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p53 Immunoreactivity in biopsy specimens of T1G3 transitional cell carcinoma of the bladder — a helpful parameter in guiding the decision for or against cystectomy?☆

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Abstract

The aim of this study was to determine whether p53 is helpful in making the decision to undergo cystectomy in T1, G3 transitional cell carcinoma (TCC) of the bladder, by prospectively comparing the p53 status of bladder biopsies with the histology and p53 status of the corresponding cystectomy specimens. From January 1996 to August 1997, 38 consecutive patients with T1G3 TCC at 6 different centres were enrolled into the study. Bladder biopsies and cystectomy specimens were examined with three different antibodies against p53. The p53 status of each bladder biopsy was compared with p53 status, tumour stage and grade of the cystectomy specimen. An independent evaluation of the histology and immunohistochemistry was carried out by two pathologists. 15 of 38 patients (39%) were found to have a higher tumour stage in the cystectomy specimen compared with the staging by transurethral resection of the bladder tumour (TUR-B). 3 patients did not show residual tumour in the cystectomy specimen. No differences in p53 positivity were noted between the different antibodies. 14 of 31 evaluable tumours (45%) were p53 positive at the time of the TUR-B. p53 staining of the TUR-B specimen did not correctly predict the residual tumour in the cystectomy specimen. We, therefore, concluded that compared with standard histopathology, the p53 status of the TUR-B specimen does not provide additional relevant information with regard to local tumour staging and, thus, is not helpful in making the decision for or against a cystectomy.

Keywords: p53; T1G3 transitional cell carcinoma; Bladder cancer; Staging

1. Introduction

Standard therapy of transitional cell carcinoma (TCC) of the bladder is based upon histological findings. Whereas superficial (pTa and pT1), low and intermediate-grade lesions are well controlled by transurethral resection of the bladder tumour (TUR-B), with or without instillation of chemotherapeutic agents or Bacillus Calmette-Guerin (BCG), invasive tumours (pT2 and higher), regardless of their grade, are usually treated by cystectomy. However, for superficial (pT1), high-grade (G3) tumours, no generally accepted therapeutic guidelines exist. Whether this tumour entity should be treated by TUR-B/instillation (conservative management) or by cystectomy (radical management) is still a matter of debate. More than half of these patients do not progress, initial cystectomy, therefore, represents overtreatment. However, observation alone following TUR-B is associated with a progression rate of 48% [1].

To date, different prognostic factors have been proposed including p53 overexpression [2–4]. *TP53*, a tumour suppressor gene located on chromosomal band 17p13.1 is known to be involved in cell cycle regulation. The wild-type protein arrests cells in the G1 phase, thus allowing cellular DNA-repair or apoptosis to occur [5]. Missense mutation of the *TP53* gene leads to the accumulation of a protein with an extended half-life which is detectable by immunohistochemistry [6]. Mutant p53 has been shown to correlate well with DNA molecular

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defects in bladder cancer [7] and with disease progression in stage pTis and pT1 bladder cancer [8]. Furthermore, it has been reported in retrospective studies that p53 is an independent predictor of recurrence and survival in patients with stage T1-T3a TCC of the bladder [9]. However, there are only a few retrospective series on T1G3 TCC patients [9] and, thus, it remains unclear whether p53 may be helpful to guide the treatment decision in T1G3 tumours. The treatment strategy of the participating centres favours cystectomy for (recurrent) T1G3 bladder tumours and, therefore, it was not possible to prospectively test p53 with regard to recurrence and survival using a conservative treatment approach. It is known, that staging by TUR-B often proves to be incorrect and that a considerable number of patients with T1G3 TCC will not show residual tumour in the cystectomy specimen. Approximately 30% of patients, however, will have a higher tumour stage in the cystectomy specimen compared with the TUR-B staging [10]. Hence, the current study was designed to investigate p53, in addition to histopathology, to better predict local tumour stage to see if this was potentially helpful in making the decision of whether to treat patients with a cystectomy.

2. Patients and methods

2.1. Patients, standard histology

From January 1996 to August 1997, 38 consecutive patients, initially treated by TUR-B for stage T1 G3 TCC at six different institutions were enrolled into the study. Following histological diagnosis all patients underwent cystectomy without further adjuvant therapy. One representative paraffin-embedded tissue block from both the TUR-B and the cystectomy specimen was chosen to confirm histological diagnosis by haematoxylin-eosin staining by two reference pathologists. After standard histological diagnosis, 3 μm sections were cut for immunohistochemical analysis.

