Urol Res (1999) 27:169 – 173

ORIGINAL PAPER

Hiroshi Kanamaru · Benyi Li · Yoshiji Miwa
Hironobu Akino · Kenichiro Okada

Immunohistochemical expression of p53 and bcl-2 proteins is not associated with sarcomatoid change in renal cell carcinoma

Received: 8 June 1998 / Accepted: 14 August 1998

Abstract An immunhistochemical study was conducted to examine the expression of p53 and bcl-2 proteins in RCC (renal cell carcinoma) with sarcomatoid change in order to determine whether abnormalities in those proteins are associated with an enhanced malignant potential of RCC. Paraffin-embedded tissues from 11 patients with RCC, in which sarcomatoid change was prominent, were stained using anti-p53, bcl-2 and Ki-67 antibodies. Immunoreactivities for these antibodies were compared between the sarcomatoid components and corresponding basic histologic (clear or papillary) components in individual cases. Measurement of the mean nuclear areas of each component was also performed using an image analyzer system. There was no substantial increase in immunoreactivity for p53 or bcl-2 proteins in sarcomatoid components as compared with basic components. In contrast, the percentage of Ki-67-positive cells and the mean nuclear area were signficantly larger in sarcomatous components than in basic components. The expression of p53 and bcl-2 proteins was not likely to play a major role in the sarcomatoid change of RCC.

Key words Renal cell carcinoma · Sarcomatoid · p53 · bcl-2

Introduction

Recent cytogenetic and molecular genetic studies have elucidated several specific genetic changes that are associated with the development of renal cell carcinomas (RCC) [10, 12]. However, relatively little is known of the mechanisms which induce the progression of disease in patients with RCC. In a previous study on the tumor-size-related histologic change of RCC, we demonstrated that the presence of sarcomatoid histology was strongly correlated with tumor growth as well as with a poor prognosis for the patients [8]. We therefore hypothesized that as the tumor grows larger, more genetic changes occur which result in the development of invasive histologic phenotypes such as sarcomatoid histology. The present study was conducted to examine the expression of p53 and bcl-2 proteins in sarcomatoid RCC in order to determine whether abnormalities in these proteins, which are considered to regulate cell cycle or apoptosis of cells, are associated with the sarcomatoid change of RCC.

H. Kanamaru · B. Li · Y. Miwa · H. Akino · K. Okada
Department of Urology, Fukui Medical University,
Matsuoka, Fukui, 910-1193, Japan

Materials and methods

Patients and tumor samples

Tumor specimens of 85 RCC cases, which were obtained by nephrectomy at Fukui Medical University Hospital from 1985 to 1995, were histologically examined for the presence of a sarcomatoid component, and 11 tumor samples in which sarcomatoid change was prominent were selected for the following immunohistochemical and morphometric studies.

Immunohistochemistry

The following three primary antibodies were used in this study: anti-p53 antibody (DO-1, 1:60, Calbiochem, Cambridge, UK), anti-bcl-2 antibody (clone 100/D5, 1:50, Novocastra Laboratories, New Castle, UK) and anti-Ki-67 antibody (MIB-1, 1:60, Immunotech, Marseille, France). The paraffin-embedded tissue sections, in which the co-existence of sarcomatoid and basic (clear cell or papillary) components had been microscopically confirmed by hematoxylin-eosin staining, were subjected to immunostaining using a streptavidin-peroxidase method (Dako-LSAB kit, Dako, Carpinteria, USA). Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol. Antigen retrieval procedure was done by heating the sections in citrate buffer (pH 6.0) in an autoclave at 120°C for 20 min. After cooling to room temperature, all the sections were routinely rinsed in 2% milk solution for 20 min followed by blocking agent (Dako-LSAB kit) for 15 min. at 37°C. The sections were then incubated with the primary antibodies at 4°C overnight, followed by a second biotinylated antibody and the ABC complex (Dako-LSAB kit) for 40 min. Careful rinses were done with several changes of PBS (pH 7.6) between each step of the

procedure in all immunostaining. The color was developed with 3′3-diaminobenzidine tetrahydrochloride (DAB) as chromogen; after that, the sections were lightly counterstained with hematoxylin. Negative controls were carried out by replacing the primary antibody with nonimmune immunoglobulins. Bladder cancer specimens known for p53 or bcl-2 imunoreactivities were used as positive controls. The immunoreactivities for p53, bcl-2 and Ki-67 proteins were microscopically evaluated, and the number of stained cells were scored and given as a percentage of all cells counted.

