TP53 Gene Mutations in Prostate Cancer Progression

THORSTEN H. ECKE[1*], HORST H. SCHLECHTE[2*], KATRIN SCHIEMENZ[2], MARKUS D. SACHS[2], SEVERIN V. LENK[2], BIRGIT D. RUDOLPH[3] and STEFAN A. LOENING[2]

[1]*HELIOS Hospital, Department of Urology, Bad Saarow, Germany;*
[2]*Department of Urology, Charité – Universitätsmedizin Berlin, Germany;*
[3]*Institute of Pathology, Charité – Universitätsmedizin Berlin, Germany*

Abstract. *Background: We assessed the predictive value of TP53 mutations and prostate-specific antigen (PSA) for tumor progression in prostate cancer (PCa) patients. Materials and Methods: Ninety tumor tissue samples of patients with PCa from radical prostatectomy were used. Tumor progression was estimated biochemically by the PSA level (> 0.2 μg/l) or by detection of metastases. Screening for TP53 mutations was performed by temperature gradient gel electrophoresis (TGGE) in exon-specific manner. Follow-up data were collected from medical protocols. Statistical analysis was performed by uni- and multivariate techniques. Results: In 32 out of 90 patients (35.6%), TP53 mutations were detected. Thirteen out of 32 patients (40.6%) with TP53 mutations and nine out of 58 patients (15.5%) with TP53 wild-type showed tumor progression after 25 and 45 months, respectively. Conclusion: TP53 mutations in exon 7 and exon 8 are factors of tumor progression in PCa. Their contribution to tumor recurrence is more significant than tumor stage and pretherapeutic PSA level.*

It has been suggested that a subset of mutant alleles acquired by a subclone of tumor cells early in tumorigenesis leads not only to a selected replication advantage, but also, later in tumorigenesis, to the ability to metastasize (1). Clinical tumor stage, Gleason score, and pretherapy serum prostate-specific antigen (PSA) in descenting order were independently associated with clinical or biochemical relapse of PCa (2).

PCa is one of the malignancies with the highest frequency of genetic variations (3). Renan has calculated a probability of 12 mutated genes from epidemiological data (4). The *TP53* tumor suppressor gene is one of the most frequently mutated genes in human malignancies (5). However, the mutation frequency of *TP53* in PCa has a low level of about 30% (6). Furthermore, the *TP53* mutation frequency in prostate tumor tissue does not show a significant rising level in correlation with rising tumor grading and staging, as for example in bladder cancer. Mutations of *TP53* influence the activation of cell proliferation and suppression of DNA repair, and apoptosis (7). Therefore, an acceleration of tumor progression by TP53 mutations was claimed (8, 9). Kuczyk *et al*. have described a correlation between overexpression of p53 protein and tumor progression in PCa patients (10): during univariate analysis, p53 overexpression, histological grading, and tumor stage were significant prognostic factors for survival, among which only p53 overexpression remained an independent significant predictor in multivariate analysis.

In an earlier study, we found a low *TP53* mutation frequency of between 16.5 and 19.0% in benign prostatic hyperplasia, with a higher rate of later occurence of PCa in patients with mutations (6, 11). Recently, regulation of PSA by *TP53* was suggested (12, 13). In the diagnosis the rate of PCa patients with a serum PSA less than 4.0 μg/l is very high (14). However, the PSA level is the most specific tumor marker for PCa (15, 16). After radical surgical treatment of PCa the PSA level declines to less than 0.1 μg/l. An increase of PSA of 0.2 μg/l after curative PCa treatment is assumed to be indicative of tumor progression (17, 18). A high level of pretreatment PSA was considered also as a risk factor for tumor progression (19). In this communication we present follow-up data. Examining the influence of *TP53* mutation status, pretreatment PSA level, patients age, tumor grading and staging on tumor progression in PCa patients.

Materials and Methods

Ninety patients who were treated for clinically organ confined primary PCa between 1993 and 2000 by either radical retropubic prostatectomy (until 06/1999) or by laparoscopic radical prostatectomy (after 06/1999) were followed for 22.5 (range 3-108) months. The Gleason score was not considerd in this study. All samples were analyzed according to histopathological standard methods (20).

*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: +49 3363173170, Fax: +49 3363173136, e-mail: thorsten.ecke@helios-kliniken.de

Key Words: Prostate cancer, progression, p53, mutation, tumor suppressor-gene.

