

Abnormal Expression of MDM2 in Prostate Carcinoma

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Mutation of *p53* is rare in localized prostate carcinoma. The oncoprotein MDM2, whose gene has a response element for *p53*, promotes the degradation of *p53* protein and inhibits its transcriptional activation of genes related to cell cycle arrest and apoptosis, constituting a negative feedback control. We studied *p53* and MDM2 expression by immunohistochemistry and looked for mutations in *p53* exons 5 to 8 by polymerase chain reaction-single strand conformational polymorphism in 118 patients submitted to radical prostatectomy for localized prostate cancer. In 28 cases, we studied cell proliferation by immunohistochemistry, using antibody for Ki-67, and apoptosis by the deoxynucleotidyl transferase mediated dUTP biotin nick end labeling technique. Although no *p53* mutations were found, *p53* protein was detected in 31.4% of the cases, and these cases had higher Gleason scores ($P = .03$) and more advanced tumor stages ($P = .02$). MDM2 was overexpressed in 40.7% of the cases, and these cases had greater tumor volumes ($P = .001$). Tumors that were positive for both *p53* and MDM2 were larger ($P = .003$) and of more advanced stage ($P = .03$). Within the 28-case subset, the proliferative index was higher among MDM2-positive tumors ($P = .046$), and the apoptotic index was lower among *p53*-positive tumors ($P = .01$). We conclude that, although *p53* mutation is a rare event in prostate carcinogenesis, the detection of *p53* protein by immunohistochemistry is common and is associated with decreased apoptosis and increased histologic grade and tumor stage. We also conclude that the overexpression of MDM2 has a role in prostate carcinogenesis, being frequently detected and asso-

ciated with increased cell proliferation and tumor volume. Finally, we propose that the MDM2-positive/*p53*-positive phenotype identifies prostate cancers with aggressive behavior.

KEY WORDS: Prostate cancer, Immunohistochemistry, *p53*, MDM2, Prognosis, Polymerase chain reaction, Single strand conformation polymorphism, Gene mutation, Cell proliferation, Apoptosis.

Mod Pathol 2001;14(5):428–436

The *p53* protein, which activates genes involved in cell cycle arrest (1, 2) and apoptosis (3), is missing or inactive in more than 50% of human tumors as a result of point mutations or deletions (4, 5) or interactions with proteins (6–8). One such protein is MDM2, a cellular oncoprotein encoded by a gene located on chromosome 12q13-14 (9). MDM2 binds to *p53*, promoting its degradation via ubiquitin, masking its transactivation domain, and inhibiting its transcriptional activation of genes related to cell cycle arrest and apoptosis (9). The transcription of MDM2 is promoted by *p53* (8, 9), creating a negative feedback control of *p53* by MDM2 (9).

Amplification of the *MDM2* gene or overexpression of the MDM2 protein have been implicated in the development of tumors (8, 10), and MDM2 overexpression has been related to more aggressive disease and poorer survival. Amplification of *MDM2* has been found in 35% of soft tissue sarcomas (11), one third of malignant fibrous histiocytomas (12), 42% of liposarcomas (13), and 7% of osteosarcomas (14). Overexpression of MDM2 is common in lymphoblastic leukemia (15) and bronchogenic carcinoma (16), and has been related to more aggressive disease or poorer survival in these diseases as well as in lymphoma (17) and bladder cancer (18). A recent study showed MDM2 overexpression in all cases of salivary gland carcinomas, where *p53* mutations are rare (19).

p53 mutations are also rare in prostate cancer. They occur only late in the development of the disease and have been related to metastasis and androgen-independent growth (20–22). In one series, *p53* mutations were described in 4.4% of cases of localized adenocarcinoma, but in 45% of cases of bone marrow metastasis (20). However, even in the absence of *p53* mutations, it could be that the function of *p53* is abrogated by interactions with an oncogenic protein such as MDM2. The half-life of *p53* is short, and it is not normally detected by immunohistochemistry (IHC), but *p53* that has been inactivated by missense mutation or, sometimes, by interaction with other proteins is abnormally stable and, therefore, detectable (9, 23).

The aim of this study was to evaluate the roles played by *p53* mutation, *p53* expression, and MDM2 overexpression in 118 cases of localized prostatic adenocarcinoma. Mutations of *p53* were detected by polymerase chain reaction-single strand conformational polymorphism analysis of exons 5, 6, 7, and 8 (24), and *p53* and MDM2 were detected by IHC. The results were correlated with the stage, grade, and volume of the tumors. To further evaluate the roles of *p53* and MDM2, cell proliferation and apoptosis were studied in a subset of 28 cases selected so that both normal and abnormal expression of *p53* and MDM2 would be represented. A proliferative index was determined by IHC, using antibody to Ki-67, a protein that is expressed only by dividing cells (25). An apoptotic index was determined by the deoxynucleotidyl transferase mediated dUTP biotin nick end labeling technique (26).

