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Chromosome 17 Abnormalities and TP53 Mutations in Adult Soft Tissue Sarcomas

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This study was designed to determine the frequency of structural genetic abnormalities of chromosome 17 and the incidence of TP53 mutations as they relate to the biological behavior of adult soft tissue sarcomas. We analyzed a group of 73 soft tissue sarcomas of adults that were clinically and pathologically well characterized using molecular genetic techniques and expression studies. We then correlated genotype and phenotype with pathological parameters. Overall, allelic loss of 17p and 17q was identified in 53 and 29% of informative cases, respectively. p53 nuclear overexpression was detected in 34% of the tumors analyzed. We observed an association between 17p deletions and tumor presentation being more frequent in recurrent and metastatic tumors than primary lesion. p53 nuclear overexpression was associated with tumor grade, size, and more frequently detected in metastatic than primary sarcomas. The 11 intragenic mutations characterized included 10 cases of single base substitution and one single base deletion; 8 were of the missense type and 3 were nonsense. It is concluded that 17p deletions and TP53 mutations are common events in adult soft tissue sarcomas and that due to the trends observed with the cohort of patients analyzed they may become prognostic markers for patients affected with these tumors. (Am J Pathol 1994, 145:345–355)

Genetic alterations of the TP53 gene, such as structural rearrangements, homozygous deletions, and point mutations, are frequent events in human cancer.[1–7] In addition to somatic mutations affecting TP53, certain inherited alterations of this gene have been identified in cancer patients. Germ line mutations of the TP53 gene have been characterized in members of families affected with the Li-Fraumeni syndrome, a rare autosomal dominant trait that predisposes these individuals to develop a variety of tumors, including soft tissue sarcomas (STS).[8,5] More recently, TP53 germ line mutations were also detected in cancer patients with no apparent family history of cancer,[10] as well as a subset of patients presenting with a second primary neoplasm.[11]

Grade and tumor size are the most important pathological factors predicting clinical outcome of STS.[12–14] Grade is assigned based on microscopic evaluation of differentiation, cellularity, amount of stroma, extent of necrosis, and mitotic count.[14] The histological type of sarcoma (eg, a leiomyosarcoma or fibrosarcoma) has not been shown to be a consistent independent prognostic factor.[13,15] Moreover, presentation of STS as undifferentiated tumors is not an infrequent event. Most of the tumor-associated markers related to differentiation characterized represent mid to late stages. Consequently, they are not expressed in poorly differentiated tumor cells, presumed to reflect early developmental phenotypes. Biochemical and molecular markers that correlate with the behavior of human sarcomas are being identified. Altered expression of the RB gene product (pRB) and deletions of the RB gene were found to be frequent events in sarcoma patients.[16,17] The characterization of tumor markers related to prognosis would be important adjuncts in stratifying patients affected with STS of similar histological types but distinct biological behavior.

The TP53 gene encodes a 53-kd nuclear phosphoprotein (p53), which appears to be involved in transcriptional control[18–20] and may act as a regulatory check point in the cell cycle, arresting cells in G1.[21–23] Altered patterns of p53 expression and TP53 muta-

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tions have been reported to occur in STS,[24–26] however, these studies lacked clinicopathological correlations and did not compare genotypic *versus* phenotypic alterations. We have studied a well characterized cohort of 73 STS adult patients with the objectives of determining 1) the frequency of allelic loss affecting chromosome 17, primarily 17p loss of heterozygosity (LOH); 2) the incidence of p53 nuclear overexpression as a reflection of detecting TP53 mutations; 3) the concordance between 17p LOH and mutations of the contralateral allele; and 4) the correlation between genotype and phenotype abnormalities and the pathological parameters of the tumors studied.

Materials and Methods

Source of Tissue

A cohort of 73 patients affected with STS were used for this study. The tumor lesions analyzed included 8 synovial sarcomas, 25 liposarcomas, 13 leiomyosarcomas, 2 rhabdomyosarcomas, 9 malignant fibrous histiocytomas (MFH), 7 fibrosarcomas, 6 peripheral nerve sheath tumors (PNST), and 3 undifferentiated sarcomas. Forty-eight patients presented with primary tumors, whereas 14 cases studied were recurrent lesions and 11 cases were metastatic STS.

