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ORIGINAL ARTICLE

Relationship of TP53 and Ki67 expression in bladder cancer under WHO 2004 classification

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Summary

Purpose: *Tumor markers TP53 and Ki67 are currently common labels used in the diagnosis of bladder cancer throughout the world. In light of the co-existence of both WHO1973 and 2004 classifications for bladder cancer, it is necessary to establish different quantification standards for both labels to better cater for the grading and staging.*

Methods: *We investigated the immunohistochemical profiles of 280 bladder cancer samples classified under WHO 2004 standards. TP53 was scored semi-quantitatively whilst Ki67 was scored by label index.*

Results: *We found that expression of TP53 was not correlated to either grade or stage, a finding that doesn't agree with most of the literature. Expression of Ki67 was correlated with grade and stage. Expressions of TP53 and Ki67 were correlated with each other. Interestingly, Ki67 expression was higher in females.*

Conclusion: *The expression of TP53 could be modified to better suit the WHO 2004 classification.*

Key words: *esophageal carcinoma, meta-analysis , prognostic factor, vascular endothelial growth factor*

Introduction

Bladder cancer is the fourth most common malignancy in the Western world and the first leading cause of cancer-related deaths among Chinese patients [1,2]. Known prognostic factors for bladder cancer survival include tumor stage and tumor grade. Histological tumor grade is considered an important prognostic factor in particular for the non-muscle-invasive urothelial bladder neoplasms (NMIBC), a category that includes tumors either with only lamina propria invasion or without invasion. Tumor stage also has association with prognosis of bladder cancer. Ta and Tis tumors account for 75–85% of the bladder neoplasms, while the remaining 15–25% are invasive (T1, T2–T4) or metastatic lesions at the time of initial presentation [3]. Survival at 5 years is less than 50% for stage T2–T3 tumors and less than 10% for T4 or N+/M+ tumors [4].

In bladder cancer, expression of several biological markers correlates with tumor stage and grade, and such markers have been proven to be significant prognostic factors of survival mostly in univariate analyses. According to the literature, the cell proliferation marker Ki67 and the cell cycle regulator TP53 are most important. Ki67 is a protein that is encoded by the MKI67 gene in humans and is a cellular marker for proliferation [5]. It is strictly associated with cell proliferation, which is present during all active phases of the cell cycle (G_1, S, G_2, and mitosis), but is absent from resting cells (G_0). Likewise, TP53 mutations frequently occur in the progression of bladder cancer; moreover, a relationship has been noted between gelsolin and TP53 status, tumor stage, and clinical outcome [6]. Urothelial cancer has been described as a tumor type in which the TP53 gene is frequently mutated [7]. This gene plays an essential role in regulating the cell cycle and apoptosis and therefore contributes to both cellular transformation and malignancy [8].

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In this study, we have analyzed the relationship among tumor grades, clinical stages, Ki67 and TP53 expressions and the tumor category with clinical outcome in invasive and noninvasive papillary urothelial neoplasia. Our aim was to evaluate and compare the clinicopathological relevance of the 2004 WHO classification.

Methods

General information

Patients with bladder cancer operated on between January 2007 and December 2009 at Huashan hospital were included in this study. All patients had confirmed urothelial malignancy. There were 230 specimens acquired from transurethral resection of the bladder (TURBT), 27 specimens acquired from radical cystoprostatectomy, 11 specimens from partial cystectomy, and 12 specimens from cystoscopic biopsy. All sections were investigated twice by 2 independent pathologists for consistency in grade and stage according to the WHO/ISUP (2004) and UICC-TNM (2002) [9,10]. Tumor samples of Ta were classified as non-invasive papillary and of T1 as tumor invading subepithelial connective tissue, T2 as tumor invading the muscle layer and T3 to T4 as tumor invading perivesical tissue and the prostate, uterus, vagina, pelvic wall and abdominal wall. Ta-T1 were defined as NMIBC and T2-T4 as muscle invaling tumors (MIBC). Nodal and metastatic profiles were not included due to the limited number of patients. Tumors were graded as papillary urothelial neoplasms of low malignant potential (PUNLMP), low-grade and high-grade urothelial carcinomas. Informed consent was obtained from all patients and the study was approved by the Huashan institutional review board (HIRB).

Immunohistochemistry

All samples were formalin-fixed and subsequently paraffin-embedded. Slices were cut at 4μm, and tissues were mounted on polylysine-coated glass slides. Endogenous peroxidase of deparaffinized sections was blocked through incubation with 3% hydrogen peroxide for 15 min. Before further processing, heat-mediated antigen retrieval was performed by boiling the slides in 0.01M citrate buffer, pH 6.0, for 20 min in a microwave oven. The primary antibodies were diluted, including TP53 (Novocastra, Newcastle, UK) at 1:50 and Ki67 (Novocastra, Newcastle, UK) at 1:100. The slides were stained immunohistochemically using the avidin-biotin-complex method for both antibodies. DAB (diaminobenzidine tetrahydrochloride) solution was then used for color developing. Then, all slides were counterstained with Mayer's hematoxylin blue in 0.3% ammonia. Then, the slides were dehydrated through graded alcohols to xylene and mounted in mounting medium. For positive controls we used colon carcinoma for TP53. For negative controls we omitted the primary antibodies.