MATERIALS AND METHODS

Case Selection and Pathologic Examination

One hundred eighteen patients with prostate cancer, diagnosed by biopsy and clinically staged T1c-T2, were submitted to radical prostatectomy between August 1997 and June 1998 at the Hospital Sirio Libanes of Sao Paulo, Brazil. The surgical specimens were fixed in 10% buffered formalin for no longer than 4 hours. The entire surgical margin was stained with India ink, the left and right lobes were separated, 3-mm transverse serial sections were taken from each lobe, and the entire gland was submitted for histologic examination. Sections of the bladder neck, prostatic apex, seminal vesicles, and pelvic lymph nodes were also submitted. From 16 to 20 slides of each gland were examined. The Gleason score was used for histologic grading (27). The tumor volume was evaluated as described by Humphrey *et al.* (28). Briefly, a grid was placed below the slides, on which the area involved by the tumor had been previously sketched out. The per-

centage of tumor on a slide was determined by dividing the number of squares involved by tumor by the number of squares occupied by the whole section on the slide. Tumor volume was defined as the mean percentage of tumor in the prostate gland (the percentage of tumor on each slide divided by the number of slides from the prostate gland). Extraprostatic involvement was defined as tumor infiltration of the adipose tissue, the neurovascular plexus, or the parenchyma of the seminal vesicles. The TNM system was used for tumor staging (29).

p53 Mutation Analysis

DNA Extraction

DNA was extracted from three 10- μ m sections of the paraffin block that best represented each tumor (previously selected from hematoxylin- and eosin-stained slides). Disposable microtome blades were used, and instruments were cleaned with xylene after each tissue section to avoid cross-contamination. Three baths in 500 μ L of xylene at 95°C followed by three baths in 500 μ L of 99% ethanol were used to dewax the tissue. Next, the tissue samples were centrifuged for 5 minutes at 13,000 rpm and 4°C. The resulting pellets were incubated overnight at 37°C in 500 μ L of digestion buffer (10 mM Tris [pH 7.4], 100 mM NaCl, 25 mM disodium EDTA, 0.5% sodium dodecyl sulfate) containing 10 μ L of proteinase K (200 μ g/mL final concentration). The enzyme was inactivated by heating the specimens for 10 minutes at 95°C, protein was removed by adding an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), and nucleic acid was precipitated from the aqueous phase by adding $\frac{1}{4}$ volume 8 M ammonium acetate and an equal volume of isopropanol. The nucleic acid samples were then incubated at -20°C for 1 hour and centrifuged for 10 minutes at 13,000 rpm and 4°C. The resulting pellets were washed in 70% ethanol to remove salt, air dried, and resuspended in 50 μ L of TE/RNase (30).

Polymerase Chain Reaction Amplification

The polymerase chain reaction (PCR) amplification and nonisotopic detection of exons 5 to 8 of the *p53* gene were described by Soong *et al.* (31) and will be summarized here. The PCR reactions were carried out in 25 μ L of 1 x Reaction Buffer containing 0.2 mM deoxynucleotide triphosphates, 2.5 mM magnesium chloride, each primer at a concentration of 0.4 μ M, 0.25 units of *Taq* polymerase, and 1 μ L of extracted DNA. Thirty-five thermal cycles of 30 seconds at 94°C, 1 minute at 60°C, and 1 minute at 72°C were completed, and the cycling was concluded by a 10-minute extension at 72°C, all in a thermocycle Gene Amp PCR System 2400 (Perkin Elmer, Foster City, CA). All procedures to prevent contamination were followed.