Tumor specimens were obtained from the Department of Pathology embedded in cryopreservative solution (ornithine carbonoyltransferase compound; Miles Laboratories, Elkhart, IN) and snap-frozen in isopentane and stored at −70 C. Representative hematoxylin and eosin-stained sections of each block were examined microscopically to confirm the presence of tumor and to evaluate percentage of tumor cells comprising these lesions and extent of tumor necrosis. Adjacent tumor and normal tissue specimens were also collected for molecular genetic assays. These tissue samples were immediately frozen after surgical removal and stored at −70 C before DNA extraction.

Monoclonal Antibodies and Immunohistochemistry

Three mouse monoclonal antibodies detecting different epitopes on p53 proteins were used for this study. Anti-p53 antibody PAb1801 (Ab-2; Oncogene Science, Manhasset, NY) recognizes an epitope located between amino acids (aa) 32 to 79 of both wild-type and mutant human p53 proteins.[27] Antibody PAb240 (Ab-3; Oncogene Science) recognizes a conformational epitope located between aa 156 to 335 characteristic of certain mutant p53 products.[28] Antibody PAb1620 (Ab-5; Oncogene Science) reacts specifically with wild-type p53.[29] MIgS-Kp I, a mouse monoclonal antibody of the same subclass than the anti-p53 antibodies, was also used as a negative control at similar working concentrations.

The avidin-biotin peroxidase method was performed on 5-μ thick frozen tissue sections fixed with cold methanol acetone (1:1 dilution). Briefly, sections were incubated for 15 minutes with 10% normal horse serum (Organon Tecknika Corp., Westchester, PA), followed by a 2-hour incubation with appropriately diluted primary antibodies (Ab-2 was used at 200 ng/ml, Ab-3 at 250 ng/ml, and Ab-5 at 3 mg/ml). After extensive washing, sections were subsequently incubated for 30 minutes with biotinylated horse anti-mouse IgG antibodies at 1:200 dilution (Vector Laboratories, Burlingame, CA) and avidin-biotin peroxidase complexes (Vector Laboratories at 1:25 dilution for 30 minutes). Diaminobenzidine (0.06%) was used as the final chromogen and hematoxylin as the nuclear counterstain.

Immunohistochemical evaluation was done by two independent investigators (EL and CCC) scoring the estimated percentage of tumor cells that showed nuclear staining. p53 nuclear overexpression was classified into three categories defined as follows: negative (<20% tumor cells displaying nuclear staining), heterogeneous (20 to 70% tumors cells with nuclear reactivities), and homogeneous (>70% tumor cells with intense nuclear staining).

Restriction Fragment Length Polymorphism Analysis

Probes used in this study included PYNZ22 (17p13.3, D17S5, TaqI), php53B (17p 13.1, TP53, BglII), and PTHH59 (17q23-q25.3 DI7S4, TaqI) (American Type Culture Collection, Rockville, MD) and NM23-HI (17q21, NM23, BglII) (kindly provided by Dr. Patricia Steeg, Laboratory of Pathology, National Cancer Institute, Bethesda, MD). Southern analysis was performed as described.[6,7] Briefly, DNA was extracted by the nonorganic method developed by Oncor (Gaithersburg, MD) from paired normal and tumor samples, digested with the appropriate restriction enzymes, electrophoresed in 0.7% agarose gel, and blotted onto nylon membranes. The membranes were prehybridized with Hybrisol I (Oncor) at 42 C for 1 hour and hybridized with probes labeled to high specific activity with [^{32}P]dCTP overnight. Membranes were then washed and subjected to autoradiography

using intensifying screens at −70 C. LOH was defined as a more than 40% decrease in signal intensity of an allele in the tumor samples.[6,7,30] Densitometry using an Ultrascan XL Laser Densitometer (Pharmacia LKB, Biotechnology) was performed in selected cases to confirm the results. Cases were considered noninformative if all the probes for a particular arm were noninformative, nondeleted if they were nondeleted or one was nondeleted and the other noninformative, and deleted if at least one probe showed LOH.

Polymerase Chain Reaction (PCR)-Single Strand Conformation Polymorphism (PCR-SSCP) Analysis

These studies were performed according to a slight modification of the method reported by Orita et al.[31] Amplifications were performed using 100 ng of genomic DNA extracted from the samples described above. The primers used were obtained from intronic sequences flanking exons 2 through 9 of the human TP53 gene, sequences being previously published.[32] DNA was amplified after 30 cycles of PCR (30 seconds at 94 C, 30 seconds at the appropriate annealing temperature, and 60 seconds at 72 C) (annealing temperatures were: 60 C for exons 2 to 3, 68 C for exon 4, 63 C for exons 5 to 7, and 58 C for exons 8 and 9) using a Thermal Cycler (Perkin Elmer Cetus, Norwalk, CT). Amplified samples were then denatured and loaded onto a nondenaturing acrylamide gel containing 5% glycerol and run at room temperature for 12 to 16 hours at 10 to 12 watts. Gels were dried at 80 C under vacuum and exposed to X-ray film at −70 C for 4 to 16 hours.