Nuclear morphometry

The morphometric analysis with an image analyzer system was performed as described by Tosi et al. [21]. The microscopic images of the hematoxylin-eosin stained tumor lesions were transported to a video screen through a video camera (ICD-740, Olympus, Japan) attached to the microscope. The boundaries of the nuclei were outlined, and the mean nuclear areas of basic and sarcomatoid components were automatically calculated using the Mac SCOPE computer software (Mitani Corporation, Japan).

Statistical analysis

The average values of the percentage of positive cells and the mean nuclear area were compared between sarcomatoid components and corresponding basic components by Student's paired *t*-test.

Results

Demographics and clinical outcome of the patients

The clinicopathological characteristics and outcomes of the 11 patients are summarized in Table 1. Nine of the 11 patients died of disease progression, while the other two were alive with metastatic disease at the time of investigation.

Expression of p53 protein

Positive p53 stainings of basic histologic components and sarcomatoid components were observed in 2 (18%) and 3 (27%) out of 11 RCC patients, respectively (Table 2). However, the percentage of tumor cells expressing p53 protein was generally small (less than 10%) in both components, except for one case in which more than 20% of cells in the sarcomatoid component were positive for p53 (Fig. 1). As a whole, there was no significant increase in the percentage of p53-positive cells in sarcomatoid components when compared with corresponding basic components (2.2 ± 6.3% and 0.2 ± 0.4%, respectively).

Expression of bcl-2 protein

Positive bcl-2 stainings of basic and sarcomatoid components were observed in two (18%) and one (9%) cases, respectively (Table 2, Fig. 2). There was no significant increase in the percentage of bcl-2-positive cells in sarcomatoid components when compared with corresponding basic components (0.3 ± 1.1% and 1.6 ± 3.6%, respectively).

Expression of Ki-67 antigen

The percentage of Ki-67-positive cells in sarcomatoid components was higher than that in basic components in

Table 2 Results of immunohistochemistry for p53 and bcl-2

Patient	Histologic component	% positive cells	
		p53	bcl-2
1	Clear cell	0	0
	sarcomatoid	0	0
2	Clear cell	0	0
	sarcomatoid	0	0
3	Clear cell	0	0
	sarcomatoid	0	0
4	Clear cell	0	0
	sarcomatoid	0	0
5	Clear cell	0	0
	sarcomatoid	0	0
6	Clear cell	0	0
	sarcomatoid	2.1	0
7	Clear cell	0	0
	sarcomatoid	0	0
8	Papillary	0.7	0
	sarcomatoid	21.2	0
9	Papillary	0	8.6
	sarcomatoid	0	0
10	Papillary	1.1	0
	sarcomatoid	1.2	0
11	Papillary	0	9.3
	sarcomatoid	0	3.7

Table 1 Clinicopathological features and outcomes of the patients with sarcomatoid RCC

Patient	Age	Gender	Stage	Basic histologic component	Outcome (months after surgery)
1	61	M	T2N0M0	Clear cell	Alive with metastasis (32)
2	75	M	T3N0M1	Clear cell	Alive with metastasis (24)
3	79	F	T3N1M1	Clear cell	Cancer death (6)
4	71	M	T3N0M1	Clear cell	Cancer death (19)
5	70	F	T3N2M0	Clear cell	Cancer death (22)
6	38	F	T4N0M1	Clear cell	Cancer death (1)
7	70	M	T3N0M0	Clear cell	Cancer death (12)
8	68	F	T4N0M0	Papillary	Cancer death (6)
9	45	M	T3N0M1	Papillary	Cancer death (21)
10	66	F	T3N0M0	Papillary	Cancer death (17)
11	65	M	T3N0M1	Papillary	Cancer death (36)