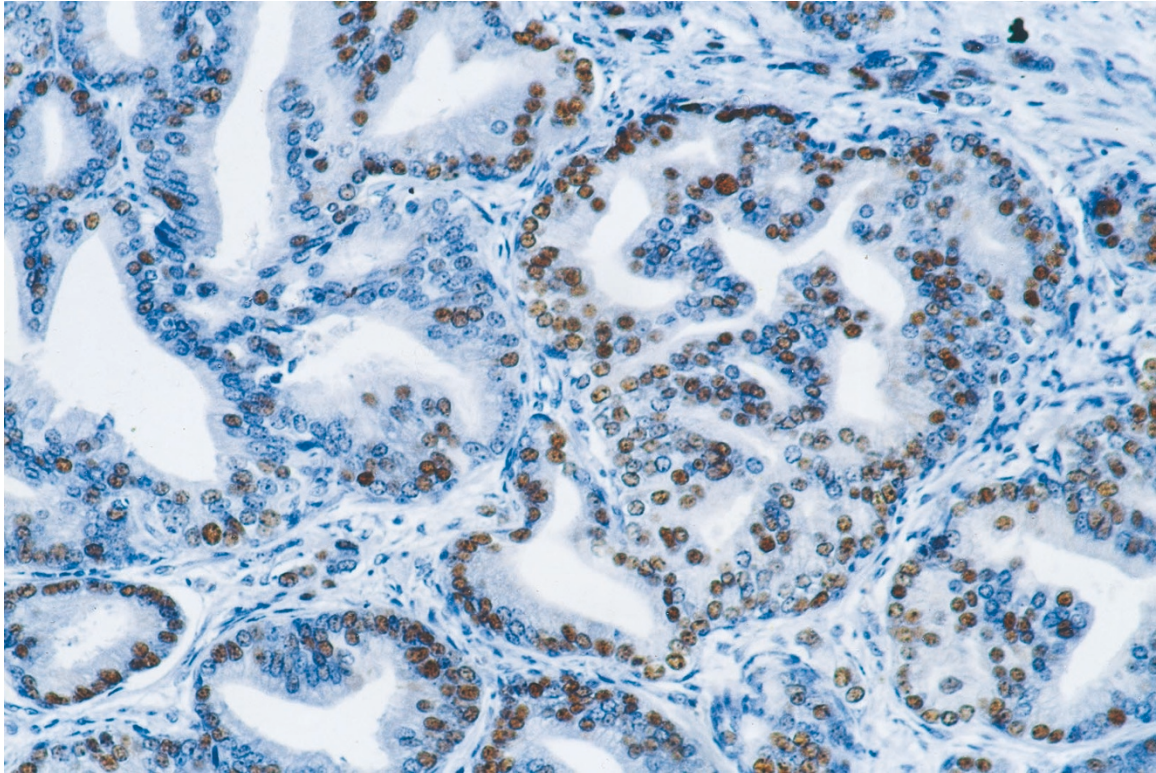


FIGURE 1. p53 protein expression in prostate adenocarcinoma. StreptABC technique using anti-p53 clone DO7 (original magnification, 200 \times).

SSCP Screening for p53 Mutations

Single-stranded DNA for SSCP analysis was produced by combining equal 5- μ L volumes of PCR

product and formamide loading buffer (95% formamide, 10 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) and heating at 95 $^{\circ}$ C for 10

