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Mdm2 and p53 expressions and Ki-67 proliferative index in fibrohistiocytic tumors

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Objective: Various genetic alterations including inactivation of tumor suppressor genes and activation of oncogenes, are described in different types of neoplasms as well as soft-tissue tumors. The purpose of the present study is to evaluate the expressions of mdm2 and p53, and Ki-67 proliferative activity and to investigate their interrelations with the clinicopathologic parameters in fibrohistiocytic soft tissue tumors.

Study design: Of the 43 patients diagnosed as fibrohistiocytic tumors including 30 fibroblastic neoplasms and 13 undifferentiated pleomorphic sarcomas were studied. All slides were stained with mdm2, p53 antibodies and Ki-67 by using the standard biotin immunoperoxidase method. Nuclear staining was scored semiquantitatively for all of the cases.

Results: p53 and mdm2 expressions were observed in 47.5% and 20% of the whole tumors, respectively. No association was found between p53 and mdm2 expressions and the clinicopathologic parameters; whereas, there was statistically significant difference between fibroblastic neoplasms and undifferentiated pleomorphic sarcomas in terms of p53 and mdm2 expressions. Ki-67 index showed a statistically significant relation with tumor grade and expression of p53 protein. Ki-67 values were also significantly different between fibroblastic neoplasms and undifferentiated pleomorphic sarcomas.

Conclusions: Our results support the idea that mdm2 and p53 expressions indicate tumorigenic potential in soft tissue tumors. The expressions of p53 and mdm2 proteins and Ki-67 proliferative index in fibrohistiocytic tumors seem to be useful markers for differential diagnosis.

Keywords: fibrohistiocytic tumors, p53, mdm2, Ki-67

Introduction

Various genetic alterations including inactivation of tumor suppressor genes and activation of oncogenes are described in different types of neoplasms as well as soft-tissue tumors (STT).[1–3] However, the association between the prognostic parameters of the tumor and the genetic alterations in STT has not been fully understood.[3,4]

The p53 tumor-suppressor gene, located on chromosome 17p13.1, is involved in cell cycle checkpoints by virtue of its transcriptional activity. The mutation of p53 gene by homozygous allelic loss leads to increased genetic instability. Mutant p53 protein may be identified by immunohistochemical methods, related to the longer time required for the destruction of the mutant protein compared with the wild type. It has been reported that mutant p53 was expressed among a number of different human tumors as well as STTs and associated with unfavorable prognosis.[1]

Murine Double Minute gene 2 (mdm2), a cellular oncogene product with the ability to bind p53, has been shown to inhibit the transcriptional activity of p53. It has been thought that the mdm2 gene amplification is an alternative mechanism to inactivate the p53 gene leading to development of tumor.[5]

Recently, mdm2 expression has been reported in a variety of STT.[6,7]

Ki-67 is a proliferation marker which recognizes a nuclear antigen present only in proliferating cells in G1, S, G2 and M phases, giving additional prognostic information in a variety of tumors as well as STT.[8,9] On the other hand, data concerning altered expression of cell-cycle regulators in STT are not still clear.

The purpose of the present study was to evaluate the immunohistochemical expressions of p53 and mdm2, and Ki-67 proliferative activity in fibrohistiocytic tumors of soft tissues and to investigate their interrelations with the clinico-pathologic parameters such as tumor grade, tumor size, sex, and age. Also, the importance of p53 and mdm2 expressions and Ki-67 proliferative activity in the differential diagnosis of fibrohistiocytic tumors was evaluated.

Materials and methods

Paraffin blocks of 43 fibrohistiocytic tumors including 30 fibroblastic neoplasms (FN) [previously classified as cutaneous fibrous histiocytoma (n=24) and dermatofibrosarcoma protuberans (n=6)] and 13 undifferentiated pleomorphic sarcoma (UPS) (previously classified as malignant fibrous histiocytoma-MFH) diagnosed between 1993 and 2001, were obtained from the archival surgical specimens. The excisional biopsy specimens were originally fixed in formalin, routinely-processed and embedded in paraffin. H&E-stained slides of each case were collected from pathology archives and reviewed in a blind manner to confirm the original histological diagnosis by two pathologists (BL and BT). The tumors were classified according to the WHO Classification of Tumors of Soft Tissues[10] and graded according to the 'Federation Nationale des Centres de Lutte Centre le Cancer' (FNLCC) criteria.[11]

