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SHORT COMMUNICATION

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

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Summary

Purpose: *To reevaluate the expression levels of p53, p63, c-myc, $p21^{WAF1/cip1}$ and $p27^{kip1}$ 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 $p21^{WAF1/cip1}$ and $p27^{kip1}$ 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 $p27^{kip1}$ 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*

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, $p21^{WAF1/cip1}$ and $p27^{kip1}$)

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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, $p21^{WAF1/cip1}$ and $p27^{kip1}$ 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 $p21^{WAF1/cip1}$ and $p27^{kip1}$ 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).

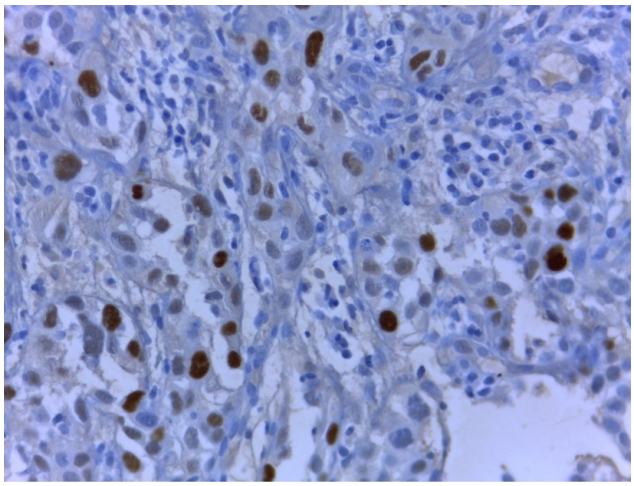

Figure 1. Nuclear p53 overexpression in urinary bladder carcinoma (x 400).

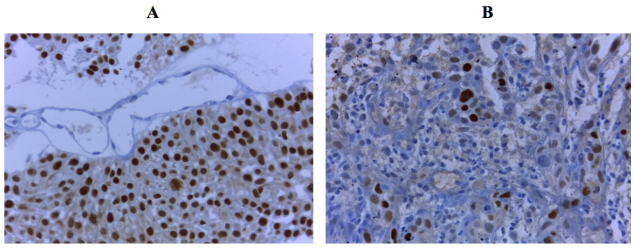

Figure 2. Nuclear p63 **(A)** and c-myc **(B)** overexpression in urinary bladder carcinoma (x 400).

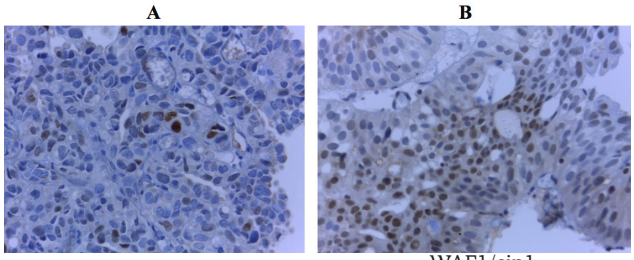

Figure 3. Positive expression of $p21^{WAF1/cip1}$ **(A)** and $p27^{kip1}$ **(B)** in urinary bladder carcinoma (x 400).

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). $p21^{WAF1/cip1}$ and $p27^{kip1}$ 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, $p21^{WAF1/cip1}$ and $p27^{kip1}$ proteins and the clinicopathological variables evaluated (gender, age, tumor stage and

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)

	Total N	*p53*		*p value*	*p63*		*p value*	*c-myc*		*p value*	*p21*		*p value*	*p27*		*p value*
		-	+		-	+		-	+		-	+		-	+	
Gender																
Male	79	58	21		47	9		17	62		59	20		73	6	
Female	21	16	5		32	59		8	13		16	5		15	6	
Total	100	74	26	0.797	12	41	0.846	25	75	0.119	75	25	0.887	88	12	0.009
Age (years)																
<65	36	27	9		21	56		10	49		25	11		34	2	
≥65	64	47	17		15	59		26	25		50	14		54	10	
Total	100	74	26	0.864	38	41	0.919	15	75	0.630	75	25	0.336	88	12	0.137
T stage																
Superficial	70	56	14		39	31		16	54		51	19		62	8	
Invasive	30	18	12		20	10		9	21		24	6		26	4	
Total	100	74	26	0.037	59	41	0.308	25	75	0.450	75	25	0.450	88	12	0.788
Grade																
Low	21	18	3		9	12		7	14		15	6		17	4	
High	79	56	23		50	29		18	61		60	19		71	8	
Total	100	74	26	0.169	59	41	0.091	25	75	0.321	75	25	0.671	88	12	0.263

grade) are summarized in Table 1. Positive staining for p53 was associated with increased tumor stage (pT2) (p=0.037), while altered expression of $p27^{kip1}$ 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 $p21^{WAF1/cip1}$ and $p27^{kip1}$ [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 $p27^{kip1}$ expression were associated with increased tumor stage and male gender, respectively, while the expression levels of the remaining markers (p63, c-myc and $p21^{WAF1/cip1}$) 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-

rospective analyses.

In conclusion, the results of this prospectively 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

1. Burger M, Catto JW, Dalbagni G et al. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 2013;63:234-241.
2. Mathsushita K, Cha EK, Matsumoto K et al. Immunohistochemical biomarkers for bladder cancer prognosis. Int J Urol 2011;18:616-629.
3. Baffa R, Letko J, McClung C, LeNoir J, Vecchione A, Gomella LG. Molecular genetics of bladder cancer: targets for diagnosis and therapy. J Exp Clin Cancer Res 2006;25:145-160.
4. Shariat SF, Zlotta AR, Ashfag R, Sagalowsky AI, Lotan Y. Cooperative effect of cell-cycle regulators expression on bladder cancer development and biologic aggressiveness. Mod Pathol 2007;20:445-459.
5. Shariat SF, Karakiewicz PI, Ashfag R et al. Multiple biomarkers improve prediction of bladder cancer recurrence and mortality in patients undergoing cystectomy. Cancer 2008;112:315-325.
6. Visca P, Alo PL, Del Nonno F et al. Immunohistochemical expression of fatty acid synthase, apoptotic-regulating genes, proliferating factors, and ras protein product in colorectal adenomas, carcinomas, and adjacent nonneoplastic mucosa. Clin Cancer Res 1999;5:4111-4118.
7. Comperat E, Camparo P, Haus R et al. Immunohistochemical expression of p63, p53 and MIB-1 in urinary bladder carcinoma. A tissue microarray study of 158 cases. Virchows Arch 2006;448:319-324.
8. Shariat SF, Tokunaga H, Zhou J et al. p53, p21, pRB, and p16 expression predict clinical outcome in cystectomy with bladder cancer. J Clin Oncol 2004;22:1014-1024.
9. Chatterjee SJ, Datar R, Youssefzadeh D et al. Combined effects of p53, p21 and pRb expression in the progression of bladder transitional cell carcinoma. J Clin Oncol 2004;22:1007-1013.
10. Urist MJ, Di Como CJ, Lu ML et al. Loss of p63 expression is associated with tumor progression in bladder cancer. Am J Pathol 2002;161:1199-1206.
11. Schulz WA, Jankevicius F, Gerharz CD et al. Predictive value of molecular alterations for the prognosis of urothelial carcinoma. Cancer Detect Prev 1998;22:422-429.
12. Netto GJ. Molecular biomarkers in urothelial carcinoma of the bladder: are we there yet? Nat Rev Urol 2011;9:41-51.