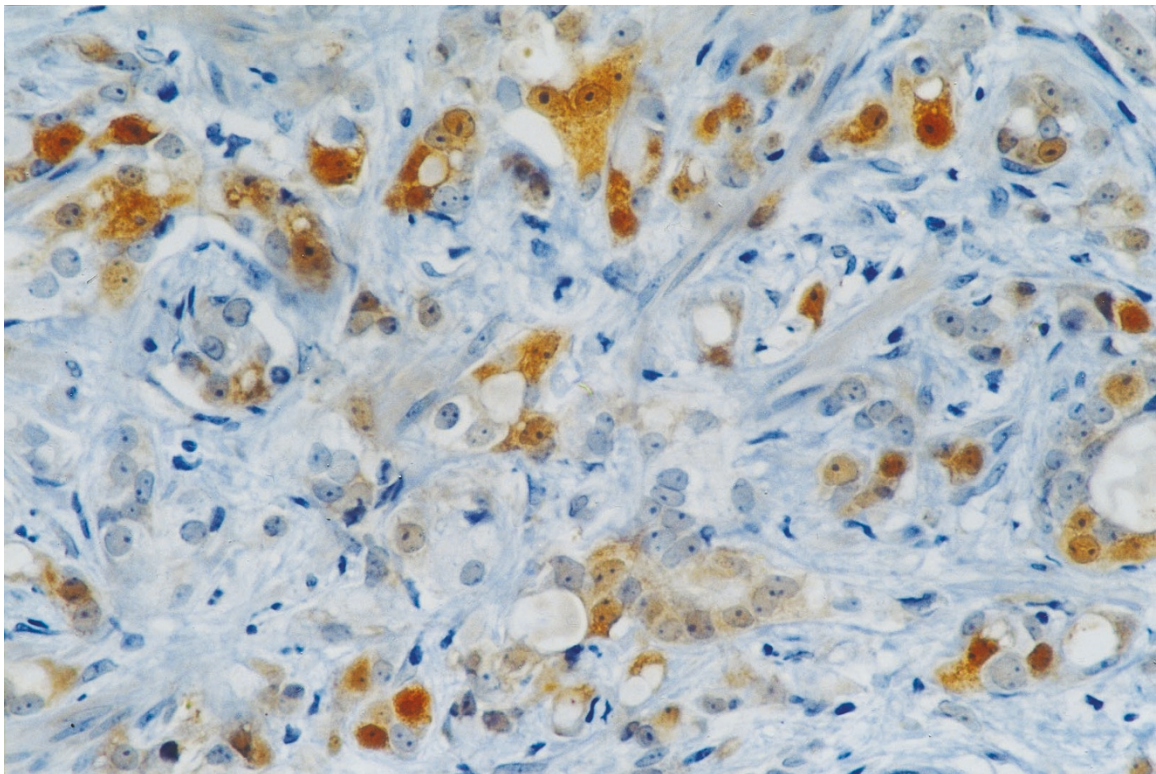


FIGURE 2. Overexpression of MDM2 protein in prostate adenocarcinoma. StreptABC technique using anti-MDM2 clone 1B10 (original magnification, 200 \times).

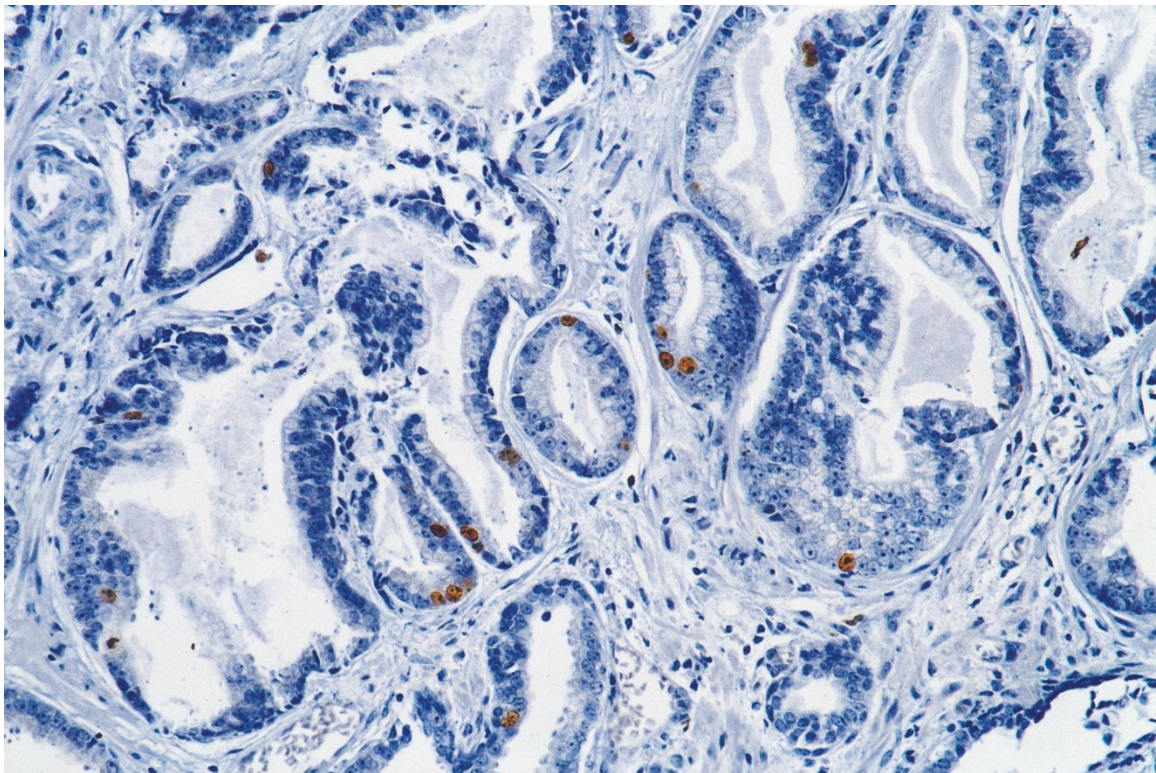


FIGURE 3. Proliferating cells in prostate adenocarcinoma. StreptABC technique using anti-Ki-67 clone MIB-1 (original magnification, 200 \times).