Immunohistochemistry

The tissue blocks containing the most representative areas in H&E stained tissue sections fullfilling the histological criteria were chosen from each case and 5 µm sections were cut into poly-L-lysin coated slides for immunohistochemical staining. Standard streptavidin-biotin immunoperoxidase method was performed with p53 antibody (Clone D0-7, Code No: M7001, Dako, Denmark, dilution 1/50), mdm2 antibody (Cone SMP14, MS-291-P, NeoMarkers, Fremont, CA, dilution 1/50) and Ki-67 (Ab-2, MB67, MS-1006-A, NeoMarkers, Fremont, CA, dilution 1/50).

Briefly, the tissue sections were deparaffinized in xylene, rehydrated in alcohol series and immersed in distilled water. Endogen peroxidase activity was blocked using a 0.3% solution of hydrogen peroxide in phosphate buffered saline (PBS) at room temperature for 10 minutes and rinsed with TRIS buffer. Primary antibodies were applied for 60 minutes at room temperature and washed in TRIS buffer. Linking antibody and streptavidin peroxidase complex (DAKO LSAB Kit, K-0675, Carpintera, USA) were added consecutively for ten minutes at room temperature and washed in TRIS buffer. Peroxidase activity was visualized with 0.03% 3,3-diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemical Co, St. Louis, Missouri, USA), which was applied for 5 minutes. The sections were than washed in deionized water, counterstained with Mayer's hematoxylin and mounted. Control tissues included in each experiment consisted of tissues previously demonstrated to express the antigen of interest as positive controls (invasive breast carcinoma for p53, fibrosarcoma for mdm2 and tonsilla palatina for Ki-67), while the primary antibody was replaced by TRIS buffer in the case of negative controls.

Evaluation of immunostaining

Evaluation of p53 and mdm2 immunostaining

Tumor cells with nuclear immunohistochemical staining were accepted as positive. The extent of positive p53 and mdm2 staining was graded semiquantitatively for intensity and distribution as follows: <5%; 5% to 19%; 20% to 49%; and >50%. The cases that have less than 5% intensity grade were accepted as negative, while remaining were accepted as positive.[12]

Evaluation of Ki-67 index

Nuclear immunostaining in tumor cells were accepted as positive. At least 10 fields of approximately 500–1000 tumor cells per case were counted at 400x magnification. The number of Ki-67–staining tumor

cells in each field was determined as a percentage of the total number of tumor cells counted.[13]

Statistical analysis

Results were analyzed by a computer software (SPSS, 10.0, Inc, Chicago, Illinois, U.S.A). The probability level of 0.05 or less represented statistical significance. Immunohistochemical scores were compared among prognostic parameter subgroups by the Chi-square and independent samples-t tests.

Results

Patients

The study involved 20 (46.5%) men and 23 (53.4%) women, age 9-72 years (mean age, 46.5± 13.3 years). The cases of UPS were measured from 3 to 20 cm (mean, 8.45±5.50 cm).

Five (11.6%) tumors were located in the head and neck region, 6 (14%) in the trunk, 9 (21%) in the lower extremity, 21 (49%) in the upper extremity, and 2 (4.4%) in the retroperitoneum (Table 1).

Table 1. The distribution of tumors according to locations.

Histopathology	Trunk	LE	UE	Head&Neck	RP	Total
FN	4	5	18	3	–	30
UPS	2	4	3	2	2	13
Total	6	9	21	5	2	43

FN: Fibroblastic neoplasms, UPS: Undifferentiated pleomorphic sarcomas, LE: Lower extremity, UE: Upper extremity, RP: Retro-peritoneum.

Of the forthy-three tumors 30 (69.7%) were FN, 13 (30.3%) were UPS. Histologic differentiation of UPS (n=13) were determined according to the grading schema proposed by FNCLCC (11). Nine of 13 (69%) UPS cases were grade 2 and 4 (31%) were grade 3.

Table 2. The distribution of cases according to p53, mdm2 expressions and Ki-67 scores.

