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Analysis of p53 and mdm2 proteins in malignant fibrous histiocytoma in absence of gene alteration: prognostic significance

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Abstract *TP53* and *MDM2* genes and their protein expression were evaluated in frozen and paraffin-embedded tissue from 27 patients with malignant fibrous histiocytoma to elucidate the relationship between them, their implication in tumor progression mechanisms and their possible diagnostic-prognostic value in malignant fibrous histiocytoma. Single-strand conformation polymorphism analysis and direct sequencing of polymerase chain reaction-amplified DNA were used to establish two *TP53* mutations (7.4%): a point mutation and a 63-bp duplication. Amplification of the *MDM2* gene was observed in two tumors (7.4%) by means of Southern-blot analysis, one of them also carrying the TP53 point mutation. Immunohistochemical and Western-blot techniques were used to study nuclear accumulation of p53 and mdm2 proteins: 11 cases (40.7%) with p53 protein expression and thirteen cases (48.1%) with mdm2 protein expression were detected. We confirmed overexpression of mdm2 protein in eight of ten cases (80%) with p53 protein expression without *TP53* gene mutation. Statistical analysis shows that simultaneous co-expression of p53 and mdm2 in malignant fibrous histiocytoma is significantly correlated with survival in absence of gene alteration in contrast to the lack of statistical correlation with survival of p53 protein expression alone.

Key words Malignant fibrous histiocytoma · p53 · mdm2 · Prognosis and immunohistochemistry

Introduction

Malignant fibrous histiocytoma (MFH) is the most common type of soft tissue sarcoma (STS) in adulthood, comprising 25–40% of all soft-tissue sarcomas in this population [8, 67]. Despite this, the diagnosis is controversial because of its uncertain histogenesis. Generally, MFHs have an aggressive biologic behavior with high incidence of local recurrences and distant metastases. According to different series, the 5-year survival rate varies between 50% and 70% [30, 51, 54, 56, 60, 71]. Important prognostic factors are tumor size, depth and grade. Although histological grade is the most important prognostic factor in STS, in the case of MFH (especially the subtype storiform-pleomorphic), it is difficult to evaluate as an independent variable because the majority of MFHs are grade III or IV [8]. In addition to morphologic and immunohistochemical parameters, the introduction of molecular biology techniques can contribute to biological characterization of MFH and could provide the identification of new prognostic variables.

Mutations in the *TP53* gene are the most common genetic changes found in human cancer and have been associated with poor prognosis in a large number of neoplasms [14, 16, 65]. *TP53* plays an essential role in DNA transcription [17, 22] and cell cycle regulation [6, 34]. *TP53* mutations may neutralize the tumor-suppressive effect and modify the structure of the 53-kDa nuclear phosphoprotein, leading to its stabilization and inactivation. Mutant p53 protein has a longer half-life than wild-type p53 and can be immunohistochemically demonstrated. However, investigations comparing p53 immunoreactivity in human tumors with molecular genetic alterations in *TP53* showed divergent results [4, 12, 13, 15, 23, 33]. Stabilization and inactivation of p53 protein could also be due to interaction with other viral or cellular proteins [9, 27, 48, 58].

In vitro experiments have demonstrated that the product of the *MDM2* gene is able to bind and functionally inactivate the transactivating domain of p53 protein [42, 46, 47]. By this interaction, mdm2 may stabilize p53 protein [21, 37]. The first intron of the *MDM2* gene contains a p53 DNA-binding site, which can stimulate the expression of the *MDM2* gene in a p53-dependent way. This provides an autoregulatory feedback of p53 function [19, 68]. The critical role of this feedback is supported by in vivo experiments with transgenic mice:

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MDM2 – mice are viable only when *TP53* is absent [18, 43].

Previous studies investigated simultaneously both genes in human sarcomas [4, 10, 21, 24, 31, 40, 49, 55]. The *MDM2* gene has been widely studied in human sarcomas because of its localization in an often aberrant region in STS (12q13-q14) [43] and its interaction with p53, which leads these tumors to escape from p53-regulated growth control. In the majority of the studies, the authors have not found an alteration of *TP53* and *MDM2* genes in the same tumor, supporting the existence of an alternative mechanism of inactivation of the same regulatory pathway for suppressing cell growth [10, 24, 31, 40, 55]. However, these studies describe tumors with coexpression of p53 and mdm2 proteins without gene alterations. This fact may be explained by the feedback between these proteins. In these cases, overexpression of both proteins is a predictor for a poor prognosis and short survival times [4, 7, 20, 63]. Regarding MFH, this concordance does not exist. Previous studies in MFH have not found correlation between survival and p53 protein expression and have obtained different results when contrasting immunohistochemistry and sequencing analysis [20, 55, 70].

The objective of the present study was to analyze *TP53* and *MDM2* genes and the expression of p53 and mdm2 proteins in a series of 27 cases of MFH. We attempted to elucidate the relationship between them, their implication in tumor progression mechanisms and their possible diagnostic-prognostic application in MFH.

