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 descending 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). Kuczyn 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 occurrence 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 considered in this study. All samples were analyzed according to histopathological standard methods (20).
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 40.1 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. Preparation of PCR products and TGGE analysis was carried out as previously described. Mutation sequence data were the results of analyses of single eluted TGGE bands in case of mutation detection.

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 specimens. 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 intermediate 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.

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

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

Figure 1a 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.
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 described 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).
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 TP53 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 III. Tumor progression data of 22 patients with PCa.

<table>
<thead>
<tr>
<th>Patient number*</th>
<th>Age (years)</th>
<th>Pretreatment PSA (μg/l)</th>
<th>T/G</th>
<th>Mutation in affected map position</th>
<th>Codon</th>
<th>Tumor progression after months</th>
<th>PSA after follow-up (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>252 (1994)</td>
<td>66</td>
<td>5.90</td>
<td>pT3a G3a exon 6</td>
<td>13397-99 CGA ==&gt; TGG</td>
<td>213 Arg ==&gt; Trp</td>
<td>53</td>
<td>1.24</td>
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<tr>
<td>754 (1994)</td>
<td>68</td>
<td>8.24</td>
<td>pT2a G1 exon 6</td>
<td>13399 A ==&gt; G</td>
<td>213 Arg silent</td>
<td>6</td>
<td>0.25</td>
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<tr>
<td>796 (1995)</td>
<td>64</td>
<td>6.80</td>
<td>pT2b G2 exon 6</td>
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<td>160 Met ==&gt; Val</td>
<td>43</td>
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<tr>
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<td>6.48</td>
<td>pT2a G1 exon 5 &amp; 7</td>
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</tr>
<tr>
<td>1027 (1995)</td>
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<td>pT2b G3 exon 7</td>
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<td></td>
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<tr>
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<td>247 Asp ==&gt; Ser</td>
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<td>0.26</td>
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<tr>
<td>1432 (1996)</td>
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<td>pT3c G3b exon 7</td>
<td>14050 C ==&gt; T</td>
<td>241 Ser silent</td>
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<td>71</td>
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<td>245 Gly ==&gt; Asp</td>
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<td>244-5 Gly-Gly ==&gt; Val-Arg-Ala</td>
<td>5</td>
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<tr>
<td>2155 (1999)</td>
<td>58</td>
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<td>pT3 G2a exon 8</td>
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<td>245 Gly ==&gt; Asp</td>
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<td>10.10</td>
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<tr>
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<td>50</td>
<td>3.20</td>
<td>pT2 G2a exon 8</td>
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<td>245 Gly ==&gt; Asp</td>
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<td>240 (1994)</td>
<td>68</td>
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<tr>
<td>256 (1994)</td>
<td>72</td>
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<td>pT3c G3a wild-type</td>
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<tr>
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<td>52</td>
<td>17.70</td>
<td>pT3c G3a wild-type</td>
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<tr>
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<td>pT2a G2 wild-type</td>
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<td>2780 (2000)</td>
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<td>pT2b G3a wild-type</td>
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</table>

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

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References


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