DNA Sequencing

Amplification of genomic DNA was independent of the PCR amplification used for SSCP analysis (1 μg) using 35 cycles (60 seconds at 94 C, 60 seconds at the appropriate annealing temperature depending on the exon, see above, and 90 seconds at 72 C). DNA fragments were isolated from 2% low melting point agarose gels then purified and sequenced by the dideoxy method.[33] Both strands were sequenced for each DNA analyzed, and genomic DNA from control samples containing wild-type TP53 were sequenced in parallel to confirm the mutations.

Statistical Analysis

The association between 17p, 17q, or p53 nuclear overexpression and the pathological parameters studied were assessed by the Fisher's exact test.[34]

Results

Tables 1 to 3 summarize pertinent laboratory results and clinicopathological data. Figure 1 illustrates patterns of allelic deletions and immunohistochemical evaluation of p53 nuclear immunoreactivities. Figure 2 illustrates representative allelotypes and corresponding p53 immunohistochemistry profile in four cases of this study. Figure 3 displays the molecular characterization of TP53 mutations in a selected case.

Allelic Losses of Chromosome 17 and Rearrangements of the TP53 Gene

We examined 73 pairs of somatic and tumor DNA with four different probes for both arms of chromosome 17. Table 1 summarizes the association of allelic deletions with grade, size, and site of the tumors studied.

Deletions of the short arm of chromosome 17 were found in 27 of 51 (53%) informative cases examined. The 17p LOH was more frequently observed in high grade (21 of 37 cases, 57%) than in low grade (6 of 14 cases, 43%) tumors. Similarly, there was an association between 17p LOH and tumor presentation. Deletions of 17p were more commonly found in metastasis (6 of 8 cases, 75%) than in primary sarcomas (14 of 33 cases, 42%). Data did not reach statistical significance.

Four homozygous tumors for probe php53 were found to present rearrangement of the TP53 gene. The presence of additional fragments of abnormal

Table 1. *Association Between Deletions of Chromosome 17 and Pathological Parameters*

	17p	17q
Primary tumors		
Grade		
Low	2/8*	2/6
High	12/25	8/30
Size		
<5 cm	1/7	0/4
>5 cm	13/26	10/32
Site		
Superficial	4/8	4/14
Deep	10/25	6/21
Recurrent tumors		
Grade		
Low	4/6	1/6
High	3/4	4/7
Size		
<5 cm	1/1	0/2
>5 cm	6/9	5/11
Site		
Superficial	2/3	1/4
Deep	5/7	4/9
Metastatic tumors		
	6/8	2/9

* Number of cases showing deletions/total number of informative cases.

Table 2. *Association Between p53 Expression and Pathological Parameters*

	−*	+/−†	+‡	Total
Primary tumors				
Grade				
Low	7	1	0	8
High	26	9	5	40
Size				
<5 cm	7	0	0	7
>5 cm	26	10	5	41
Site				
Superficial	12	2	2	16
Deep	20	8	3	31
Recurrent tumors				
Grade				
Low	5	1	0	6
High	4	2	2	8
Size				
<5 cm	1	1	0	2
>5 cm	8	2	2	12
Site				
Superficial	3	2	0	5
Deep	6	1	2	9
Metastatic tumors	6	2	3	11

* −, <20% cells stained.
† +/−, 20 to 70%.
‡ +, >70% cells stained.

size was detected in these cases. Three of these tumors displayed the same extra band of approximately 14 kb (Figure 1, cases 9, 20, and 31) (data not shown).

