OnLine Journal of Biological Sciences 13 (2): 66-71, 2013
ISSN: 1608-4217

doi:10.3844/ojbssp.2013.66.71 Published Online 13 (2) 2013 (http://www.thescipub.com/ojbs.toc)

IMMUNOHISTOCHEMICAL ANALYSIS OF UROTHELIAL BLADDER CANCERS

[1]Katarina Bevizova, [2]Jan Jakubovsky, [3]Jan Luha, [3]Zuzana Varchulova Novakova and [2]Stefan Polak

[1]Department of Anatomy,
[2]Institute of Histology and Embryology,
[3]Institute of Medical Biology, Genetics and Clinical Genetics,
Faculty of Medicine, Comenius University, Bratislava, Slovakia

Received 2013-07-13, Revised 2013-07-16; Accepted 2013-07-17

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

Corresponding Author: Katarina Bevizova, Department of Anatomy, Faculty of Medicine, Comenius University, Bratislava, Slovakia

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 histo-pathological 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 (5,9)	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 = 0.0.79).

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.

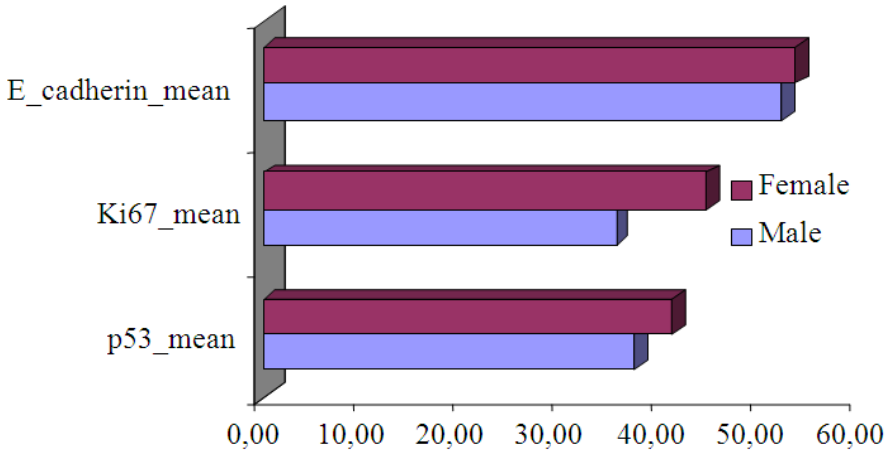

Fig. 1. Comparison of markers according to gender

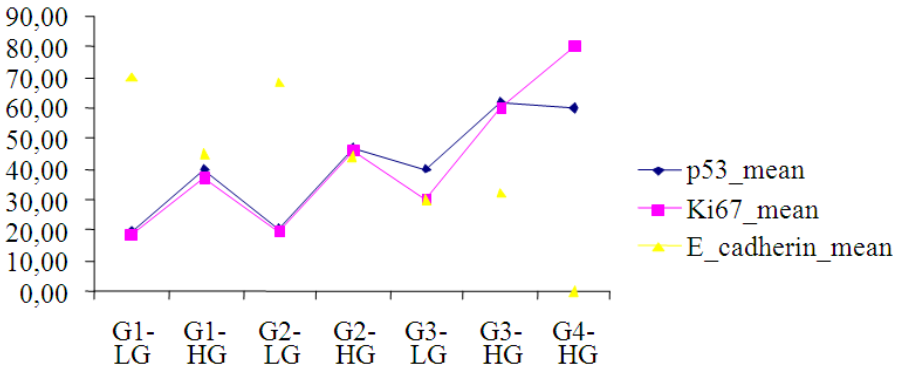

Fig. 2. Differentiation of urinary bladder cancer according to malignity grade with the classification of the LG and HG – comparison of mean values of p53, Ki-67 and E-cadherin expression

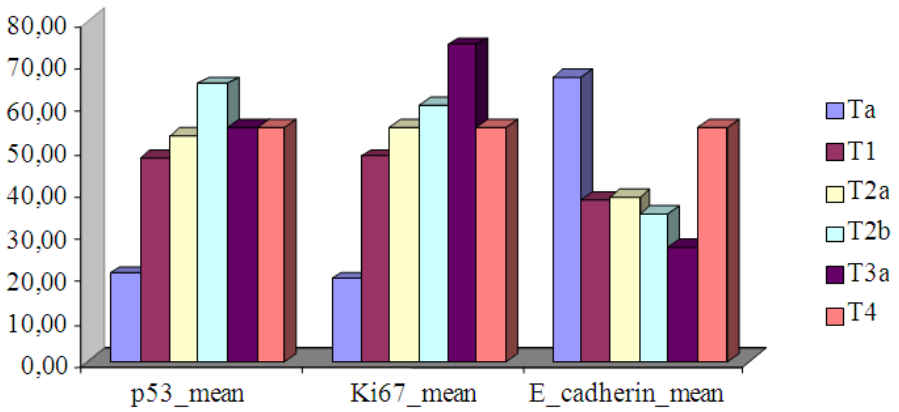

Fig. 3. Differentiation of urinary bladder cancer according to stage-comparison of mean values of p53, Ki-67 and E-cadherin expression