PSA level was determined by the Department of Laboratory Medicine of the Charité Hospital. A post treatment PSA rise of 0.2 μg/l and more was considered as progression. For the patients case history clinical data documented in charts were reviewed. Selection criteria were clinically organ confined disease, PSA decrease to <0.1 μg/l after surgery, and available follow-up data. As time to progression the first detection of PSA of 0.2 μg/l or higher was used. As nonprogression the time of last outpatient or clinical consultation in case of PSA level less than 0.2 μg/l was considered.

Genomic DNA was isolated by standard technique from fresh tumor tissue or paraffine material of prostatectomy specimens. *TP53* mutation analysis was carried out by temperature gradient gel electrophoresis (TGGE) of GC-clamped PCR products for *TP53* exons 5, 6, 7, 8 in different reaction steps (21). Preparation of PCR products and TGGE analysis was carried out as previously described (22).

Mutation sequence data were the results of analyses of single eluated TGGE bands in case of mutation detection (23).

Completing the definitions of prostate cancer risk groups according to D'Amico *et al*. (24), we included this factor in our calculations.

Statistical analyses were carried out using SPSS 12.0.1 (Mann-Whitney test, Kaplan-Meier technique, Cox regression modeling). 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

In 32 out of 90 patients (35.6%), one or more *TP53* mutations (5 times in exon 6 and exon 7, 2 times in exon 5 and exon 7, 2 times in exon 7 and exon 8) were detected by TGGE (Table I) in tumor tissue of prostatectomy material. Seven out of 16 mutations were sequenced for 13 patients with detectable tumor progression (Table III). Table I shows the mutation frequency of all tumors indicating no clear correlation between rising histopathological classification (G1: 54.5%. G2: 33.3%, G3: 32.4%) and *TP53* mutations.

The overall frequency of tumor progression was 24.4% (22/90) after 3-75 (median 28.5) months. Thirteen out of 32 patients (40.6%) with *TP53* mutations and nine out of 58 patients (15.5%) with *TP53* wild-type showed tumor progression after a median of 25 and 45 months respectively. Available data of all patients with PCa progression are summarized in Table II.

Regarding the risk groups, we calculated the overall frequency of tumor progression according to D'Amico *et al*. (24) for the low risk group with 14.3% (4/28), the intemediate risk group with 17.4% (4/23), and the high risk group with 35.9% (14/39) after 3-75 (median 28.5) months. Calculating the *TP53* mutation status with D'Amico risk groups, we found an overall frequency of *TP53* mutation for the low-risk group with 35.8% (10/28), the intermediate-risk group with 26.1% (6/23), and the high-risk group with 41.0% (16/39) after 3-75 (median 28.5) months. Although the Chi-square test is in all cases $p>0.05$, it is clear that a higher risk group after D'Amico shows a higher overall frequency of tumor progression, but not a higher overall frequency of *TP53* mutation in this group.

Table I. *TP53 mutation frequency (%) of 90 PCa samples dependent on histopathological tumor classification.*

	pT2	pT3	pT4	Total [%]
G1	*5/10=50.0% (2)	1/1=100% (0)	-	6/11 (2) [54.4]
G2	11/35=31.4% (5)	4/10=40.0% (3)	-	15/45 (8) [33.3]
G3	5/14=35.7% (3)	6/19=31.6% (8)	0/1 (1)	11/34 (12) [32.4]
Total [%]	21/59 (10) [35.6]	11/30 (11) [36.7]	0/1 (1) [0]	32/90 (22) [35.6]

*Mutations/samples in indicated classification; the numbers in parentheses represent the number of patients with tumor progression in this classification.

Figure 1 shows a correlation between PCa progression and *TP53* mutation status. In Figure 1a the whole patient population is analyzed, showing a significant correlation. Furthermore, the interval until PCa progression is significantly shorter (15 *versus* 45 months) in mutation-bearing tumors. Figure 1b shows the results for the 40 patients with a follow-up period of more than 11 months. Figure 1c shows the analysis of the subgroup with less than 12 months' follow-up. Even in this shorter interval, a significantly worse prognosis is evident.

If calculated separately, mutations in exon 5 and 6 do not seem to influence progression significantly, while exon 7 and 8 mutations do, as shown by the Kaplan-Meier method (Figure 2) and by Cox regression modelling (Table IV).

Other factors potentially influencing tumor progression are given in Tables II and IV: preoperative PSA, tumor stage, tumor grade, and age. Pretreatment PSA shows a nonsignificant tendency to be lower in patients with tumor tissue mutations, and to be higher in patients with tumor progression in comparison with non-progressing tumors. Median PSA was 9.10 μg/l in patients with progression, *versus* 7.88 μg/l in patients without progression. In the Cox regression modeling, PSA has non-significant *p*-values in 90 patients followed up for 3-108 months, and in a 40 patient subgroup followed up for 12-108 months. In the 50 patients with 3-11 months' follow-up, PSA is the most significant progression factor. In this subgroup, patient age becomes significant ($p=0.046$) too. Only two patients with tumor progression in case of *TP53* wild-type, and age of 56 and 55 years (note Figure 1c) were found. Overall, the age did not differ significantly between patients with or without PSA progression.

