Original Article

P53 Protein Expression in Transitional Cell Carcinoma of the Bladder — Experience of the University of Malaya Medical Centre

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OBJECTIVES: To study the incidence of p53 oncoprotein overexpression and its relationship to tumour grade, stage and clinical prognosis in a cohort of local Malaysian patients.

METHODS: All cases of transitional cell carcinoma (TCC) of the bladder diagnosed and treated at the University of Malaya Medical Centre from January 1995 to December 2000 were retrieved from the hospital records. Sections from paraffin-embedded tissues were retrieved and stained for p53 oncoprotein using immunohistochemistry techniques. P53 oncoprotein results were analyzed in relation to tumour grade, stage and clinical prognosis. Fisher’s exact test was used to evaluate the relationship between categorical variables and the Kaplan-Meier procedure was used to assess survival outcomes. The Cox regression model was used for multivariate analysis.

RESULTS: A total of 64 cases were studied. The mean follow-up period was 23.7 months. The number of p53 positive cases was significantly higher in high-grade (G3) (p = 0.006) and muscle-invasive tumours (≥ T2, p = 0.035). The status of p53 expression had no significant association with recurrence-free (p = 0.594) or overall survival (p = 0.955). In multivariate analysis, a multiplicity of tumours at presentation (p = 0.004) and a history of cigarette smoking (p = 0.016) were independent predictors of recurrence. Tumour stage (p = 0.024) was the single independent predictor for poor overall survival.

CONCLUSIONS: Overexpression of p53 is associated with TCC of higher grade and tumour stage. It had no significant impact on prognosis in this cohort of TCC cases. (Asian J Surg 2003;26(1):31–6)

Introduction

P53, a tumour suppressor gene localized to chromosome 17p13.1, is the most thoroughly characterized gene of human bladder cancer. The gene consists of 11 exons that encode for p53, a 393 amino-acid, 53 kDa nuclear protein that functions as a transcription factor regulating the expression of many downstream genes such as p21, bax and bcl-2. This leads to cell growth arrest, apoptosis or other cellular mechanisms allowing the cell to adapt to mutational insults. The normal p53 gene and its protein product are important in regulating cell proliferation. It is called the “gate-keeper gene” because it arrests the cell cycle at the G1 phase in the event of DNA damage, allowing the damage to be repaired and reducing the probability of a mutation. In neoplastic cells containing mutant p53 protein, the cells might have an increased resistance to radiation, chemotherapeutic drugs and enhanced frequency of other mutational events.

Various studies report that mutant p53 protein is commonly detected in bladder carcinomas, especially in more advanced cancers. This suggests that the p53 gene has probably mutated in the later stage of carcinogenesis. The mutant p53 protein is stable and has a long half-life compared to the normal protein, with a half-life of 5 to 40 minutes. This phenomenon results in the accumulation of the mutant protein in the nucleus, which renders it detectable by immunohisto-
chemical (IHC) methods. P53 gene mutations can also be studied at the genomic level using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing. Most of the genetic mutations are detected in high-grade tumours. Detection of p53 overexpression using IHC is reported to correlate well with p53 mutations, as determined by PCR-SSCP and DNA sequence analysis.

P53 mutation has been studied for its potential as a marker for prognosis and survival outcomes, chemosensitivity, as a predictor of treatment failure after Bacillus Calmette-Guerin therapy, and as a target for genetherapy. Recent evidence indicates that the level of mutant p53 protein in transitional cell carcinoma (TCC) is important for the management of urinary bladder cancer, in that advanced stage cancers that overexpress p53 protein may benefit from adjuvant chemotherapy after cystectomy. Nonetheless, the question remains whether or not p53 overexpression can be used as a predictive marker for TCC tumour progression, recurrence and prediction of poor survival.

There are many reports elucidating the relationships between the loss of p53 protein function and tumour grade, stage and survival outcomes (recurrence, progression and overall survival) in TCC. Nonetheless, no conclusive evidence has emerged from these studies. Most of the studies applied immunohistochemical techniques for the detection of p53 oncoprotein because they are inexpensive, easy to perform and applicable as a routine laboratory procedure compared to molecular methods. The majority of the studies found that most of the aggressive and advanced stage cancers overexpress p53 protein, and a number of these studies concluded in favour of p53 overexpression as an independent prognostic marker. On the other hand, there are other studies reporting that p53 overexpression does not predict recurrence and survival in TCC of the urinary bladder.

We analyzed the overexpression of p53 oncoprotein in TCC of the bladder in a cohort of multiracial Malaysian patients, with the aim of adding to the pool of knowledge about p53 protein expression and its relationships to tumour stage, grade and survival outcomes in Asian bladder cancer.