2.2. Immunohistochemistry

The following three different monoclonal antibodies (MAbs) were utilised: DO1 (Dianova, Hamburg, Germany), dilution 1:50; DO7 (DAKO, Hamburg, Germany), dilution 1:400; and 1801 (Dianova, Hamburg, Germany), dilution 1:200. All antibodies react with both wild-type and mutant p53, however, they recognise different epitopes. DO1 reacts with an epitope located near the amino-terminus (amino acids 21–25) of all known forms of p53. The antigen for DO7 is known to reside between amino acids 19 to 26 in the N-terminus of the human p53 protein. The 1801 epitope is found within the amino terminal region 46 to 55.

Paraffin was removed from the 3 μm sections by treatment with xylenes, which was repeated twice. Sections were then brought into water by decreasing concentrations of ethanol. Endogenous peroxidase activity was quenched by incubating the specimens for 5 min in 3% hydrogen peroxide in water and carefully rinsing with water.

Microwave treatment was applied in a 10 mM sodium citrate buffer (pH 6.0) at 600 W for 30 min. Sections were cooled down for approximately 1 h, before rinsing thoroughly with water and buffering in TBS (Tris-buffered saline, 50 mM Tris/HCl, 150 mM NaCl, pH 7.6) for 5 min. Non-specific binding sites were blocked by covering the tissue with normal swine serum (normal swine serum, DAKO, Hamburg, Germany, dilution 1:5) for 15 min and discarding the serum thereafter. 200 μl of specific antibody solution or normal control serum was applied and the sections were incubated in a humidified chamber at 4°C overnight. Antibodies were diluted in TBS buffer containing 1% bovine albumin (Albumin, Fraction V, Boehringer Mannheim, Mannheim, Germany) and washed three times with TBS buffer. Incubation with the secondary antibody (biotinylated antimouse immunoglobulin) was performed for 30 min at room temperature. The sections were rinsed three times with TBS buffer and streptavidin peroxidase conjugate was applied for another 30 min. After rinsing with TBS, staining was completed by AEC chromogen solution (AEC Substrate System, DAKO, Hamburg, Germany) for 10 minutes at room temperature. Sections were then rinsed with water and counterstained with haematoxylin and mounted with Aquatex (Merck, Darmstadt, Germany).

A p53 positive breast carcinoma was used as positive control. As negative control, an irrelevant mouse immunoglobulin (mouse IgG, negative control, DAKO, Hamburg, Germany, dilution 1:200) was used.

2.3. Assessment of p53 status

Immunohistochemical analysis was performed in a blinded fashion, i.e. without knowledge of the tumour stage in the cystectomy specimen by two independent pathologists. The reaction of p53 was considered to be positive only in the case of nuclear staining. Grading was done within the most representative tumour area of each specimen, assessing 500 tumour cells. In accordance to other studies, only samples demonstrating at least 20% nuclear reactivity of tumour cells were considered to be p53 positive [8,9].

2.4. Statistical analysis

Statistical analysis was carried out by using the Fisher's exact test.

3. Results

We examined bladder biopsies of 38 patients with the initial diagnosis of T1G3 TCC and corresponding cystectomy specimens in the time from January 1996 to August 1997. The mean age at time of diagnosis was 63 (38–75) years, the male to female ratio was 4.4:1. 22 patients (58%) had recurrent disease with previous low- and intermediate grade superficial lesions. Tumours with associated carcinoma *in situ* were excluded from the analysis. All TUR-B specimens contained tissue of the complete bladder wall including the muscular layers. In 7 patients (18%), p53 staining results of the TUR-B specimens were not available: in 3 patients (8%) only histopathological reports of the biopsy specimens were available and in 4 patients (11%) p53 staining of the TUR-B specimens was technically impossible. In the remaining 31 tumours (82%), all three p53 antibodies showed identical results. Staging and grading of cystectomy specimens are shown in Figs. 1 and 2. Assessing all tumours, 17 (45%) had the same stage (TUR-B compared with cystectomy), 15 (39%) were under- and 6 (16%) were overstaged by TUR-B including 3 cystectomy specimens without tumour (Fig. 3). In the current context, 'overstaged by TUR-B' is defined as the presence of a more advanced tumour stage in the

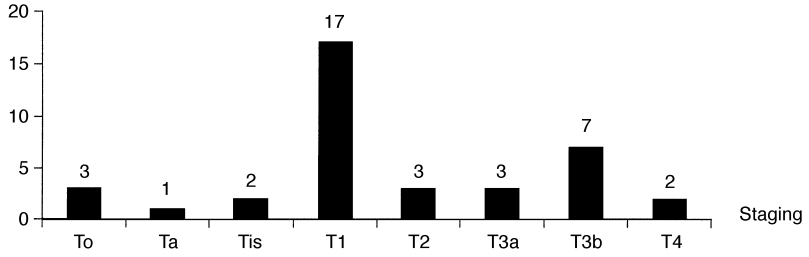

Fig. 1. Staging of cystectomy specimens of 38 patients who were initially (after TUR-B) diagnosed to have an pT1, G3 tumour.