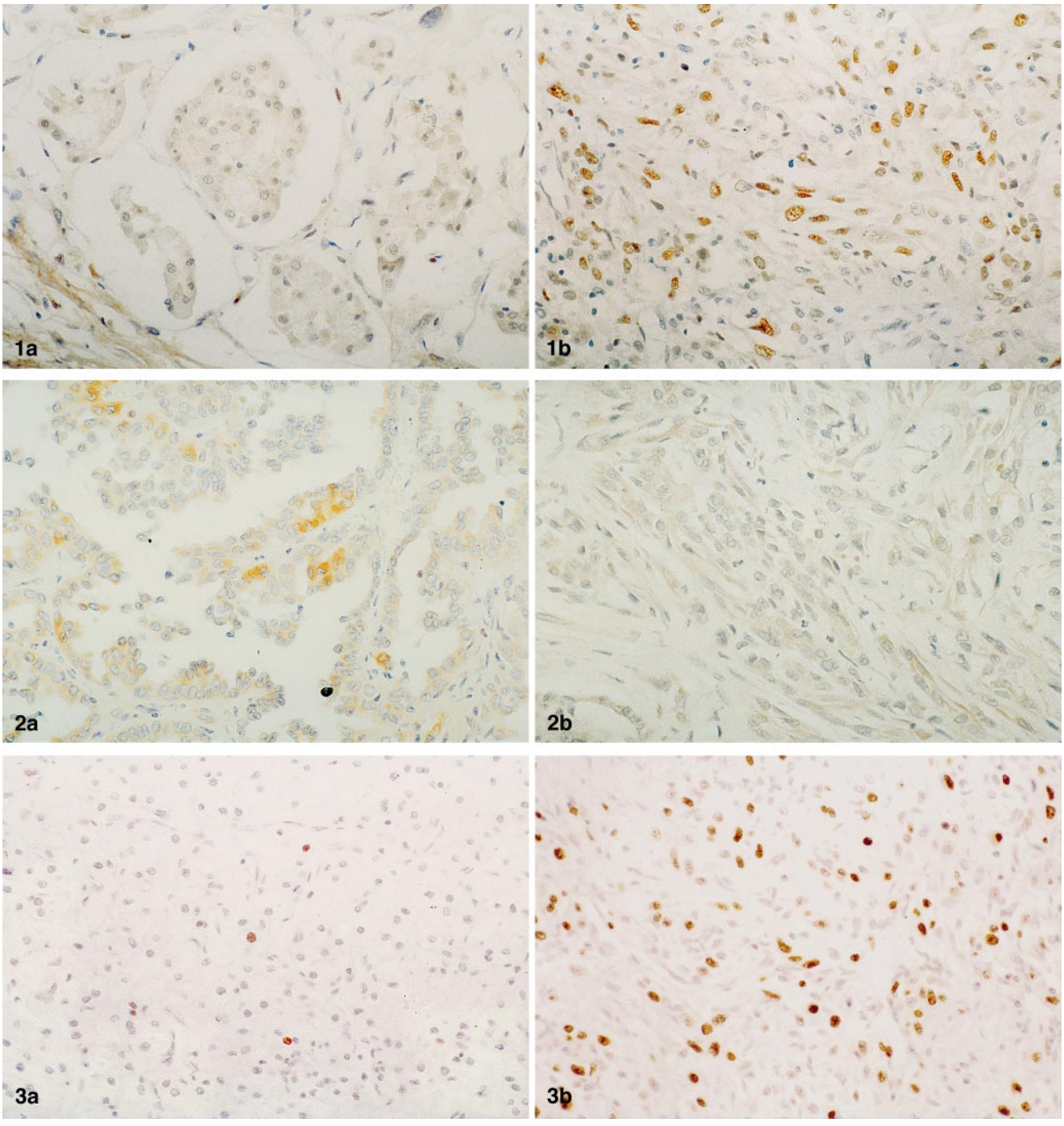

Fig. 1 Immunoreactivities for p53 protein. In patient 8, the staining was (**a**) almost negative in the papillary component and (**b**) positive in the sarcomatoid component

