

## TP53 Mutation in Prostate Needle Biopsies – Comparison with Patients Follow-up

THORSTEN H. ECKE<sup>1\*</sup>, HORST H. SCHLECHTE<sup>2\*</sup>, ANNE HÜBSCH<sup>2</sup>, SEVERIN V. LENK<sup>2</sup>,  
KATRIN SCHIEMENZ<sup>2</sup>, BIRGIT D. RUDOLPH<sup>3</sup> and KURT MILLER<sup>2</sup>

<sup>1</sup>HELIOS Hospital, Department of Urology, Bad Saarow;

<sup>2</sup>Department of Urology and

<sup>3</sup>Institute of Pathology, Universitätsmedizin Berlin, Charité, Berlin, Germany

**Abstract.** *Background:* The predictive value of TP53 mutations and prostate-specific antigen (PSA) was assessed in prostate needle biopsies of samples without signs of malignancy for later affliction by prostate cancer (PCa). Comparison of mutation frequency and PSA level in prostate needle biopsies with data of patients with benign prostate hyperplasia (BPH) treated by transurethral resection (TURP), patients with prostatic intraepithelial neoplasia (PIN), and patients with PCa, was made. *Materials and Methods:* A total of 466 samples were analysed from patients with benign and malignant diseases of the prostate, including 52 samples of needle biopsies of the prostate with repeated benign histopathological diagnosis. Analysis of TP53 state by temperature gradient gel electrophoresis of TP53 exon-specific PCR products of exons 5, 6, 7 and 8 was performed. Clinical follow-up of 100 patients with benign diseases of the prostate and with PIN was available. *Results:* Needle biopsy samples with repeated benign diagnosis resemble BPH specimens taken by TURP in TP53 mutation frequency (TURP: 16.1%, needle biopsy: 21.2%) and later affliction by PCa (TURP: 3/32 = 9.4%, needle biopsy: 8/51 = 15.7%,  $p=0.409$ ). Patients with TP53 mutations in needle biopsy samples showed a significantly reduced disease-free survival time for affliction by PCa (log rank:  $p=0.0149$ ). This significance is raised by computing exon 6 mutations only with respect to affection by PCa ( $p=0.0002$ ). In TURP patients, exon 7-mutations were also significant ( $p=0.0086$ ). Needle biopsy

TP53 mutations ( $p=0.029$ ) had significant predictive values for later affliction by PCa in multivariate analysis. PSA level and patient age had no predictive value for PCa. *Conclusion:* TP53 mutations reduce the PCa-free survival time in patients with needle biopsy of the prostate and primary benign diagnosis. Exon 6 mutations enhance the risk of being affected by PCa 32-fold.

TP53 mutations are present in tumour tissues with varying frequencies (1). In prostate cancer (PCa) a moderate TP53 mutation frequency in the range of 30% without evident relationship to tumour grade or stage has been reported (2). This may be an indication of an early stage of mutagenesis during tumorigenesis of the prostate. TP53 plays a role as cell cycle regulator and in the stress response. Its contribution in case of mutation to the development of PCa is questioned (3). However, in PCa tissue, the overexpression of the p53 gene product is a risk factor for tumour progression (4, 5). Contribution of TP53 mutation to progression of PCa has been discussed (6-8). Berner *et al.* have discussed a TP53 exon 8 mutation in codon 273 as a hot spot for tumour progression of PCa (9). In isolated benign cells located near foci of high-grade adenocarcinoma of the prostate one mutation of exon 7 was detected in 20 specimens (6). Navone *et al.* (1999) found two exon 7 mutations in PCa tissue and also in lymph nodes (8).

In patients with benign prostate hyperplasia (BPH), TP53 mutations are detectable in approximately 19.0% (10). We have analyzed a high proportion of silent mutations in benign prostate tissue samples (recent data: 19/35=54.3%). It is questioned whether TP53 mutations in benign prostate disease contribute to the incidence of PCa. BPH is not considered as a precancerous disease (11), and BPH is treated by transurethral resection (TURP). In the Charité Hospital the prevalence of PCa is 6.6% (10 out of 151) in the case of TURP treatment of patients suspected for BPH.

