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Mdm2 gene expression in adipose-tissue tumors: association with tumor progression in liposarcomas

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Objective: Multiple genetic defects, such as inactivation of tumor suppressor genes and activation of oncogenes, play important roles in the development of various neoplasms including soft tissue tumors. The purpose of this study is to evaluate the expression of mdm2 and to determine whether it may be an additional prognostic variable in the prediction of tumor progression in liposarcomas.

Study design: Lesions from 43 patients, (20 men, 23 women) were diagnosed as adipocytic tumors. These included 27 lipoma, 5 atypical lipomatous tumor/well differentiated liposarcoma (ALT/WDLS), 11 liposarcomas (LS). 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: There was no staining with p53 and mdm2 in the 27 lipomas and 5 ALT/WDLS while, p53 and mdm2 expressions were observed in 4 (30.7%) and in 3 (23%) of the 11 LS cases, respectively. No association was found between p53 expression and tumor grade whereas, mdm2 expression showed a statistically significant relation with the tumor grade ($p=0.04$, $r=0.479$). Ki-67 values were significantly different between lipomas and ALT/WDLS ($p<0.001$) and ALT/WDLS and LS ($p<0.001$).

Conclusion: p53 expression may be involved in the tumorigenesis of liposarcomas. However, it does not seem to be related to tumor grade and other prognostic factors. On the other hand, mdm2 expression may be associated with dedifferentiation.

Keywords: mdm2, p53, Ki-67, proliferative activity, lipoma, atypical lipomatous tumor, liposarcoma

Introduction

Pathologists are interested in soft tissue sarcomas (STS) for several reasons. The morphology of tumors and reactive lesions of soft tissues shows great variability with divergent clinical behavior. Treatment of STS is problematic because of ubiquitous localization possibilities, histologic diversity, and different biologic behaviour.[1] Factors like tumor grade, tumor size and tumor depth can provide valuable information about the behavior of most soft tissue sarcomas.[2]

Multiple genetic defects, such as inactivation of tumor suppressor genes and activation of oncogenes, play important roles in the development of various neoplasms including soft tissue tumors. Although, genetic alterations in the p53 and mdm2 genes have been reported to occur in some STSs, the importance of individual gene mutations for tumorigenesis of STSs are not fully established.[3]

The mdm2 gene was found as an amplified sequence in a spontaneously transformed murine cell line and shown to have tumorigenic potential. Mdm2 gene is located in chromosome 12q13-14, a region frequently altered in certain types of STSs. Although the function of mdm2 gene product is still unclear, it has been demonstrated that the mdm2 protein bind directly to the p53 protein and inactivate its transcription factor activity.[4] Its amplification and

overexpression may down-regulate the p53 function and afford an effect equivalent to the loss-of-function mutations of the p53 gene. Amplification of mdm2 human neoplasms was first demonstrated in various types of sarcomas.[4] Mdm2 expression was found to be a predictor of poor prognosis and short survival in various STS types like liposarcoma.[3]

Ki-67 is a proliferation marker that is synthesized throughout the cell cycle. Although its function is unknown, nuclear overexpression of this marker has been associated with tumor progression.[5,6] Recent reports have indicated that semiquantitative proliferative indicators such as Ki-67 correlates with the prognosis of STS.[7]

The aim of this study was to evaluate the expressions of p53, mdm2 and Ki-67 proliferative activity in lipomatous tumors and their role in predicting tumor progression of liposarcomas.

Material and methods

Forty-three patients with lipomatous tumors were included in this study. Histologic diagnosis of each specimen was made by standard light microscopic evaluation of routinely-processed and paraffin-embedded tissues. H&E stained slides of each case were taken from the pathology archives and reviewed by two authors (A.K. and K.Y). The tumors were classified as benign, intermediate and malignant according to the 'World Health Organization Classification of Tumors of Soft Tissue'[8] and graded according to the 'Federation Nationale des Centres de Lutte Centre le Cancer' (FNLCC) criteria.[9]