Fig. 2 Immunoreactivities for bcl-2 protein. In patient 9, the staining was (**a**) positive in a small percentage of cells of the papillary component and (**b**) negative in the sarcomatoid component

Fig. 3 Immunoreactivities for Ki-67 protein. In patient 5, a larger number of cells were stained in the sarcomatoid component (**b**) than in the clear cell component (**a**)

all the cases, and the difference was statistically significant ($P = 0.0003$) (Figs. 3, 4).

Nuclear morphometry

The mean nuclear area of the sarcomatoid component was larger than the corresponding basic component in all the cases (Fig. 5). The average value of the mean

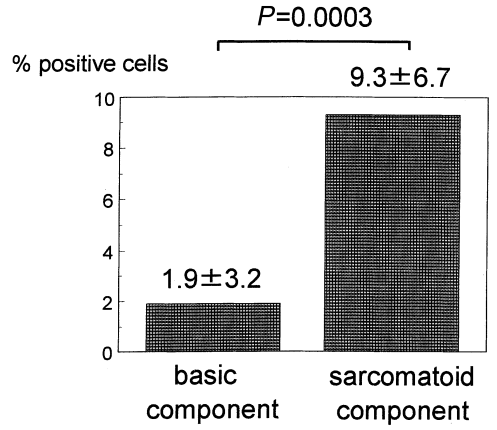

Fig. 4 The percentage of Ki-67-positive cells in the sarcomatoid components was significantly higher than in basic components

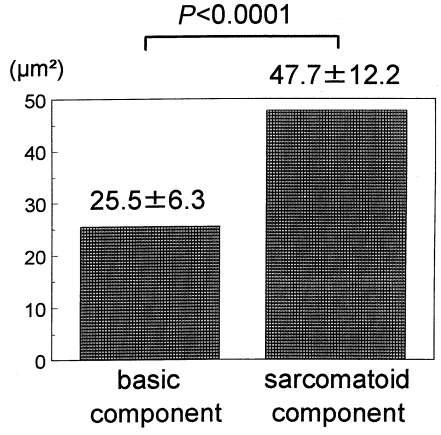

Fig. 5 The mean nuclear area of the sarcomatoid components was significantly larger than the basic components

nuclear areas for the sarcomatoid components was significantly larger than that for the basic components, ($P < 0.0001$).

Discussion

It has been well recognized that sarcomatoid renal carcinoma is a highly malignant tumor with an extremely poor prognosis [17]. There is now a consensus that sarcomatoid histology is not a distinct histologic type, but a manifestation of high grade carcinoma which can arise in all types of RCC [18]. It seems, therefore, to be very important to search for genetic alterations associated with the sarcomatoid change in order to clarify the mechanisms of disease progression in RCC.

As far as the authors are aware, there have been only a few studies focusing on the genetic changes related to sarcomatoid histology of RCC. Oda et al. [15] reported that a high mutation rate for the p53 gene (11/14, 79%) was observed in sarcomatoid portion as compared with a low mutation rate (2/14,14%) in carcinomatous portions of the same tumors. In a case report of sarcomatoid RCC, Dijkhuizen et al. [4] performed cytogenetic and molecular genetic studies using tumor tissue, and found a p53 gene mutation. On the other hand, Contractor et al. [3] could not find any p53 mutations in all five sarcomatoid RCC examined.