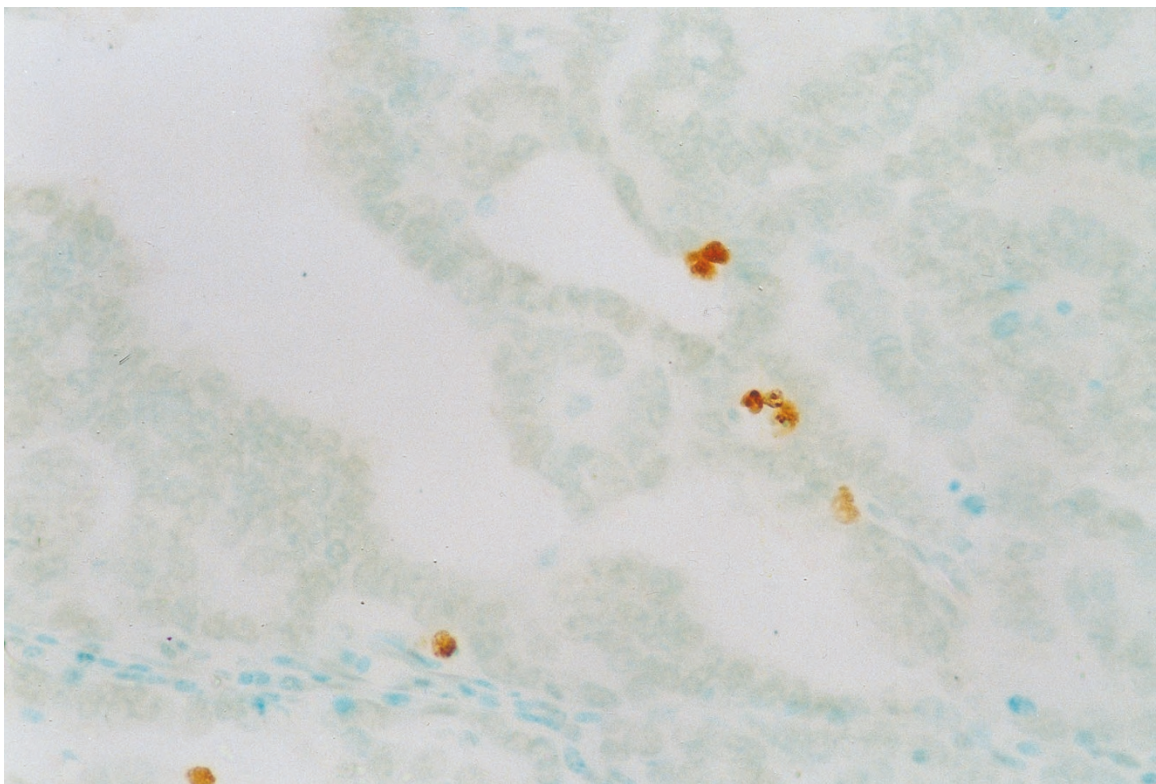


FIGURE 4. Apoptotic cells in prostate adenocarcinoma, stained brown by the deoxynucleotidyl transferase mediated dUTP biotin nick end labeling technique (original magnification, 200 \times).

minutes. The DNA was subjected to electrophoresis at 200 V for 2 hours at 4°C within a Mighty-small apparatus (Pharmacia, Uppsala, Sweden) containing a 15% polyacrylamide gel with 5% glycerol. Silver staining was done by the method of Bassam *et al.* (32). The gels were soaked for 5 minutes in 10% ethanol, 5 minutes in 1% nitric acid, and 10 minutes in an impregnating solution (0.1 g of silver nitrate and 150 μ L of formaldehyde in 100 mL water); then soaked in a developing solution (3 g of sodium carbonate, 150 μ L of formaldehyde, and 100 μ L of sodium sulfate in 100 mL water) until bands were visible; and then fixed in 10% acetic acid for 5 minutes. The gels were analyzed under white light in a computerized imaging system (Gel Doc 1000, Biorad, North Ryde, Australia) and compared with samples of wild-type *p53* from normal tissue extracted and amplified by the same methods to detect *p53* mutations, which would appear as extra bands or mobility shifts.

Immunohistochemical Analysis

Three-micrometer sections from the paraffin block that best represented the tumor (the same block used for PCR) were placed on adhesive-coated slides. In a heat antigen retrieval process (33) the slides were placed in a citrate buffer (1 mM, pH 6.0) and heated three times for 8 minutes each time in a domestic microwave oven at high power. The slides were incubated overnight at 4°C with monoclonal antibodies to p53 (clone DO7, Dako, Glostrup, Denmark), MDM2 (clone 1B10, Novocastria, Newcastle upon Tyne, UK), or Ki-67 (clone MIB1, Immunotech, Marseille, France) at respective dilutions of 1:50, 1:50, and 1:100 in phosphate-buffered saline (PBS). Biotinylated antimouse immunoglobulin G was applied at 1:200 dilution for 60 minutes at room temperature. The slides were rinsed with PBS for 30 minutes, incubated with peroxidase-conjugated streptavidin (streptABC Kit, Dako) at 1:400 dilution in PBS for 45 minutes at room temperature, and then rinsed again with PBS for 30 minutes. Color was developed by incubating the slides in 0.06% diaminobenzidine in PBS for 15 minutes, and the slides were then rinsed in tap water, counterstained with Harris hematoxylin, dehydrated, coverslipped, and reviewed under a light microscope (34). For p53 and MDM2, any dark, brown nuclear staining was considered positive, indicating abnormal stabilization of p53 or overexpression of MDM2 (Figs. 1 and 2, respectively). For Ki-67, at least 500 cells were counted, and the percentage of cells with dark, brown nuclear staining (Fig. 3) was considered the proliferative index.