	FN	UPS
p53	10.3%	31%
mdm2	45.4%	91%
Ki67	12.13 ± 5.26	51.25 ± 20.96

FN: Fibroblastic neoplasms,
UPS: Undifferentiated pleomorphic sarcomas

Immunohistochemical findings

Expression of p53 and clinicopathologic correlations

In the whole group, 47.5% of the tumors showed positive nuclear staining with p53 antibody (Figure 1). Thirty-one percent of the cases of FN and 91% of the cases of UPS demonstrated nuclear accumulation of p53 protein (Table 2). No correlation was found between p53 expression and the other clinicopathologic parameters such as age, sex, tumor size, and tumor grade. On the other hand, there was statistically significant difference between FN and UPS cases in terms of p53 expression (p=0.005).

Expression of mdm2 and clinicopathologic correlations

Although 20% of the cases showed positive nuclear staining with mdm2 antibody in the whole group (Figure 2), mdm2 expressions were found in 10.3% of the cases of FN and 45.4% of the cases of UPS. No correlation was found between mdm2 expression and the other clinicopathologic parameters. There was statistically significant difference between FN and UPS cases in terms of mdm2 expression (p=0.018).

Ki-67 proliferative index

The mean proliferative indexes of FN and UPS (Figure 3) were 12.13 ± 5.26 and 51.25 ± 20.96, respectively.

There was statistically significant difference between FN and UPS cases in terms of mean proliferative index (p=0.000, r=0.706).

When the statistical analysis was performed for the tumor grades, the mean proliferative index of grade

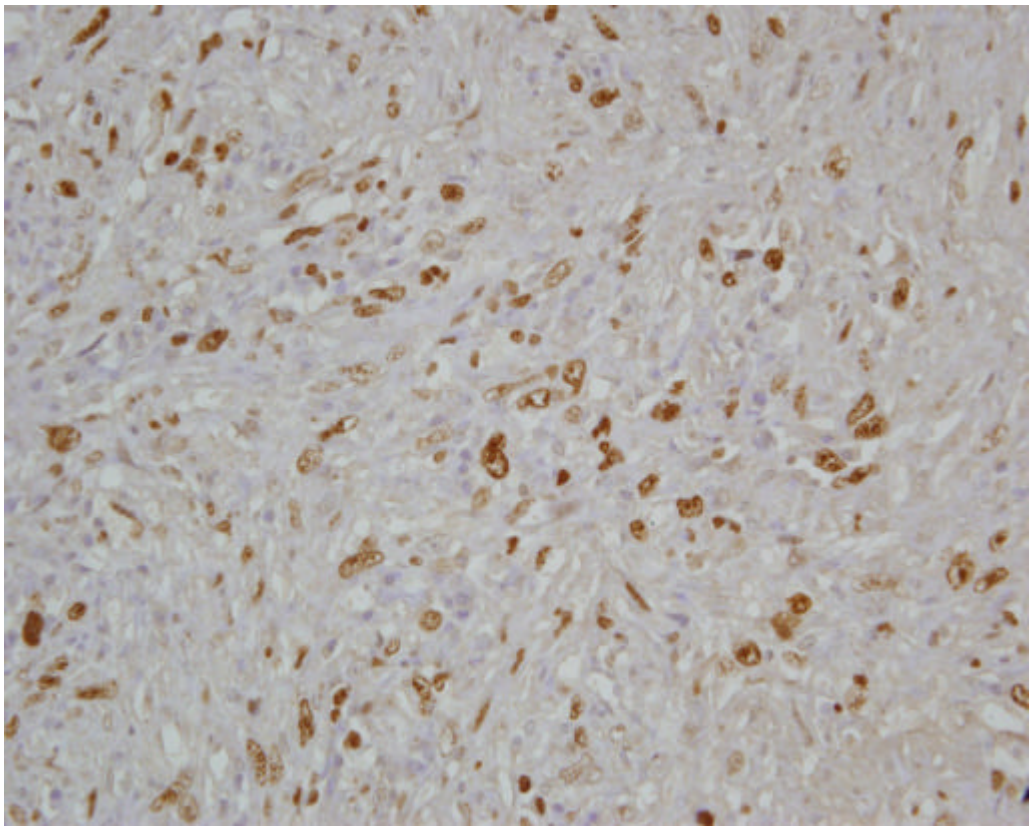

Figure 1. Nuclear immunostaining of UPS with p53 antibody (immunoperoxidase staining, original magnification, x 400).