Material and methods

Samples

We studied 37 MFH samples from 27 patients diagnosed (World Health Organization Classification Soft Tissue Tumors, 1994) between 1985 and 1996 at the Department of Pathology, Medical School, University of Valencia. Tumor specimens were fixed in 10% formalin and embedded in paraffin. Representative tissue samples were recovered and cryopreserved at –70°C. All were xenotransplanted subcutaneously into athymic Balb-c nude mice [35]. Growth was positive in 20 cases. Xenografted tissue was analyzed in 11 cases. Clinical and histopathologic data are listed in Table 1.

Polymerase chain reaction/single-strand conformation polymorphism analysis and direct sequencing

Polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) analyses were performed as previously described [50]. DNA was isolated from frozen samples using standard procedures [57]. DNA from exons 5–9 of the p53 gene was amplified using the PCR performed in 25 µl reaction mixture containing 200–300 ng genomic DNA, 0.5 µM each primer, 200 µM each dNTP and 0.5 U *Taq* polymerase (Gibco BRL). The primers used were commercially available (Human p53 amplimers Clontech). The PCR was carried out in a thermal cycler for 30 cycles under the following conditions: 30 s at 94°C, 45 s at 65°C and 90 s at 72°C. A negative DNA template was run with each set of samples. After 2.5% agarose gel electrophoresis to check the quality of the PCR product, we took 3–5 µl of this product and mixed it with an equal volume of formamide loading dye. The mixture was heated at 95°C for 10 min, chilled on ice and applied to a 10% non-denaturing polyacrylamide gel containing 5% glycerol. Electrophoresis was performed at 20 V/cm in 0.5× Tris-boric acid/eth-

Table 1 Clinical and histopathologic data in malignant fibrous histiocytoma (MFH). *T* original tumor; *xen* xenograft; *R* recurrent tumor; *IR* incomplete tumor resection; *NG* not given; *D* died, *?* unknown

Case	Location	Size	Histologic subtype	Grade	Recurrence (months)	Metastasis (months)	Survival (months)
1	Elbow	NG	Storiform-pleomorphic	II	–	–	60 D
2	Knee	2.5×3	Storiform-pleomorphic	III	–	–	60 D
3	Proximal humerus	10×15	Storiform-pleomorphic	III	NG	NG	NG?
4	Proximal femur	16×10	Storiform-pleomorphic	III	–	12	14 D
5	Proximal femur	18×16	Storiform-pleomorphic	III	24	25	36 D
6	Proximal femur	NG	Storiform-pleomorphic	II	24	–	60 D
7	Proximal femur	NG	Storiform-pleomorphic	III	NG	NG	NG?
8	Proximal femur	10×11	Storiform-pleomorphic	III	12	24	36 D
9	Proximal femur	6×6	Storiform-pleomorphic	II	17	30	44 D
10	Retroperitoneum	20×17	Inflammatory	II	15	–	17 D
11	Rib	6×4	Storiform-pleomorphic	III	1	–	60 D
12	Proximal humerus	6×5	Storiform-pleomorphic	II	19	19	28 D
13	Knee	8×7	Storiform-pleomorphic	II	12	–	30 D
14	Head	4×1	Myxoid	I	–	–	60 D
15	Proximal femur	5.4×4 5	Myxoid	I	–	–	60 D
16	Proximal humerus	12×10	Storiform-pleomorphic	III	3	–	60 D
17	Proximal femur	9×8	Storiform-pleomorphic	II	NG	NG	NG?
18	Knee	4×4	Storiform-pleomorphic	II	IR	–	60 D
19	Distal humerus	15×10	Storiform-pleomorphic	III	3	–	60 D
20	Proximal femur	22×5	Myxoid	II	7	–	60 D
21	Proximal femur	8.5×7	Giant cell	III	1	7	15 D
22	Trunk wall	8×5.4	Storiform-pleomorphic	II	–	–	60 D
23	Foot	4×2	Storiform-pleomorphic	III	–	–	60 D
24	NG		Storiform-pleomorphic	II	NG	NG	NG?
25	Trunk wall	10	Storiform-pleomorphic	III	17	–	60 D
26	NG	NG	Storiform-pleomorphic	II	NG	NG	NG?
27	Proximal femur	8×6.5	Storiform-pleomorphic	III	5	6	8 D

ylene diamine tetraacetic acid (EDTA) for approximately 5 h. The temperature was maintained at 20°C with constant recirculation of the buffer between the upper and lower chambers. The gels were stained using the Silver Stain Kit (Bio Rad). Samples that showed anomalous migrating bands were purified with Centricon columns and sequenced on an Applied Biosystem model 373 automated sequencer using the dideoxy dye terminator method with the *Taq* polymerase and the PCR primers.

Southern-blot analysis

DNA (10 µg) was digested with the restriction enzyme *Eco*RI or *Hin*dIII, electrophoresed in 1% agarose gel and blotted onto nylon membranes (Hybond-N, Amersham). The membranes were prehybridized with Hybrisol I (Oncor) at 42°C for 2 h and hybridized with randomly primed labeled probes overnight. Two MDM2 probes were synthesized by means of PCR as described by Ladanyi et al. [25] using normal kidney tissue as template and were used in Southern blots to assess gene amplification. An actin probe (Oncor) served as control. Quantification of gene copy number was performed by optical transmission densitometry (LBK2202 Ultroscam-HP3390). Amplification was not considered to be present if the number of *MDM2* gene copies was below three.