Detection of Apoptosis

The apoptotic index was assessed by the deoxynucleotidyl transferase mediated dUTP biotin nick end labeling technique using the TDT-FragEL kit (Oncogene, Cambridge, MA), as described by Gavrieli *et al.* (26). Briefly, 3- μ m sections of the paraffin block that best represented the tumor (the same block used for PCR and IHC), were placed on adhesive-coated slides, deparaffinized in xylene, and rehydrated in alcohol. After cell membranes were made permeable by treating the slides with proteinase K diluted 1:10 in 10 mM Tris, pH 8.0, for 20 minutes at room temperature, endogenous peroxidase was inactivated by applying 3% H₂O₂ for 5 minutes at room temperature. Labeling was performed by incubating the slides in a humidified chamber at 37°C for 1.5 hours with the terminal deoxynucleotidyl transferase reaction mix from the kit, which contains labeled and unlabeled deoxynucleotides as well as terminal deoxynucleotidyl transferase enzyme. The reaction was stopped by applying 0.5 M EDTA, pH 8.0, for 5 minutes at 37°C and blocked by applying 4% bovine serum albumin in PBS for 10 minutes at room temperature. The peroxidase-conjugated streptavidin, diluted 1:50 in blocking buffer, was applied for 30 minutes at room temperature in a humidified chamber. The slides were then incubated with diaminobenzidine, counterstained with methyl green, and coverslipped. At least 200 cells were counted, and the percentage of stained cells (Fig. 4) was considered to be the apoptotic index.

Statistical Analysis

To verify significant relationships between variables, the non-parametric Mann-Whitney and Kruskal-Wallis tests were used for quantitative variables, and the χ^2 test was used for qualitative variables. *P* values were two-sided and were considered significant when less than 0.05. The tests were performed with the computer program SPSS, version 8 (SPSS, Inc., Chicago, IL).

RESULTS

Patient and Tumor Characteristics

The median age of the patients was 63 years (range, 46 to 75 yr). The median Gleason score was 7 (range, 4 to 10) in the entire study group of 118 patients, and 8 (range, 6 to 10) in the 28-patient subset for the apoptosis and proliferation studies. The median tumor volume was 22% (range, 1 to 100; mean, 25.0; SD, 19.4) in the entire group and 26% (range, 1 to 90; mean, 33.2; SD, 24.4) in the subset for apoptosis and proliferation studies. In the entire study group, the pathologically deter-

mined tumor stage was pT2N0 in 69 cases (58.5%), pT3N0 in 44 cases (37.3%), and pT3N1 in five cases (4.2%). In the subset for apoptosis and proliferation studies, the stage was pT2N0 in 17 cases (61%), pT3N0 in nine cases (32%), and pT3N1 in two cases (7%).

p53 Mutation

No mutations were detected; electrophoresis of the amplified products of exons 5, 6, 7, and 8 of the *p53* gene revealed no abnormalities of band migration in any of the 118 cases studied.

p53 Expression and Grade, Volume, and Stage of Tumors

Thirty-seven cases (31.4%) were positive for p53. Among the positive cases, brown staining was present in a median of 18% of cells. Characteristics of the p53-positive and p53-negative tumors are compared in Table 1. Positivity for p53 was related to the histologic grade of the tumor, with higher Gleason scores among the p53-positive cases than among the p53-negative cases. A relationship to tumor stage also was demonstrated: 23% (16/69) of the stage pT2 tumors were p53-positive, while 43% (21/49) of the stage pT3 tumors were p53-positive. No statistically significant relationship was demonstrated between p53 and tumor volume.

MDM2 Expression and Grade, Volume, and Stage of Tumors

MDM2 was overexpressed in 48 patients (40.7%). In most cases, only the nuclei of tumor cells were stained; cytoplasmic staining occurred rarely, mainly in Gleason 4 or 5 patterns. There was no background staining, and staining was often focal in the tumor. Characteristics of the MDM2-positive and MDM2-negative tumors are compared in Table 1. Overexpression of MDM2 was related to tumor volume: the median volume was 26% for MDM2-positive tumors and 17% for MDM2-negative tumors. A relationship of borderline significance was observed between MDM2 and tumor stage: 33% (23/69) of the stage pT2 tumors were MDM2-positive, while 51% (25/49) of the stage pT3 tumors were MDM2-positive. There was no relationship between MDM2 and the Gleason score.