LOH of the long arm of chromosome 17 was found in 17 of 58 (29%) informative cases analyzed. There was no significant correlation between 17q LOH and pathological parameters of poor clinical outcome. However, we were able to study a recurrent tumor (case 51) and a metastatic lesion (case 69) from the same patient. Although the recurrent STS had an intact 17q (nm23 locus), the metastasis showed 17q LOH.

p53 Nuclear Overexpression

Table 2 summarizes the association of p53 nuclear immunoreactivities with grade, size, and site of the tumors studied. To assess the pattern of expression of p53, we used three mouse monoclonal antibodies detecting different epitopes on p53 proteins. In this study, 25 of 73 (34%) sarcomas showed over 20% of the tumor cells with nuclear immunoreactivities for antibodies PAb1801 and/or PAb240. There was 85% concordance when comparing staining characteristics of these two antibodies. There were seven cases considered positive for PAb1801 and negative for PAb240 and two cases scored negative for PAb1801 that were considered PAb240 positive. In all samples analyzed both PAb1801 and PAb240 were unreactive with normal mesenchymal cells. No immunoreactivities were observed for the wild-type specific antibody PAb1620 in either normal or tumor cells.

Twenty-three of 59 (39%) high grade tumors showed intense nuclear staining, whereas only 2 of 14 (14%) low grade sarcomas were found to be positive. As for the relationship of p53 nuclear overexpression and tumor size, we observed that none of the seven primary sarcomas classified as small tumors (<5 cm) showed positive p53 nuclear staining, however, 15 of 41 primary large sarcomas displayed p53 nuclear overexpression. Moreover, the p53-positive phenotype was more frequently observed among metastatic lesions (5 of 11 cases, 45%) than in primary tumors (15 of 45 cases, 31%), although the difference was not significant.

Characterization of TP53 Mutations

To further characterize the specific intragenic mutations of TP53 because they may relate to p53 nuclear overexpression, we analyzed all 73 STS by PCR-SSCP (exons 2 through 9), followed by sequencing assays in all cases showing PCR-SSCP altered patterns. Shifts in mobility were observed in 13 cases (6 liposarcomas, 5 leiomyosarcomas, 1 PNST, and 1 MFH). Confirmation of the presence of a mutation was revealed in 11 of these 13 STS by sequencing analysis. In two cases we were not able to further detect the

Table 3. *TP53 Mutations in Soft Tissue Sarcomas*

Tumor	Exon	Codon	Mutation nucleotide	Change
4	4		Not found	
63	5	163	TAC (Tyr) → TGC (Cys)	AT → GC
72	5	163	TAC (Tyr) → TGC (Cys)	AT → GC
39	5	165	CAG (Gln) → TAG (stop)	GC → AT
24	5	168	CAC (His) → CGC (Arg)	AT → GC
37	5	Intron	ATgg → ATag	GC → AT
40	6	193	CAT (His) → TAT (Tyr)	GC → AT
48	6	214	CAT (His) → CGT (Arg)	AT → GC
54	7	246	ATG (Met) → GTG (Val)	AT → GC
3	7	248	CGG (Arg) → TGG (Trp)	GC → AT
30	7		Not found	
66	8	266	GGA (Gly) → GAA (Glu)	GC → AT
18	8	278	CCT (Pro) → CTG (Leu)	C deletion; stop at 344