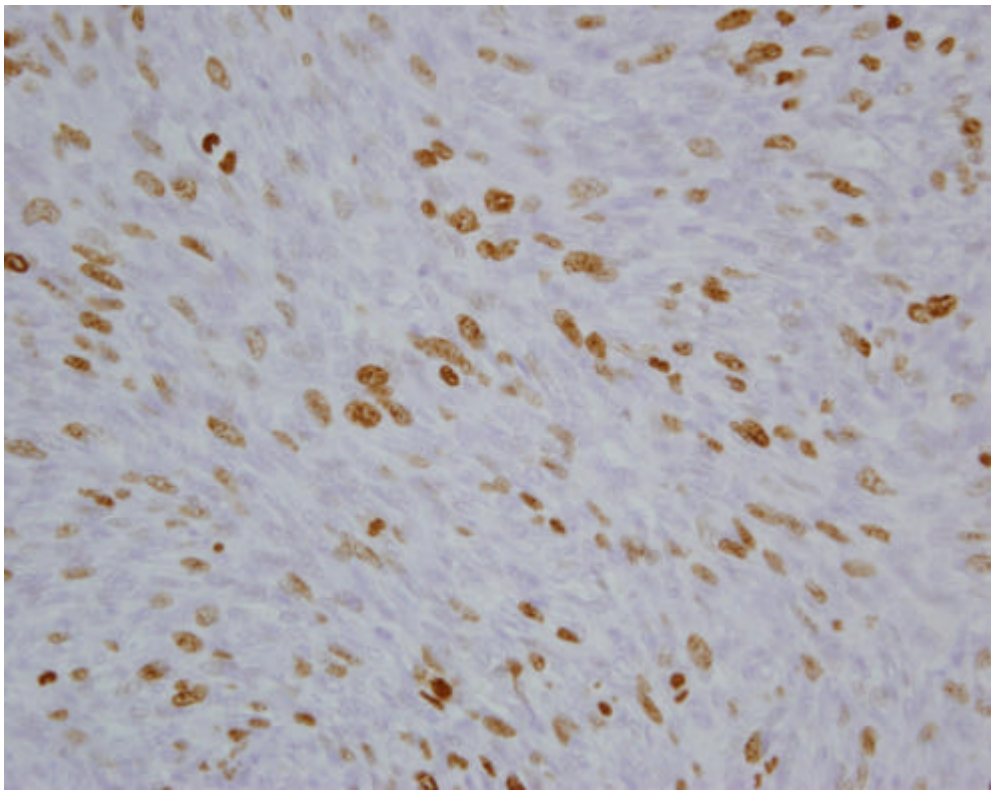

Figure 2. Immunostaining of UPS with mdm2 antibody (immunoperoxidase staining, original magnification, x 400).