\*Both authors contributed equally to this work.

*Correspondence to:* Dr. Thorsten H. Ecke, HELIOS Hospital, Department of Urology, Pieskower Strasse 33, D-15526 Bad Saarow, Germany. Tel: +493363173170, Fax: +493363173136, e-mail: tho\_ecke@hotmail.com

*Key Words:* Needle biopsy, benign, prostate, cancer, TP53, mutation, PSA, follow-up.

A needle biopsy is carried out in patients suspected for PCa. The incidence of PCa determined from needle biopsies of patients without any history of PCa is 28.4% (19 out of 67) at the Charité Hospital. A high preoperative serum PSA level one of the main risk factors for PCa in needle biopsies (12). It is known that the correlation between the outcome of needle rebiopsy and PSA is insignificant, even if the serum PSA level is less than 4 ng/ml (13, 14).

In this study, we screened needle biopsy samples of patients with repeated benign diagnosis. The advantage of using such samples consists of better conditions for outpatient follow-up in comparison with TURP patients. The predictivity of *TP53* mutations in benign prostatic tissue for later affliction by PCa was evaluated. The aim of this study was to investigate if *TP53* mutations could reduce the PCa-free survival time in patients with needle biopsy of the prostate, and if *TP53* mutations could enhance the risk of being affected by PCa.

**Materials and Methods**

Analytical data and summarized follow-up results were available for 466 patients. The patients were sampled in groups according to disease:

i) *BPH group*. A total of 218 tissue samples of benign prostate hyperplasia, taken by transurethral resection (TURP), were available, in five cases by adenectomy. Thirty-two patients of this group were followed up for 34 months, including seven patients with *TP53* mutations for 38 months (range 3-64) months, and 25 patients with wild-type *TP53* for 32 months (range 2-97 months).

ii) *Needle-benign group (NB)*. Samples were taken from 1994 to 1999 at the Outpatient Clinic Department of Urology of the Charité Hospital by ultrasound-controlled prostate needle biopsy of 52 patients suspected for PCa, but without signs of malignant disease in their histopathological examination of all needle cores. Forty-four of these patients had a repeated benign diagnosis 6-58 months after first examination and *TP53* analysis. The remaining seven patients had already been diagnosed using TURP for BPH or with needle biopsy without PCa in the past (1-108 months). Fifty-one patients of this group were followed up for 31 months, including eleven patients with *TP53* mutations for 29 months (range 14-62), and 40 patients with wild-type *TP53* for 32 months (range 2-82 months).

iii) *PIN group*. This group comprised samples with histopathological signs of intraepithelial neoplasia of the prostate (21 cases) or with atypic adenomatous hyperplasia (two cases) but without signs of PCa. Eighteen samples were taken by needle biopsy, five by TURP. Seventeen patients of this group were followed for 20 months, including seven patients with *TP53* mutations for 22 months (range 4-40), and ten patients with wild-type *TP53* for 20 months (range 6-35 months).

iv) *Needle-PCa group (NPCa)*. This group composed 50 prostate tissue samples taken by needle biopsy of patients suspected for PCa. Histopathological diagnosis confirmed malignancy in at least one core.

v) *Surgery-PCa group (SPCa)*. A total of 123 PCa tissue samples of first surgical treatment made up this group.

Table I. Statistical comparison of *TP53* mutation frequencies.

	<i>p</i> -value <sup>1</sup>
BPH 36/218=16.5% vs. NB 11/52=21.2%	0.428
BPH 36/218=16.5% vs. SPCa 38/123=30.9%	0.002
NB 11/52=21.2% vs. NPCa 17/50=34.0%	0.146
NB 11/52=21.2% vs. PIN 9/23=39.1%	0.105
NB 11/52=21.2% vs. SPCa 38/123=30.9%:	0.190

<sup>1</sup>Pearson's Chi-square test (asymptotic significance), patients with *TP53* mutations / all patients of indicated groups.