Patients and methods

All cases of first-time diagnosed TCC of the urinary bladder were retrieved from the medical records of the University of Malaya Medical Centre (UMMC), from January 1995 to December 2000, and were included in this study. The demographic data, clinical management and progress of these patients were obtained from the patient records.

Formalin-fixed, paraffin-embedded tissues from the primary tumours were retrieved from the archives of the Pathology Department at UMMC. The haematoxylin and eosin slides of the tissue samples were reviewed, independently graded according to M. Stojilkić et al’s grading scheme and staged according to the T staging system of tumours-nodes-metastasis (TNM) by two pathologists (PSC and MN).

Immunohistochemistry

Mouse monoclonal antibody DO 7 (Dako, A/S, Golstrup, Denmark) was used as the primary antibody for the detection of p53 oncoprotein. The immunohistochemical stains were performed on 4-µm-thick tissue sections, microwaved in citrate buffer (pH 6.0) at 99°C for 25 minutes to retrieve the antigen. A secondary goat anti-mouse antibody conjugated with peroxidase-labelled dextran polymers was used (Dako Envision®, Carpinteria, CA, USA). Reactivity was visualized by adding diamino-benzidine (Dako Liquid DAB Substrate-Chromagen System, Carpinteria, CA, USA). A section of squamous cell carcinoma of the lung was used as a positive control for every batch of samples stained.

A tissue sample was considered positive for p53 oncoprotein overexpression when at least 20% of the tumour cell population showed positive nuclear reactivity. Samples demonstrating less than 20% nuclear reactivity were considered as non-expressors.

Statistical analysis

The correlation of p53 nuclear staining with tumour stage, grade and other clinical variables was analyzed using Fisher’s exact test. The Kaplan-Meier procedure was used for univariate survival analysis. The recurrence-free survival (RFS) was defined as the time period (in months) from the date of diagnosis to the date of the first documented clinical recurrence. The overall survival (OS) was defined as the time period from the date of diagnosis to the date of death from any cause. The relationship between the different survival curves was assessed using the log-rank test. Cases with no follow-up data were excluded from the analysis. For multivariate analysis, the Cox regression model was used. All p values reported are two-sided and values of p < 0.05 are considered as significant. All analyses were performed using SPSS (Statistical Package for the Social Sciences).
Results
A total of 64 cases of TCC of the urinary bladder were included in this study (from January 1995 to December 2000). All of the cases studied were newly diagnosed and were treated at UMMC, following the standard treatment protocol for TCC. The demographic and clinical data are presented in Table 1.

P53 overexpression was present in 42 out of the 64 cases (66%). The distribution of the proportion of tumours with p53 overexpression according to tumour stage and grade is shown in Table 2.

There was one case in which the tumour stage could not be ascertained from the available data and the archival specimen.

A statistically significant relationship was noted between p53 overexpression and tumours of higher stage (T ≥ T2 vs T1; p = 0.035). A similar trend was also observed for tumour grade, in which tumours of higher grade were associated with a higher incidence of p53 overexpression (grades 1/2 vs grade 3; p = 0.006). The overexpression of p53 did not appear to be associated with age at presentation (p = 0.220), patient gender (p = 1.000), ethnic group (p = 0.405), history of cigarette smoking (p = 0.570) or multiplicity of tumours at presentation (p = 1.000).

For the study of survival pattern, complete data of 53 patients were available for analysis. The mean follow-up was 23.7 months (standard deviation, 20.9 months; median, 14.0 months). RFS did not differ between the p53 overexpression and non-expression groups (p = 0.594) (Figure 1). The mean duration to recurrence was 35.4 months (95% confidence interval, CI, 23.7–47.0) for tumours that overexpressed p53 and 35.5 months (95% CI, 24.6–46.4) for tumours that did not.

In a multivariate analysis of RFS taking into consideration age at presentation, p53 positivity, tumour stage, grade, multiplicity of tumours at presentation and history of cigarette smoking, were independent predictors of recurrence (p = 0.004 and 0.016, respectively).

Univariate analysis for overall survival revealed that p53 overexpression was not significantly associated with a decreased probability of survival (p = 0.955) (Figure 2). The mean time to death was 52.5 months (95% CI, 42.0–63.0) for tumours that overexpressed p53 and 52.8 months (95% CI, 39.7–65.9) for tumours that did not overexpress p53. Multivariate analysis considering age at presentation, p53 positivity, and tumour stage and grade showed that high tumour stage was the independent predictor for unfavourable overall survival (p = 0.024). Further analysis showed no significant difference in RFS between tumours with or without p53 overexpression for T1 and T2 tumours (p = 0.355) (Figure 3).