Immunohistochemical procedure

The tumor tissues cut from the paraffin blocks were taken to poly-L-lysin coated slides. The standard streptavidin biotin immunperoxidase method was performed for immunostaining with p53 (NeoMarker, 738P007, California, USA; dilution 1/200), mdm2 (NeoMarker, 291P906, California, USA; dilution 1/50) antibodies and Ki-67 (Neomarker, 1006-A, California USA; dilution 1/50). The sections were deparaffinized, rehydrated and endogenous peroxidase activity was blocked using a 0.3% solution of hydrogen peroxide at room temperature for ten minutes. The sections were heated in citrate buffer (0.01 mol/l, pH=6) for epitope retrieval in a microwave oven (3 times for 10 minutes at 700 W), then allowed to cool to room temperature for 20 minutes. Primary antibodies were applied for 30 minutes at room temperature and washed in TRIS buffer. Linking antibody and streptavidin peroxidase complex (DAKO LSAB Kit, K0675, Carpintera, California, USA) were added consecutively for 10 minutes at room temperature and washed in TRIS buffer. Peroxidase activity was visualized with 3,3'-diaminobenzidine tetrachloride (DAB; Sigma Chemical Co, St. Louis, Mo., USA). The sections were counterstained with Mayer's hematoxylene. Known positive controls (breast carcinoma section for both p53 and mdm2, tonsil section for Ki-67) were also stained simultaneously. Negative controls were stained by omitting the primary antibody incubation.

Evaluation of the staining

Any dark, brown nuclear staining was considered positive. For statistical analysis, the labeling index for p53, mdm2 and Ki-67 expressions were obtained by counting the number of positive nuclei versus the number of the total neoplastic nuclei (Figures 1–3). Cut-off levels was stratified at 20% for p53 and mdm2 indices while, Ki-67 immunostaining was scored by counting positive cells per 1,000 tumor cells and expressed as the percentage of positively stained cells.[10]

Statistical analysis

The data of immunohistochemical evaluation were statistically analyzed using computer software (SPSS 10.0, Chicago, IL, U.S.A). The probability level of 0.05 or less was chosen to represent statistical significance. The correlation between p53 and mdm2 expressions with histologic grade and localization were evaluated by χ^2 test. The relation of Ki-67 proliferative activity with various parameters including histologic grade, age, sex, tumor size and localization were investigated by independent samples t tests.

Results

Of the 43 patients, 20 were men, 23 were women and the mean age was 47.2 (ranging from 17 to 75). The size of the tumor ranged between 1 and 20 cm (median=5.9). Five (11.6%) tumors were located in the head and neck region, 23 (53.2%) in the trunk, 7

Table 1. The distribution of tumors according to locations.

Histopathology	Trunk	LE	UE	Head&Neck	RP	Total
Benign						
Lipoma	18	-	4	5	-	27
Intermediate						
ALT	1	-	2	-	-	3
WDLS	-	-	-	-	2	2
Malignant						
Myxoid LS	4	1	-	-	-	5
Pleomorphic LS	-	2	-	-	-	2
DDLS	-	4	-	-	-	4
Total	23	7	6	5	2	43

LE, lower extremity; UE, upper extremity; RP, retroperitoneum; ALT, atypical lipomatous tumor; LS, liposarcoma; WDLS, well differentiated LS; DDLS, dedifferentiated LS.

(16.3%) in the lower extremity, 6 (14%) in the upper extremity and 2 (4.6%) in the retroperitoneum (Table 1). Of the 43 tumors, 27 (63%) were lipomas (L), 5 (11.5%) were atypical lipomatous tumors/well differentiated liposarcomas (ALT/WDLS) and 11 (25.5%) were liposarcomas (LS). Of the 11 liposarcomas, 5 (45.4%) were myxoid liposarcomas (MLS), 4 (36.4%) were dedifferentiated liposarcomas (DDLS), 2 (18.2%) were pleomorphic liposarcomas (PLS). Histologic differentiation of liposarcomas was grade I in 5 (45.5.8%), grade II in 1 (9%), and grade III in 5 (45.5%) cases according to the grading schema proposed by FNCLCC.[9]

There was no p53 and mdm2 expression in 27 lipomas and 5 ALT/WDLS while, p53 and mdm2 expressions were observed in 4 (30.7%) and in 3 (23%) of the 11 LS cases, respectively. The distribution of p53 and mdm2 expressions is given in Table 2.

There was no correlation between p53 expression and tumor grade (p=0.28) whereas, mdm2 expression showed a significant relation with the tumor grade (p=0.04, r=0.479). There was no association between the expressions of p53 and mdm2, and sex, age, tumor size and localization.

The number of Ki-67 immunoreactive neoplastic nuclei varied from 0% to 85 % with a mean±SD of 0.62±29.4%, 17.25±24.25% and 34.76±28.01% in L, ALT/WDLS and LS, respectively. Ki-67 values were significantly different between L and ALT/WDLS ($p<0.001$) and ALT/WDLS and LS ($p<0.001$).

The rate of positivity for the Ki-67 score was not different between p53 positive and negative cases. Similarly, there was no significant difference when mdm2 positive cases were compared with mdm2 negative cases in terms of Ki-67 scores.