Western-blot analysis

Proteins were isolated from frozen samples using standard procedures [48]. Proteins were extracted and denatured in sample buffer [25 mM Tris/HCl, pH 7.5, 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 50 mM NaCl, 50 mM Fna, 0.5% dithiothreitol (DTT), 10 µg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride (PMSF)], vortexed and centrifuged at 15,000×*g* for 20 min. Protein extracts were resolved by means of SDS polyacrylamide gel electrophoresis (SDS-PAGE) – 7.5% (for p53 protein) or 10% (for mdm2 protein) – and then electrically transferred to a poly(vinylidene difluoride) (PVDF) membrane (Inmobilon-P) in a 0.25 mM Tris/192 mM glycine buffer. The blots were probed with p53-monoclonal antibodies (mAbs) (DO7-DAKO and PAb1801-BIOMEDA) or mdm2-mAbs (1B10-NOVOCASTRA) and proteins were detected using a chemiluminescent detection system with CSPD (Tropix) according to manufacturer's instructions. The mAbs DO7 and PAb1801 recognize both normal and mutant forms of human p53. The mAb 1B10 recognizes the C-terminal portion of the MDM2 molecule.

Immunohistochemistry

The avidin-biotin complex method (ABC) was used on 5 µm paraffin-embedded sections. Endogenous peroxidase was inhibited with 3% hydrogen peroxide in methanol for 15 min, then washed several times in phosphate buffered saline (PBS). The microwave antigen retrieval technique (700 W, 10 min) preceded immunohistochemical staining. Sections were blocked with 20% horse serum in PBS, incubated with primary antibody overnight at 4°C, washed with PBS and incubated for the secondary antibody and avidin-biotin complexes 30 min at room temperature. The immunoreaction was developed using DAB (0.05% 3'3'diamino-bencidine in 0.1% hydrogen peroxide). Positive and negative sections were included in each run. Immunohistochemical evaluation was carried out according to the intensity of staining (negative, light, moderate and intense) and to the percentage of positive cells (<10% staining cells –; 10–40% staining cells +; 40–70% staining cells ++; and >70% staining cells +++). p53 Protein expression was assessed from ++/moderate.

Statistical analysis

Overall survival and disease-free survival were estimated using the Kaplan-Meier method. Correlation of all the results with overall survival, local recurrences and distant metastases were analyzed using the log-rank test. Statistics were performed using SPSS software.

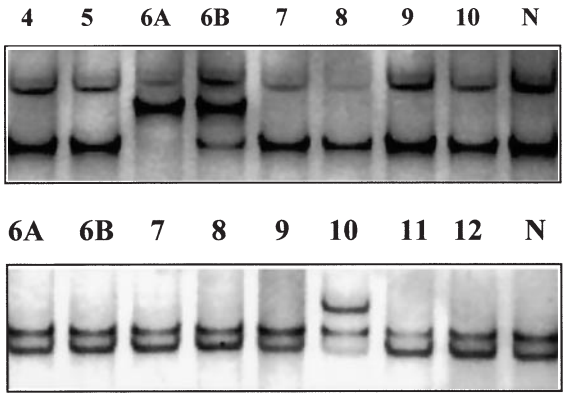

Fig. 1 Single-strand conformation polymorphism (SSCP) analysis of the *TP53* gene. Cases 6A and 6B (primary and xenotransplanted tumor) (*top*) and case 10 (*bottom*) show an anomalous migrating of bands. DNA sequencing confirms the presence of a point mutation (G/C) in codon 178 (His/Asp) in exon 5, in cases 6A and 6B, and a 63-bp in-frame duplication, affecting codons 199–219 of exon 6 in case 10

Results

Clinicopathologic features

We studied 27 cases of MFH and their corresponding xenotransplanted tumors in athymic nude mice. Histologic subclassification includes:

- 22 storiform-pleomorphic type (81%): 12 grade III (48%), 10 grade II (33.33%)
- 2 myxoid type: 1 grade II (3.7%) and 2 grade I (3.7%)
- 1 giant cell type: grade III (3.7%)
- 1 inflammatory type: grade II (3.7%)

Diagnosis was confirmed by means of immunohistochemistry. Clinical data and outcome are detailed in Table 1.

TP53 gene mutations and p53 protein expression

SSCP analysis showed band shifts in four tumors (14.8%): two in exon 5 and two in exon 6. Direct sequencing confirmed two mutations (7.41%) and one polymorphism. Both point mutation and polymorphism presented in original and xenografted tumors. DNA sequencing, despite successive procedures, could not confirm one of the exon-5 mutations observed by SSCP. The two mutations that we found were: a point mutation (G/C) in codon 178 (His/Asp) of exon 5 and a 63-bp in-frame duplication, affecting codons 199–219 of exon 6. The polymorphism (A/G) concerned the codon 213 (Arg/Arg) of exon 6. Most representative results of PCR-SSCP and direct sequencing analysis are shown in Fig. 1.