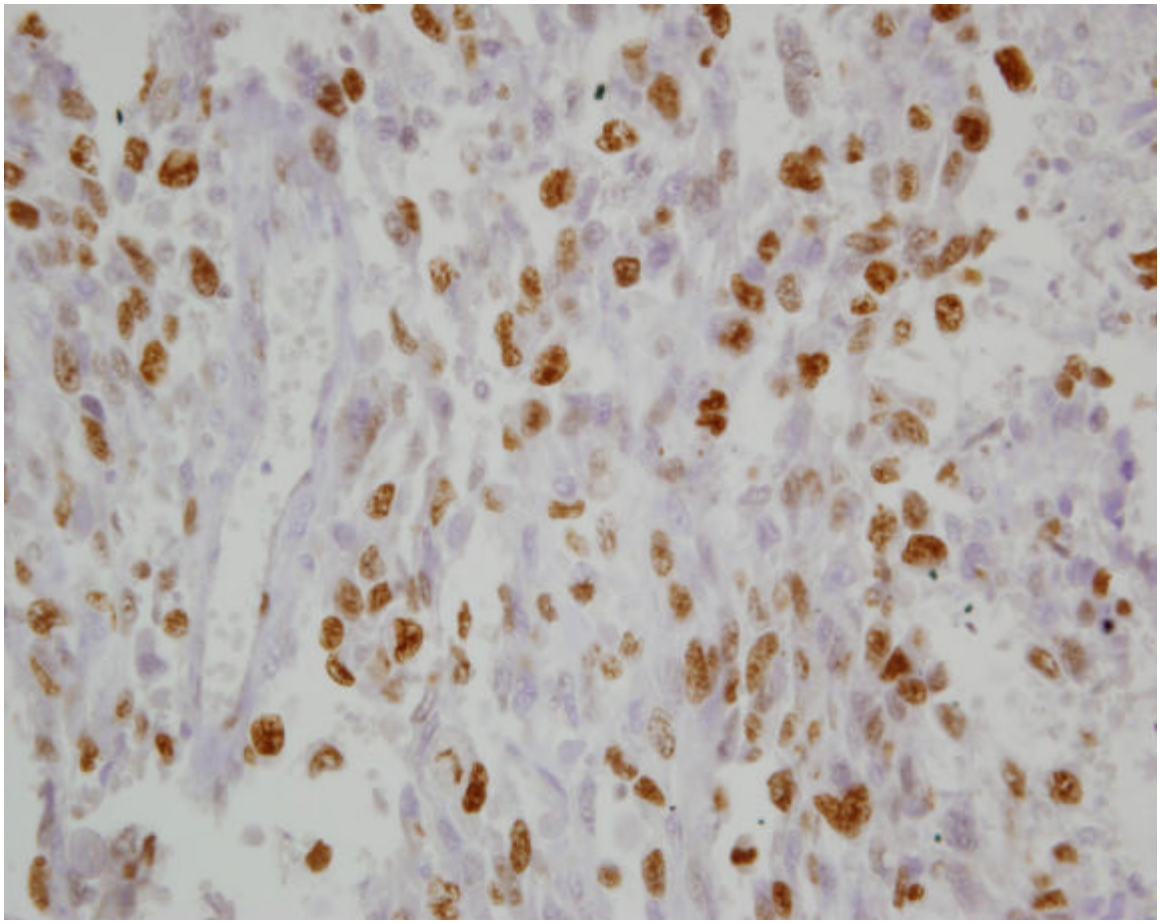

Figure 3. Nuclear immunostaining of UPS with Ki-67 (immunoperoxidase staining, original magnification, x 400).

1 (n=6), grade 2 (n=9), and grade 3 (n=3) tumors were 10.00 ± 2.75, 53.77 ± 18.64, and 59.33 ± 10.59, respectively. Mean proliferative index of the tumors was significantly different among different tumor grades. The mean proliferative index of grade 1 tumors was significantly lower than grade 2 and grade 3 tumors (p= 0.000, r=0.766). However, no significant difference was found between grade 2 and grade 3 tumors in terms of mean proliferative index.

In the whole group, the mean proliferative index of p53 positive and negative cases (32.55 ± 25.85, and 15.13 ± 13.05, respectively) was statistically significant (p= 0.009). On the other hand, there was no significant difference between mdm2 positive and mdm2 negative cases in terms of Ki-67 scores (p=0.175).

The relationship between the expression of p53 and mdm2

In the whole group, there was a significant and direct, but low correlation between p53 and mdm2 expressions (r = 0.289, p= 0.05).

Discussion

Inactivation and mutation of tumor suppressor gene p53, which is an important step in the development of soft tissue tumors (STT), were detected in various soft tissue tumors such as leiomyosarcomas, liposarcomas[14] and malignant fibrous histiocytomas.[15] In different studies, p53 gene mutations have been demonstrated for less than 3% of all mutations in STT.[16,17]

There have been several studies dealing with p53 expression of FN.[18–22] Takahira et al[18] found more frequent missense p53 mutation in the cases of DFSP with fibrosarcomatous component than the cases of dermatofibrosarcoma protuberans. In a study of Sasaki

et al[19], the p53 expression was observed in 3 of 19 cases of DFSP by immunohistochemistry, while no expression was observed in all cases of dermatofibroma. Similarly Lee et al[20] found increased p53 immunoreactivity in DFSP, but not in dermatofibroma, and they suggested that increased expression of p53 protein might be important in the pathogenesis of the more aggressive group of fibrohistiocytic tumors.

In the present study, p53 expression was observed in 31% of FN and 91% of UPS. This difference was found statistically significant. p53 expression was found to be relatively higher in UPS, being 91% in our study, an incidence markedly higher than the previous studies of Yang et al[23] (39%) and Kawai et al[2] (32.2%). We also found no significant correlation between p53 expression and clinicopathologic parameters. These results are in accordance with the study of Yang et al except regarding the primary tumor size.[23] On the other hand, contrasting with our result, Kawai et al[2] demonstrated significant association between p53 expression and clinicopathologic features of soft tissue sarcomas. These observations indicate that p53 expression is related with the malignancy in STT. A possible explanation of this discordance may be the limited number of UPS cases in our study and/or the differences in the primary antibody and/ or immunohistochemical procedures used.