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

	L *(n=27)*	**ALT/WDLS** *(n=5)*	**LS** *(n=11)*		
			GI *(n=5)*	**GII** *(n=1)*	**GIII** *(n=5)*
P53	-	-	2/5	1/1	1/5
Mdm2	-	-	0/5	0/1	3/5
Ki-67	0.62±0.9%	17.25±24.25%	34.76±28.01		

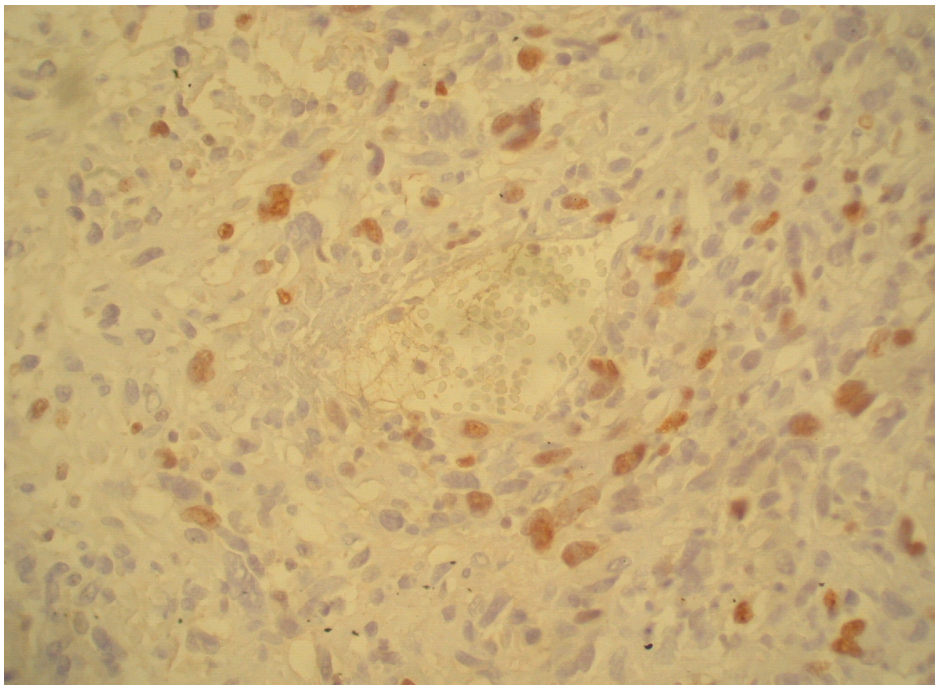

Figure 1. Nuclear immunostaining of p53 protein in liposarcoma (Immunoperoxidase staining, original magnification x 200).

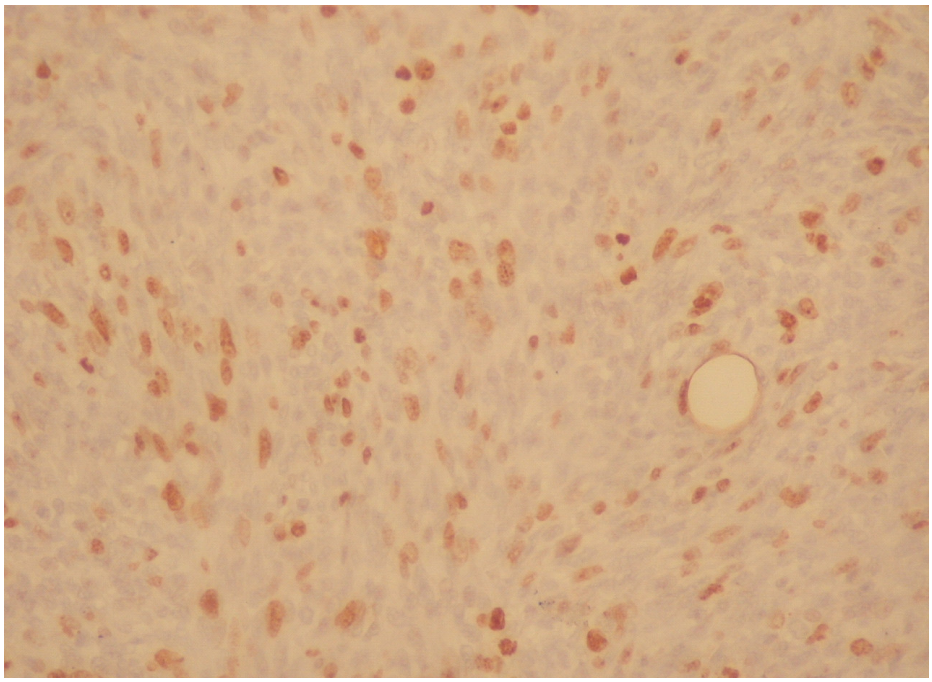

Figure 2. Nuclear immunostaining of liposarcoma with mdm2 antibody (Immunoperoxidase staining, original magnification x 200).