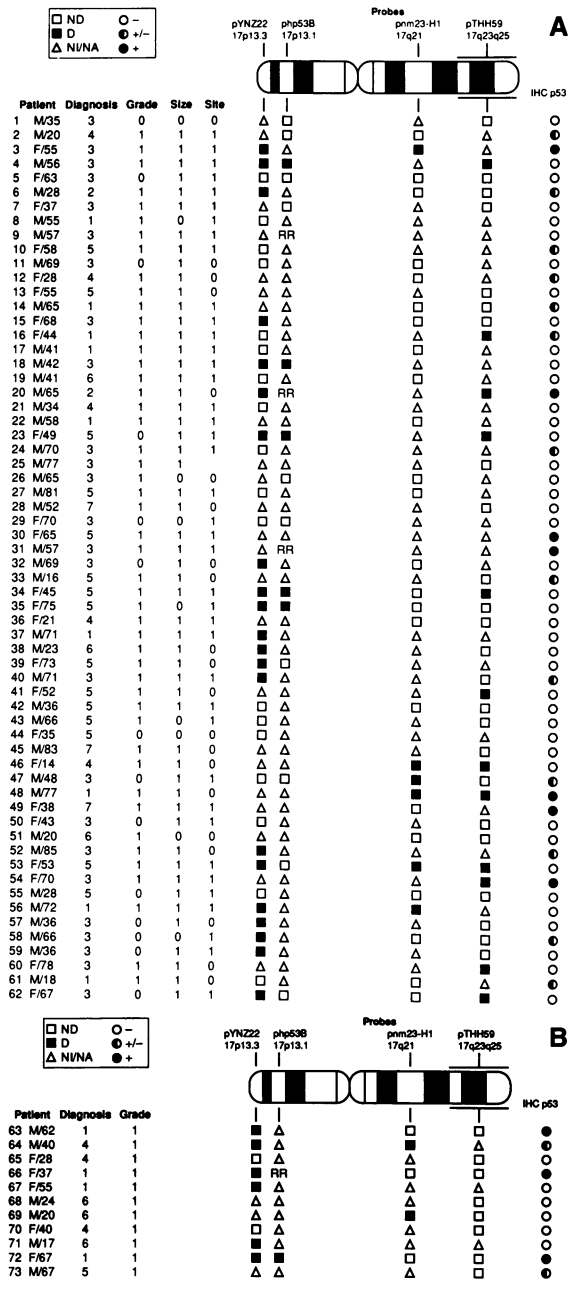


Figure 1. *Pathology, restriction fragment length polymorphism, and immunohistochemistry results. Data of primary (cases 1 to 48) and recurrent (cases 49 to 62) STS is summarized in* **A**, *whereas data for metastatic lesions (cases 63 to 73) is displayed in* **B**. *Demographic information includes sex (M, male; F, female) and age. Pathology diagnosis was codified as follows: 1, leiomyosarcoma; 2, rhabdomyosarcoma; 3, liposarcoma; 4, synovial, sarcoma; 5, fibrosarcoma and malignant fibrous histiocytoma; 6, PNST; 7, unclassified. Grade 0, low grade; grade 1, high grade; size 0, <5 cm; size 1, >5 cm; site 0, superficial lesion; site 1, deep lesions. ND, nondeleted; D, deleted; NI/NA, noninformative/nonavailable; RR, rearrangement. Identification of p53 nuclear overexpression by PAB1801 and/or PAb240 was scored as follows: −, <20% tumor cell nuclei positive; +/−, 20 to 70% tumor cell nuclei positive; +, >70% tumor cell nuclei positive.*

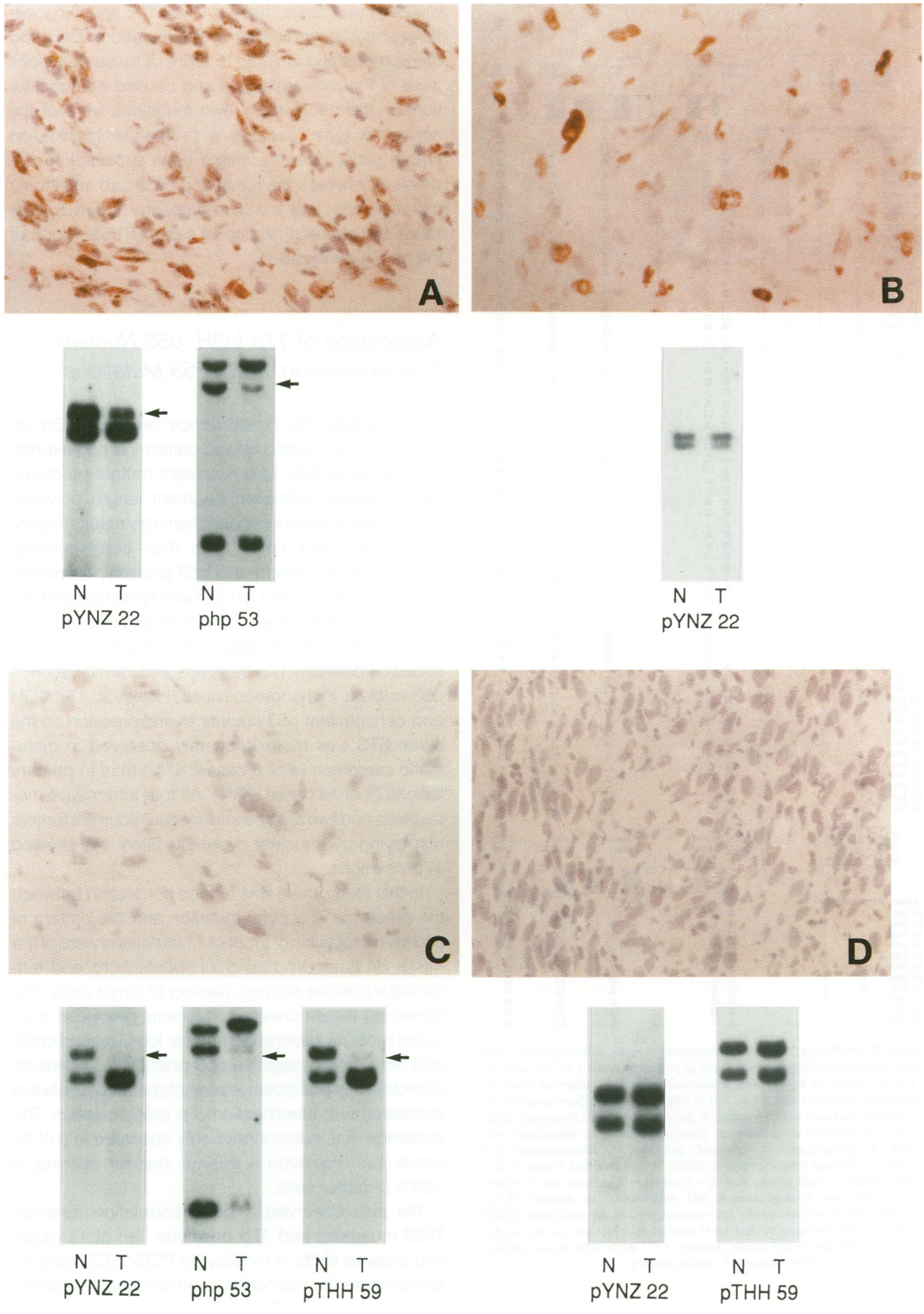
mutation due to shortage of DNA. The specific locations and characteristics of the mutations identified are summarized in Table 3. Eight of these mutations were of the missense type and caused aa substitutions in the p53 protein. Two mutations were of the nonsense type, causing a premature termination codon either due to a single base substitution or a deletion, whereas the remaining case had an intronic mutation affecting a splice junction. Figure 3 illustrates the characterization of mutant TP53 in one of the cases analyzed.

