

Urological Oncology

Correlation between Apoptosis and Histological Grade of Transitional Cell Carcinoma of Urinary Bladder

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ABSTRACT

Purpose: To evaluate the relationship between histological grade and apoptotic index (AI) in transitional cell carcinoma (TCC) of urinary bladder.

Materials and Methods: Formalin-fixed and paraffin-embedded tissue blocks from 75 patients with TCC, who undergone transurethral resection (TUR) were studied. One 3-micron section was provided from each TUR samples. In one section after hematoxylin and eosin (H&E) staining, tumor grade was determined according to World Health Organization/International Society of Urology and Pathology (WHO/ISUP) criteria. The apoptotic cells were determined using a terminal deoxynucleotidyl transferase (TdT) mediated dUTP biotin nick end labeling (TUNEL) technique. Apoptotic index was then obtained as the percent of TUNEL positive cells from observations of at least 1000 cells in each section.

Results: Forty-nine patients were men and 26 were women. Mean age was 56.34 ± 9 years. Mean AI was 2.30 ± 0.50 . The relationship between grade and AI was significant ($p=0.000$, $r=0.551$); a higher grade was associated with a higher AI.

Conclusion: Apoptosis index has a positive correlation with bladder TCC's grade. Further studies are needed to better determine the effect of apoptosis index on prognosis.

KEY WORDS: transitional cell carcinoma, grade, apoptotic index, TUNEL

Introduction

Transitional cell carcinoma (TCC) of urinary bladder is the second most common genitourinary cancer.⁽¹⁾ Patient's prognosis depends on several clinicopathological findings. Some of the related variables are stage, histological grade, age, site of the tumor, and simultaneous mucosal lesions in bladder.⁽²⁾ It is well known that the growth of tumor is determined by cell proliferation and cell loss (apoptosis). Recent studies revealed important role for apoptosis in the tumor progression and the prediction of patient survival in a variety of cancers,^(3,4,5) but little is known concerning the role of tumor apoptosis in TCC of urinary bladder. In the current study, we investigated the frequency of apoptosis by termi-

nal deoxynucleotidyl transferase (TdT) dUTP biotin nick end labeling (TUNEL) technique, and evaluated the relationship between apoptosis and tumor grade.

Materials and Methods

Formalin-fixed and paraffin-embedded tissue blocks from 75 patients with TCC of urinary bladder who had undergone transurethral resection (TUR) were studied retrospectively. None of the patients had any treatment before TUR. The tissue blocks were cut into 3-micron thick sections. Representative sections were stained with routine hematoxylin and eosin (H&E) and evaluated. Histological grading was done using the WHO/ISUP criteria. For the detection of apoptot-

ic cells, in 3-micron thick sections of the formalin-fixed and paraffin-embedded tissue, the TUNEL method was used according to the procedures included in the In Situ Cell Detection Kit, POD (Roche Diagnostics GmbH).⁽⁶⁾ In brief, after deparaffinization and blocking steps, the sections were exposed to Trypsin (0.25 gr) in 100 ml HCl (0.01 normality) and incubated for 30 minutes at 37 degrees centigrade. Incubation with 50 microliters TUNEL reaction mixture (50 microliters terminal deoxynucleotidyl transferase with 450 microliters nucleotide mixture) was performed in a moist chamber for 60 minutes at 37 degrees centigrade, followed by exposure to 50 microliters converter-peroxidase for 30 minutes at 37 degrees centigrade. The sections were then exposed to diaminobenzidine tetrahydrochloride. For negative controls, TdT was omitted.⁽⁶⁾ The apoptotic index (AI) was obtained as the ratio of TUNEL-positive cells to the total number of counted tumor cells and calculated from observations of at least 1000 cells in each section.

Spearman's Rho test was used to analyze the data by SPSS 9.0 package.

Results

We studied 75 samples from patients with TCC of the urinary bladder, of whom 49 were men and 26 were women. The median age was 56 (range 39 to 80) years. The frequency of the samples' grades is shown in figure 1. Mean AI was 2.30 ± 0.50 (range 1.10 to 3.60) percent. Mean AI in each grade is shown in figure 2. AI ranged

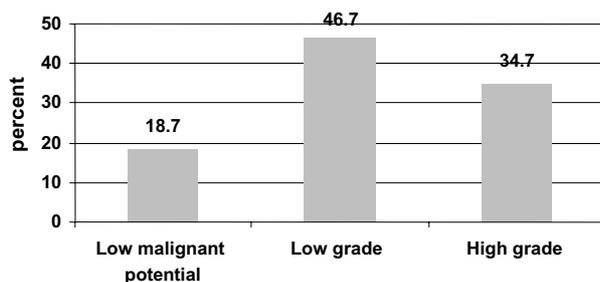


FIG. 1. Frequency of different Histological grades

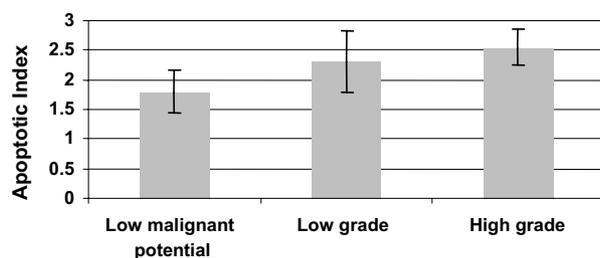


FIG. 2. Mean apoptotic index (AI) in different grades

from 1.10% to 2.30% in low malignant potential (LMP) grade, from 1.40% to 3.60% in low grade, and from 1.80 to 3.20% in high grade tumors. AI was significantly related to the tumor grade ($p=0.000$, $r=0.551$).

Discussion

Most previous studies have demonstrated significant relationship between the presence of apoptosis and tumor grade in TCC of the bladder,^(7,8,9) using the TUNEL method. These studies reported that a higher tumor grade was associated with a higher percent of AI. Our study also agrees with this finding.

However, Lavezzi et al reported an opposite result.⁽¹⁰⁾ They observed a worse prognosis in the presence of low or absent apoptosis. Their study involved 177 patients, which were more than the current and previous studies.^(7,8,9,11)

Zhang et al reported a mean value of 2.26% for AI.⁽⁹⁾ Our report agrees with their study. Nevertheless, according to Shiina et al,⁽⁸⁾ mean AI was 0.96%. The TUNEL method was used for detecting apoptotic cells in these studies, so the differences in performing several steps of the TUNEL technique is probable.

Korkolopoulou et al demonstrated that the assessment of apoptotic potential is more informative than standard prognostic factors in predicting overall survival in patients with TCC of the urinary bladder.⁽¹¹⁾ For more comparable results our patients are needed to be followed up for long-term.

The advantage of this study, compared with the previous ones,⁽⁷⁻¹¹⁾ is the usage of WHO/ISUP criteria in histological grading, which is the newest suggested grading criteria.

Conclusion

We demonstrated a close association between apoptosis and tumor grade in TCC of urinary bladder. We believe that the evaluation of apoptosis may be useful in predicting prognosis of the TCC of the urinary bladder. Other prospective studies are needed for better determination of the correlation between apoptosis and tumor grade in TCC of bladder.

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