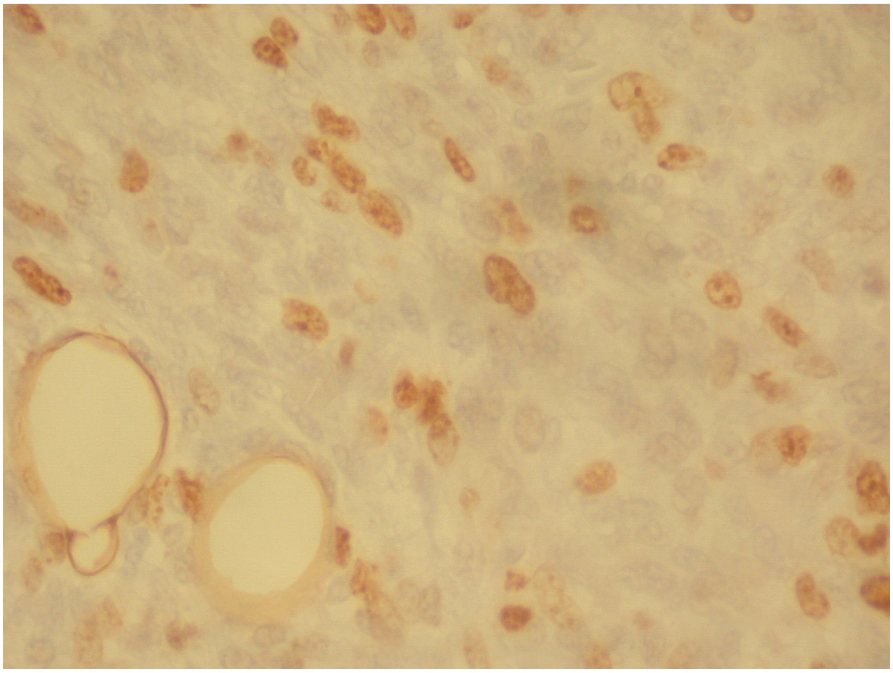

Figure 2. Nuclear immunostaining of liposarcoma with mdm2 antibody (Immunoperoxidase staining, original magnification x 200).

Discussion

Soft tissue sarcomas are malignant mesenchymal lesions with a high degree of prognostic variability. Staging, grading, tumor type and localization are the known prognostic factors. Soft tissue sarcomas constitute a heterogeneous group of tumors and tumorigenesis of STSs are not fully known. Altered cell-cycle regulation may underlie the development or progression of human malignancies.[11] Normal cellular proliferation is regulated by cell cycle associated protein complexes. p53 is a tumor suppressor gene and has a vital role in the regulation of the cell cycle. Alterations in p53 gene result in the production of an abnormal and usually dysfunctional protein and consequently causing cell cycle arrest.[12] Loss of cell cycle control provides a significant growth advantage to uncontrolled proliferating cells and appears to occur in early stages of tumorigenesis and subsequently in tumor progression.[13]

Mutation of p53 plays an important role in carcinogenesis.[7] In vitro studies demonstrated that under γ-irradiation or actinomycin-D inhibition of DNA synthesis, cells showed accumulation of p53 protein in nuclei that correlated with cell arrest in G1 phase. Several viral and cellular proteins have been shown to interact with p53 protein and alter its function. In addition, a cellular oncogene product, mdm2 has been shown to bind to p53 and inactivate its transcriptional factor activity.[3] On the other hand, mdm2 protein was demonstrated to interact with RNA and to bind many cellular proteins among which the most investigated and intriguing is p53. Recent studies have shown that the physical interaction between the two proteins results in a rapid p53 degradation.[14]

Lipoma which is one of the benign lipomatous tumors was characterized by lack of p53 protein and mdm2 expressions[4,15] whereas, alterations in the p53 and mdm2 genes have been reported to occur in STSs.[3,15] There are studies dealing with the function of p53 protein in various STS such as liposarcomas, with contradictory results as a prognosticator. It has been demonstrated that p53 alterations existed among a large variety of human malignancies and were associated with an unfavorable prognosis in patients with colon and breast carcinomas.[16,17] In various tumor types such as breast, colorectal, lung, gastric and brain tumors, expression of high level of p53 protein is a significant predictor of a shorter patient survival time.[3] There is evidence that p53 protein is correlated with histologic grade in various STS, like liposarcomas.[7]