The mdm2 gene was first described as an amplified sequence in a spontaneously transformed murine cell line by Cahilly-Synder et al.[24] Then its tumorigenic potential was demonstrated.[25] The overexpression of mdm2 oncogene is an alternative mechanism for p53 gene inactivation and tumor development.[5] mdm2 gene amplification was found in a substantial portion of various types of human STT and osteosarcomas. It has been suggested that mdm2 binds to p53, inhibits its transcriptional activity in dose-dependent manner and lead to tumor formation.[6, 7,26,27] The human homologue of mdm2 has been located to chromosome 12q13-14 which is a special site frequently changed in various types of STT.[28] It was also found that mdm2 amplification was correlated with tumor grade in bone and soft-tissue tumors[29] and lipomatous tumors.[30] Cordon-Cardo et al[31] have investigated the expressions of mdm2 and p53 in adult soft tissue sarcomas. They found that the expression of the mdm2 and p53 proteins was a predictor for poor prognosis and survival. The authors also found a significant correlation between p53 positive and mdm2 positive phenotypes and tumor grade.

In the current study, we found mdm2 expression in 25% of all fibrohistiocytic tumor group. Although no significant correlation was found between mdm2 expression and the other clinicopathologic parameters, statistically significant difference was observed between FN and UPS cases in terms of mdm2 expressions. Our results support the idea that mdm2 expression indicates the tumorigenic potential in soft tissue tumors.

We also found statistically significant correlation between p53 and mdm2 expressions in all tumors. Our findings are in agreement with those of Cordon-Cardo et al.[31] These results suggest that mdm2 expression is likely to play a significant role in tumorigenesis in fibrohistiocytic soft tissue tumors in p53-dependent pathways.

Ki-67 nuclear antigen is a proliferative marker, expressed in S, G1 and M phases but not in G0 phase in the cell cycle.[8] In a study of Yang et al[23], it has been found that the number of Ki-67 positive nuclei of the tumor varied from 5.35% to 90.8% (mean, 42.7%) in MFHs. They also found that the high Ki-67 labeling index was correlated significantly with tumor size, tumor grade, recurrence and metastasis, disease free interval and survival in patients with MFHs. Similarly Heslin et al[32] demonstrated Ki-67 overexpression was an independent prognostic factor associated with increased risk of distant metastasis and tumor mortality in high grade extremity soft tissue sarcomas.

In our study, the mean proliferative indexes of FN and UPS were 12.13 ± 5.26, and 51.25 ± 20.96, respectively. In statistical analysis, there was a significant difference between FN and UPS cases in terms of Ki-67 index. We also found a significant correlation between Ki-67 proliferative index and tumor grades as previously described by Yang et al.[23] The mean proliferative index of grade 1 tumors was significantly lower than grade 2 tumors and grade 3 tumors. However, no significant difference was found between grade 2 and grade 3 tumors in terms of mean

proliferative index. Our results indicate that Ki-67 proliferative index may be helpful in the histopathological differential diagnosis of fibrohistiocytic tumors of soft-tissue. We also found the statistically significant difference between p53 positive and negative cases in terms of proliferative index. This result was in contrast to the study reported by Lonardo et al. who found a relation between high tumor grade and Ki-67 index, but no correlation between p53 protein expression and Ki-67 proliferative index in osteosarcomas.[33] Taking into consideration our results, p53 protein expression appears to be the sole determinant of the proliferative index.

In conclusion, although the number of our cases is limited, the expressions of p53 and mdm2 proteins are frequent in fibrohistiocytic tumors, particularly in UPS. On the other hand, no significant correlation was found between p53 and mdm2 expressions and clinicopathologic parameters. Only Ki-67 proliferative index was significantly associated with tumor grade. In the whole group, there was statistically significant difference between FN and UPS cases in terms of the expressions of p53 and mdm2 and Ki-67 proliferative index. The expressions of p53 and mdm2 proteins and Ki-67 proliferative index in fibrohistiocytic tumors seem to be useful markers for differential diagnosis.

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