SUMMARY

Purpose: To reevaluate the expression levels of p53, p63, c-myc, p21\(^{\text{WAF1/cip1}}\) and p27\(^{\text{kip1}}\) proteins and their potential association with standard clinicopathological parameters, including tumor stage and grade, in urothelial bladder carcinoma (UBC).

Methods: Immunohistochemistry was performed in 100 transurethral resection specimens obtained from prospectively identified patients with primary UBC.

Results: Overall, 26, 41 and 75% of the cases showed positive staining for p53, p63 and c-myc, respectively, while p21\(^{\text{WAF1/cip1}}\) and p27\(^{\text{kip1}}\) expression levels were altered in 75 and 88% of the cases, respectively. Positive staining for p53 was associated with increased tumor stage (pT2) (p=0.037), while altered expression of p27\(^{\text{kip1}}\) was strongly associated with male gender (p=0.009).

Conclusion: The results of our study imply that p53 overexpression may be a useful marker of tumor invasion in UBC. In contrast, we failed to demonstrate any statistically significant correlation between the remaining markers evaluated and tumor stage or grade.

Key words: c-myc, cyclin-dependent kinase inhibitors, p53, p63, urinary bladder carcinoma

SHORT COMMUNICATION

Immunohistochemical expression of p53, p63, c-myc, p21\(^{\text{WAF1/cip1}}\) and p27\(^{\text{kip1}}\) proteins in urothelial bladder carcinoma: correlation with clinicopathological parameters

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Summary

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Introduction

Urothelial (transitional cell) bladder carcinoma UBC is the most common malignancy of the urinary tract and the seventh leading cancer in men worldwide [1]. Although tumor stage and grade remain the most important clinicopathological variables for predicting prognosis in this kind of cancer, there is also increasing recognition that UBC is a genetically heterogeneous malignancy and that conventional clinicopathological parameters alone cannot safely predict disease prognosis and treatment response in the individual patient [2].

Numerous previous studies have evaluated the expression and clinical value of cell cycle proteins in UBC, often in association with various oncogenes or apoptosis biomarkers, mainly using immunohistochemical methods [2,3]. The results of these investigations have largely revealed that some cell cycle regulators may have a significant value in predicting prognosis and that combinations of multiple markers may provide more accurate prognostication as compared with the standard single-marker approach [2,4,5]. However, additional data are needed to confirm these promising findings, while the considerable discrepancy observed among various studies as regards the exact prognostic relevance of individual markers warrants further investigation.

The aim of the present study was to reevaluate the expression levels of various proteins involved in cell cycle regulation, including both widely studied markers (p53, p21\(^{\text{WAF1/cip1}}\) and p27\(^{\text{kip1}}\)
as well as markers that have not received as much attention to date (c-myc and p63) in UBC, and to explore their potential association with standard clinicopathological parameters, including gender, age, tumor stage and grade.

Methods

Our study population comprised 100 prospectively-collected consecutive cases with primary UBC who were diagnosed and treated at our Institution. The mean patient age was 70.4 years (range 49-93) and the majority (77%) were males. Tissue samples in all cases were obtained during initial transurethral resection of the bladder tumor (TUR-BT). Tumors were staged according to the 2002 TNM classification and graded according to the 2004 World Health Organization (WHO) classification. For the statistical analysis, clinicopathological variables were dichotomized as follows: gender; age: <65 years vs ≥65 years; tumor stage: superficial (pTa-pT1) vs invasive (≥pT2); tumor grade: low vs high. A total of 70 tumors were classified as superficial and 30 as invasive, whereas 21 tumors were low grade and 79 high grade. Follow-up data were available only in 38% of our studied cases and were not included in the analysis.

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded sections obtained from each tumor specimen. Briefly, tissue sections were deparaffinized and rehydrated using standard procedures. Heat-induced antigen retrieval was performed in citrate buffer (pH=6). Commercially available monoclonal antibodies were used for the immunohistochemical detection of p53, p63, c-myc, p21WAF1/cip1 and p27kip1 proteins (clones: D07, 7JUL, 9E11, 4D10 and 1B4, respectively; dilutions: 1:50, 1:25, prediluted, 1:25 and 1:20, respectively; source: YLEM). Immunostaining was performed on a Bond-X automated immunostainer (Vision biosystems, Newcastle, UK). Known positive controls were included in each run of immunostaining. Negative controls were also run by replacing the primary antibody with PBS.