Interaction of p53 and MDM2 Effects on Grade, Volume, and Stage of Tumors

When the cases were grouped according to their expression of both MDM2 and p53 (Table 1), there were statistically significant differences among the four groups with respect to tumor volume and tumor stage. The median tumor volume was 38% for

tumors that were positive for both MDM2 and p53, but only 17% for tumors that were negative for both proteins. Extraprostatic invasion (stage pT3) was present in 62% (10/16) of tumors that were positive for both p53 and MDM2, but only 27% (13/49) of tumors that were negative for both proteins. Lymph node status did not appear to be related to p53 or MDM2 expression. There was no statistically significant variation in the Gleason score among the four groups, although the median Gleason score was 8 for tumors that were positive for both p53 and MDM2 and 6 for those that were negative for both proteins.

Apoptosis, Cell Proliferation, and p53 or MDM2 Expression

In the subset of 28 cases in which apoptosis and cell proliferation were studied, the median apoptotic index was 3.4% (range, 0.5 to 9.7; mean, 3.8; SD, 2.5), and the median proliferative index was 8% (range, 0.5 to 23.9; mean, 9.1; SD, 6.2). Table 2 compares the apoptotic and proliferative indexes for tumors grouped according to their expression of p53 and MDM2. Positivity for p53 was negatively associated with the apoptotic index. There was no statistically significant relationship between p53 and cell proliferation. Overexpression of MDM2 was positively associated with the proliferative index, but there was no statistically significant relationship between MDM2 and the apoptotic index.

Interaction of p53 and MDM2 Effects on Apoptosis and Cell Proliferation

When the cases were grouped according to their expression of both MDM2 and p53 (Table 2), there was statistically significant variation among the four groups in the apoptotic index, with lower indexes occurring in the two p53-positive groups, and the highest median apoptotic index in the p53-negative/MDM2-positive group. There was no statistically significant variation in proliferative index among the groups, although the median proliferative index was higher in the two MDM2-positive groups, with the highest median index in the group that was positive for both p53 and MDM2.

DISCUSSION

Although we detected no *p53* mutations in our 118 cases of localized prostate carcinoma, IHC indicated that p53 was abnormally stabilized in 31% of the tumors. Among these tumors, the histologic grade was higher ($P = .03$), the tumor stage was more advanced ($P = .02$), and the apoptotic index was lower ($P = .01$) than among the p53-negative tumors. Overexpression of MDM2 was detected in

TABLE 1. Characteristics of Tumors Grouped by p53 and MDM2 Expression

Characteristic	Group							
	p53+	p53-	MDM2+	MDM2-	p53+/MDM2+	p53-/MDM2+	p53+/MDM2-	p53-/MDM2-
Number of cases (n = 118)	37	81	48	70	16	32	21	49
Gleason score								
Median	7	6	7	6.5	8	6.5	7	6
Range	4-10	4-10	4-10	4-10	4-10	4-10	4-10	4-10
Test statistic	U = 1137.5		U = 1570.5		Kruskal-Wallis $\chi^2_3 = 4.9$			
P value	.03		.54		.18			
Tumor volume ^a								
Median (%)	26	19.5	26	17	38.5	23	18	17
Range (%)	1-90	1-100	2-84	1-100	9-84	2-65	1-90	1-100
Test statistic	U = 1190.5		U = 1076.5		Kruskal-Wallis $\chi^2_3 = 14.0$			
P value	.09		.001		.003			
Tumor stage ^b								
pT2	16 (43)	53 (65)	23 (48)	46 (66)	6 (38)	17 (53)	10 (48)	36 (73)
pT3	21 (57)	28 (35)	25 (52)	24 (34)	10 (62)	15 (47)	11 (52)	13 (27)
Test statistic	$\chi^2_1 = 5.2$		$\chi^2_1 = 3.7$		$\chi^2_3 = 8.8$			
P value	.02		.054		.03			

p53+, p53 detected; p53-, p53 not detected; MDM2+, MDM2 overexpressed; MDM2-, MDM2 not overexpressed; U is the Mann-Whitney statistic.

^a Tumor volume is percentage of prostate gland infiltrated by tumor.

^b Values are number of cases (%). Denominator of percentages is the number of cases in the p53/MDM2 group.

