IMMUNOHISTOCHEMICAL ANALYSIS OF UROTHELIAL BLADDER CANCERS

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ABSTRACT

Malignant cancers of urinary bladder are the second most common malignancy of the urinary tract and the fourth most common malignancy in general, especially in men. The aim of this study was a retrospective analysis of selected markers (p53, Ki-67 and E-cadherin) of urinary bladder cancers from the Department of Urology in Bratislava, Slovak Republic between years 2007 and 2009. We analysed 244 patients (202 males, 42 females) with diagnosed bladder cancer via cystoscopy and subsequent transurethral resection. Patients' age varied from 36 to 98 years. Obtained samples were fixed by 10% buffered formalin for 24 to 48 h. Subsequently, they were dehydrated in ascending ethanol series and embedded in paraffin. The parafin sections of 5 µm were prepared by microtome and they were stained by haematoxylin and eosin. The antibodies against to p53, Ki-67 and E-cadherin were used in immunohistochemical analysis. Statistical evaluation was performed via SPSS using non-parametric Kruskal-Wallis test and p values<0.05 were considered statistically significant. No significant differences in the expression of selected markers were found between genders. Expression of p53 and Ki-67, in G1 and G2 of low grade tumours was lower in comparison to their expression in G3 tumors. Expression of E-cadherin was the opposite in this case. The expression of p53 and Ki-67 positively correlated with tumor’s depth of invasion, while the expression of E-cadherin significantly decreased. In case of T4 tumors, the expression of all markers exhibited consistently high values. When analysing tumor multiplicity, the expression of p53 and Ki-67 significantly decreased, while the expression of E-cadherin significantly increased. Based on the obtained results it can be concluded that the analysis of p53, Ki-67 and E-cadherin expression is essential for diagnostics and prognostics of bladder cancer and should be routinely used in daily practise together with “conventional” markers to improve prognosis estimation.

Keywords: Urinary Bladder Cancers, Immunohistochemistry, p53, Ki-67, E-Cadherin

1. INTRODUCTION

Urinary bladder cancer is seventh most common cancer worldwide and fourth in the developed countries (Jemal et al., 2011). Urinary bladder cancer is the second most frequent malignancy, after prostate cancer, of the urinary tract. The incidence of bladder cancer of men is higher than that of women; approximately 4:1 (Chu et al., 2013; Ziaran et al., 2011).

In Slovak population, the majority of urinary bladder cancers are from transitional urothelium. Approximately 70-75% of them do not infiltrate muscle of bladder wall. The rest of them (approximately 25%) infiltrate some layer of urinary bladder muscle. Approximately 600
cases of newly diagnosed urinary bladder cancer are recorded per year and it has slightly increasing tendency (Tomaskin et al., 2008).

Bladder cancer is a complex disease, which is affected by various internal and external factors, including genetical predisposition, age, gender, smoking, professional exposure to chemical carcinogens (Chu et al., 2013).

Significant impact on treatment and prognosis of urinary bladder cancer has early and accurate diagnosis. Recently, the transurethral cystoscopy followed by histopathological and immunohistochemical analysis represent “gold standard” in diagnostics. However, these techniques are supplemented by methods of molecular biology and genetics (Böhmer et al., 2010).

The expression of p53, Ki-67 and E-cadherin is extensively studied in process of finding of new prognostic markers. p53 and Ki-67 belong to regulatory proteins of cell cycle and proliferation and E-cadherin is involved in process of cell adhesion. Mutation of genes for these proteins may lead into malignant transformation (Priya et al., 2010; Shariat et al., 2009).

The aim of this study was a retrospective analysis of selected prognostic markers in patients with diagnosed bladder cancer in relation to gender, grade, stage of malignancy and multiplicity and relapses.

2. MATERIALS AND METHODS

2.1. Patients

In the present study, we analyzed 244 patients (202 males, 42 females) with an established diagnosis of bladder cancer, who were hospitalized at the Department of Urology, Faculty of Medicine in Bratislava, from January 2007 until the end of 2009. The basic characteristics are presented in Table 1.

2.2. Immunohistochemistry

The tissue samples were obtained from patients during planned transurethral cystoscopy, always following patient’s informed consent. All sampling procedures were performed in accordance with The Helsinki Declaration.

Obtained samples of tumors were fixed by 10% buffered formalin for 24-48 h. Subsequently, they were dehydrated in ascending ethanol series and embedded in paraffin. The parafin sections of 5 µm were prepared by microtome. The antibodies against to p53, Ki-67 and E-cadherin (Dako, Glostrup, Denmark) were used for immunohistochemical analysis according to manufacturer’s recommendations.

| Table 1. Basic characteristics of patients |
|-------------------------------------------|
| Age (years) | n (244%) | Males (202%) | Females (42%) |
| -50 | 14 (5,7) | 12 (6,0) | 2 (4,8) |
| 50-70 | 114 (46,7) | 100 (49,5) | 14 (33,3) |
| 70- | 116 (47,5) | 90 (44,6) | 26 (61,9) |

Finally, preparations were stained by Mayer’s hematoxylin and eosin. The mammary gland tissue was used as a positive control for p53 expression, tonsil tissue for Ki-67 and liver tissue for E-cadherin.