Discussion
TCC is a common form of urinary bladder cancer. Its treatment demands a substantial amount of resources due to its propensity to recur after primary treatment of transurethral resection. Long-term follow-up is an essential component of the management plan. Many predictive and prognostic factors have been studied and evaluated for their usefulness in predicting clinical progression and outcome for this cancer, with the aim of identifying the subgroup of patients who would require more aggressive primary or adjuvant treatment and closer, more vigilant, follow-up. This is especially true in superficial, non-muscle invasive cancers, as tumours of similar
grade might behave very differently. In the extensive search for additional prognostic factors, p53 has emerged as one of the most promising candidates to fulfill the role. As far as we know, this study represents the first in-depth analysis of p53 overexpression in TCC of the urinary bladder in a multiracial Malaysian population.

The overexpression of p53 protein was noted in 66% of the cases studied. The incidence of p53 positivity in non-muscle invasive (Ta/T1) and invasive (≥T2) tumours was 56% and 88%, respectively. Tumours of all stages in this cohort demonstrated a high incidence of p53 positivity compared to that of other studies, despite a relatively high cut-off point of 20% for the evaluation of p53 overexpression. In general, the incidence of p53 positivity for non-muscle invasive and invasive tumours ranged from 15% to 50% for non-muscle invasive tumours and from 50% to 85% for invasive tumours in other reports. The biological and clinical significance of this finding is uncertain. It might imply that tumours in our series of cases were more aggressive in nature and, hence, would be an interesting aspect for future studies.

Tumours of higher stage and grade demonstrated higher incidence of p53 positivity in our study. This finding is in accordance with most other studies to date. It supports the hypothesis that p53 mutation and subsequent production of mutant p53 protein probably occurs at a later stage in the process of TCC carcinogenesis of the bladder. As a “gate-keeper” gene, the loss of p53 function gives the tumour a survival advantage, as the normal cell-regulatory functions of G1 arrest and the initiation of apoptosis are lost in face of carcinogenic insults. Furthermore, there is evidence that normal p53 has an anti-angiogenic function. Thus, in the case of p53 mutation, the angiogenesis inhibition function would be lost and potential for better growth and distant metastasis might be enhanced through angiogenesis.

Analysis of the results showed that p53 overexpression was not related to clinical variables such as age at presentation, patient sex, ethnic group, history of smoking or multiplicity of tumours at presentation. It is worthy to note that previous studies also found no difference in the incidence of p53 overexpression between smokers and non-smokers. Nonetheless, the p53 mutation spectrum is different between smokers and non-smokers. The three main ethnic groups (Malay, Chinese, Indian) in this study appeared to be no different in terms of the incidence of p53 overexpression. The relatively small number of patients, especially ethnic Indians, made further meaningful analysis of patient subgroups according to tumour stage impractical.
The aim of defining the role of p53 overexpression as a prognostic factor in TCC of the bladder remains to be ascertained. In this study, no significant relationship was found between p53 overexpression and RFS or OS when cases of all stages were analyzed. For the clinically important subgroup of superficial, non-muscle invasive cases, analysis showed that RFS was independent of the p53 status of the primary tumors. Thus, p53 status does not appear to predict which superficial tumours would be more likely to recur. Controversy still prevails on the role of p53 as a marker for bladder cancer progression. Our findings concur with many others that failed to show the benefit of p53 IHC studies, which contradicts the reports that demonstrated a positive correlation between p53 positivity and a higher risk of tumour recurrence and a poorer survival.

There is little doubt that IHC can identify most cases in which the cells had mutated p53 genes, thus, providing a reliable method to study p53 abnormalities in various cancers. However, 15% to 20% of tumours with p53 gene mutation fail to show overexpression by IHC studies. Furthermore, some tumours with nuclear expression of p53 lacked evidence of p53 gene mutation. The common problems faced in the interpretations of the various IHC studies of p53 in bladder cancer are the use of different antibodies, different cut-off points for determination of overexpression, case selection criteria, and length of follow-up. Hence, in this study, we used DO-7, which is a well-established antibody for p53 in IHC, and the 20% cut-off point was chosen based on previous studies that suggested that more than 20% staining of tumour cell nuclei for p53 was an independent predictor of tumour progression. Moreover, the number of patients available for analysis was certainly compatible with other studies of similar nature.

In summary, this study on TCC of the bladder in Malaysian patients demonstrated a high incidence of p53 oncoprotein overexpression. While p53 overexpression was associated with tumours of higher tumour stage and grade, this study failed to support the useful role of p53 overexpression for predicting disease progression. Since there is a lack of clear consensus or definitive treatment protocol regarding the use of p53 as a prognostic factor, traditional prognostic variables such as tumour stage remain the main factors to be considered in the management of bladder cancer. Refining and standardizing current research methodologies and international collaboration to enrol larger numbers of patients would certainly help to resolve the current impasse.

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References