Only two samples without any sign of prostate disease were available for analysis. These samples had wild-type *TP53*, but PSA analysis results were not available. Therefore comparison with PSA or mutation analysis data of other patients was not possible, and these patients were excluded from further examination.

PSA level data were taken from medical laboratory results (Immulte procedure) of the Charité Hospital.

*TP53* mutation analysis was carried out by temperature gradient gel electrophoresis (TGGE) screening of mobility shifts of GC-clamped PCR products of *TP53* exons 5, 6, 7, and 8, in single reactions as described elsewhere (10). Sequence analysis was performed from TGGE extracted lanes by reamplification and solid-phase sequencing reaction (2).

Statistical analyses were carried out using SPSS 12.0.1. A type I error level of *p*=0.05 was used for all statistical tests. Cox regression was performed using the "Forward Conditional" method.

**Results**

*TP53 mutation analysis*. Samples of needle biopsy in the NB group contained mutations in 21.2% of cases. *TP53* mutations were found in exon 5 (4 pts), exon 6 (3 pts), in exon 7 (5 pts), but not in exon 8. One sample (patient 63 years old, PSA 9.71 ng/ml, follow-up 42 months) contained mutations in exon 5 and in exon 7. The mutation frequency of the NB was compared with those of other patient groups. The results of this computation by Chi-square test showed no significant difference against any other patient groups. This is outlined in Table I. Significant differences in *TP53* mutation frequency between BPH (16.5%) and SPCa (30.9%) groups are reported in Table I (*p*=0.001). BPH exon 6 contained more mutations than other exons. But exon-specific differences of mutation frequencies between groups were not significant. All other patient groups have most mutations in exon 7.

Result of the sequence analysis of patient 1909 is given in Figure 1, showing a silent mutation in codon 213 of exon 6 at the time of benign histopathological diagnosis of the needle biopsy.

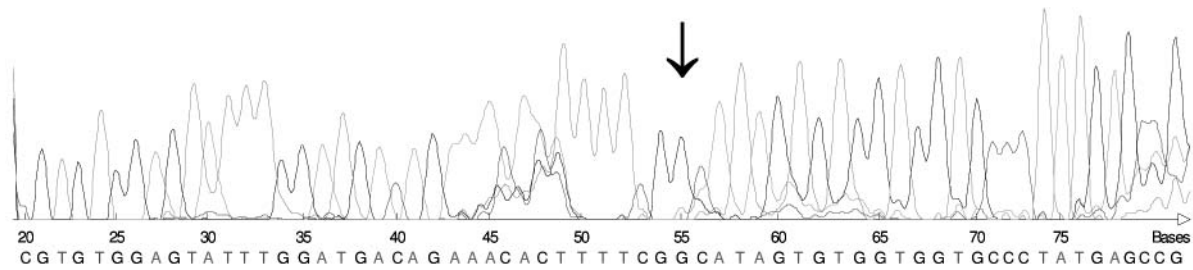


Figure 1. BPH patient No.1909: Sequence analysis of TP53 exon 6, region of codons 202-222. Mutation A  $\Rightarrow$ G (arrow) in map position 13399 in third position of codon 213 Arg-silent.

**PSA level.** Metric values of PSA are never normally distributed in any patient group. Median values are indicated as followed: NB (7 ng/ml), BPH (3.7 ng/ml), PIN (9.9 ng/ml), NPCa (11.55 ng/ml) and SPCa (8.65 ng/ml). In the NB, BPH and PIN groups, the PSA median levels were lower in patients with wild-type TP53 in their prostate tissue compared to those of the same group found to have mutated TP53. This tendency was reversed in the NPCa and SPCa groups. However, any significance of this different tendency of PSA in comparison with TP53 state was demonstrated only in the NPCa group ( $p=0.018$ : Table II).

**Clinical follow-up.** One patient (63 years old, PSA 9.71 ng/ml) of the NB group showed a TP53 mutation in exon 5 and in exon 7. However, this patient had no sign of malignancy after 42 months follow-up. One patient (89 years old, PSA 146.90 ng/ml) with wild-type TP53 in their needle biopsy tissue was followed up for 2 months only, but without affliction by PCa.