In Table II a rising progression frequency with increasing tumor stage and with tumor grade 3 *versus* grades 1 and 2 is shown. In the Cox regression model however, this tendency is not significant. The column Exp(B) in Table IV refers to the increase of probability to suffer from tumor progression in case of mutation.

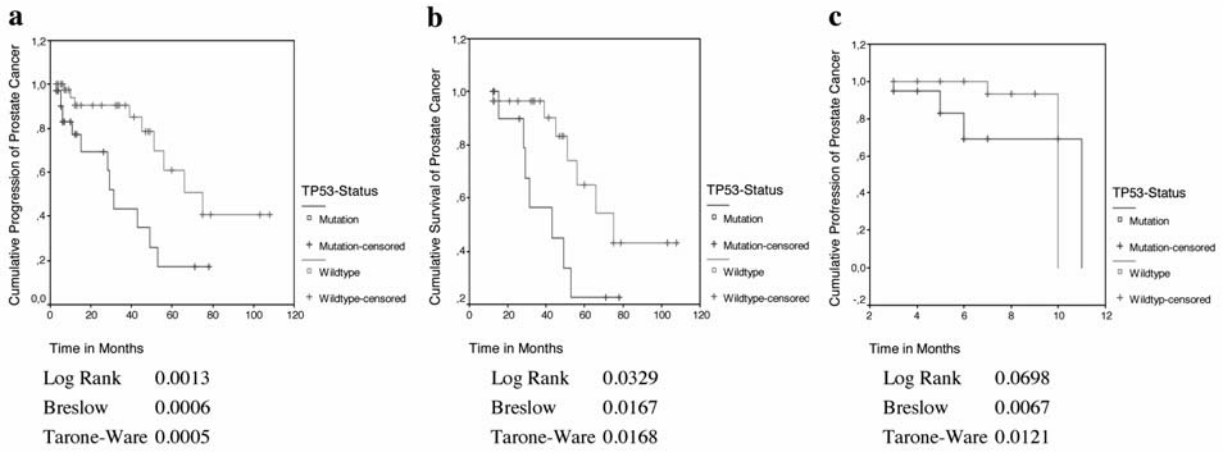

Figure 1. *Kaplan-Meier analysis of tumor progression of patients with PCa. Mutation analysis of TP53 exons 5-8 by TGGE. a: 90 patients: progression in 9 out of 58 patients (15.5%) with TP53 wild-type in tumor tissue, and in 13 out of 32 patients (40.6%) with TP53 mutation. b: Analysis of a 40 patients subgroup with follow-up of 12-108 months. Progression in 7 out of 27 patients (25.9%) with wild-type in tumor tissue, and in 7 out of 13 patients (53.8%) with TP53 mutation. c: Analysis of a 50-patient subgroup with follow-up of 3-11 months. Progression in 2 out of 31 patients (6.5%) with wild-type in tumor tissue, and in 6 out of 19 patients (31.6%) with TP53 mutation.*