Eight MFH cases showed over 40% of the tumor cells with immunoreactivities for antibody DO-7. p53 staining was exclusively nuclear. Western-blot analysis (DO-7,

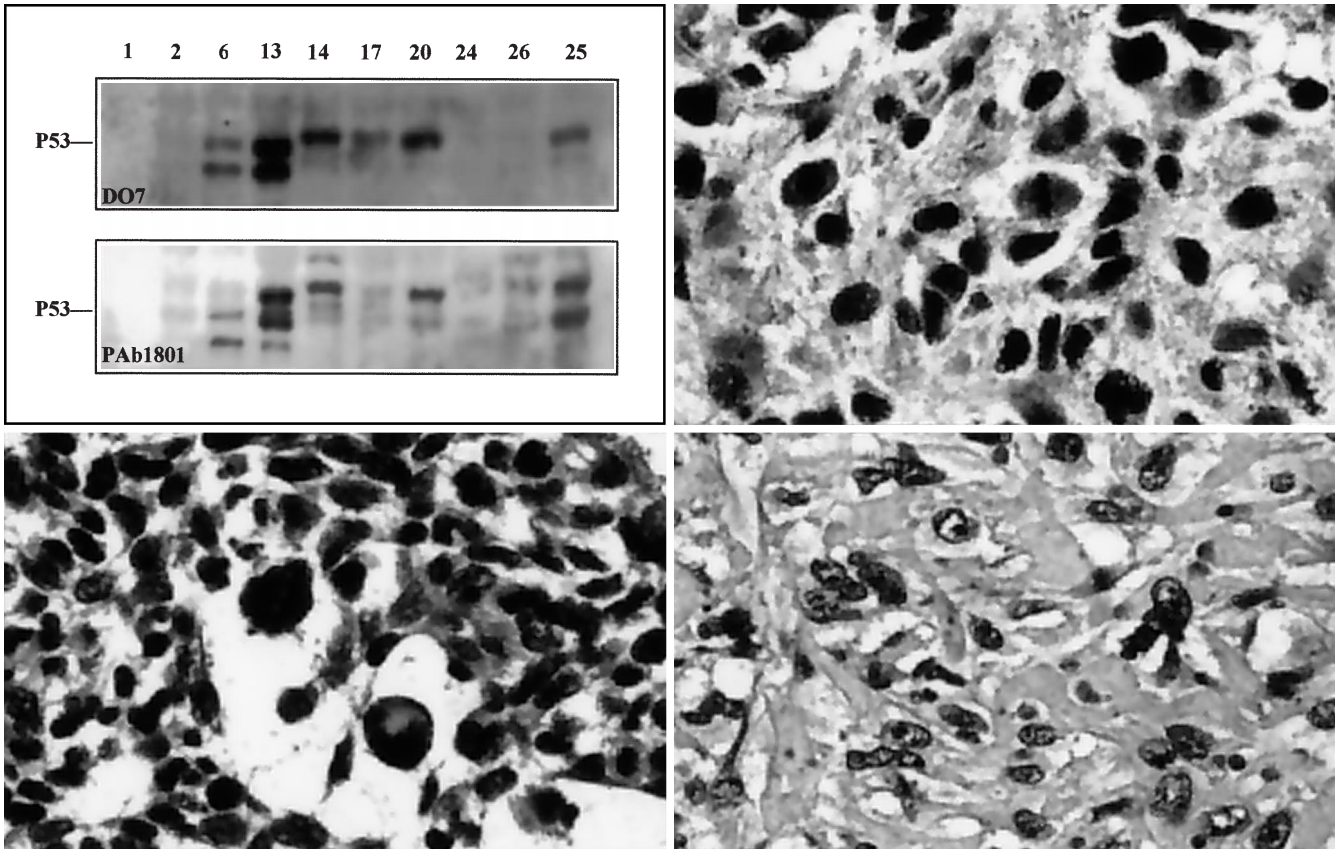

Fig. 2 p53 Protein overexpression in a series of malignant fibrous histiocytomas. *Top left*: Western-blot analysis for p53 detection with DO7 and PAb1801 antibodies. *Lanes 1* and *2* correspond to normal human tissue, liver and kidney. Three cases with strong (*top right*, *bottom left*) and moderate (*bottom right*) nuclear immunopositivity for p53 using DO7 antibody. Original magnification, ×100

PAb1801) confirmed immunohistochemical results and detected three other cases in which paraffin-embedded tumor samples were not available. High concordance was seen when comparing p53 protein detection by Western-blot analysis with both antibodies. Figure 2 shows some cases with p53 protein expression as detected by immunohistochemistry and Western-blot techniques.

In summary, we found two cases with *TP53* gene mutations and 11 cases with p53 protein overexpression. The tumor sample with point mutation in codon 178 overexpressed p53 protein, demonstrated by immunohistochemistry and Western-blot analysis, as expected. Conversely, in the case with +63-bp duplication, we did not detect p53 protein with either antibody. The remaining ten tumors had elevated levels of p53 protein without gene alteration.