Assessment of Ki67 and TP53 staining

For Ki67, at least 1000 tumor cells (×400 magnification) from the most immunopositive regions of the noninvasive part of each neoplasm were visually counted, and the percentage of positive cells (labeling index, LI) was calculated. For TP53 however, a semi-quantitative method was used. Each slide was given a value composed of the sum of staining intensity and the proportion of the stained cells. This proportion was graded as follows: 0 for 0-10% of tumor cells stained, 1 for 11–25% of cells stained, 2 for 26–50% of cells stained and 3 for >50% of cells stained. Staining intensity was graded as follows: 1 for light yellow, 2 for dark yellow and 3 for brown. The final staining quantification value was as follows: 0 for negative (1-2), 1+ for mild (3), 2+ for moderate (4), and 3+ for strong (5-6) [11]. Immunohistochemical positivity was defined as nuclear staining for Ki67 and TP53.

Statistics

The SPSS 17.0 for Windows program was used for statistical analyses. All data were presented as mean ± standard deviation (SD). The Student's t-test was applied to compare scores of TP53 and Ki67 between 2 categories. The Kruskal-Wallis test was used for comparisons in more than 2 categories. Correlations between expressions of TP53 and Ki67 were evaluated with Spearman's correlation test. A p value of <0.05 was considered statistically significant.

Results

Staining of TP53 (Figure 1) and Ki67 (Figure 2) was immunopositive in the nucleus. Included were 207 (74%) males and 73 (26%) females, with average age 66.5 years (range 20-92). There were 24 (9%) samples graded as PUNLMP, whereas 170 (61%) samples were low-grade and 86 (70%) high-grade carcinomas. There were 240 (86%) cases with Ta stage, 3 (1%) cases with T1 stage, 25 (9%) cases with T2 stage, 9 (3%) cases with T3 stage and 3 (1%) cases with T4 stage. There were 240 noninvasive cases (Ta, 86%) and 40 invasive cases (T1-4, 14%) and 243 (87%) cases of NMIBC and 37 (17%) cases of MIBC.

Comparison of the expression levels of both factors is summarized in Table1. The expression of TP53 did not change significantly with progression of grades (Table 1). There was also no significant change in the expression of TP53 when cases were grouped according to invasion of the lamina propria or muscle and TP53 expression was not related to gender or age (Table 1).

Table 1. TP53 and Ki67 expression in relation to clinicopathological parameters (mean ± standard deviation)

Parameters	*TP53*	*Ki67*
PUNLMP	1.2±0.7	13.8±16.9
Low grade	1.1±0.8	21.7±21.8
High grade	1.2±1.0	46.0±27.2
p-value	*0.7851*	*< 0.0001*
Non-invasive	1.1±0.8	27.0±25.2
Invasive	1.1±1.1	36.8±29.2
p-value	*0.6204*	*0.0290*
NMIBC	1.1±0.8	26.9±25.2
MIBC	1.1±1.1	38.2±29.2
p-value	*0.3177*	*0.0712*
Males	1.1±0.8	26.4±25.4
Females	1.2±0.9	33.5±26.8
p-value	*0.5851*	*0.0455*
Correlation with age		
p-value	*0.8170*	*0.9602*

PUNLMP: papillary urothelial neoplasm of low malignant potential, NMIBC: non-muscle invasive bladder cancer, MIBC: muscle invasive bladder cancer

Concerning Ki67, its expression was significantly higher in the female population and was higher in cases with higher grade. Ki67 expression level was also higher in cases with infiltration of the lamina propria (Table 1). When cases were grouped by muscle invasion (NMIBC vs MIBC) a trend for higher expression was noted. Expression of Ki67 was also found to correlate with gender, as it was significantly higher in females (Table 1).

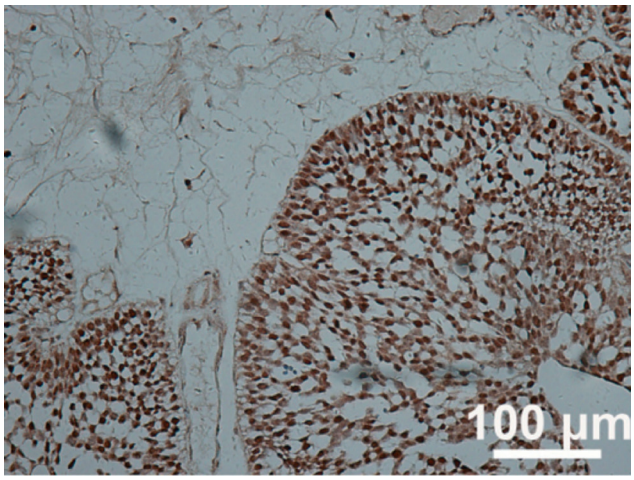

Figure 1. Immunohistochemistry of Ki67 in low grade bladder cancer (× 200).