In all groups and subgroups with benign disease some patients were diagnosed with PCa later. In the BPH group two out of 17 patients followed (2 out of 4 with TP53 mutation, 0 out of 13 with wild-type TP53) were later affected by PCa. In the NB group 7 out of 51 (5 out of 11 with TP53 mutation, 2 out of 40 with wild-type TP53) were diagnosed with PCa during the follow-up. In the PIN group 4 out of 16 (2 out of 7 with TP53 mutation, 2 out of 9 with wild-type TP53) were affected by PCa at a later time. Data of all patients later affected by PCa are given in Table III. The effect of PCa during follow-up was computed by the Kaplan-Meier method. Results of computation are shown in Figure 2. Results of given statistical tests for the Kaplan-Meier method show significantly reduced tumour-free survival of patients with TP53 mutation in the NB group, but not of all in the BPH or PIN groups (Figure 2 a-c). In the case of available computation of the mutation status of single TP53 exons by the Kaplan-Meier method, significance was found for exon 6 and exon 7. Exon 7 results in better statistical

Table II. Statistical comparison of median PSA levels (ng/ml) between subgroups of patients with prostatic TP53<sup>WT</sup> against TP53<sup>Mutated</sup> in indicated patient groups.

	TP53 <sup>WT</sup>		TP53 <sup>Mut</sup>	<i>p</i> -value <sup>1</sup>
BPH	3.68	vs.	5.80	0.486
NB	6.90	vs.	8.80	0.179
PIN	9.65	vs.	9.90	0.777
NPCa	15.60	vs.	8.03	0.018
SPCa	9.49	vs.	6.80	0.358
NPCa + SPCa	10.18	vs.	7.35	0.051

<sup>1</sup>Mann-Whitney *U*-Test (asymptotic significance), median PSA level is given in parentheses.

significance in comparison with the summarized TP53 status of exons 5-8. These statistical results are shown in Figure 2 d-f, indicating significance for exon 7 in the BPH group, too. Cox regression was carried out to analyze the impact of the factors TP53-mutation, PSA-level and patient age on later affliction by PCa in the NB group. The output of the beginning block 0 (univariate analysis) by the “Forward Conditional” method shows a significance for TP53 ( $p=0.002$ ), but no significance for PSA ( $p=0.371$ ) or for age ( $p=0.658$ ). The same tendency results from computation of the effect of exon 6, and exon 7 (Table III). In block 1 (multivariate analysis) of same computations of Cox regression, significance is given with  $p=0.009$  for TP53 only,  $p=0.012$  for exon 6, and  $p=0.001$  for exon 7, respectively. This is outlined in Table IV. In the case of a one-step categorical factor, as mutation and significance for this factor, the head Exp(B) in Table IV refers to the increased probability of developing PCa. Thus Exp(B) is 15.141 for exon 7 mutations, and 8.956 for any TP53-mutation.

The difference between affliction by PCa in patients with primary benign disease (BPH+NB groups: 9/68=13.2%) versus PIN (4/16=25.0%) is not significant (Chi-square test:  $p=0.242$ ) with our data.

Table III. Follow-up of patients later affected by prostate cancer (PCa). TP53-status: - = wild-type in indicated exon, x = mutation in indicated exon.

Patient groups	Patient code	Age (Years)	PSA (ng/ml)	TP53-status (exon)				Affection of PCa (months)	Tumour classification	
				5	6	7	8		T/G	Gleason score
NB	557	67	19.10	-	-	x	-	8	T2G1	n.d.
	1475	66	9.37	-	-	x	-	23	T2aG1	1 + 1
	1909	73	6.90	-	x	-	-	11	T1cG1b	2 + 2
	1947	68	5.00	-	x	-	-	32	T2G3a	4 + 5
	406	73	5.55	-	-	-	-	83	T2a	2 + 3
	457	61	3.57	-	-	-	-	58	T2G2	n.d.
	929	69	16.65	-	-	-	-	26	T2G2	n.d.
	1722	64	7.30	-	-	-	-	57	T2aG2	2 + 3
BPH	13	75	n.d.	-	-	x	-	18	T1G3	n.d.
	705	76	10.50	-	-	x	-	40	T2aG3	n.d.
	1009	60	1.30	-	-	-	-	83	T3aG3	4 + 5
PIN	36	79	9.90	-	x	-	-	30	T2cG2a	n.d.
	509	60	8.09	x	-	-	-	60	deceased	
	963	76	25.22	-	-	-	-	28	T2aG3	n.d.
	1328	72	15.20	-	-	-	-	16	G3	4 + 4