MDM2 amplification and mdm2 protein expression

Amplification of the *MDM2* gene was detected in 2 of 27 cases of MFH (Fig. 3). In one of them, the amplification was also detected in its homologous xenotransplanted tumor. Both of them showed mdm2 protein overexpression. Thirteen cases displayed high levels of mdm2 protein, detected by immunohistochemistry and/or Western-blot analysis, with no evidence of gene amplification.

Correlation between *TP53*/*MDM2* gene alterations and p53/mdm2 protein expression

Table 2 summarizes these results. Two cases presented *TP53* gene mutation; one of them (point mutation in codon 178) simultaneously showed *MDM2* amplification and overexpression of both proteins. The other tumor with *MDM2* amplification had elevated levels of p53 and mdm2 proteins. Seven cases showed mdm2 and p53 protein expression without detectable gene alteration. Isolated expression of p53 protein was found in two tumors and, exclusively, mdm2 protein was detected in four cases.

Survival analysis

Figure 4A illustrates the overall survival of this series of patients. Survival was significantly decreased in those cases with p53 and mdm2 protein co-expression or isolated mdm2 protein overexpression (Fig. 4B). However, independent analysis of nuclear p53 immunoreactivity did not show significant differences in overall survival.

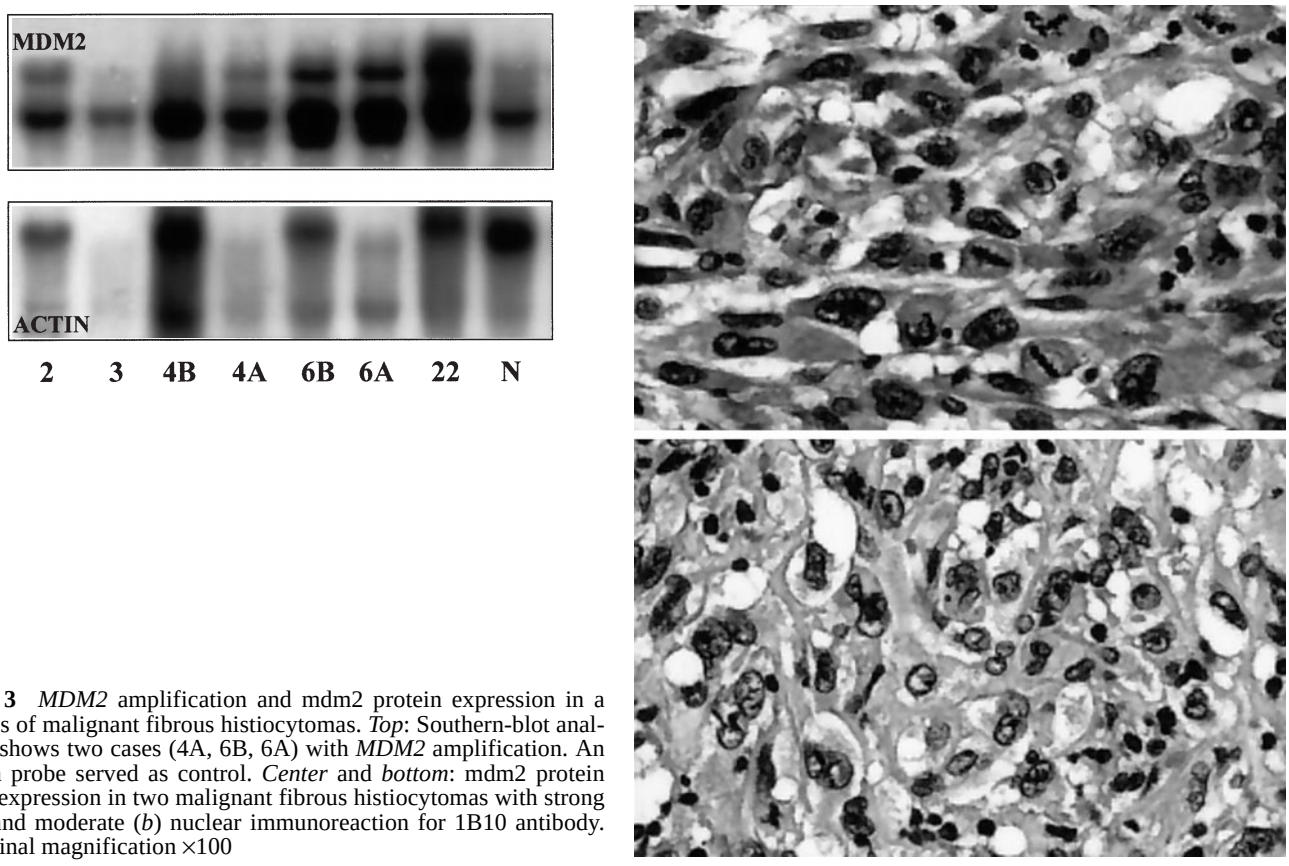

Fig. 3 *MDM2* amplification and mdm2 protein expression in a series of malignant fibrous histiocytomas. *Top*: Southern-blot analysis shows two cases (4A, 6B, 6A) with *MDM2* amplification. An actin probe served as control. *Center* and *bottom*: mdm2 protein overexpression in two malignant fibrous histiocytomas with strong (*a*) and moderate (*b*) nuclear immunoreaction for 1B10 antibody. Original magnification ×100