Association of 17p LOH, p53 Nuclear Overexpression, and TP53 Mutations

To investigate the concordance between LOH on chromosome 17p and altered patterns of p53 expression, because they may represent mutant products, we compared restriction fragment length polymorphism data and immunohistochemistry results. Figure 2 illustrates four cases with their corresponding Southern hybridization and p53 phenotype profiles. We noted that overall 17p LOH was more frequent (27 of 51 informative cases, 53%) than p53 nuclear overexpression (25 of 73 cases, 34%). There was no association between 17p deletions and identification of p53 nuclear immunoreactivities. However, 17p LOH and concomitant p53 nuclear overexpression on the same STS was more frequently observed in metastatic sarcomas (4 of 6 cases, 67%) than in primary lesions (4 of 14 cases, 28%). All four informative metastases and two of three informative recurrent tumors displaying p53 nuclear overexpression also showed 17p deletions.

In this study, there was a good correlation between the presence of a point mutation and the pattern of p53 immunostaining. Eight of 11 mutations were of the missense type, producing aa substitutions and rendering a positive nuclear staining of tumor cells. The remaining three nonsense mutations produced truncated proteins, lacking the nuclear localization signal, and rendering a negative p53 phenotype. However, detection of a p53-positive phenotype was not always correlated with identification of a point mutation. The presence of a mutation was only observed in 9 of 25 cases that displayed a positive nuclear staining in >20% of tumor cells.

We also observed a good correlation between TP53 mutations and 17p deletions. Ten of 13 cases that showed shifts in mobility by PCR-SSCP were informative for the restriction fragment length polymorphism analysis on 17p. Allelic losses of the short arm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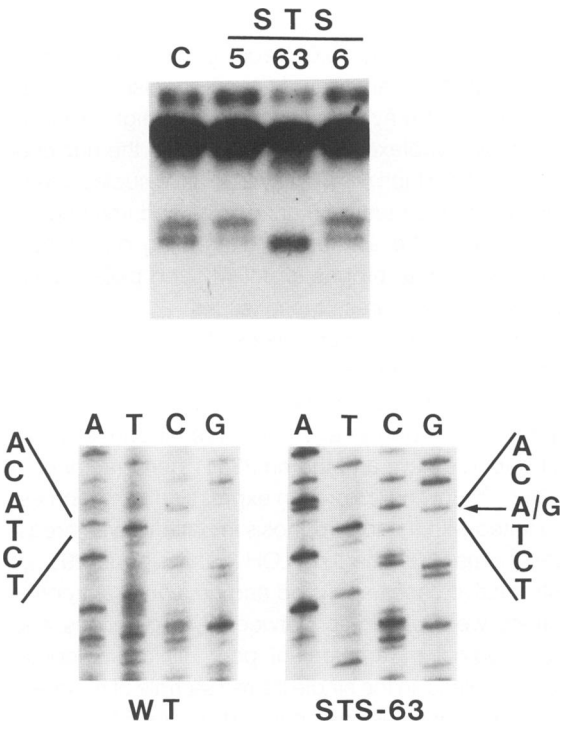

