 22q11-12 region and the chromosome 10q21.1 region may reveal genes involved in the formation of this rare tumor and possible other more common tumors.

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