These results were similar in relation to local recurrences or distant metastases (Fig. 4C–D). Similarly, we obtained identical results analyzing three groups: tumors without p53/mdm2 expression, tumors with p53 or mdm2 protein expression, and tumors with p53 and mdm2 protein expression. Although there was a higher tendency of p53 and/or mdm2 protein expression in high grade MFH than in low grade MFH, the difference was not statistically significant. In relation to patient age, a higher p53 and/or mdm2 expression was observed in younger patients. In the case of p53, this correlation is statistically significant. The median age for p53 protein expression was 55.37 years and for mdm2, 61.81 years. Statistical analysis of gene alterations could not be carried out because of the short number of cases showing such mutations. Nevertheless, all three patients with molecular abnormalities presented local recurrence, distant metastasis and overall survival less than 5 years.

Discussion

Studies of *TP53* gene in MFH identify point mutations in approximately 15% of cases, but a specific site of presentation or hot spot does not exist [1, 10, 29, 31, 55, 63, 64]. In the current study, we detected two *TP53* gene mutations (7.41%) in 27 cases of MFH analyzed: a duplication and a point mutation. We also found a neutral mutation (A/G) in codon 213 (Arg/Arg) of exon 6. Interestingly, the genetic duplication detected in case 10 comprises 63 nucleotides spanned through a DNA region that codes for amino acids 199–219 in a precise and well-defined region in the core domain of p53 protein – from the c-end of the S5 β-strand through the c-end of the S7 β-strand located in the two antiparallel β-sheets. This region is not close to DNA, but can affect the structural integrity of the core domain, thus inactivating p53 most likely by unfolding of this structure [3]. This is a very infrequent rearrangement in TP53 gene. A 9-bp duplication (exon 5, codons 165–166) in a carcinoid tumor of lung [41] and a 42-bp duplication (exon 5) in a liposarcoma have been described [63]. The point mutation of case 6 is a G/C transversion in codon 178 that changes histidine to asparagine. This codon is located in a conserved region of the *TP53* gene and frequently appears mutated in others tumors, but not in the MFH so far analyzed [32].

We found 40.7% (11 of 27) of tumors with p53 protein expression by immunohistochemistry and Western-blot analysis. Similar results were found in several studies regarding STS and MFH [1, 4, 7, 20, 29, 55, 62, 63, 66, 69, 70]. Only one of the 11 p53-positive tumors had a gene mutation – a point mutation (G/C) in codon 178 (His/Asp) of exon 5. p53 protein expression without detecting a *TP53* gene mutation (10 of 27 tumors) warrants some explanations, such as: (1) presence of mutations in

Table 2 Relationship between *TP53*/*MDM2* gene abnormalities and p53-mdm2 protein expression. *IHC* immunohistochemistry; *Seq* sequencing; *ND* not determined; *nm* neutral mutation; *dp* duplication. Percentage is given in parentheses

Case	*TP53* mutations		p53 Protein expression IHC	Western blot		*MDM2* amplification	mdm2 Protein expression	
	PCR-SSCP-Seq		DO7	DO7	PAb1801	Southern blot	IHC	Western blot
1			–	ND	ND	–	–	ND
2			–	–	–	–	–	–
3	E6-codon 213-nm		–	–	–	–	–	–
4			+	–	–	+	+	–
5			ND	–	–	–	ND	–
6	E5-codon 178 (G/C)		+	+	+	+	+	–
7			ND	+	+	–	ND	+
8			–	–	–	–	+	–
9			+	+	+	–	+	–
10	E6-codons 199–219		–	–	–	–	–	–
11			–	ND	ND	–	+	ND
12			+	ND	ND	–	–	ND
13			+	+	+	–	+	–
14			+	+	+	–	+	+
15			–	ND	ND	–	+	ND
16			ND	+	–	–	ND	–
17			–	+	+	–	+	+
18			–	–	–	–	–	–
19			–	–	–	–	–	–
20			+	+	+	–	–	+
21			–	–	–	–	–	–
22			–	–	–	–	–	–
23			ND	–	–	–	ND	–
24			–	–	–	–	–	–
25			+	+	+	–	+	+
26			–	–	–	–	+	+
27			–	–	–	–	–	–
Status subtotal		2	8 (29)	9 (33)	8 (29)	2 (7)	11 (40)	6 (22)
Status total		2		11 (40)		2 (7)	13 (48)	

non-analyzed exons; (2) presence of non-detected missense mutations in the initial screening with PCR-SSCP analysis; and (3) stabilization of non-mutated p53 protein through cellular proteins or increased synthesis as a response to DNA damage. In these cases, the protein should be wild type. This fact has been demonstrated by antibodies that detected only one of the two forms of the protein [66]. In this study, the antibodies we used detected both wild and mutant p53 proteins. Most comparative studies between *TP53* gene and p53 protein expression in MFH and STS describe similar results. These reports describe a high proportion of cases with p53 protein detection without gene mutation: 4 of 39 (10%) [1], 16 of 56 (28%) [66], 13 of 73 (17%) [4], 16 of 73 (22%) [29], 20 of 62 (32%) [63] and 6 of 52 (11%) [55]. The variability in the results could be due to the different methods used by the authors: type of antibody, immunohistochemical score, histological type and tumor grade, not mentioned in some reports.

