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BCL-2, TP53 and BAX protein expression in superficial urothelial bladder carcinoma

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Abstract

Whether TP53, BCL-2 and BAX expressions add independent prognostic information in patients with Ta/T1bladder urothelial carcinoma remains unclear. TP53 overexpression correlated with high tumor grade ($p = 0.004$), WHO grading categories (0.045), BAX expression ($p = 0.043$) and pathologic stage ($p = 0.05$). BCL-2 immunostaining was inverse associated with tumor grade ($p = 0.008$). Lack of BAX expression was related to reduced patient's survival ($p = 0.028$). Mortality was higher in patients with BCL-2+/TP53+ ($p = 0.023$) or TP53+/BAX− ($p = 0.027$) phenotype. BAX and pathologic stage were independent predictors of progression-free and overall survival, respectively. Therefore, BAX expression might be relevant in patient's prognosis.

Keywords: TP53; BCL-2; BAX; Bladder cancer

1. Introduction

It is known that TP53, BCL-2 and BAX genes code for proteins which have pro- or anti-apoptotic actions. Cells with mutated TP53 fail to undergo apoptosis following DNA damage, leading to genomic instability; on the other hand, BCL-2 and BAX have opposite effects on cell death; while BCL-2 overexpression may result in cell proliferation, BAX overexpression promotes cell death [1]. In bladder urothelial neoplasms the influence of these proteins on prognosis has been studied [2–35] with often conflictive results that might be related to the lack of uniform criteria in patients selection, staining procedure and cut-off values, as pointed out in a recent TP53 meta-analysis [3]. So far, little

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attention has been paid to their role in superficial tumors graded according to the recent 2004 World Health Organization (WHO) grading scheme [36].

Our aim was to investigate the prognostic and predictive value of TP53, BAX and BCL-2 immunohistochemical expressions in a consecutive series from a single institution of Ta/T1bladder urothelial carcinomas graded according to 2004 WHO grading scheme. Conventional clinico-pathological variables entered the analysis for comparison purposes.

2. Material and methods

The study group was a sequential cohort series of 147 patients with primary bladder tumor treated between 1991 and 1996 by complete transurethral resection of bladder (TURB) followed in case of high grade carcinoma by intravesical BCG (*Bacillus* Calmette–Guerin) as current protocol. Patient's follow-up, calculated as the number of months from the date of the diagnostic surgical procedure to the date of the most recent cystoscopy (or the last visit or death) was 75 ± 28 (mean $\pm$ SD) months (range 6–120 months). Tumor recurrence was defined as reappearance of tumor after the initial treatment with at least one tumor-free cystoscopy interval. Tumor stage progression was defined as a shift to stage T1–T4 or the appearance of metastasis; survival time was the period between diagnosis and the time of death. Cancer-related death was defined as that caused by bladder carcinoma. The end point of the study was disease-free, progression-free and overall cancer-specific survival. Tumor size was defined as the largest tumor measured with the resection loop that is 1 cm long. Available hematoxylin and eosin stained slides including primary tumors and their recurrences were re-evaluated by three dedicated pathologists (RGC, ALB and AGE) without knowledge of the clinical status. The resulting grade and stage was according with the 2004 WHO [36] and the TNM (Tumor, Node and Metastasis), 2002 revision.

2.1. Quantitative and qualitative assessment of immunohistochemistry

Representative paraffin blocks were serially cut at 4 μm thick, deparaffinized in xylene, rehydrated in graded ethanol and washed for 5 min with phosphate-buffered saline. For antigen retrieval, the sections were boiled immersed in 10 mM citrate buffer (pH 6.0). Endogenous peroxidase was blocked by incubation of the slides for 30 min with 3% hydrogen peroxide in methanol. Sections were then incubated with primary mouse monoclonal antibodies (Novocastra, Newcastle upon Tyne, UK) at room temperature; TP53 (BP 53-12 at 1:50 dilution), BCL-2 (100/B5 at 1:80 dilution) and BAX (AHP 471 at 1:1000 dilution). Immunohistochemical stains were performed using the high sensitive polymer-based system (EnVision, DakoCytomation, Denmark) with Diaminobenzidine substrate solution as chromogen. Sections were counterstained with Mayer's hematoxylin, dehydrated and mounted following the standard procedure.

Quantitative analysis was conducted using a Nikon Labophot optical microscope (Tokyo, Japan). Three dedicated pathologists independently evaluated all immunohistochemical slides in a blinded fashion. The same area on each slide was examined. Each marker was quantitated by using random fields measuring 62,500 μm^2 delineated by 1 cm^2 graded ocular grid attached to the eyepiece of the microscope. The regions were chosen inside high immunoreactive areas and were examined under high power (400×) view counting a mean of 1000 cells per case. For statistical evaluation the following cut-off and categories were selected: TP53, 5% (negative: $<5\%$; positive: $\geqslant 5\%$); BCL-2, 1% (negative: $<1\%$; positive: $\geqslant 1\%$); BAX, 25% (negative: $<25\%$; positive: $\geqslant 25\%$).

2.2. Statistical analysis

Bivariate and multivariate analysis was undertaken by Fisher exact test and χ^2 analysis and comparison of the means by ANOVA and Kruskal–Wallis test. Univariate survival analysis was conducted using the Kaplan–Meier method, and differences among groups were tested for significance using the Log-rank test; significant parameters entered a multivariate analysis of probable prognostic factors for survival using Cox's proportional hazard regression analysis and calculated the relative risk (R.R.) with 95% confidence interval. All were two-sided tests. All of the statistical analysis was performed using the SPSS for Windows Software (SPSS Inc, Chicago, IL). A p-value of less than 0.05 was considered as significant.

3. Results

Mean patient's age at diagnosis of the 147 (20 were female) cases included in this study was 66 years (range 30–95, years). Sixty three (42.9%) patients had non-invasive tumors classified as papilloma (3.4%), papillary urothelial neoplasm of low malignant potential urothelial (PUNLMP) (16.3%) and low grade urothelial carcinoma (LGTa) (23.1%). Eighty four (57.1%) were stage T1 tumors: low grade urothelial carcinoma (LGT1) (39.5%) and high grade urothelial carcinoma (HGT1) (17.7%). Tumor size ranged 1–8 cm (mean 3.1 cm). During follow-up period 79 (53.63%) patients remained free of disease and 68 (46.37%) had recurrences. Stage progression was observed in 22 (15.0%) cases and 16 (10.9%) patients died of disease. Stage correlated with recurrence ($p = 0.046$), but dead ($p = 0.059$) and progression (0.060) had marginal significance.