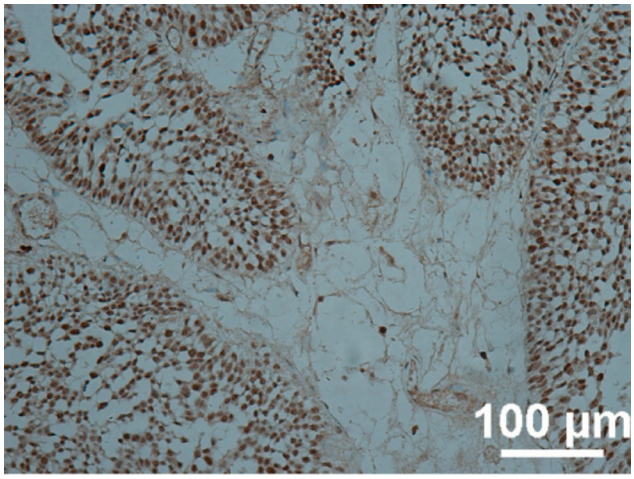

Figure 2. Immunohistochemistry of TP53 in low grade bladder cancer (× 200).

Discussion

Histological grade and stage are two powerful prognostic factors for recurrence and progression of bladder cancer [12,13]. At the time of initial diagnosis, approximately 70% of tumors are noninvasive, and only 5–10% of these will progress to invade the muscle layer [14].

TP53 expression is one of the most studied prognostic markers, indicating poor prognosis in bladder cancer [15,16]. Some researchers assert that TP53 accumulation in the tumor cell nuclei detected by immunohistochemical methods is an independent predictor of increased risk of recurrence and death in patients with bladder cancer [17]. Recent evidence demonstrates that TP53 is able to identify patients with high risk of recurrence in pTa and pT1 tumors, and that it is an independent predictor of increased recurrence rate and death in patients with urothelial neoplasms [18,19]. Some studies that used univariate analysis support this opinion [20,21], but others reject the predictive value of TP53 immunostaining for recurrence [22,23]. In our research we found that TP53 was not associated with bladder cancer grade or stage, similar with some studies that have shown a lack of association between TP53 expression and disease progression [24,25]. Our results do not agree with studies that have described a relationship of bladder cancer to TP53 expression in univariate analyses [26,27]. This discrepancy may result from either the difference of the grading systems (1973 vs 2004 version), or the semi-quantitative scoring pattern we applied. In some centers, expression of TP53 is quantified as Ki67 using expression fractions, whilst we have adopted a traditional pattern of scoring. It is possible that our pattern is more applicable under the WHO 1973 classification, while the fractional scoring suits the WHO 2004 version better [28].

The proliferative activity of tumors determined by Ki67 labeling index was found to cor-

relate with aggressive behavior of many tumor types including bladder cancer. Although tumor grade and stage are considered signs of aggressive behavior for bladder cancer, several reports in the literature describe a correlation between Ki67 labeling index with well-known prognostic factors, such as grade and stage [29]. In our series, Ki67 expression was also correlated with higher tumor grade as well as muscle invasion, which corresponds with most reports. We have, however, found a novel and interesting phenomenon that Ki67 is significantly higher in female patients. Given the fact that the incidence of bladder cancer is higher in males, this warrants further investigation on whether women are more susceptible to more aggressive Bladder Cancer by population-based studies. Currently, the mainstay of reports indicates that Ki67 expression is parallel to grade and stage status of bladder cancer both under the 1973 and 2004 classifications [30]. Nonetheless, the multivariate analysis of Yurakh et al. did not confirm the independent prognostic value of Ki67 for recurrence-free survival [31], and Mhawech et al. failed to confirm any value of Ki67 in predicting tumor progression [32]. All these discrepancies indicate that immunohistochemical quantification is prone to subjective judgment and it is thus hard to designate unanimous standards for different observers. However, no matter how much the expressions vary, the positive correlations between Ki67 and TP53 are documented in almost every study that involves these 2 factors, which was also found in our study.

Conclusion

We found that the semi-quantitative evaluation of TP53 does not correlate with grade or stage, whilst Ki67, quantified by label index, correlates with grade and stage. The expressions of TP53 and Ki67 are positively correlated. This indicates that quantification of TP53 should be modified according to WHO 2004 classification.

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