n.d. = analysis not available. PIN: The four patients of this group had the primary diagnose PIN, i.e. no AAH-patient is known to be later affected by PCa. Age, PSA and TP53-status at time of primary benign diagnosis.

Table IV. Cox regression with method "Forward Conditional" of data of group NB. Exp(B) is taken from the result of "variables in the equation" of computation block 1. Variables TP53, Exon 6, Exon 7, are categorical. Variables PSA, patients age, are numerical.

Computation number	Variables	Block 0 beginning block significance	Block 1 significance	Exp(B)	95.0% Confidence interval for Exp(B)	
					Lower	Upper
1	TP53	0.015	0.029	5.309	1.181	23.864
	PSA	0.162				
	Age	0.728				
2	Exon 6	0.000	0.003	32.646	3.371	316.162
	PSA	0.162				
	Age	0.728				
3	Exon 7	0.013	0.033	7.049	1.172	42.403
	PSA	0.162				
	Age	0.728				

**Discussion**

Assessment of p53 and PSA in combination for the outcome in prostate diseases, potentially offering improved prediction, has not yet been performed. In this study of 52 patients, using multivariate analysis to evaluate the predictive value of TP53 mutations and PSA level, TP53 was strongly predictive of PCa in patients who had a needle biopsy and a diagnosis of primary benign disease. PSA was not a significant factor for PCa affection in univariate analysis either.

In breast cancer patients a combination analysis of p53 expression and PSA in serum was done by Yu *et al.* in 1999

(15). It was shown that PSA(-)/p53(+) patients have a significantly higher risk for tumour relapse and death than PSA(+)/p53(-) patients. The main result of the analysis was the demonstration of the advantage of combined PSA and p53 expression status.

Poor prognosis is a well known phenomenon in tumour patients with TP53 mutations in their malignant cells (16-23). This is confirmed by our results for patients with benign prostate disease. The reason for the high significance of exon 7 mutations for this predictive value cannot be answered with our data. Exon 7 contains the region for the L3-loop of p53 (codons 236-251), partly responsible for DNA-binding by p53