All samples were investigated by using laboratory microscope Nikon Eclipse 80i. Observations were digitally recorded by Nikon DS-Fi1 camera (Nikon, Japan).

2.3. Statistical Analysis

Statistical evaluation was performed in program SPSS using non-parametric Kruskal-Wallis test. P values <0.05 were considered statistically significant. Graphs were constructed in Microsoft ® Office Excel 2003.

3. RESULTS

No significant differences in the expression of selected markers were found between genders (p53, p = 0.629; Ki-67, p = 0.651; E-cadherin, P = 0.752) (Fig. 1). For markers p53 and Ki-67, in the case of G1 and G2 tumors, the average expression was significantly lower (p = 0.000) when compared with the average expression in HG tumors (Fig. 2). Expression of E-cadherin was the opposite in this case.

The analysis of the depth of invasion of the bladder in relation to the selected marker, it was found that expression of the p53 and Ki-67 increased with increasing depth of invasion (T1 →T3a), but the expression of E-cadherin decreased. In case of T4 tumors, all markers showed stable values of marker expression (Fig. 3).

In the analysis of tumors according to their multiplicity in relation to the selected markers is presented in Fig. 4. The expression of p53 and Ki-67 was lower than in case of E-cadherin expression. It was found that only the expression of p53 was at the threshold of statistical significance (p = 0.79).

The expression of p53 and Ki-67 was lower in case of newly diagnosed and relapsed urinary bladder cancers. On the contrary, the expression of E-cadherin was higher. However, these results were not statistically significant (p53, p = 0.629; Ki-67, p = 0.651; E-cadherin, p = 0.752) (Fig. 5).

Figure 4 differentiation of urinary bladder cancer according to multiplicity-comparison of mean values of p53, Ki-67 and E-cadherin expression.
Fig. 1. Comparison of markers according to gender

Fig. 2. Differentiation of urinary bladder cancer according to malignity grade with the classification of the LG and HG – comparison of mean values of p53, Ki-67 and E-cadherin expression

Fig. 3. Differentiation of urinary bladder cancer according to stage-comparison of mean values of p53, Ki-67 and E-cadherin expression
4. DISCUSSION

The new markers, necessary for more precise diagnostics and prognosis of bladder cancer (especially T1-T2) are intensively studied. Besides to classical (conventional) markers (grade, stage), some regulatory proteins (e.g., p53 and Ki-67) or intercellular adhesion markers (e.g., E-cadherin) seem to be promising in mentioned respect.

The overexpression of TP53 belongs to most frequently examined. TP53 gene mutations are the most common genetic abnormality in invasive papillary urothelial tumors which lead into upregulation of p53 (Lee et al., 2013). This analysis is usually completed by testing the expression of Ki-67 and E-cadherin (Priya et al., 2010; Ancuta et al., 2009). Ki-67 is a regulatory protein associated with the cell division. During interphase it can be detected in the nucleus. It is also present in all active phases of the cell cycle. But it is not occurred in quiescent cells. E-cadherin is a transmembrane glycoprotein that plays a key role in cell adhesion. Loss or reduction of E-cadherin expression is related to invasive phenotype...
of many cancers, including urinary bladder cancer (Khorrami et al., 2012).

We showed that expression of p53 and Ki-67 was significantly lower in case of LG when compared HG tumors, while in case of expression of E-cadherin it was opposite. Similar results were obtained by Jang et al. (2010), who found that the expression of E-cadherin decreases in case of HG tumors.

When analyzing tumors according to depth of invasion, we found that of p53 and Ki-67 expression increase in the case of muscle-infiltrating tumors (T2-T3), while the expression of E-cadherin decreased. Only in case of T4 tumors, the expression of all markers showed stable high values. These observations are in agreement with the results of Lim et al. (2011). Divergence in the case of E-cadherin expression in T4 tumors can be explained by the fact that in our group we had only one patient in this stage.

In case of analysis according to multiplicity, we divided tumors into 2 groups. First ones were multiple tumors and second ones were solitary. Results showed that the expression of p53 and Ki-67 had lower values when compared with the expression of E-cadherin. By comparison, it was found that only the expression of p53 was on the threshold of significance (p = 0.079). Our results are consistent with the results published Calvo et al. (2006), who also found a statistically significant increase in the expression of Ki-67 in the case of recurrent tumors. Moreover, it was found that patients with low expression of E-cadherin have lower risk for recurrence of the tumor.

Expression of p53 and Ki-67 was lower, both, in the case of newly diagnosed tumors and relapses. On the contrary, the expression of E-cadherin was higher in both cases. Those correlates with the results published Khorrami et al. (2012).

All of the above findings should be verified in a larger group of patients using the other potential markers (e.g., PCNA, FGFR3, AKT1). Extensive study allow definitively determine the diagnostic and prognostic markers with the highest informative value suitable not only for urinary bladder cancer, but also for other malignancies (Cariaga-Martinez et al., 2013; Ziaran et al., 2013).

5. CONCLUSION

In summary, based on obtained results it should be concluded that the analysis of expression of p53, Ki-67 and E-cadherin have a important prognostic value in the case of bladder cancer.

6. ACKNOWLEDGEMENT

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