TABLE 2. Apoptotic and Proliferative Indexes of Tumors Grouped by p53 and MDM2 Expression

Index	Group							
	p53+	p53-	MDM2+	MDM2-	p53+/MDM2+	p53-/MDM2+	p53+/MDM2-	p53-/MDM2-
Number of cases (n = 28)	13	15	13	15	5	8	8	7
Apoptotic index								
Median (%)	2.4	4.7	4	2.7	2.9	6.7	2.3	3.9
Test statistic	U = 44.000		U = 62.500		Kruskal-Wallis $\chi^2_3 = 8.5$			
P value	.01		.11		.04			
Proliferative index								
Median (%)	8.6	7.5	10.6	6.4	15.6	9.3	7.3	6.3
Test statistic	U = 76.000		U = 54.500		Kruskal-Wallis $\chi^2_3 = 5.6$			
P value	.34		.046		.13			

p53+, p53 detected; p53-, p53 not detected; MDM2+, MDM2 overexpressed; MDM2-, MDM2 not overexpressed; U is the Mann-Whitney statistic.

41% of the tumors and was positively related to the tumor volume ($P = .001$) and the proliferative index ($P = .046$).

Although the frequency of p53 detection was somewhat higher in our study than in previous reports, in which p53 was detected in 5% to 17% of prostate cancers, our results agreed with these reports that p53 expression is positively related to tumor stage and tumor grade (20, 22). This relationship of p53 to tumor grade was dramatic in another series, in which p53 was expressed in only 4.5% of well-differentiated prostatic carcinomas, but in 80% of poorly differentiated tumors (35). The frequency of MDM2 overexpression in our study was similar to the frequency in other reports that describe MDM2 overexpression in almost one third to almost one half of prostate cancers (36, 37). In a recent report (37), MDM2 overexpression was related to tumor stage, a relationship that was of marginal significance ($P = .054$) in our study.

Our results confirmed other reports that p53 mutations are rare in localized prostate cancer. In several studies, p53 mutations were found in only 2% to 6% of cases (20, 21, 38). A possible explanation for this low frequency of mutation is that in our study and most of the other studies with low p53 mutation rates (20, 38, 39), only exons 5 through 8 were examined. When primers to amplify the region extending from exon 4 through exon 11 were used to study a series of 48 cases of localized and metastatic prostate cancer (40), p53 mutations were found in 77% of the cases, suggesting that exons 4, 9, 10, and 11 ought to be evaluated in addition to exons 5 through 8.

To interpret our results, it is important to realize the situations that can cause a tumor to be p53-positive or p53-negative by IHC. As mentioned before, p53 normally has too short a half-life to be detected by IHC (24). Missense mutations are the most common p53 mutations, and because they

stabilize the p53 protein, they are detected by IHC (24). Nonsense or frame-shift mutations, gene deletions, and mutations that cause truncation of the protein may not be detected by IHC. The IHC method can also detect p53 that has been temporarily stabilized by some mechanism other than mutation (41), such as the action of the product of the *p19arf* gene (42) or the formation of complexes with proteins such as MDM2 (9, 11). Stabilized p53 that is detected by IHC method is inactive and does not function normally in the control of the cell cycle and apoptosis induction (24).

MDM2-positivity by IHC, on the other hand, signifies that a tumor overexpresses MDM2, whether as a result of increased transcription (43), enhanced translation (44), or gene amplification. Amplification is the least common of these mechanisms (43, 45); in one prostate carcinoma series (46), no cases of *MDM2* gene amplification were found.

The relationship we found between p53 and apoptosis appeared to be partially independent of MDM2 expression, suggesting that the ability of p53 to induce apoptosis cannot always be abrogated by MDM2.

The relationship we found between MDM2 overexpression and cell proliferation was interesting. It may explain the relationship between MDM2 overexpression and tumor volume, and it indicates to us that the overexpression of MDM2 has a role in prostate carcinogenesis.

Because MDM2 overexpression had a significant relationship to the proliferative index while p53 positivity did not, it would seem that MDM2 can affect cell proliferation not only by interfering with the p53's control of the cell cycle, but also by some other mechanism. The cell cycle is negatively controlled by the Rb protein as well as by the p53 protein, and MDM2 may interfere with that control by interacting with the Rb protein directly (47) or by inhibiting the ability of transforming growth factor β to maintain the Rb protein in its active form (48). Also, a RING finger domain suggests that MDM2 could interact with rRNA, suggesting possible roles for MDM2 in translation control, RNA transport, or ribosome biogenesis (8).

Whatever the additional mechanism of MDM2 control over the cell cycle may be, it may add to the effect of p53 inactivation. In the four-group analysis, the group in which MDM2 and p53 were positive had a higher median proliferative index than the other groups, although we were not able to show this difference to be statistically significant. This group also had greater tumor volumes ($P = .003$) and more advanced tumor stages ($P = .03$) than the other groups. It may be that the MDM2-positive/p53-positive phenotype identifies an aggressive subset of prostate adenocarcinoma. It has been suggested that the results of IHC investigation

for both proteins, rather than just one, would better reflect the behavior of tumors (49), and we believe that assessment of both p53 and MDM2 by IHC should be part of the evaluation of prostate cancer.

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