The purpose of the parallel study of *MDM2* in MFH was to elucidate the status of this gene in the cases with supposed-wild p53 protein expression. p53 and mdm2 proteins participate in an autoregulatory feedback [2, 68]. mdm2 binds to transactivating domain of p53 and modulates its transcriptional activity. Any circumstance that modifies the relationship between each protein should lead to an autoregulatory dysfunction with the subsequent cascade of pathological events. We have found *MDM2* gene amplification in 2 of 27 cases of MFH and mdm2 protein expression in 13 of 27 MFH by means of immunohistochemistry and Western-blot analysis. The frequency of *MDM2* amplification in MFH is more variable and depends on the methodology applied and the predominant subtype and histological grade. *MDM2* amplification in about 4–30% of MFH cases has been described [31, 43, 44, 49, 55]. However, MDM2 amplification was not found in two series of 20 cases of MFH [10, 11]. The fact of no correlation between gene and protein alteration (2/13) has been described in well-documented reports: Cordon-Cardo et al. demonstrated mdm2 overexpression in 76 of 207 sarcomas analyzed and *MDM2* gene amplification in 11 of them. In these 11 tumors, only 6 expressed mdm2 protein [4]. Patterson et al. identified mdm2 overexpression in 21 of 39 sarcomas and gene amplification in 11 [49].

We found one case displaying a *TP53* point mutation with *MDM2* gene amplification and another with *MDM2* gene amplification and overexpression of both proteins. This coexistence is rarely reported even though there are cases described in sarcomas [10, 36, 40, 53] as well as in

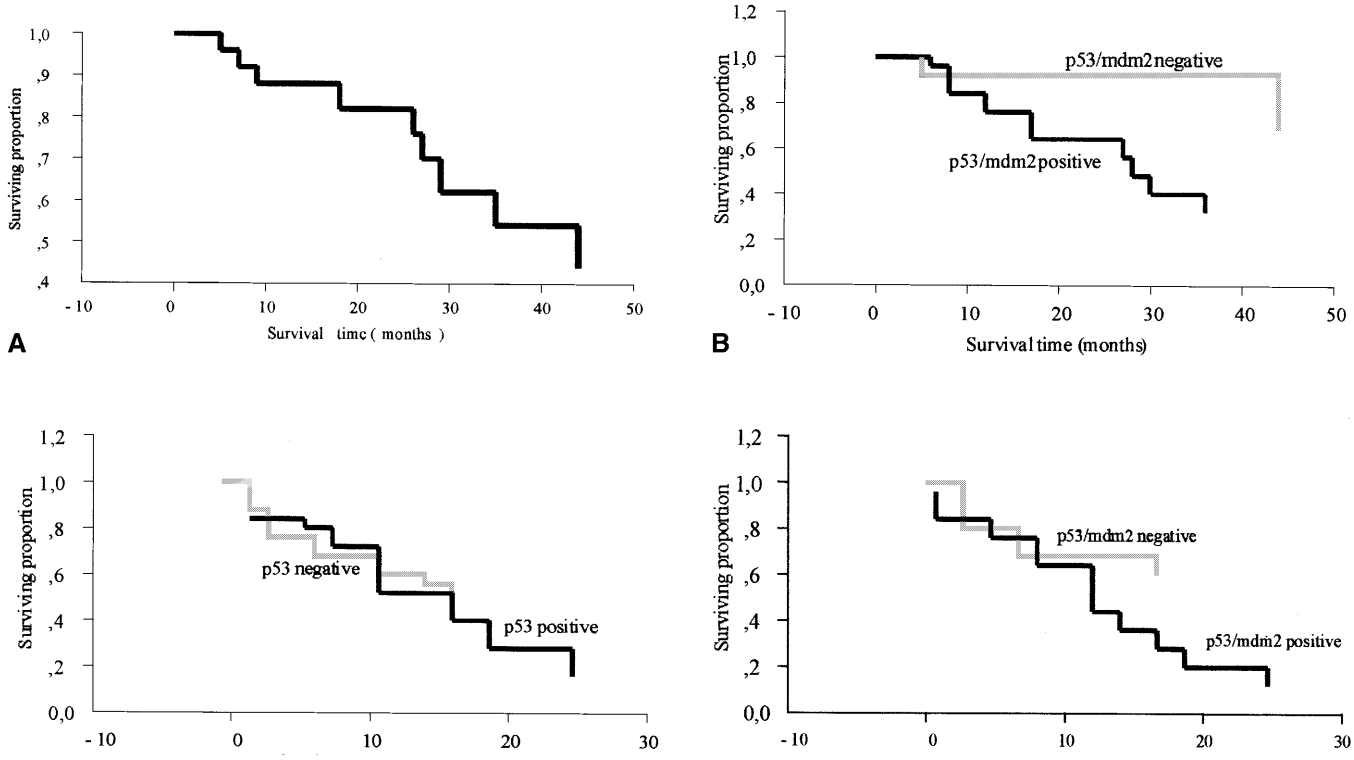

Fig. 4. Patient survival related to p53 and mdm2 protein expression. **A** Overall survival of this cohort of patients. Survival was significantly decreased in those cases with p53 and mdm2 protein co-expression (B). These results were similar in relation to local recurrence or distant metastasis (D). Independent analysis of nuclear p53 immunoreactivity did not show significant differences in overall survival (C)