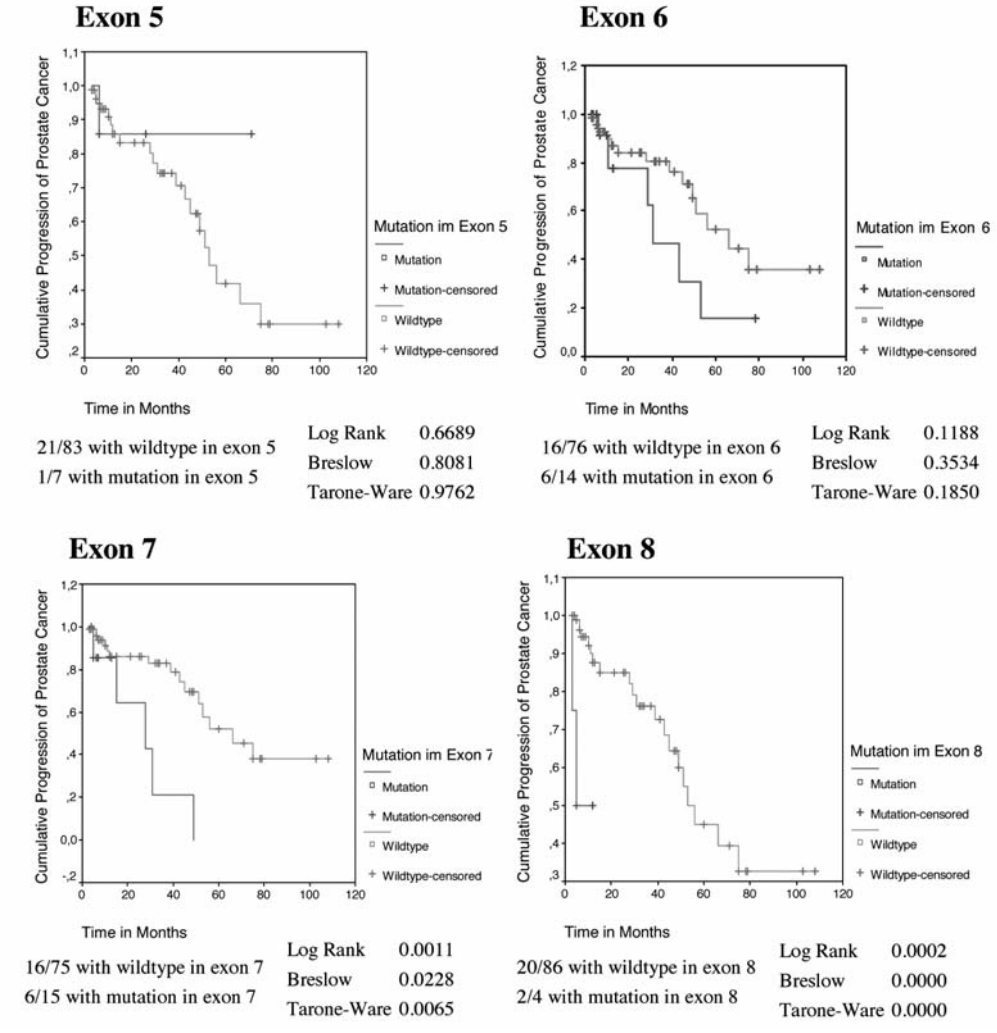

Figure 2. *Kaplan-Meier analysis of tumor progression of 90 patients with PCa, specified for genetic status of TP53 exons.*

Table II. *Summarized data of 90 PCa patients with follow-up. Treatment by radical prostatectomy, PSA levels before surgical treatment. Pretreatment PSA levels were not significantly different between patients with TP53 mutation or with wild-type (Mann-Whitney-U-test: p=0.125).*

Follow-up of all patients	n=90	3-108 months, mean 22.5, median 8.5			
Age (years)		50-72, mean 62.4, median 63			
Pretherapeutic PSA (μg/l)		1.00-59.70, mean 10.88, median 7.79			
Tumor staging		mutations in exon 5,	exon 6,	exon 7,	exon 8
pT2 n=59 (10 with progression=16.9%)		5	11	9	4
pT3 n=30 (11 with progression=36.7%)		2	3	7	0
pT4 n=1 (1 with progression)		0	0	0	0
Tumor grading					
G1 n=11 (2 with progression=18.2%)		4	2	2	0
G2 n=45 (8 with progression=17.8%)		3	5	8	3
G3 n=34 (12 with progression=35.3%)		0	7	6	1
Patients with tumor progression	n=22	3-75 months, mean 29.3, median 28.5			
Age (years)		50-72, mean 62.8, median 63.5			
Pretherapeutic PSA (μg/l)		1.45-59.70, mean 15.02, median 8.42			
Patients with no signs of tumor progression	n=68	3-108 months, mean 20.2, median 7			
Age (years)		53-72, mean 62.3, median 63			
Pretherapeutic PSA (μg/l)		1.00-25.30, mean 9.54, median 7.88			
TP53 status					
Patients with mutation in tumor tissue	n=32	mutations in exon 5,	exon 6,	exon 7,	exon 8
		7	14	16	4
Age (years)		50-72, mean 63.7, median 64			
Pretherapeutic PSA (μg/l)		1.45-59.70, mean 11.80, median 6.55			
Patients with tumor progression after 3-53 months, mean 21.8, median 15	n=13	1	6	7	1
Age (years)		50-71, mean 63.3, median 64			
Pretherapeutic PSA (μg/l)		1.45-59.70 μg/l mean 16.45, median 6.89			
Patients with mutations in two exons	n=9	(2 with progression=22.2%: 1 x exon 5 + 7, 1 x exon 7 + 8)			
Patients with wild-type in tumor tissue	n=58				
Age (years)		50-72, mean 61.7, median 61.5			
Pretherapeutic PSA (μg/l)		1.00-32.00, mean 10.37, median 8.61			
Patients with tumor progression after 7-75 months, mean 40.1, median 45	n=9				
Age (years)		52-72, mean 62.1, median 63			
Pretherapeutic PSA (μg/l)		4.20-22.90, mean 12.95, median 9.90			

Discussion

General remark. A remarkable rise of tumor progression in PCa patients with *TP53* mutations in their tumor tissue was shown by frequency, Kaplan-Meier analysis and multifactor analysis. However, pretreatment PSA level was also a progression influencing factor of abated contribution in our analysis. *TP53* mutations of exons 7-8 could be identified as progression factors of PCa, and this effect was more important than pretherapeutic PSA level.