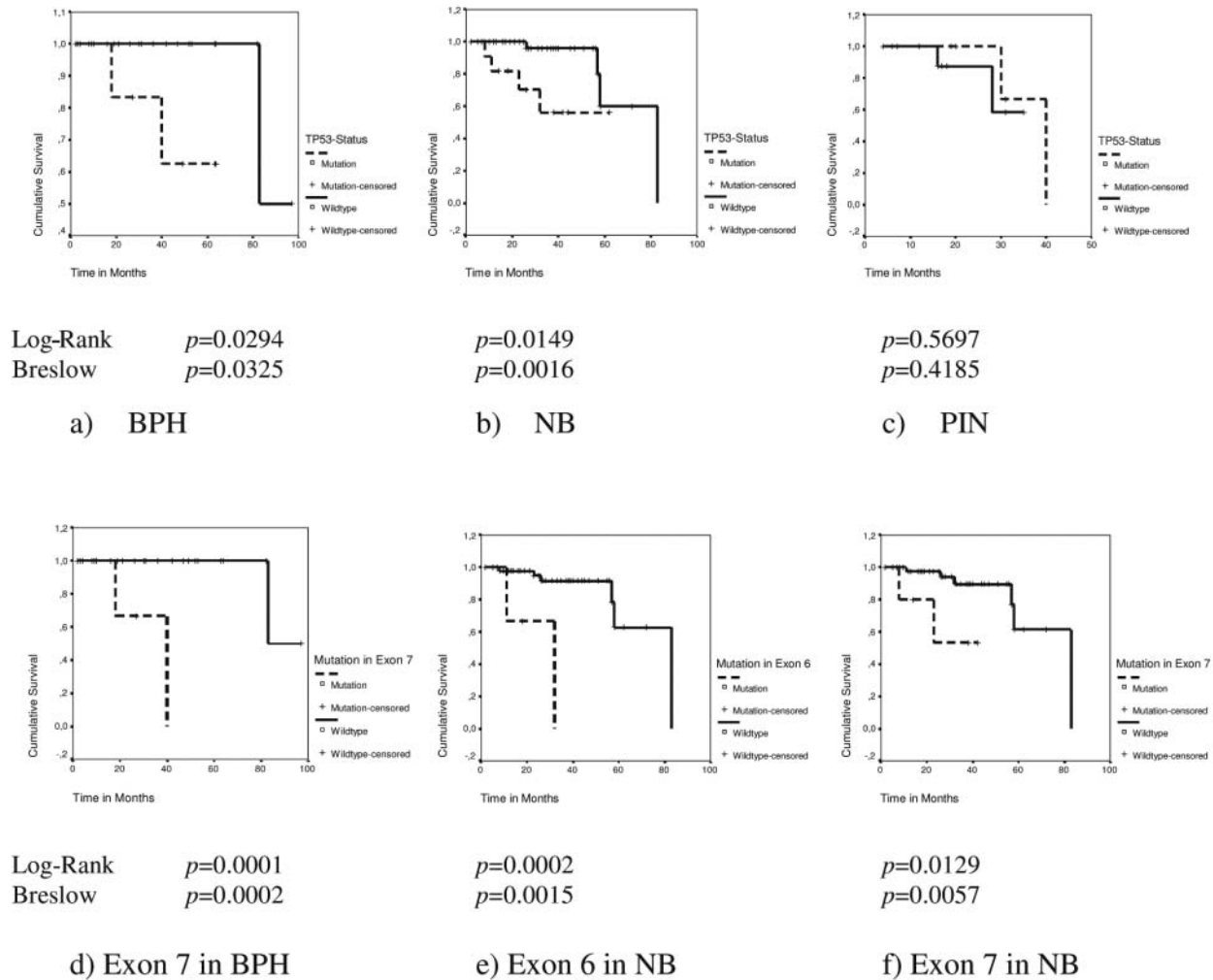


Figure 2. Appearance of prostate cancer during follow-up of patients with benign diagnosis at time 0.

(24). Kucera *et al.* did not find a relationship between prognosis and TP53 mutations in L2/L3-loop regions in breast cancer patients (22). Barnabas *et al.* (2001) describe exon 7 mutations conferring six out of seven patients with chronic lymphocytic leukemia to a clinically aggressive disease (25).

PSA is the best tumour marker currently available and used for screening of PCa (14, 26-28). As in breast cancer the *KLK3* gene encoding PSA is downregulated in PCa cells. This gene is suspected to be a tumour suppressor. Serum levels of PSA, as reported in this study, are never indicators of gene regulation. Therefore, PSA as a prognosis factor, should be questioned for the prostate.

TP53 mutations may be factors of cell proliferation and angiogenesis in prostate tissue (29). It would be interesting to examine histological differences between wild-type and mutated TP53 prostate tissues including PSA expression.

## Conclusion

TP53 mutations reduce the PCa-free survival time in patients with needle biopsy of the prostate and diagnosis of primary benign disease. TP53 mutations, especially the exon 7 mutations, enhance the risk of being affected by PCa 15-fold. Therefore, a TP53 mutation analysis should be carried out on needle biopsy tissues of the prostate of urological outpatients with a benign histopathological result.

## Acknowledgements

We thank Cornelia Stelzer for her excellent technical assistance in carrying out the TGGE analyses.

This work was supported by a research grant from the German Cancer Aid / Dr. Mildred Scheel-Foundation (Project No 10349).

