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Short Communication

Molecular Aberrations of the G1-S Checkpoint in Myxoid and Round Cell Liposarcoma

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Myxoid and round cell liposarcoma represents a morphological spectrum in which tumor progression from low-grade myxoid to high-grade round cell areas is frequently observed. A distinctive t(12;16)(q13;p11) reciprocal translocation rearranges the CHOP gene localized to 12q13 in most cases. Data concerning the occurrence of cell cycle aberrations in this subset of mesenchymal malignancies are very limited. Therefore, we analyzed a histologically homogeneous series of 21 cases of myxoid and round cell liposarcoma. The p53 pathway was studied by investigating the TP53 gene and protein, mdm2 protein, and $p21^{Waf1}$ protein. The Rb-cyclin D pathway was analyzed by studying the pRb protein, the $p16^{MTS1}$ gene, cyclin D1, cyclin D3, $p27^{Kip1}$, cdk4, and cdk6 proteins. In contrast with the rare involvement of the TP53 gene in well differentiated liposarcoma, aberrations of the TP53 gene were observed in approximately 30% of cases of myxoid and round cell liposarcoma. Notably, mdm2 overexpression was seen in 56% of cases and correlated with histological grade, therefore indicating a possible role in tumor progression. Abnormalities involving the Rb-cyclin D pathway were observed in more than 90% of cases. pRb loss was present in one-third of cases and, at variance with that observed in other subsets of sarcoma, overexpression of cyclin Ds represented a rare event. Interestingly, upregulation of either cdk4 or cdk6 was demonstrated in 85% of cases. *(Am J Pathol 1997, 151:1531–1539)*

Liposarcomas represent one of the commonest subsets of sarcoma of adult life. They are currently classified into five main subgroups: well differentiated, myxoid, round cell, pleomorphic, and dedifferentiated.[1] The well differentiated group is further subclassified into adipocytic, inflammatory, and sclerosing types, and recently a spindle cell variant of well differentiated liposarcoma has been described[2].

Myxoid and round cell liposarcoma, even if still sometimes classified as two distinct subtypes, represents a morphological spectrum in which tumor progression from low-grade myxoid to high-grade round cell or hypercellular areas is relatively frequent. Clinically, pure myxoid lesions are characterized by a 5-year survival rate of approximately 70%, which in purely round cell tumors drops to approximately 20%.[3] Importantly, the presence of a round cell component ranging between 5 and 25% seems to be associated with a significant worsening of prognosis.[4,5] Myxoid and round cell liposarcoma share the same characteristic chromosome change, t(12;16)(q13;p11), which, at the molecular level, fuses the CHOP gene on 12q13, which encodes for a transcription factor involved in growth arrest and adipocyte differentiation, to the FUS (or TLS) gene on 16p11, which encodes for a RNA-binding protein.[6] The resulting chimeric product fails to induce exit from the cell cycle. These distinctive chromosomal changes probably represent early etiological molecular events, but additional targets for molecular abnormalities exist. It is accepted that oncogenic processes exert their greatest effect by targeting genes (and their proteins) that regulate the G1-S cell cycle checkpoint.[7,8] Several studies have analyzed heterogeneous series of soft tissue sarcomas,[9–16] and most recently, data concerning aberrations of the G1-S checkpoint in specific subsets of sarcomas have started to be collected that indicate the existence of a certain degree of tumor specificity.[17–20]

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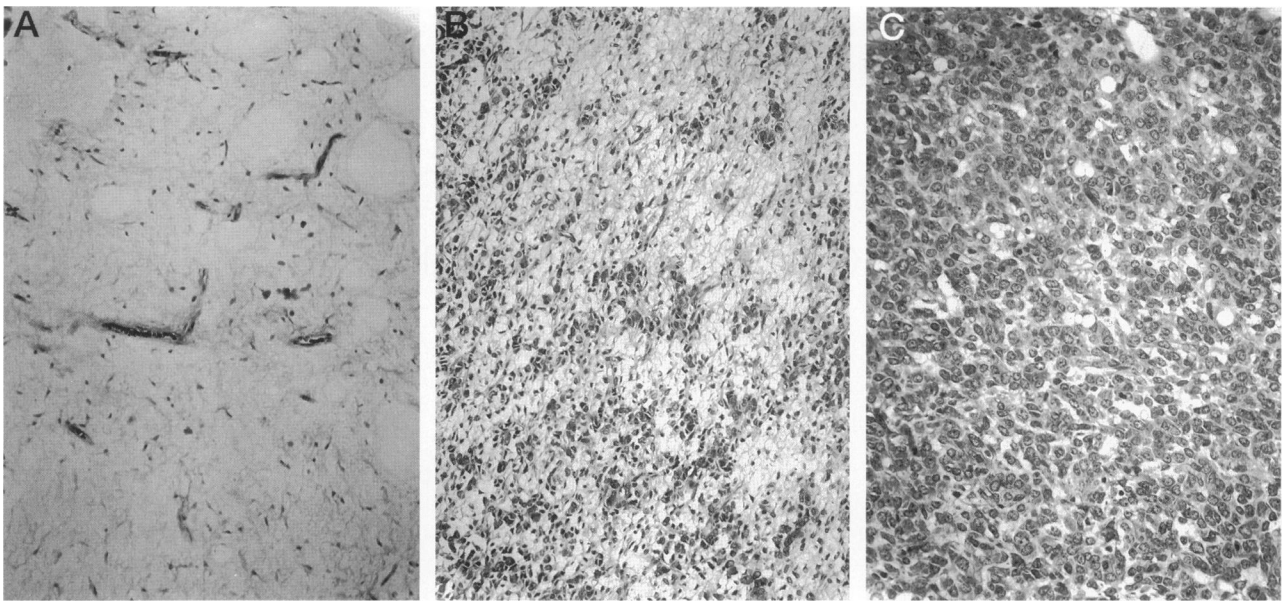