other tumoral types [15]. This infrequent combination could represent various implicit phenomena described: p53-independent oncogenic activity of the *MDM2* gene [52, 61], stabilization and accumulation of p53 protein, wild or mutated, by high levels of *MDM2* or gene amplification facilitated by p53 inactivation [39].

Seven cases of MFH showed p53 and mdm2 overexpression without evidence of gene alteration. Similar results have been obtained in several series of sarcomas and carcinomas: 10.6% of 207 cases of STS [4], 18% of 87 bladder tumors [33], 51% of 41 and 30% of 201 lung tumors [12, 15], 28% of 61 gliomas [23] and 12.9% of 31 breast carcinoma [13]. These results suggest the existence of biological mechanisms of tumorigenesis and tumor progression related to both proteins. A study in rhabdomyosarcoma cell lines shows overexpression and prolonged half-life of wild-type p53 in cell lines that do not carry p53 mutations. In these cell lines, co-localization and co-immunoprecipitation of p53 and mdm2 implicating a physical association was evident [21]; in this study, the authors observed high expression of *MDM2* mRNA and protein in absence of gene amplification, suggesting a transcriptional enhancement. Other authors have reported a post-transcriptional mechanism associated with an enhanced translation of the gene, identifying a choriocarcinoma cell line with mdm2 protein expression in absence of gene amplification, increased mRNA levels or altered protein stabilization [26]. In this cell line, high levels of wild-type p53 were present. Comparable results were obtained in human sarcomas [53].

In the present study, isolated mdm2 protein expression was detected in four cases. p53-independent mdm2 protein expression has been reported in normal tissue and F9 embryonal carcinoma cells [5, 38], and p53-independent alternative functions of mdm2 have been described with implication of its N-terminal and C-terminal domains [61]. In parallel, mdm2 immunohistochemical detection could be due to *MDM2* gene mutations that stabilize the protein [59]. A CGH analysis of soft tissue MFH reveals gains at the 12q13–q15 region in only 5% of the samples, suggesting that oncogenes usually involved in this amplicon (SAS, MDM2 and CDK4 among others) were activated by mechanisms other than amplification (e.g., by point mutation, transcriptional gene activation or chromosomal translocation) [28].

In relation to p53 and mdm2 overexpression as prognostic factors, we did not find a statistically significant correlation between isolated p53 protein expression and local recurrence, distant metastasis or survival. Although several studies on sarcomas found that survival is significantly reduced in those cases with p53 protein expression [7, 20, 63], in MFH series, the results are not comparable. There are no linear correlations between p53 expression and survival of patients with MFH [20, 55, 70]. These results suggest that isolated p53 protein expression has no prognostic implications, although it is a frequent event in MFH. Alternatively, survival was significantly reduced in those cases with p53 and mdm2 protein co-expression or isolated mdm2 protein overexpres-

sion. These results were similar in relation to local recurrence or distant metastasis and they are in concordance with the results obtained in a study of 211 sarcomas [4].

A higher p53 and/or mdm2 expression is observed in younger patients. In the case of p53, this correlation is statistically significant. The patient distribution age is similar to previous studies performed in sarcomas [36, 50] if taking into account the age groups established in each statistical analysis. A greater tendency of p53 and/or mdm2 protein expression is observed in high-grade MFH than in low-grade MFH, but this difference is not statistically significant. In that respect, there are contradictory results: while there are authors who obtained significant correlation with the tumoral grade [20, 29] others failed to find any relationship [49, 63, 70].

Our three patients with molecular abnormalities presented local recurrences, distant metastasis and an overall survival of less than 5 years. These results are in concordance with previous studies [4, 29, 31, 44, 63] and suggest a poor prognosis in tumors with *TP53* gene mutations or *MDM2* gene amplifications.

In summary, the present study shows the prognostic importance of p53 and mdm2 immunohistochemical codetection in MFH in the absence of gene alterations, in contrast to the lack of statistical correlation with survival of p53 protein expression. Isolated mdm2 immunostaining detected in this study and in previous reports [4, 69] constitutes an independent prognostic factor and makes more evident its widely reported oncogenic activity [61, 52]. Various authors corroborate the high percentage of tumors with p53 and/or mdm2 overexpression without gene alteration. This fact probably evidences an autoregulatory feedback deregulation under little understood circumstances and may provide a key for future therapeutic approaches in these types of tumors.

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