Expression of p53 protein in high grade tumors was significantly higher than in low grade tumors whereas, no relationship was observed between nuclear p53 immunoreaction and sex, tumor size, or nodal or distant metastasis in STS.[7] In a study of Yang et al., p53 protein was correlated significantly with the primary tumor size but not with histologic grade in MFH.[18] In contrast, Taubert et al. reported that p53 positive tumors were associated with an increasing malignancy grade in liposarcomas and MFH. Furthermore, they demonstrated that p53 mutations did not necessarily correlate with higher grades.[19] Similar results were found by Cordon-Cardo et al. and Kawai et al., who suggested p53 immunoreactivity as a marker for poor prognosis.[3,20]

We found no association between p53 expression and tumor grade. This finding is in contrast with the result of Tauberg et al. A possible explanation for this finding may be related to the limited number of patients in our study. On the other hand, we also found no correlation between p53 status and tumor size, age, sex and localization as reported by Kawai et al.[7]

The mdm2 gene is a proto-oncogene that binds to p53, inhibiting its transcriptional activity. Overexpression or amplification of mdm2 gene is common in various tumors such as soft tissue sarcomas, lymphoblastic leukemia and less common in bronchogenic carcinoma and bladder cancer[21] and has been related to more aggressive behavior and poor survival in these diseases. Mdm2 binds to p53 creating negative feed-back control of p53. Mdm2 gene codes for a protein that is tumorigenic when overexpressed. This property is presumably secondary to the ability of the mdm2 protein to bind p53 and Rb, inhibiting the transcriptional activity of p53.[22] Mdm2 overexpression has been demonstrated in various sarcoma types and seems to be a common phenomenon in well differentiated liposarcoma, in which it may also play a role in dedifferentiation.[23,24] Orvieto et al. demonstrated p53 gene mutations to occur relatively frequently while, mdm2 alteration was observed in more than half of the cases and appeared to correlate with progression to higher grade in well-differentiated LS. In a study dealing with myxoid and round cell liposarcomas, there was positive correlation between overexpression of mdm2 and tumor grade.[25] In another study, expressions of p53 and mdm2 were found to be tumor-progression-associated markers in dedifferentiated LS.[26] According to several authors, histogenetically, the pathway that explains the phenomenon of dedifferentiation of a well-differentiated LS is unclear, and it seems that p53 mutations play no role in such pathogenetic mechanisms.[27–29] In a study of Nakayama et al.[3], inactivation of p53 function was a critical event in dedifferentiation of mesenchymal tumors and in tumors of adipocyte lineage, this inactivation was accomplished by mdm2 amplification rather than by mutations of the p53 gene itself. In our study, a statistically significant association was observed between high-grade morphology and expression of mdm2. This finding was similar to those reported by Orvieto, Dei Tos and Hasegawa. Although the number of the patients is limited for a definitive conclusion, our results support the idea suggesting a role for mdm2 upregulation in tumor progression.

Boltze et al. reported no p53 and mdm2 protein expressions in lipomas, in contrast, mdm2 protein expression was found to be associated with malignancy and dedifferentiation, while p53 expression was slightly increased in liposarcomas.[15] We also found no p53 and mdm2 expressions in lipomas and atypical lipomas.

The assessment of the proliferative activity has a prominent role in the differential diagnosis between normal and neoplastic lesions, like uterine smooth muscle tumors and as a prognostic indicator in malignancies such as soft tissue sarcomas and breast carcinomas. A correlation between Ki-67 proliferative activity and tumor grade and risk of distant metastasis was demonstrated in STS including liposarcomas.[2,22] Lonardo et al. reported an association between high tumor grade with Ki-67 scores, but no correlation between the proliferative activity and the p53 status.[22] We have also found an association between proliferative activity and tumor grade. However, no significant difference between p53 status and proliferative activity as reported by Lonardo et al. was found.[22] This observation indicate that p53 protein does not appear to be the sole determinant of the proliferative activity.

In conclusion, p53 expression is involved in the tumorigenesis of liposarcomas, while, it does not seem to be related to tumor grade and other prognostic factors. However, mdm2 expression may have a role in the dedifferentiation. Ki-67 scores were also found to be higher in liposarcomas than lipomas and ALT/WDLS. High proliferative activity in all subtypes of liposarcomas may be one of the important factors leading the similarity of their clinical behavior. These observations need to be confirmed by further studies.

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