Figure 1. The morphological spectrum of myxoid and round cell liposarcoma. **A:** A neoplastic spindle cell proliferation set in a myxoid background is observed at the low-grade end of the spectrum. A rich, plexiform vascular network is appreciable. **B:** Round cell, hypercellular areas admixed with hypocellular ones characterize the combined myxoid and round cell liposarcoma. **C:** A solid, undifferentiated round cell population is observed at the high-grade end of the spectrum. H&E; original magnification, ×200.

Here we analyze a histologically homogeneous series of myxoid and round cell liposarcoma by investigating both the p53 and the Rb-cyclin D pathways in both low- and high-grade components to verify whether aberrations at the G1-S checkpoint may play a role in the molecular oncogenesis as well as in tumor progression of this subset of liposarcoma.

Materials and Methods

Twenty-one cases of myxoid and round cell liposarcoma (7 pure myxoid (low grade), 9 combined myxoid and round cell, and 5 round cell (high grade)) classified according to well established histological criteria[3] (Figure 1, A–C) and with available tissue blocks were retrieved from the files of one of the authors (C.D.M. Fletcher). More than 5% round cell component was considered sufficient for inclusion in the mixed myxoid/round cell category, whereas cases with more than 80% round cell or hypercellular areas were classified as pure round cell. In 10 cases included in this series, the presence of the CHOP-TLS fusion product had been previously demonstrated by the reverse transcriptase polymerase chain reaction (PCR) technique.[6]

Immunohistochemistry

The following panel of monoclonal and polyclonal antibodies were used: anti-p53 antibody DO7 (dilution 1/100; Dako, Glostrup Denmark), anti-p53 antibody BP53-12-1 (dilution 1/100; Biogenex, San Ramon, CA), anti-p53 antibody DO1 (dilution 1/100; Neomarkers, Freemont, CA), anti-$p21^{waf1}$ antibody EA10 (dilution 1/100; Oncogene Science, Manhasset, NY), anti-mdm2 antibody IF2 (dilution 1/100; Oncogene Science), anti-mdm2 antibody SMP14 (dilution 1/100; Neomarkers), anti-$p27^{Kip1}$ clone 57 (1/1000; Transduction, Lexington, KY), anti-pRb antibody 245 (dilution 1/200; PharMingen, San Diego, CA), anti-cyclin D1 antibody DCS6 (dilution 1/100; Neomarkers), anti-cyclin D1 antiserum SC753 (polyclonal, dilution 1/2000; Santa Cruz Biotechnology, Santa Cruz, CA), anti-cyclin D3 antibody DCS22 (dilution 1/100; Neomarkers), anti-cyclin D3 antiserum SC182 (polyclonal, dilution 1/1000; Santa Cruz), anti-cdk4 antiserum SC260 (polyclonal, 1/5000; Santa Cruz), and anti-cdk6 antiserum SC177 (polyclonal, dilution 1/1000; Santa Cruz). Immunostaining was performed by a sensitive peroxidase-streptavidin method on formalin-fixed paraffin-embedded material. Sections were pretreated with a heat-induced epitope retrieval method. Negative controls were obtained by incubating the slides with antibodies of the same isotype and of unrelated specificity. Absorption tests for polyclonal antisera (SC753, SC182, SC260, and SC177) were performed using the corresponding peptides. p53- and mdm2-positive controls were represented by sections of TP53 mutated laryngeal cancer[21] and of MDM2-amplified breast cancer,[22] respectively. $p21^{Waf1}$-positive control was represented by sections of normal colonic mucosa.[23] The percentage of immunoreactive cells was evaluated semiquantitatively by counting at least 1000 neoplastic cells in the most representative well differentiated and high-grade areas.

DNA Extraction

Ten-micron-thick paraffin-embedded tissue sections were deparaffinized by serial washes in xylene followed by an ethanol rinse and drying. Genomic DNA was purified from the tissue pellets by digestion with proteinase K and RNAse and extracted with phenol chloroform as

described previously.[24] Molecular analysis of the TP53 gene was performed on the region of the gene encompassing exons 4 to 9.