Mutation frequency. The *TP53* mutation frequency (35.6%), reported in this study, was close to the range of 25-30% in published results (22, 25). TGGE detected mutation frequency of PCa samples in our laboratory was more than 30% (6). In contrast to another report (26) in our material, where we found *TP53* exon 5 mutations with corresponding tumor progression in PCa in one of seven cases only. This patient (Table III: No. 1017) had a mutation in exon 7, too. Dahiya *et al*. have descibed PCa specific *TP53* mutations in exon 7 only (27). In an earlier study we found 16.7% (4/24) exon 5 mutations *versus* 8.3% (2/24) exon 7 mutations in PCa (22). In the present report we described exon-specific mutation frequencies (Table II: exon 5=7.8%, exon 6=15.6%, exon 7=17.8%, exon 8=4.4%). These counts were quite different from mutation frequencies in benign prostate hyperplasia for example, where the highest mutation frequency of *TP53* belonged to exon 6 in the range of 8% (6, 11). TGGE was more sensitive in *TP53* mutation detection than sequencing in our laboratory (22, 28).

Table III. *Tumor progression data of 22 patients with PCa.*

Patient number*	Age (years)	Pretreatment PSA (µg/l)	T/G	Mutation in	*TP53*-status affected map position	Codon	Tumor progression after months		PSA after follow-up (µg/l)
252 (1994)	66	5.90	pT3a G3a	exon 6	13397-99 CGA ==> TGG	213 Arg ==> Trp	53		1.24
754 (1994)	68	8.24	pT2a G1	exon 6			6		0.25
796 (1995)	64	6.80	pT2b G2	exon 6	13399 A ==> G	213 Arg silent	43		0.20
1017 (1995)	65	6.48	pT2a G1	exon 5	13157 A ==> G	160 Met ==> Val			
				exon 7			6		1.90
1027 (1995)	63	4.54	pT2b G3	exon 7	14058 ins T				
					14061 ins A				
					14063 ins T	244-5 Gly-Gly ==>	Val-Arg-Ala		
				exon 8			5		1.90
1054 (1993)	60	1.45	pT3a G3a	exon 7			15		2.05
1432 (1996)	69	59.70	pT3c G3b	exon 7			28		6.94
1435 (1996)	71	50.00	pT3a G2	exon 7	14067 A ==> G	247 Asp ==> Ser	5		0.26
1447 (1996)	68	43.20	pT2c G2b	exon 6					
				exon 7	14050 C ==> T	241 Ser silent	31		5.00
2155 (1999)	58	7.40	pT3 G2a	exon 7	14061 G ==> A	245 Gly ==> Asp	49		2.30
2231 (1999)	59	10.10	pT2b G3a	exon 6			29		6.10
2353 (1999)	50	3.20	pT2 G2a	exon 8			3	metastasis	n.d.
2564 (1995)	62	6.89	pT3 G3	exon 6			11		2.00
223 (1994)	66	8.60	pT3a G3	wild-type			56		4.74
231 (1993)	61	9.90	pT3c G3b	wild-type			75		3.00
240 (1994)	68	4.20	pT4a G3a	wild-type			66		0.80
256 (1994)	72	5.11	pT3c G3a	wild-type			45		0.36
275 (1994)	52	17.70	pT3c G3a	wild-type			51		88.0
755 (1994)	66	9.60	pT2a G2b	wild-type			39		6.50
757 (1994)	55	15.80	pT3b G3b	wild-type			10		2.12
759 (1994)	63	13.60	pT2a G2	wild-type			12		0.23
2780 (2000)	56	32.00	pT2b G3a	wild-type			7		1.00

*Patient number (in parentheses: year of surgical treatment); pT=tumor staging; G=tumor grading.