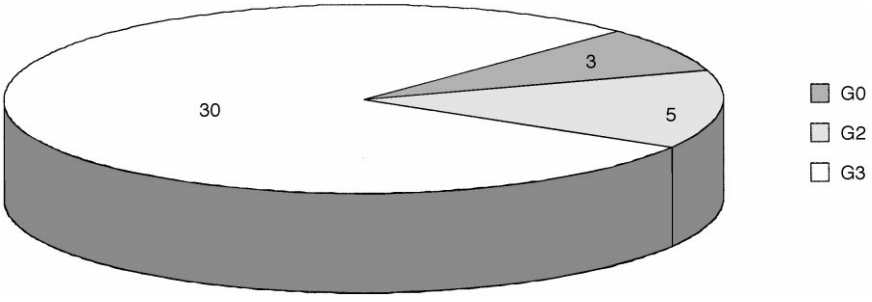

Fig. 2. Grading of cystectomy specimens of 38 patients who were initially (after TUR-B) diagnosed to have an pT1, G3 tumour.

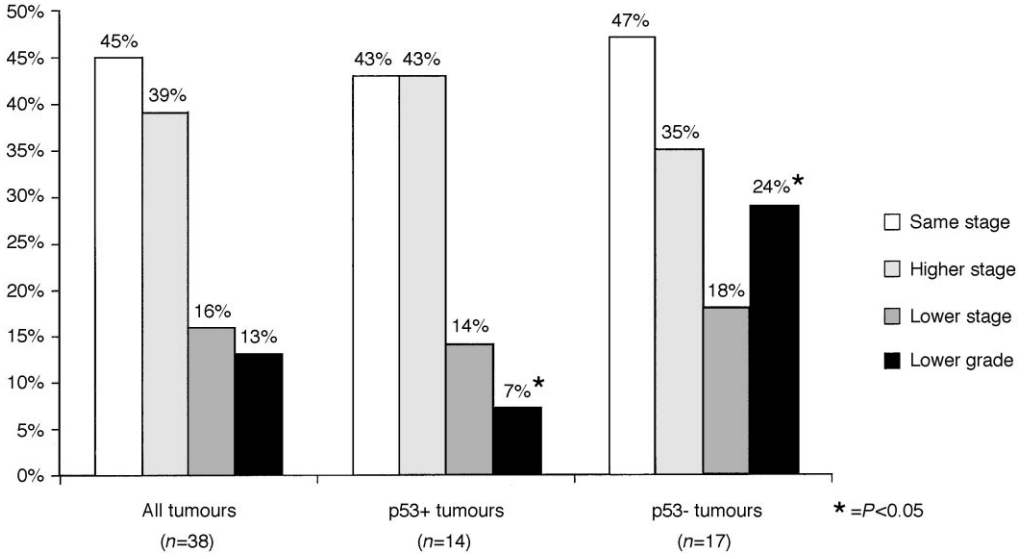

Fig. 3. Comparison of the predictive value of p53 immunostaining considering staging and grading between TUR-B and cystectomy specimens.

TUR-B tissue as compared with the cystectomy specimen. 14 tumours (37%) were p53 positive at the time of TUR-B. Of these, six (43%) had the same stage, six (43%) were understaged and two (14%) were overstaged by TUR-B. Of all 17 p53 negative tumours at the time of TUR-B, 8 (47%) were found to be of the same stage, six (35%) were understaged and three (18%) were overstaged, respectively. Lower grading (cystectomy compared with TUR-B) was found in 5/38 (13%) cystectomy specimens, one (7%) in the p53 positive group and four (24%) amongst the p53 negative tumours (Fig. 3). In 10/14 (71%) p53 positive tumours (TUR-B) and in 11 of 17 (65%) p53 negative tumours (TUR-B) the same p53 status was found in the corresponding cystectomy specimen.

The comparison of each group regarding either the equal, under- or overstaging of tumours (TUR-B versus cystectomy specimens) revealed no statistically significant difference in the p53 positive and p53 negative tumours. However, in p53 negative TUR-B biopsies, the corresponding cystectomy specimens showed a more lower grade tumour than the p53 positive TUR-B biopsy tumours and this difference was significant ($P < 0.05$; Fig. 3).