P53 is known as a tumor-supressor gene, and a mutation of the p53 gene has been considered to play a major role in many kind of cancers [6]. However, the overall mutation rates for the p53 gene in RCC have been reported to be generally low [2, 9, 19, 20], and the role of p53 gene alteration in sarcomatoid RCC remains unclear. In the present study, we therefore evaluated the role of p53 and another apotosis-related protein, bcl-2, in relation to the sarcomatoid change of RCC using an immunohistochemical approach. Tumor specimens from 11 patients, in which sarcomatoid change was prominent, were selected for the present study. The poor clinical outcomes of all the cases suggested that the tumors used in the present analysis were of a highly malignant character.

Our immunohistochemical study for p53 and bcl-2 proteins demonstrated that the percentage of positively stained cases as well the percentage of positive cells in individual cases were not substantially increased in sarcomatoid components as compared with the basic components. On the other hand, an analysis using a proliferation marker (Ki-67) and a morphometric parameter (mean nuclear area), both of which have been recognized to be reliable prognostic factors in RCC [5], showed a higher proliferative index and an increased nuclear area of sarcomatoid components than basic components, indicating an increase in invasive nature associated with sarcomatoid change.

These results are in contrast to the previous report by Oda et al. [15], in which the immunoreactivity for p53 protein using DO-7 antibody was higher in sarcomatoid compornents than in carcinomatous components. One possible explanation for the differing results is that DO-1 antibody, which we used, could not detect an overexpression of p53 effectively in RCC. However, such an explanation seems rather unlikely, since DO-1 as well as DO-7 have been widely used to recognize both wild-type and mutant p53 proteins [23]. Furthermore, a high rate of positive staining by DO-1 antibody could be obtained when we performed the same immunohistochemical procedure to detect p53 overexpression in bladder cancers, in which p53 mutation has been reported to occur frequently [13]. Further studies using a larger number of sarcomatoid RCC cases are required to clarify the discrepancy between the two studies.

According to the previous immunohistochemical studies on RCC not specified for sarcomatoid histology, the overall positive-staining rate for p53 protein varied widely from 3% to 51% [1, 2, 7, 11, 14, 16, 20, 22], and that for bcl-2 protein varied from 6% to 68% [7, 14, 16, 20], although the criteria of positivity and antibodies used were not consistent among these studies. There is also no agreement on the clinical usefulness of the two

apotosis-related markers as a prognostic indicator of RCC [1, 5, 7, 11, 14]. However, when looking at the present results, that no substantial overexpression of p53 and bcl-2 proteins was detected in either sarcomatoid or basic components of selected RCC cases with poor prognosis, the immunoreactivity for p53 and bcl-2 proteins seems not to be a good prognostic indicator of RCC.

In conclusion, the present study demonstrated that although the sarcomatoid histology had a higher proliferative index and larger mean nuclear area, the overexpression of p53 and bcl-2 proteins was not related to the sarcomatoid change of RCC, suggesting the possibility that other mechanisms with unknown genetic alterations might be responsible for it.