Special mutations. *TP53* mutations as tumor progression factors have been discussed for many different tumors (29-33). Recently, *TP53* mutations were suspected to speed up PCa progression (34). These authors have shown a progression towards androgen independence by gain of function mutations in LNCaP cells. One affected codon number 245 of the four mentioned gain of function codons was also detected in our material (Table III). In our experiments, we have found *TP53* mutations with correlations to tumor progression in exons 7 and 8 only (one patient no. 1017 with mutations in exon 5 and exon 7). This confirms results of Dahiya *et al*., but is at variance with results of Shi *et al*. in some respects (25, 27). This group has described 12 mutations with different transactivation capabilities for p53 responsive genes, five of them in exon 5, but none of them in exon 6. Surprisingly, several mutations with partial transactivation function were temperature sensitive.

p53 and regulation. Functional significance of *TP53* mutations in PCa has to be questioned. Expression of p53 is induced in response to DNA damage (35). In general, *TP53* missense mutations repress p53 wild-type function. Several *TP53* missense mutations, reported as hotspots in tumor cells, have dominant negative effects on transactivation of other genes containing p53-specific responsive elements. However, Forrester *et al*. (36) generally attributed minimal dominant negative effects only to codons 143ala-, 175his-, 248trp-, 249ser-, 273his-mutations in PCa cell line PC-3, lacking one basepair in codon 138. In human lung adenocarcinoma and mesothelioma cell lines these mutations had strong transactivation inhibition effects. Transactivation may depend on the presence of *p53* wild-type. In PCa with *TP53* mutation the *TP53* wild-type alleles are lost (loss of heterozygosity) and mutated p53 is usually overexpressed. Loss of heterozygosity of chromosome 13q33 sequences could be associated with loss of a region containing the DNA repair gene *XPG/ERCC5* in PCa (37).

Table IV. *Cox regression modeling with method "Enter" of data of 90 PCa patients. 58 patients with TP53 wild-type in PCa tissue, 32 patients with TP53 mutation. Variables TP53, pT (tumor staging), G (tumor grading), are categorical. Variables PSA, patients age, are metric. Exp(B) is taken from the result of "variables in the equation" of computation by the program SPSS11.0.*

Variables	Significance *p*-Value	Exp(B)	95.0% Confidence interval for Exp(B)	
			Lower	Upper
90 patients				
TP53	0.001	5.421	1.929	15.193
PSA		0.075		
Age		0.235		
pT		0.719		
pG		0.129		
40 patients (13 with mutation) with follow-up 12-108 months				
TP53	0.032	4.147	1.130	15.217
PSA		0.077		
Age		0.936		
pT		0.808		
pG		0.769		
50 patients (19 with mutation), follow-up 3-11 months				
TP53	0.038	51.443	1.244	2128.153
PSA		0.011		
Age		0.044		
pT		0.699		
pG		0.143		
90 patients, status of exon 7, exon 8 (18 with mutation)				
Exon 7+8 status	0.001	5.942	2.034	17.361
PSA		0.376		
50 patients, status of exon 7, exon 8 (12 with mutation), follow-up 3-11 months				
Exon 7+8 status	0.021	8.272	1.379	49.634
PSA		0.044		
40 patients, status of exon 7, exon 8 (6 with mutation), follow-up 12-108 months				
Exon 7+8 status	0.008	7.560	1.691	33.803
PSA		0.428		

PSA. Only few cases of PCa recurrence without PSA increase have been reported (38-39). PSA is positively regulated by the androgen receptor (40). In a recent publication by Freeland *et al.*, 0.4 μg/l PSA was suggested as an ideal cut-off for determining PCa recurrence after radical prostatectomy (41). Computing our results with this cut-off led to similar results (17 patients with tumor progression; p=0.027 for TP53, p=0.261 for pretherapy PSA in Cox regression) as reported for 0.2 μg/l PSA.

Gurova *et al*. found a negative control function of p53 expression on PSA secretion and PSA mRNA level in PCa cell line LNCaP (13). Thus, PSA is likely to be a tissue specific indicator of transformation-associated p53 suppression in prostate cells. This conclusion provides a plausible explanation for a frequent increase of PSA levels in advanced PCa. We have observed lower PSA levels in patients with *TP53* mutations in their prostate tissue in comparison with *TP53* wild-type.

Progression and metastasis. In general, poor prognosis is a well-known phenomenon in tumour patients with *TP53* mutations in their malignant cells (42). The predictive value of p53 overexpression for PCa patients prognosis, reported by Kuczyk *et al*. (10) and other groups (43-44) has been confirmed by our results of *TP53* mutation analysis. This effect would be influenced mainly by mutations in exon 7 and exon 8.