Single-Strand Conformation Polymorphism (SSCP) Analysis

p53 and $p16^{INK4a/MTS1}$ mutation analysis was performed by SSCP using the primers listed below. Briefly, 10-μl PCRs were performed using 50 ng of genomic DNA, 5 pmol of each primer, 0.2 U of *Taq* DNA polymerase (Boehringer Biochemia Robin, Mannheim, Germany), 1 μCi of [^{32}P]dCTP (3000 Ci/mmol; Amersham, Alyesbury, UK), and 2.5 μmol/L dNTP in a standard buffer (10 mmol/L Tris/HCl, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, and 0.01% gelatin). Dimethylsulfoxide (5%) and formamide (2 to 5%) were added to the PCR of p16 exon 1 and 2, respectively. The PCR consisted of 30 cycles of denaturation for 1 minute at 94°C; annealing for 1 minute at 55°C (for p53 gene exons 5 to 9 and p16 gene exon 2), at 50°C (for TP53 gene exon 4), and at 62°C (for p16 gene exon 1); and strand elongation at 72°C for 1 minute. After amplification, 1/20 of the reaction was mixed 1:1 with 95% formamide, 20 mmol/L EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol, heat denatured, and then loaded on 20 × 40 × 0.04 cm nondenaturing mutation detection enhancement gel (AT Biochem, Malvern, PA) in 0.6X Tris-buffered ethanolamine. Two sets of conditions were performed: 10 V/cm for 15 hours at room temperature and 20 V/cm for 5 hours at room temperature with fan cooling. After electrophoresis, gels were vacuum dried and autoradiographed.

Sequencing

Samples showing SSCP band shifts were selected for DNA sequencing. Briefly, a pool of three standard 100-μl PCRs, performed as described above using 0.5 μg of genomic DNA, 50 pmol of each primer, 0.25 mmol/L dNTP, and 2 U of *Taq* DNA polymerase, were purified by agarose gel electrophoresis and recovered by the Quiex gel extraction kit (Quiagen, Chatsworth, CA). Purified DNA (100 ng) was sequenced using a Sequenase kit (USB, Cleveland, OH) as detailed previously.[21] Both DNA strands were analyzed.

All of the oligos used for PCR and sequencing were synthesized using an Applied Biosystem synthesizer (Foster City, CA). The oligonucleotides used to amplify the sequences of TP53 exons 4 to 9, chosen in the intronic portion of the gene, were as follows (the primers are given 5′ to 3′): exon 4 sense, CTGGTCCTCTGACTGCTCTT, and anti, ATTGAAGTCTCATGGAAGCC; exon 5 sense, TTATCTCTTCACTTGTGCC, and anti, CCGCCCTGTCGTCTCTCCA; exon 6 sense, CCCCAGGCCTCTGATTCCTC, and anti, ACTGACAACCACCCTTAAC; exon 7 sense, TCTCCCCAAGGCGCACTGG, and anti, CAGGCCAGTGTGCAGGGTG; exon 8 sense, ACTGCCTCTTGCTTCTCTT, and anti, GGCATAACTGCACCCTTGG; and exon 9 sense, TTTATCACCTTTCCTTGCCTC, and anti, CTTTCCACTTGATAAGAGGT.

The oligonucleotides used to amplify exons 1 and 2 of the p16 gene were as follows: exon 1 sense, TCTGCGGAGAGAGGGGGAGAGCAGGC, and anti, GATCGGCCTCCGACCGTAAC; exon 2a sense, GGCTCTACACAAGCTTCCTT, and anti, GCCCTCCCGGGCAGCGTCG; exon 2b sense, CCGCCACTCTCACCGGACCC, and anti, GCAGGTACCGTGCGACATC; and exon 2c sense, CCGTGGACCTGCCTGAGGA, and anti, AGCTTTGGAAGCTCTAG.

Statistical Analysis

Statistical analysis was performed using the Epi Info (USD, Stone Mountain, GA) run on an IBM personal computer (33 P100). Association with variables was tested using the Fisher's exact test.

Results

Molecular Analysis

TP53 molecular analysis showed mutations in 6 of 21 cases (28.5%). Specifically, mutations occurred in 3 of 7 myxoid, 1 of 9 myxoid and round cell, and 2 of 5 round cell liposarcomas. Four C→T transitions were observed in cases 1, 3, 13 (round cell component), and 19. Three G→A transitions were detected in cases 2, 13 (myxoid component), and 18 (Figure 2). Case 13 presented two distinct mutations in the two components. The point mutation observed in case 9 represented a polymorphism.

$p16^{MTS1}$ molecular analysis revealed one C→T transition and one C→A transversion in cases 1 and 7, respectively. Case 5 showed a G→A mutation at codon 148 described as a polymorphism. The results of the molecular analysis are summarized in Table 1.

Immunohistochemistry

p53-positive immunoreactivity was observed in all cases with a percentage of positive cells ranging between 5 and 80% (Figure 3A). The percentage of immunoreactive cells was 20% higher with the DO7 antibody than with the other antibodies tested. DO1 and BP53 immunostained identical percentages of neoplastic cells. The pattern of p53 expression was unrelated to the TP53 gene status (Figure 3B).