References

1. Bot FJ, Godschlk JCJ, Krishnadath KK, Kwast THMVD, Bosman FT (1994) Prognostic factors in renal cell carcinoma: immunohistochemical detection of p53 protein versus clinicopathological parameters. Int J Cancer 57:634
2. Chemeris G, Loktinov A, Rempel A, Schwarz M, Bannasch P (1995) Elevated content of p53 protein in the absence of p53 gene mutation as a possible prognostic marker of human renal cell tumors. Virchows Arch 426:563
3. Contractor H, Zariwala M, Bugert P, Zeisler J, Kovacs G (1997) Mutation of the p53 tumour supressor gene occurs preferentialy in the chromophobe type of renal cell tumor. J Pathol 181:136
4. Dijkhuuizen T, Berg EVD, Berg AVD, Veen AVD, Dam A, Faber H Buys CHCM, Storkel S, Jong BD (1997) Genetics as a diagnostic tool in sarcomatoid renal cell cancer. Int J Cancer 72:265
5. Gelb AB (1997) Renal cell carcinoma: current prognostic factors. Cancer 80:981
6. Greenblatt MS, Bennett WP, Holstein M, Harris CC (1994) Mutations in the p53 tumor suppressor gene: clue to cancer etiology and molecular pathogenesis. Cancer Res 54:4855
7. Hofmockel G, Wittmann A, Dammrich J, Bassukas ID (1996) Expression of p53 and bcl-2 in primary locally confined renal cell carcinomas: no evidence for prognostic significance. Anticancer Res 16:3087
8. Kanamaru H, Muranaka K, Mori H, Akino H, Arai Y, Okada K (1996) Analysis of histological heterogeneity in renal cell carcinoma: tumor size-related histological change and its prognostic significance. Int J Urol 3:256
9. Kikuchi Y, Kishi T, Suzuki M, Furusato M, Aizawa S (1994) Polymerase chain reaction-single strand conformation polymorphism analysis of the p53 gene in paraffin-embedded surgical material from human cell carcinomas. Virchows Arch 424:229
10. Kovacs G, Szuecs S, Riese WD, Baumgaertel H (1987) Specific chromosome aberration in human renal cell carcinoma. Int J Cancer 40:171
11. Kuczyk MA, Serth J, Bokemeyer C, Jonassen J, Arndt H, Paeslack U, Werner M, Tan H-K, Jonas U (1995) Detection of p53 alteration in renal cell cancer by micropreparation techniques of tumor specimens. Int J Cancer 64:399
12. Latif F, Tony K, Gnarra J, et al. (1993) Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 260:1317
13. Li B, Kanamaru H, Noriki S, Fukuda M, Okada K (1998) Reciprocal expression of bcl-2 and p53 oncoproteins in urothelial dysplasia and carcinoma of the urinary bladder. Urol Res 26:235
14. Lipponen P, Eskelinen M, Syrjaenen K (1995) Expression of tumour-suppressor gene Rb, apotosis-suppressing protein bcl-2 and c-myc have no independent prognostic value in renal adenocarcinoma. Br J Cancer 71:863
15. Oda H, Nakatsuru Y, Ishikawa T (1995) Mutations of the p53 gene and p53 protein overexpression are associated with sarcomatoid change in renal cell carcinomas. Cancer Res 55:658
16. Paraf F, Gogusev J, Chretien Y, Droz D (1995) Expression of bcl-2 oncoprotein in renal cell tumors. J Pathol 177:247
17. Ro JY, Ayala AG, Sella A, Samuels ML, Swanson DA (1987) Sarcomatoid renal cell carcinoma: clinicopathological study of 42 cases. Cancer 59:516
18. Storkel S, Eble JN, Adlakha K, Amin M, Blute ML, Bostwick DG, Darson M, Delahunt B, Iczkowski K (1997) Classification of renal cell carcinoma. Cancer 80:987
19. Suzuki Y, Tamura G, Satodate R, Fujioka T (1992) Infrequent mutation of p53 gene in human renal cell carcinoma detected by polymerase chain reaction single-strand conformation polymorphism analysis. Jpn J Cancer Res 83:233
20. Tomita Y, Bilim V, Kawasaki T, Takahashi K, Okan I, Magnusson KP, Wiman KG (1996) Frequent expression of bcl-2 in renal cell carcinomas carryng wild-type p53. Int J cancer 66:322
21. Tosi P,Luzi P, Baak JPA, Miracco C, Santopietro R, Vindigni C, Mattei FM, Acconcia A, Massai MR (1986) Nuclear morphometry as an important prognostic factor in stage I renal cell carcinoma. Cancer 58:2512
22. Uhlman DL, Nguyen PL, Manivel JC, Aeppli D, Resnick JM, Fraley EE, Zhang G, Niehans GA (1994) Association of immunohistochemical staining for p53 with metastatic progression and poor survival in patients with renal cell carcinoma. J Natl Cancer Inst 86:1470
23. Wertz IE, Deitch AD, Gumerlock PH, Gandour-Edwards R, Chi S-G, White RWDV (1996) Correlation of genetic and immunodetection of TP53 mutations in malignant and benign prostate tissues. Hum Pathol 27:573