Bandyopadhyay *et al*. (45) and Chen *et al*. (46) have described some molecular contribution of p53 expression on genes repressing metastatic spread. Several genes have been reported to suppress tumor metastases in PCa. Other factors like contribution of androgen receptor to PCa predisposition and progression are assumed to be genetical variations of alternative signalling (47).

Conclusion

TP53 mutations in exon 7 and exon 8 are factors of tumor progression in PCa. Their contribution to tumor recurrence is more significant than tumor stage and pretherapeutic PSA level. Mutation analysis can be started with screening techniques like TGGE.

Acknowledgements

We thank Cornelia Stelzer for excellent technical assistance in carrying out the TGGE analyses and sequencing. This work was supported by a research grant from the German Cancer Aid / Dr. Mildred Scheel- Foundation (Project No 10349).

References

1 Bernards R and Weinberg RA: A progression puzzle – metastasis genes. Nature *418*: 823, 2002.

2 Pisansky TM, Kahn MJ and Bostwick DG: An enhanced progostic system for clinically localized carcinoma of the prostate. Cancer *79*: 2154-1261, 1997.

3 Boland R and Riccardiello L: How many mutations does it take to make a tumor? Proc Natl Acad Sci USA *96*: 14675-14677, 1999.

4 Renan MJ: How many mutations are required for tumorigenesis? Implications from human cancer data. Molec Carcinog *7*: 139-146, 1993.

5 Hainaut P, Hernandez T, Robinson A, Rodriguez-Tome P, Flores T, Hollstein M, Harris CC and Montesano R: IARC Database of *p53* gene mutations in human tumors and cell lines: updated compilation, revised formats and new visualisation tools. Nucl Acids Res *26*: 205-213, 1998.

6 Ecke TH, Schlechte HH, Hübsch A, Lenk SV, Schiemenz K, Rudolph BD and Miller K: *TP53* Mutation in prostate needle biopsies – comparison with patients follow-up. Anticancer Res *27(6)*: 4143-4148, 2007.

7 Sidransky D and Hollstein M: Clinical implications of the *p53* gene. Ann Rev Med *47*: 285-301, 1996.

8 Blondal JA and Benchimol S: The role of p53 in tumor progression. Semin Cancer Biol *5*: 177-186, 1994.

9 Mowat MRA: p53 in tumor progression: Life, death, and everything. Adv Cancer Res *74*: 25-48, 1998.

10 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.

11 Schlechte H, Lenk SV, Löning T, Schnorr D, Rudolph BD, Ditscherlein G and Loening SA: *p53* tumor suppressor gene mutations in benign prostatic hyperplasia and prostate cancer. Eur Urol *34*: 433-440, 1998.

12 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.

13 Gurova KV Roklin OW, Krivokrysenko VI, Chumakov PM, Cohen MB, Feinstein E and Gudkov AV: Expression of prostate specific antigen (PSA) is negatively regulated by p53. Oncogene *21*: 153-157, 2002.

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 ≤4.0 ng per milliliter. N Engl J Med *350*: 2239-2246, 2004.

15 Partin AW and Oesterling JE: Clinical use of prostatic-specific antigen: update 1994. J Urol *152*: 1358-1368, 1994.

16 Polascik TJ, Oesterling JE and Partin AW: Prostate specific antigen: a decade of discovery – what we have learned and where we are going. J Urol *162*: 293-306, 1999.

17 Moul JW: Prostate-specific antigen only progression of prostate cancer. J Urol *163*: 1632-1642, 2000.

18 Critz FA: Time to achieve a prostate-specific antigen nadir of 0.2 ng/ml after simultaneous irradiation for prostate cancer. J Urol *168*: 2434-2438, 2002.

19 Oberpenning F, Hamm M, Schmid HP, Hertle L and Semjonow A: Radical prostatectomy: Survival outcome and correlation to prostate-specific antigen levels. Anticancer Res *20*: 4969-4972, 2000.

20 Sobin LH and Wittekind C: TNM Classification of Malignant Tumours. John Wiley & Sons, 5th edn, 1997.

21 Rosenbaum V and Riesner D: Temperature gradient gel electrophoresis. Thermodynamic analysis of nucleic acids and proteins in purified form and in cellular extracts. Biophys Chem *26*: 235-246, 1987.

22 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.

23 Hedrum A, Pontén F, Ren Z, Lundeberg J, Pontén J and Uhlén M: Sequence-based analysis of the human *p53* gene based on microdissection of tumor biopsy samples. BioTechniques *17*: 118-129, 1994.

24 D'Amico AV, Whittington R, Malkowicz SB, Schultz D, Blank K, Broderick GA, Tomaszewski JE, Renshaw AA, Kaplan I, Beard CJ and Wein A: Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinical localized prostate cancer. JAMA *280(11)*: 969-974, 1998.