4. Discussion

The tumour suppressor gene protein p53 has been demonstrated to play a key role in the transition of cells from G1 to S phase in the cell cycle. It acts as a transcription factor regulating the expression of cell cycle regulators such as the cyclin-dependent kinase inhibitor *WAF1* [11]. In the absence of intact p53, cells may progress through the cell cycle despite genomic injury and give rise to daughter cells with an increasing amount of genetic alterations. Loss of a single intact allele can lead to inactivation of *TP53*. Complex formation (viral or cellular proteins) with p53 is another mechanism of inactivating p53 [12]. Genetic alterations or complex formation result in a prolonged half-life of p53 and the accumulated abnormal p53 protein may be visualised by immunohistochemistry. Approximately 90% of *TP53* mutations can be detected by this method [13].

Accumulation of p53 protein has been proposed an independent prognostic marker for superficial TCC of the bladder [14]. In the largest retrospective study with 243 patients, it has been reported to be an independent predictor of recurrence and survival in patients with stage T1-T3a TCC of the bladder [9]. In this study, however, only a small group of patients ($n = 51$) with T1 tumours was included.

p53 has not yet been prospectively tested as a prognostic marker in T1G3 TCC. At the participating institutions patients with T1G3 TCC of the bladder are treated by cystectomy. This precluded the prospective testing of p53 as a predictor of recurrence or survival. Instead, in the current study p53 was tested as predictor of muscle invasive disease. Our hypothesis was as follows: p53 negative TUR-B samples should correlate to cystectomy specimens without or with only superficial residual tumour. p53 positive TUR-B samples may indicate residual muscle invasive disease in the cystectomy specimen. Thus, p53 would be helpful in deciding whether patients should be treated with a cystectomy.

A lack of p53 immunoreactivity in the TUR-B specimen had no predictive value regarding the final stage of the cancer. However, there was a trend regarding the grading (cystectomy compared with TUR-B): only one case (7%) of all p53 positive tumours exhibited a lower grade at cystectomy, whereas 24% of p53 negative tumours did so. Of the 31 TUR-B samples, 14 showed p53 positive tumours. Of these, ten tumours (71%) still were p53 positive in the cystectomy specimens. This indicates that p53 expression might be associated with tumour cell clone. 6/17 initially p53 negative lesions (35%) exhibited p53 positive tumours in the cystectomy specimen. This change was not associated with a higher stage in the cystectomy specimen and may represent sampling error. 43% of initially p53 positive tumours were found to have the same stage at cystectomy and the same percentage was understaged by TUR-B. 3 patients had no tumour in their bladder at time of cystectomy.

Had we decided not to perform cystectomy in p53 negative patients with T1G3 TCC of the bladder, according to our initial hypothesis, 3 patients would have had a benefit because their bladder did not show cancer at cystectomy. However, 82% of patients (47% same stage and 35% understaged) would have been treated incorrectly, allowing for tumour progression in 48% [1]. In contrast, 2 patients (14%) of the p53 positive group who would have undergone cystectomy on the basis of the results of immunohistochemistry, had been overtreated.

p53 expression and progression in stage T1 TCC of the bladder have been demonstrated to correlate positively with a progression rate of 62% in p53 positive, versus 7% in p53 negative tumours [9]. However, only a small percentage of the evaluated patients had T1G3 disease. Our data suggest that there is no association between either over- or understaging and p53 status at TUR-B. Significant differences could be detected regarding the lower grade of p53 negative tumours in the analysis of the cystectomy specimens (Fig. 3). Since TUR-B was carried out by numerous surgeons at six different institutions, systematic resection errors can be excluded.

In conclusion, p53 was found not to be a prognostic marker of muscle invasive disease if used in TUR-B samples. We decided not to continue this study because

of the lack of association between p53 status and final pathology in the first 31 evaluable patients. According to our data, there are presently no unequivocal parameters to guide in making the decision for or against cystectomy in T1G3. The safe option of definitive surgery has to be discussed with the patient who has to be informed that he will lose his bladder unnecessarily in approximately 50% of cases.

Appendix

Members of the T1G3 TCC Study Group.

Name	Urological department	Consecutive patients contributed ($n = 38$)
R. Hohenfellner	Mainz University	20
S.C. Mueller	Bonn University	13
D. Frohneberg	Karlsruhe	2
J. Steffens	Eschweiler	1
M. Stoeckle	Kiel University	1
J. Thueroff	Wuppertal, Witten-Herdecke University	1

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