Figure 3. *Molecular characterization of TP53 mutations in adult STS. This figure illustrates the identification of intragenic mutations of TP53 by PCR-SSCP and sequencing. A shift in mobility was observed for case 63 in exon 5 using PCR-SSCP. Direct sequencing revealed a point mutation at codon 163 (tyrosine to cysteine) (arrow).*

of chromosome 17 were found in 9 of these 10 cases. In contrast, no apparent TP53 mutations were detected in an additional 18 cases that showed 17p deletions.

Discussion

To better assess the frequency and clinical relevance of detecting TP53 mutations in adult STS, we analyzed a well characterized cohort of 73 STS of adults. The experimental design implemented in this study has allowed us to perform a variety of molecular genetic and immunopathology techniques in all specimens, correlating morphology with phenotype and genotype.

This study demonstrates that deletions of the short arm of chromosome 17, mutations and rearrangements of the TP53 gene, and altered patterns of p53 expression are common events in adult STS. Tests of association between detection of these abnormalities and pathological parameters of poor clinical outcome (ie, tumor grade) did not reach significance probably due to the relatively small sample size of cases analyzed.

Allelic loss of chromosome 17p was observed in 53% of informative cases among the STS analyzed. Six of 14 low grade STS studied had 17p deletions. Identification of 17p LOH in low grade tumors has been also reported for astrocytoma[35] and ovarian carcinoma,[36] suggesting that the loss of gene(s) on the short arm of chromosome 17 may be an early event in certain human cancers. Contrary to this data, we have recently reported the association of 17p LOH with high grade/high stage bladder tumors. Superficial (Ta) lesions had an apparent intact 17p, whereas 55% of T1 and T2 to T4 tumors showed 17p LOH.[7] These findings suggest that different sequences of genetic abnormalities may occur in early phases of tumor progression in distinct human neoplasias. Even though identified in a subset of low grade STS, in this study 17p LOH was more frequently observed in recurrent and metastatic tumors than in primary lesions.

We used two probes to assess 17p deletions: php53 and pYNZ22 mapping, respectively, to 17p13.1 and 13.3. We noted that 3 of 12 specimens (cases 39, 53, and 62) in which both loci were informative had 17p13.3 LOH with an apparently intact 17p13.1. Coles et al[37] suggested the presence of another candidate tumor suppressor gene on chromosome 17p, telomeric to the TP53 gene, that may be involved in breast carcinogenesis. They reported two regions of LOH in bands 17p13.1 and 17p13.3, with a higher frequency of deletions for 17p13.3. Similarly, Saxena et al[38] reported allelic loss of genes distal to 17p13.3 in astrocytoma. These observations suggest the presence of a second tumor suppressor gene in the telomeric region of 17p that may play a role, either alone or in conjunction with TP53, in the initiation or tumor progression of certain human cancers, including STS.

The identification of p53 nuclear overexpression correlated with tumor grade and large tumor size but not with tumor site in this study. These associations did not reach statistical significance probably due to the small subgroup of low grade lesions studied. However, when a large cohort of patients was analyzed, there was a statistically significant association between pathological parameters of poor clinical outcome and p53 nuclear overexpression.[39] We found

Figure 2. *Representative immunohistochemistry for mutated p53 proteins and Southern blots.* A: *Patient 72, metastatic leiomyosarcoma showing >70% tumor cells with positive nuclear staining and concomitant deletions of 17p (arrows).* B: *Patient 10, primary MFH showing heterogeneous immunostaining of tumor cells but no deletion of 17p.* C: *Patient 23, primary MFH with undetectable levels of p53 but showing 17p and 17q deletions (arrows).* D: *Patient 70, metastatic synovial sarcoma with undetectable p53 and no deletions (original magnifications:* A *and* D ×100; B *and* C ×200).

excellent concordance between antibodies PAb1801 and PAb240 when comparing staining profiles. Immunoreactivities were confined to tumor cells with a nuclear pattern of staining. None of the cases showed nuclear staining for PAb1620, a wild-type specific anti-p53 antibody, on either normal or tumor cells.