$p21^{Waf1}$ expression was present in 12 of 21 cases with a percentage of positive cells ranging between 5 and 50%. Absence of $p21^{Waf1}$ immunoreactivity was observed in 3 of 6 p53 mutated cases (Figure 3C). In case 13, $p21^{Waf1}$ was abolished only in the myxoid component, which at the molecular level showed a homozygous mutation of the p53 gene distinct from the heterozygous mutation present in the round cell component.

mdm2 immunoreactivity was observed in 11 of 21 cases (56%) (Figure 3D). mdm2 overexpression was present in 5 of 16 (31%) myxoid lesions (pure myxoid and the myxoid component of combined tumors) with a percentage of cells ranging between 5 and 20% and in 8 of 14 (57%) round cell lesions (round cell component of

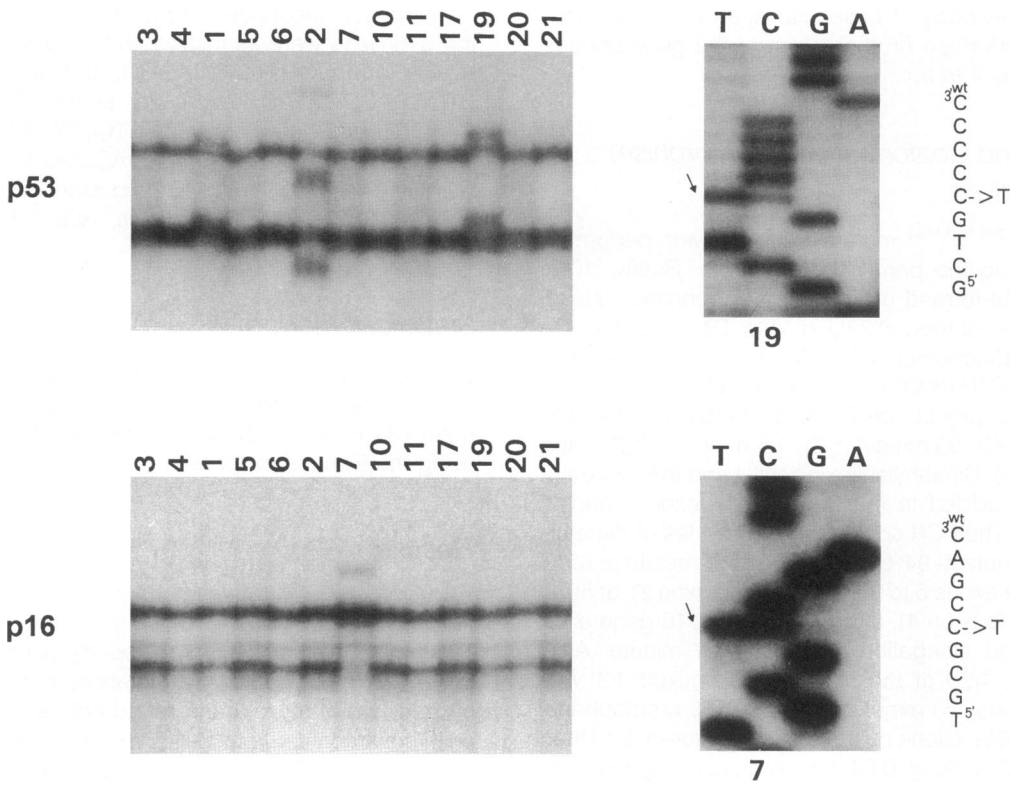

Figure 2. Molecular analysis of the TP53 and p16 genes. **Top left:** SSCP analysis of the TP53 gene (exon 8). Tumor identification numbers are given on the top of each lane. Cases 1, 2, and 19 show an an abnormal migration pattern. **Top right:** Sequence analysis of case 19, revealing the presence of a C to T homozygous transition at codon 300. The presence of a faint normal signal (C band) is derived from a slight contamination of the sample with normal cells. **Bottom left:** SSCP analysis of the p16 gene (exon 2, codons 51 to 89). **Bottom right:** Case 7 shows a band shift due a C to T heterozygous transition at codon 144.

combined lesions and pure round cell tumors) with a percentage of immunoreactive cells ranging between 20 and 40%. A statistically significant association was seen between high-grade morphology and high expression of mdm2 ($P = 0.006$).

Conserved pRb immunoreactivity was observed in 14 of 21 cases (66%; Figure 3E). Endothelial cells represented the built-in positive control in all of the pRb-negative cases.

Cyclin D1 immunopositivity was present in 2 of 21 cases (1 myxoid and 1 round cell) with a percentage of positive cells ranging between 20 and 30% (Figure 3F). Cyclin D3 expression was observed in 3 of 21 cases (1 combined and 2 round cell) with a percentage of positive cells ranging between 5 and 30%. Case 21 co-expressed both cyclin D1 and cyclin D3.