## References

- 1 Soussi T, Dehouche K and Bérout C: p53 Website and analysis of p53 gene mutations in human cancer: Forging a link between epidemiology and carcinogenesis. *Human Mut* 15: 105-113, 2000.
- 2 Schlechte HH, Schnorr D, Löning T, Rudolph BD, Pohrt UM and Loening SA: Mutation of the tumor suppressor gene p53 in human prostate and bladder cancers – investigation by temperature gradient gel electrophoresis (TGGE). *J Urol* 157: 1049-1053, 1997
- 3 Brooks JD, Bova GS, Ewing CM, Piantadosi S, Carter BS, Robinson JC, Epstein JI and Isaacs WB: An uncertain role of p53 gene alterations in human prostate cancers. *Cancer Res* 56: 3814-3822, 1996.
- 4 Grignon DJ, Caplan R, Sakar FH, Lawton CA, Hammond EH, Pilepich MV, Forman JD, Mesic J, Fu KK, Abrams RA, Pajak TF, Shipley WU and Cox JD: p53 Status and prognosis of locally advanced prostatic adenocarcinoma: a study based on RT-PCR. *J Natl Cancer Inst* 89: 158-165, 1997.
- 5 Kuczyk MA, Serth J, Bokemeyer C, Machtens S, Minssen A, Bathke W, Hartmann J and Jonas U: The prognostic value of p53 for long-term and recurrence-free survival following radical prostatectomy. *Eur J Cancer* 34: 679-686, 1998.
- 6 Kallakury BVS, Jennings TA, Ross JS, Breese K, Figge HL, Fisher HA and Figge J: Alteration of the p53 locus in benign hyperplastic epithelium associated with high-grade prostatic adenocarcinoma. *Diagn Mol Path* 3: 227-232, 1994.
- 7 Navone NM, Troncoso P, Pisters LL, Goodrow PL, Palmer JL, Nichols WW, von Eschenbach AC and Conti CJ: p53 protein accumulation and gene mutation in the progression of human prostate carcinoma. *J Natl Cancer Inst* 85: 1657-1669, 1993.
- 8 Navone NM, Labate ME, Troncoso P, Pisters LL, Conti CJ, von Eschenbach AC and Logothetis CJ: p53 mutations in prostate cancer bone metastases suggest that selected p53 mutants in the primary site define foci with metastatic potential. *J Urol* 161: 304-308, 1999.
- 9 Berner A, Geitvik G, Karlsen F, Fosså SD, Nesland JM and Børresen AL: TP53 mutations in prostatic cancer. Analysis of pre- and post-treatment archival formalin-fixed tumor tissue. *J Pathol* 176(3): 299-308, 1995.
- 10 Schlechte H, Lenk SV, Löning T, Schnorr D, Rudolph BD, Ditscherlein G and Loening SA: p53 tumour suppressor gene mutations in benign prostatic hyperplasia and prostate cancer. *Eur Urol* 34: 433-440, 1998.
- 11 Mostofi FK, Sesterhenn IA and Davis CJ Jr: A pathologist's view of prostatic carcinoma. *Cancer* 71: 906-932, 1993.
- 12 Sebo TJ, Bock BJ, Chevillie JC, Lohse C, Wollan P and Zincke H: The percent of cores positive for cancer in prostate needle biopsy specimens is strongly predictive of tumor stage and volume at radical prostatectomy. *J Urol* 163: 174-178, 2000.
- 13 Chan TY and Epstein JI: Follow-up of atypical prostate needle biopsies suspicious for cancer. *Urology* 53: 351-355, 1999.
- 14 Thompson IM, Pauler DK, Goodman PJ, Tangem CM, Lucia MS, Parnes HL, Minasian LM, Ford LG, Lippman SM, Crawford ED, Crowley JJ and Coltman CA Jr: Prevalence of Prostate Cancer among Men with a Prostate-Specific Antigen Level  $\leq 4.0$  ng per Milliliter. *N Engl J Med* 350: 2239-46, 2004.