Overall, 25 of 73 (34%) cases of STS analyzed displayed p53 nuclear overexpression. Several studies have shown that mutant p53 products have a prolonged half-life,[40–42] whereas wild-type p53 protein undergo rapid degradation.[43] The retarded degradation of abnormal p53 products yields accumulation of inactive complexes and self-aggregatory mutated molecules in the nuclei of tumor cells.[44,45] It is for these reasons that immunohistochemically based assays can be used for their detection, rendering a positive nuclear signal.[46–51] However, absence of nuclear immunostaining with anti-p53 antibodies does not rule out the presence of certain intragenic TP53 mutations that may produce truncated proteins lacking the nuclear localization signal[52] or the oligomerization domain.[53] This may be the explanation for the three p53-negative cases that had nonsense mutations in this study, as presented above. However, nonsense mutations of the TP53 gene are reported to be uncommon events in human cancer.[20,54] In contrast, the overwhelming majority of somatic intragenic TP53 mutations reported to date are of the missense type.[55–57] Moreover, most of these mutations occur in four defined hot-spot regions of TP53.[55,58] The eight confirmed mutations reported in this study belong to this category, some of them previously described by other groups.[57–60]

In addition, we found that almost all tumors with a TP53 mutation concomitantly showed 17p deletions. However, 18 cases had 17p LOH but no mutations on the TP53 gene were identified. Similar to our findings, Toguchida et al[26] have recently reported the same frequency of TP53 mutations and 17p LOH among sarcomas, however, the majority of gross alterations and point mutations reported were found in osteosarcomas and the investigators did not correlate TP53 alterations with clinicopathological parameters.

Data summarized above suggests that the identification of p53 nuclear overexpression by immunohistochemistry does not always reflect the detection of a TP53 mutation in STS. We also discussed the possible, even though unlikely, false negative results that may be obtained using these assays. False positive results may also occur under certain circumstances. Amplification of the MDM2 gene, located on the long arm of chromosome 12, has been reported in human sarcomas.[61] MDM2 amplification correlated with overexpression of its encoded protein, a nuclear phosphoprotein M_r 90,000 (p90), that binds to p53 and constitutes an alternative mechanism of inactivation.[61,62] It may be postulated that high levels of p90/p53 complexes can accumulate in the nuclei of tumor cells, which will display a positive nuclear staining when using anti-p53 antibodies and immunohistochemistry. We are further expanding our studies using molecular probes for MDM2 and p90-specific antibodies to address these issues.

Three putative candidates tumor suppressor genes are located on the long arm of chromosome 17. The neurofibromatosis gene located in 17q11.2,[63] a gene implicated in early onset breast cancer mapping to 17q21,[64] and the nm23-H1 gene residing in 17q21.[65] Low nm23 protein expression has been associated with a poor prognosis in patients with breast carcinoma.[66,67] The 17q LOH was found in 29% of informative cases analyzed and no significant correlations were observed between 17q deletions and pathological parameters of poor clinical outcome. Nevertheless, in the single paired sample of recurrent and metastatic lesions analyzed (cases 51 and 69), we found a deletion of the nm23 gene in the metastatic tumor. The lack of association between nm23-H1 deletion and pathology data does not exclude a role for this gene in tumor progression, because the techniques used in this study do not address posttranscriptional or posttranslational alterations.

Differences of results from previous studies dealing with this subject may be due to the fact that STS and osteosarcomas were grouped and analyzed together. Moreover, most reports have been conducted mixing pediatric and adult sarcomas, lacking correlations between clinicopathological variables and laboratory data. There are significant differences regarding natural history of STS *versus* osteosarcomas, as well as underlying tumorigenic and tumor progression events involved in pediatric *versus* adult cancers. Another important concern is that different methodologies have been used on a variety of clinical samples, which may also account for discrepancies between reported studies. In this study we have attempted to address these critical issues, centering in the analysis of adult STS and performing a variety of techniques on the same tissue sample from all cases studied. Data reported here indicate that 17p deletions and TP53 mutations are frequent events occurring in adult STS. Moreover, it appears that these alterations might be associated with pathological parameters of poor clinical outcome, such as tumor grade and size, as well as with the development of metastatic lesions.

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