$p27^{Kip1}$-positive immunoreactivity was observed in all of the cases in a percentage of cells ranging from 70 to 90%.

cdk4 expression was observed in 12 of 21 cases (57%; Figure 3G). cdk6 expression was detected in 7 of 21 cases (33%; Figure 3H). Immunoreactive cells ranged between 60 and 80%. In all but two cases the expression of cdks was mutually exclusive. Globally, either cdk4 or cdk6 expression was present in 84% of cases.

Table 1. Molecular Analysis of Myxoid and Round Cell Liposarcoma

Case	Histology	p53 gene exons 4 to 9	p16 (MTS1) gene exons 1 and 2
LPS1	Myxoid	Codon 301, cCa→cTa, Pro→Leu, heterozygous	Codon 144, Cgc→Tgc, Arg→Cys, homozygous
LPS2	Myxoid	Codon 307, Gca→Aca, Prol→Leu, heterozygous	
LPS3	Myxoid	Codon 166, tCa→tTa, Ser→Leu, heterozygous	
LPS5	Myxoid		Codon 148, Gcg→Acg, Ala→Thr polymorphism
LPS7	Myxoid		Codon 73, gCc→gAc, Ala→Val, heterozygous
LPS9A	Myxoid	Codon 213, cgA→cgG, Arg→Arg, homozygous polymorphism	
LPS9B	Round cell	Codon 213, cgA→cgG, Arg→Arg, homozygous polymorphism	
LPS13A	Myxoid	Codon 179, cAt→cGt, His→Arg, homozygous	
LPS13B	Round cell	Codon 170 aCg→aTg, The→Met, heterozygous	
LPS18	Round cell	Codon 248, cGc→cAg, Arg→Gln, homozygous	
LPS19	Round cell	Codon 300, Ccc→Tcc, Pro→Ser, homozygous	