- 15 Yu H, Levesque MA, Clark GM and Diamandis EP: Enhanced prediction of breast cancer prognosis by evaluating expression of p53 and prostate-specific antigen in combination. *Br J Cancer* 81: 490-495, 1999.
- 16 Bergh J, Norberg T, Sjögren S, Lindgren A and Holmberg L: Complete sequencing of the p53 gene provides prognostic information in breast cancer patients, particularly in relation to adjuvant systemic therapy and radiotherapy. *Nature Med* 1: 1029-1034, 1995.
- 17 Børresen AL, Andersen TI, Eyfjord JE, Cornelis RS, Thorlacius S, Borg A, Johansson U, Theillet C, Scherneck S, Hartmann S *et al*: Tp53 mutations and breast cancer prognosis: particularly poor survival rates for cases with mutations in the zinc-binding domains. *Genes Chromos Cancer* 14: 71-75, 1995.
- 18 Heide I, Thiede C, Sonntag T, de Kant E, Neubauer A, Jonas S, Peter FJ, Neuhaus P, Herrmann R, Huhn D and Rochlitz CF: The status of p53 in the metastatic progression of colorectal cancer. *Eur J Cancer* 33: 1314-1322, 1997.
- 19 Ichikawa A, Kinoshita T, Watanabe T, Kato H, Nagai H, Tsushita K, Saito H and Hotta T: Mutations of the p53 gene as a prognostic factor in aggressive B-cell lymphoma. *New Engl J Med* 337: 529-534, 1997.
- 20 Pollack IF, Hamilton RL, Finkelstein SD, Campbell JW, Martinez AJ, Sherwin RN, Bozik ME and Gollin SM: The relationship between Tp53 mutations and overexpression of p53 and prognosis in malignant gliomas of childhood. *Cancer Res* 57: 304-309, 1997.
- 21 Pich A: Review: p53 expression, proliferation activity and prognosis in cancer. *The Cancer J* 11: 223-228, 1998.
- 22 Kucera E, Speiser P, Gnant M, Szabo L, Samonigg H, Hausmaninger H, Mittlböck M, Fridrik M, Seifert M, Kubista E, Reiner A, Zeillinger A and Jakesz R: Prognostic significance of mutations in the p53 gene, particularly in the Zinc-binding domains, in lymph node- and steroid receptor positive breast cancer patients. Austrian Breast Cancer Study Group. *Eur J Cancer* 35: 398-405, 1999.
- 23 Serafin AM and Bohm L: Influence of p53 and bcl-2 on chemosensitivity in benign and malignant prostatic cell lines. *Urol Oncol* 23: 123-129, 2005.
- 24 Wong K-B, DeDecker BS, Freund SM, Proctor MR, Bycroft M and Fersht AR: Hot-spot mutants of p53 core domain evidence characteristic local structural changes. *Proc Natl Acad Sci USA* 96: 8438-8442, 1999.
- 25 Barnabas N, Shurafa M, Van Dyke DL, Wolman SR, Clark D and Worsham MJ: Significance of p53 mutations in patients with chronic lymphocytic leukemia. *Cancer* 91: 285-293, 2001.
- 26 Montie JE: Current prognostic factors for prostate carcinoma. *Cancer* 78: 341-344, 1996
- 27 Diamandis EP, Yousef GM, Luo LY, Magklara A and Obiezu CV: The new human kallikrein gene family: Implications in carcinogenesis. *Trend Endocrinol Metab* 11: 54-60, 2000.
- 28 Clements J, Hooper J, Dong Y and Harvey T: The expanded human kallikrein (KLK) gene family: Genomic organisation, tissue specific expression and potential functions. *Biol Chem* 382: 5-14, 2001.
- 29 Strohmeyer D, Rössing C, Bauerfeind A, Kaufmann O, Schlechte H, Bartsch G and Loening S: Vascular-endothelial growth factor and its correlation with angiogenesis and p53 expression in prostate cancer. *The Prostate* 45: 216-224, 2000.

Received July 12, 2007

Revised October 12, 2007

Accepted October 22, 2007