25 Shi XB, Nesslinger NJ, Deitch AD, Gumerlock PH and deVere White RW: Complex functions of mutant *p53* alleles from human prostate cancer. Prostate *51*: 59-72, 2002.

26 Kubota Y, Shuin T, Uemura H, Fujinami K, Miyamoto H, Torigoe S, Dobashi Y, Kitamura H, Iwasaki Y and Dannenberg K: Tumor suppressor gene *p53* mutations in human prostate cancer. Prostate *27(1)*: 18-24, 1995.

27 Dahiya R, Deng G, Chen KM, Chui RM, Haugheney PC and Narayan P: *P53* tumour-suppressor gene mutations are mainly localised on exon 7 in human primary and metastatic prostate cancer. Br J Cancer *74*: 264-268, 1996.

28 García-Delgado M, González-Navarro CJ, Napal MC, Baldonado C, Vizmanos JL and Gullon A: Higher sensitivity of denaturing gradient gel electrophoresis than sequencing in the detection of mutations in DNA from tumor samples. Biotechniques *24*: 72-76, 1998.

29 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.

30 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.

31 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(8)*: 529-534, 1997.

32 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.

33 Ecke TH, Sachs MD, Lenk SV, Loening SA and Schlechte HH: *TP53* gene mutations as an independent marker for urinary bladder cancer progression. Int J Mol Med *21*: 655-661, 2008.

34 Nesslinger NJ, Shi XB and DeVere White RW: Androgen-independent growth of LNCaP prostate cancer cells is mediated by gain-of-function mutant p53. Cancer Res *63*: 2228-2233, 2003.

35 Kastan M-B, Onyekwere O, Sidransky D, Vogelstein B and Craig RW: Participation of p53 protein in the cellular response to DNA damage. Cancer Res *51*: 6304-6311, 1991.

36 Forrester K Lupold SE and Ott VL: Effects of *p53* mutants on wild-type p53-mediated transactivation are cell type dependent. Oncogene *10*: 2103-2111, 1995.

37 Hyytinen ER, Frierson HF Jr, Sipe TW, Li CL, Degeorges A, Sikes RA, Chung LW and Dong JT: Loss of heterozygosity and lack of mutations of the *XPG/ERCC5* DNA repair gene at 13q33 in prostate cancer. Prostate *41*: 190-195, 1999.

38 Hübler J, Sükösd F and Czipri M: Tibia metastasis without prostate-specific antigen (PSA) increase following radical vesiculo-prostatectomy. Int Urol Nephrol *32*: 281-284, 2000.

39 Shinghal R, Yemoto C, McNeal JE and Brooks JD: Biochemical recurrence without PSA progression characterizes a subset of patients after radical prostatectomy. Prostate-specific antigen. Urology *61*: 380-385, 2003.

40 Balk SP, Ko YJ and Bubley GJ: Biology of prostate-specific antigen. J Clin Oncol *21*: 383-391, 2003.

41 Freeland SJ, Sutter ME, Dorey F and Aronson WJ: Defining the ideal cutpoint for determining PSA recurrence after radical prostatectomy. Prostate-specific antigen. Urology *61(2)*: 365-369, 2003.

42 Pich A: Review: p53 expression, proliferation activity and prognosis in cancer. The Cancer J *11*: 223-228, 1998.

43 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.

44 Quinn DI, Henshall SM, Head DR, Golovsky D, Wilson JD, Brenner PC, Turner JJ, Delprado W, Finlayson JF, Stricker PD, Grygiel JJ and Sutherland RL: Prognostic significance of nuclear accumulation in localized prostate cancer treated with radical prostatectomy. Cancer Res *60(6)*: 1585-1594, 2000.

45 Bandyopadhyay S, Pai SK, Gross SC, Hirota S, Hosobe S, Miura K, Saito K, Commes T, Hayashi S, Watabe M and Watabe K: The *Drg-1* gene suppresses tumor metastasis in prostate cancer. Cancer Res *63(8)*: 1731-1736, 2003.

46 Chen SL, Wu YS, Shieh HY, Yen CC, Shen JJ and Lin KH: P53 is a regulator of the metastasis suppressor gene *Nm23-H1*. Mol Carcinog *36(4)*: 204-214, 2003.

47 Buchanan G, Irvine RA, Coetzee GA and Tilley WD: Contribution of the androgen receptor to prostate cancer predisposion and progression. Cancer Metastasis Rev *20(3-4)*: 207-223, 2001.

Received January 30, 2009
Revised March 23, 2010
Accepted March 24, 2010