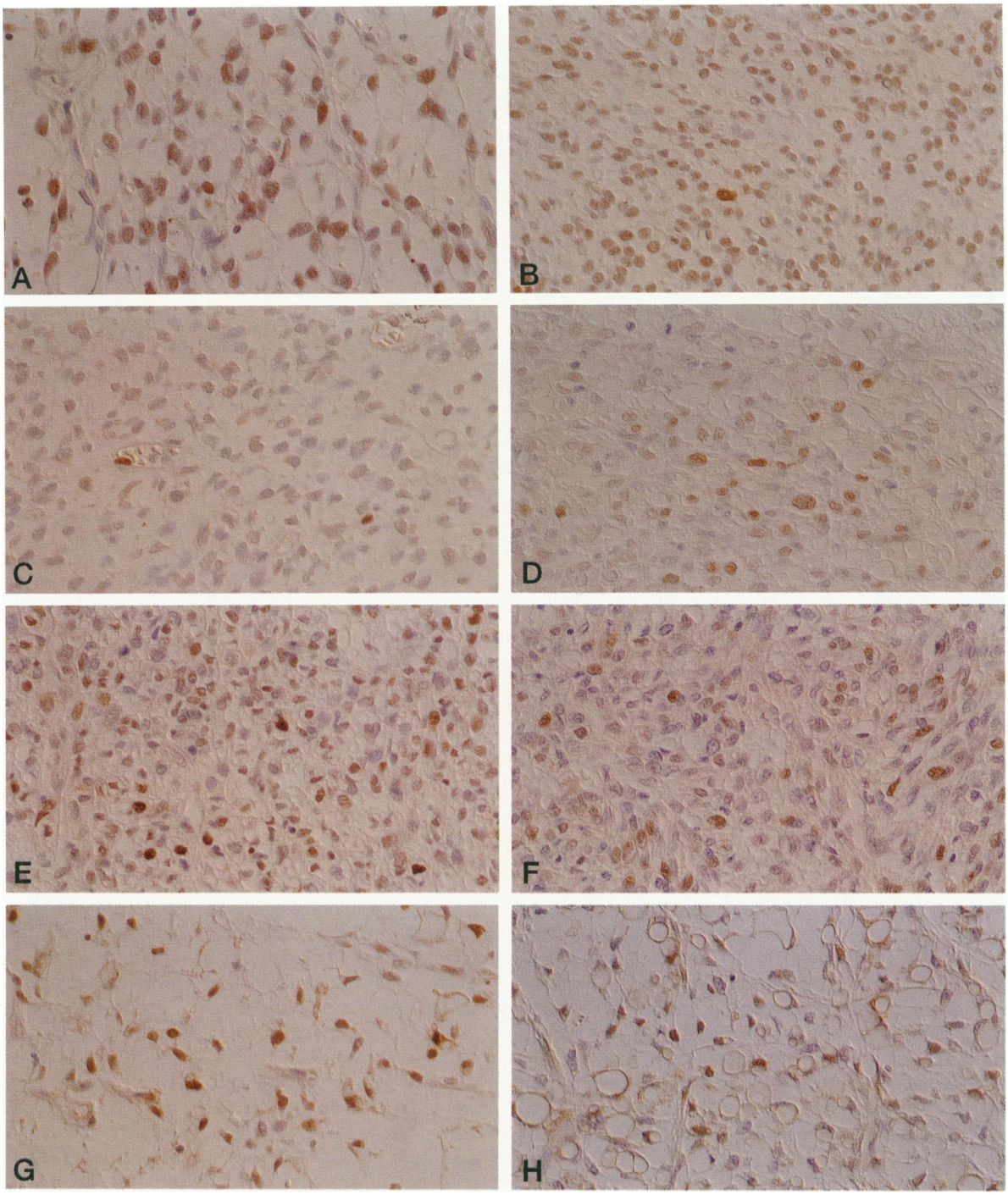

Figure 3. Immunohistochemical detection of G1-S proteins in myxoid liposarcoma. **A:** Case 13. Anti-p53 monoclonal antibody (DO7) decorates a high percentage of neoplastic cells. The TP53 gene was mutated. **B:** Case 12. High percentages of p53 immunoreactive cells were detected also in TP53 nonmutated cases. **C:** Case 12. Negative $p21^{Waf1}$ immunoreactivity is shown. **D:** Case 18. mdm2 overexpression in neoplastic cells is shown. **E:** Case 21. Conserved pRb immunoreactivity in most neoplastic cells is shown. **F:** Case 21. Cyclin D1 immunopositivity was present in two cases and was always associated to pRb conserved immunoreactivity. **G:** Case 7. cdk4 immunopositivity in most neoplastic cells is shown. **H:** Case 4. cdk6-positive immunoreactivity is present in a high percentage of neoplastic cells. Immunoperoxidase; counterstained with Mayer's hematoxylin; original magnification, ×200.

As expected, a nuclear pattern of immunoreactivity, was observed with all of the antibodies used.

Negative and positive controls showed the expected results. Absorption tests resulted in the abolition of cyclin D1, cyclin D3, cdk4, and cdk6 immunoreactivity.

The results of the immunohistochemical study are summarized in Table 2.

Discussion

Myxoid and round cell liposarcoma represent a morphological spectrum of mesenchymal neoplasias that, in the majority of cases, share a common molecular aberration represented by a specific reciprocal translocation that fuses the CHOP gene to the FUS gene. The FUS gene

Table 2. Immunohistochemical Results of Myxoid and Round Cell Liposarcoma

Case	Histology	RT-PCR	% p53$^+$ cells (Do7)	% p21$^+$ cells	% mdm2$^+$ cells	pRb	D1	D3	p27	cdk4	cdk6
LPS1	Myxoid	ND	50*†	0	0	−	0	0	+	−	+
LPS2	Myxoid	+	60*	0	0	−	0	0	+	−	−
LPS3	Myxoid	+	60*	15	5	+	30	0	+	−	+
LPS4	Myxoid	+	70	0	0	+	0	0	+	−	+
LPS5	Myxoid	ND	10	0	0	−	0	0	+	−	−
LPS6	Myxoid	ND	50	0	10	−	0	0	+	+	+
LPS7	Myxoid	ND	40†	0	20	−	0	0	+	+	−
LPS8A	Myxoid	+	60	0	0	+	0	0	+	+	−
LPS8B	Round cell	+	60	20	0	+	0	0	+	+	−
LPS9A	Myxoid	+	70	10	0	+	0	0	+	+	−
LPS9B	Round cell	+	80	20	20	+	0	20	+	+	−
LPS10A	Myxoid	+	5	0	0	+	0	0	+	+	−
LPS10B	Round cell	+	10	0	0	+	0	0	+	+	−
LPS11A	Myxoid	+	60	5	0	+	0	0	+	+	−
LPS11B	Round cell	+	70	20	20	+	0	0	+	+	−
LPS12A	Myxoid	ND	50	0	0	+	−	−	+	+	−
LPS12B	Round cell	ND	50	0	0	+	−	−	+	+	−
LPS13A	Myxoid	+	60*	0	0	+	0	0	+	−	+
LPS13B	Round cell	+	80*	30	0	+	0	0	+	+	+
LPS14A	Myxoid	ND	50	10	10	+	0	0	+	+	−
LPS14B	Round cell	ND	50	25	20	+	0	0	+	+	−
LPS15A	Myxoid	ND	60	20	0	+	0	0	+	+	−
LPS15B	Round cell	ND	60	20	20	+	0	0	+	+	−
LPS16A	Myxoid	+	20	0	0	−	0	0	+	+	0
LPS16B	Round cell	+	30	15	10	−	0	0	+	+	0
LPS17	Round cell	+	70	30	30	+	0	0	+	+	−
LPS18	Round cell	ND	80*	30	30	−	0	5	+	−	+
LPS19	Round cell	ND	70*	0	0	+	0	0	+	+	−
LPS20	Round cell	ND	10	30	0	+	0	0	+	−	+
LPS21	Round cell	ND	80	50	40	+	20	30	+	−	−

ND, not done.
*p53 mutated cases.
†p16 mutated cases.

shows great homology with the EWS gene in both structure and function, and in fact, so far the only known cytogenetic variant of myxoid liposarcoma involves a translocation between chromosome 12 involving the CHOP gene and chromosome 22 involving the EWS gene.[25,26] Clinicopathological studies have demonstrated that the clinical behavior of pure myxoid lesions differs significantly from those tumors containing hypercellular round cell areas.[4,5] The presence of the t(12;16) translocation in both myxoid and round cell tumors underlines the pathogenetic importance of this genomic change but nonetheless makes its role in tumor progression unlikely.