Immunostaining was assessed by two independent pathologists. At least 500 cells were evaluated in randomly selected high-power (400x) fields. Immunohistochemical staining for each marker was scored according to previously validated protocols [5-7]. Staining for p53 and p63 was considered positive when ≥10% of tumor cells showed strong nuclear staining. Staining for c-myc was scored as positive when >20% of tumor cells showed nuclear and/or cytoplasmic staining. Immunoreactivity for p21WAF1/cip1 and p27kip1 was considered altered when <10% and <30% of tumor cells, respectively, showed nuclear staining.

Statistics

Statistical analysis of the results was performed using Pearson’s chi-square test. All p-values were two-tailed. Statistical significance was set at p<0.05 and analyses were conducted using SPSS statistical software, version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Overall, p53 overexpression was detected in 26% of the cases (Figure 1), while 41 and 75% of the cases showed positive staining for p63 and c-myc, respectively (Figure 2A and B). p21WAF1/cip1 and p27kip1 expression levels (Figure 3A and B) were altered in 75 and 88% of all of the cases, respectively. The results of statistical analysis showing the correlations between the immunohistochemical expression of p53, p63, c-myc, p21WAF1/cip1 and p27kip1 proteins and the clinicopathological variables evaluated (gender, age, tumor stage and grade).
Expression of 5 proteins in bladder cancer

Table 1. Correlations between p53, p63, c-myc, p21 and p27 status and the clinicopathological parameters evaluated (p-values were derived using Pearson's chi-square test)

<table>
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<th>p value</th>
<th>p63</th>
<th>p value</th>
<th>c-myc</th>
<th>p value</th>
<th>p21</th>
<th>p value</th>
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<td>0.797</td>
<td>12 41</td>
<td>0.846</td>
<td>25 75</td>
<td>0.119</td>
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<td>75 25</td>
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grade) are summarized in Table 1. Positive staining for p53 was associated with increased tumor stage (pT2) (p=0.037), while altered expression of p27kip1 was strongly associated with male gender (p=0.009). No other statistically significant association was found. The results of statistical analysis regarding the correlations of all markers to each other showed no statistically significant associations either (p>0.05 in all cases).

Discussion

Mutations of cell-cycle regulatory genes, encoding a variety of inhibitory and stimulatory proteins, are among the key and most common genetic defects observed in UBC, and seem to play a major role in its development and progression [2]. Recent molecular genetic evidence has further indicated that the primary step in the pathway to abnormal cell growth and carcinogenesis in the urinary bladder involves alterations in the tumor suppressor genes responsible for cell cycle control (p53, p16 and RB1), while defects in downstream components of the cell cycle, such as cyclins, cyclin-dependent kinases (CK) and their inhibitors, have also been shown to contribute to malignant transformation of the urothelium [2,4].

Immunohistochemical detection of p53 overexpression has been previously correlated with tumor stage and grade, recurrence and progression of UBC in several studies, and is generally considered as one of the most promising biomarkers for improved risk stratification of patients [2-5,7,8]. Interestingly, recent observations have also revealed that the predictive power of p53 may be increased when this marker is evaluated in combination with other cell cycle regulators, such as the cyclin-dependent kinase inhibitors p21WAF1/cip1 and p27kip1 [4,5,8,9]. Nevertheless, the independent clinical relevance of these markers remains to be validated by prospective outcome data, while additional research is also needed to confirm preliminary evidence on the potential utility of other candidate immunohistochemical biomarkers, including the transcription factor c-myc and the p53-homologue p63, as diagnostic and prognostic tools in UBC [3,7,10-12].

In the present study, p53 overexpression and loss of p27kip1 expression were associated with increased tumor stage and male gender, respectively, while the expression levels of the remaining markers (p63, c-myc and p21WAF1/cip1) were not found to correlate with any clinicopathological parameter evaluated. Our findings are in line with some previous reports [4,7,8], thus indirectly supporting the concept that p53 may play an active role in urinary bladder carcinogenesis, potentially driving disease progression towards a more invasive phenotype.

Some important limitations of our study should nevertheless be noted, mainly including the absence of follow-up data for the majority of our patients, which practically precluded any meaningful survival analysis, as well as the relatively small sample size of patients with invasive tumors. On the other hand, the main strength of our study is its prospective design, which minimized selection and recall biases inherent in ret-
Prospective analyses.

In conclusion, the results of this prospective-collected series suggest that p53 overexpression may be a useful marker of tumor invasion in UBC. Further prospective trials are warranted to confirm and validate data derived from previous retrospective studies in order to incorporate novel molecular biomarkers into routine staging and prognostication of patients with this disease.

References