The genes (and their proteins) acting at the G1-S checkpoint represent one of the more frequent targets in molecular tumorigenesis.[8,27] The majority of previous studies in mesenchymal neoplasia have dealt with a histologically heterogeneous group of sarcomas,[9–16] but as distinctive subsets of mesenchymal malignancies are investigated, a certain degree of tumor specificity emerges among the molecular aberrations investigated.[17–20]

Analysis of the p53 pathway in myxoid and round cell liposarcoma has demonstrated TP53 gene mutation in approximately 30% of cases, a figure much higher than that observed so far in other distinct sarcoma subsets, such as well differentiated liposarcoma and leiomyosarcoma.[18,19] At the protein level, accumulation of p53 has been observed immunohistochemically in all of the cases, independently from the TP53 gene status. We find it hard to explain why these results are different from those of Smith and Goldblum[28] but assume this must be related to technical differences. In approximately 70% of cases, p53-positive immunoreactivity was related to accumulation of wild-type protein. Even if, in the large majority of these cases, the expression of p53 protein may be related to mdm2-induced stabilization, a minority of cases remain in which high levels of wild-type protein were detected despite apparent integrity of the p53 pathway. It has to be stressed that TP53 gene mutations were observed in all of the histological subgroups of myxoid liposarcoma analyzed, independent of the histological grade of the tumor, thus indicating that mutations of the TP53 gene may represent an early step in the molecular tumorigenesis of myxoid and round cell liposarcoma. Interestingly, in well differentiated liposarcomas, TP53 mutations are exceedingly rare.[19,20] Our data further underline both the biological diversity of the liposarcoma subcategories as well as the emergence of distinct patterns of molecular aberrations in soft tissue tumors.

Overexpression of mdm2 has been observed in approximately 56% of cases. mdm2 represents a nuclear phosphoprotein, the transcription of which is activated by p53 itself and which binds to and inhibits the TP53 gene transactivating domain. Such a negative feedback loop

permits release of the block of the cell cycle operated by the TP53 gene. mdm2 also inhibits pRb[29] and stimulates the E2F family of transcription factors.[30] As a consequence, overexpression of mdm2 may interfere with the two main inhibitory systems of cell proliferation that act at the G1-S checkpoint. Overexpression of mdm2 has been reported in a variety of sarcomas[8,15] and seems to be a common phenomenon in well differentiated liposarcoma (approximately 80% of cases), in which it may also play a role in the process of dedifferentiation.[19,20,31] In the context of myxoid and round cell liposarcoma, statistically significant correlation between mdm2 expression and high-grade round cell morphology has been observed, which may support a role for mdm2 in the process of tumor progression in this subset of liposarcoma also.

$p21^{Waf1}$ and $p27^{Kip1}$ are both members of a distinct family of cdk inhibitors that negatively regulate progression through the G1-S checkpoint.[32] As abundant $p27^{Kip1}$ immunoreactivity was detected in all of the cases analyzed, a role for $p27^{Kip1}$ aberrations in this subset of tumors appears unlikely. Interestingly, $p21^{Waf1}$-positive immunoreactivity was found in one-half of the TP53 mutated cases. $p21^{Waf1}$ is under transcriptional control of p53 and TP53 gene mutational inactivation should therefore lead to abolition of its expression. Although in other tumor types $p21^{Waf1}$ expression correlated with TP53 gene status and emerged as a reliable indicator of the integrity of the TP53 gene,[19] the present findings further confirm that mechanisms of p53-independent $p21^{Waf1}$ induction exist.[18,33]

The Rb-cyclin D pathway is emerging as an important target for molecular aberration at the G1-S checkpoint.[8] The normal function of cyclin Ds, coupled with their catalytic subunits cdk4 and cdk6, is to phosphorylate the pRb, which, in its unphosphorylated form, suppresses cell growth by sequestering transcription factors belonging to the E2F family and consequently by inhibiting their transactivating properties. Unphosphorylated pRb represents a major brake on the cell cycle, and as postulated earlier by Knudson,[34] its inactivation is known to be a decisive event in carcinogenesis. Additional inhibitory function is exerted by a family of four polypeptides ($p16^{MTS1/INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$, and $p19^{INK4d}$) that block the activity of both cdk4 and cdk6 by sequestering them and preventing their complexing with cyclin Ds.

As far as pRb is concerned, complete loss was observed immunohistochemically in one-third of cases. Abnormalities of the Rb gene and protein seem to be relatively frequent in soft tissue sarcomas[35]; however, the pattern of Rb gene aberrations appears to be different when distinctive subgroups of sarcomas are analyzed. The absence of Rb abnormalities in rhabdomyosarcoma[36] contrasts with the high rate of pRb loss observed in leiomyosarcoma,[18] suggesting again the possibility of tumor-specific patterns of molecular alterations.

Overexpression of cyclin Ds seems to be a rare event in myxoid and round cell liposarcoma. Yet in three pRb-positive cases, such overexpression represented an alternative mechanism of overriding the cell cycle block operated by the Rb-cyclin D pathway.[37] The cyclin D1 gene, which maps at chromosome 11q13, is amplified in a variety of cancers; it is rearranged and overexpressed in parathyroid adenoma[38] and is activated by the t(11; 14)(q13;q32) translocation in mantle-cell lymphomas.[39] With the exception of a single case in which a low percentage of immunoreactive neoplastic cells were detected, cyclin D1 overexpression was not observed in pRb-negative cases, therefore confirming at least partially that a functional Rb gene is an essential requirement for cyclin D1 expression.[40] Overexpression of cyclin D3 confirms that the different members of the cyclin family may serve as alternative targets for aberration of the Rb-cyclin D pathway.

Mutation of the $p16^{INK4a/MTS1}$ tumor suppressor gene was demonstrated in two myxoid liposarcomas. Mutations of the $p16^{INK4a/MTS1}$ gene represent an additional mechanism of cell growth deregulation, and such aberrations have been described in a variety of tumors.[41] Nonetheless, recent data indicate that mutation analysis of the $p16^{INK4a/MTS1}$ gene may underscore its actual role as a potential molecular target for aberrations occurring at the G1-S restriction points. Alternative mechanisms such as methylation of the promoter region as well as homozygous deletion exist,[42,43] which may lead to inactivation of the inhibitory function of $p16^{INK4a/MTS1}$.

Interestingly, overexpression of either cdk4 or cdk6 was detected in approximately 85% of cases. The expression of both cdks in normal tissues is limited to some developmental and proliferative compartments whereas they are undetectable immunohistochemically in terminally differentiated tissues. The CDK4 gene maps in the 12q13–15 region, which also contains important proto-oncogenes such as MDM2, SAS, CHOP, GLI, and the recently characterized HMGI-C gene, an architectural factor the rearrangement of which plays a central role in the development of distinct subsets of benign mesenchymal neoplasms.[44] In our series, overexpression of mdm2 and either cdk4 or cdk6 was present in approximately 43% of cases, raising the possibility of a synergistic effect in deregulating the G1-S checkpoint-mediated cell cycle arrest. Interestingly, co-amplification of both the CDK4 and the MDM2 genes has been reported recently in other soft tissue sarcomas.[45] It should be noted that myxoid and round cell liposarcomas are characterized by two specific variant translocations resulting in the rearrangement of the CHOP gene, which also resides in the 12q13–15 region. The genomic changes involving the CHOP gene may also be involved in the process of deregulation of both MDM2 and CDK4, but at present this remains merely speculative.

In conclusion, in contrast with the exceedingly rare involvement of the TP53 gene in well differentiated liposarcoma, TP53 gene mutations occur in approximately 30% of myxoid and round cell liposarcoma, therefore supporting the existence of a certain degree of tumor specificity for molecular aberrations even among closely related tumor types. As TP53 gene mutations were detected in both low- and high-grade lesions, it seems likely that disruption of inhibitory function exerted by the TP53 gene over the cell cycle machinery represents an early event in the molecular oncogenesis of myxoid and round cell liposarcoma. Aberrations of the Rb-cyclin D pathway

also seem to represent a common phenomenon with involvement of differing targets. Rb loss occurs in one-third of cases and, at variance from what has been observed in other subsets of sarcomas, overexpression of cyclin Ds represents a rarer event. Notably, overexpression of either cdk4 or cdk6 is present in the majority of tumors analyzed, which thus may represent an important molecular mechanism of overriding the G1-S checkpoint. Finally, high expression of mdm2 protein was found that correlated with tumor grade, suggesting a role for mdm2 upregulation in the process of tumor progression.

The G1-S restriction point is characterized by a complex molecular scenario, in which different targets for molecular aberrations exist. Most of the aberrations observed at this level lead to the inability of cells to exit the cell cycle. Earlier studies, dealing with histologically heterogeneous series, have shown that soft tissue sarcomas exhibit relatively frequent abnormalities that involve both the p53 and Rb-cyclin D pathways. As specific subsets of sarcomas are investigated, it appears that such pathways are targeted differently, indicating the existence of relatively tumor-specific patterns of molecular changes.

Aside from providing potential pathogenetic insights with regard to tumorigenesis in these lesions, such tumor-specific molecular aberrations to some extent also validate existing systems of histological subclassification among soft tissue sarcomas. In the specific context of myxoid and round cell liposarcoma, as molecular dissection of cell cycle pathways becomes more sophisticated and reproducible, it will be interesting to see whether molecular analysis can provide reliable prognostic indicators, given the considerable difficulty of undertaking this task by light microscopy.[46]

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