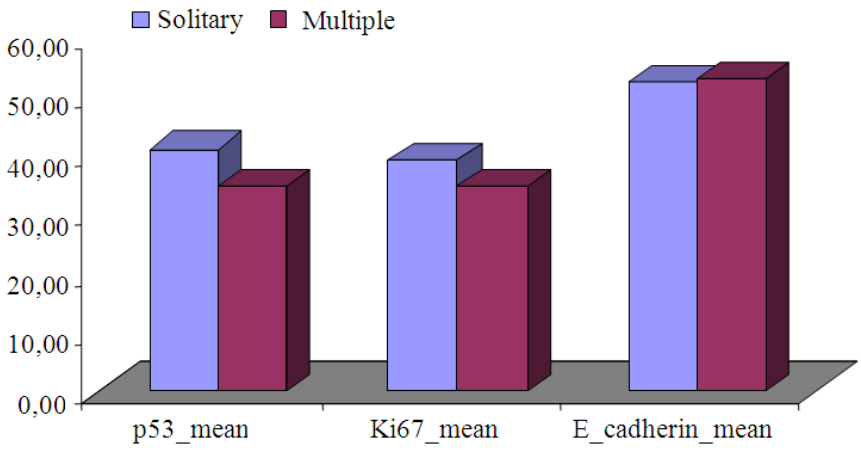

Fig. 4. Differentiation of urinary bladder cancer according to multiplicity - comparison of mean values of p53, Ki-67 and E-cadherin expression

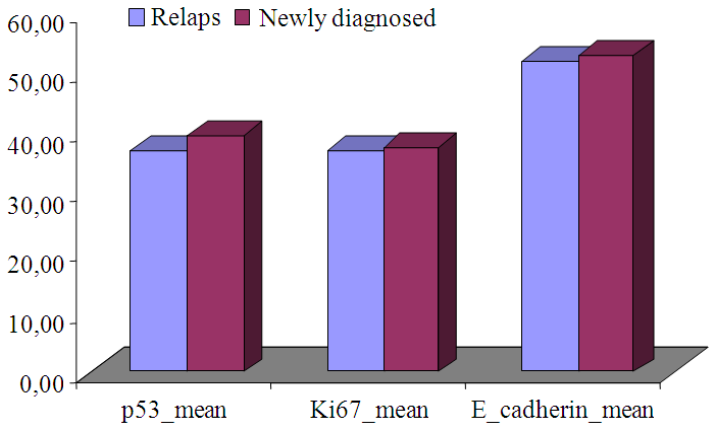

Fig. 5. Differentiation of urinary bladder cancer according to newly discovered and recurrent cases - comparison of mean values of p53, Ki-67 and E-cadherin expression

Figure 5 differentiation of urinary bladder cancer according to newly discovered and recurrent cases-comparison of mean values of p53, Ki-67 and E-cadherin expression.

4. DISSCUSSION

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 solitaire. 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

This study was partly supported by the grant VEGA no. 1/1250/12.

7. REFERENCES

Ancuta, E., C. Ancuta, L.G. Cozma, C. Iordache and I. Anghelache-Lupaşcu *et al*., 2009. Tumor biomarkers in cervical cancer: Focus on Ki-67 proliferation factor and E-cadherin expression. Rom. J. Morphol. Embryol., 50: 413-418. PMID: 19690767

Böhmer, D., V. Repiska and Ľ. Danisovic, 2010. Introduction to Medical and Molecular Biology. 1st Edn., Asklepios, Bratislava, ISBN-10: 9788071671510, pp: 95.

Calvo, J.L.M., E.B. Palenciano, A.B. Moreno and M.G. Gonzalez *et al*., 2006. Prognostic value of E-cadherina, beta catenin, Ki-67 antigen and p53 protein in the superficial bladder tumors. Actas Urol. Esp., 30: 871-878. PMID: 17175927

Cariaga-Martinez, A.E., P. Lopez-Ruiz, M.P. Nombela-Blanco, O. Motiño and A. Gonzalez-Corpas *et al*., 2013. Distinct and specific roles of AKT1 and AKT2 in androgen-sensitive and androgen-independent prostate cancer cells. Cell Signal, 25: 1586-97. DOI: 10.1016/j.cellsig.2013.03.019

Chu, H., M. Wang and Z. Zhang, 2013. Bladder cancer epidemiology and genetic susceptibility. J. Biomed. Res., 27: 170-178. DOI: 10.7555/JBR.27.20130026

Jang, T.J., W.H. Cha and K.S. Lee, 2010. Reciprocal correlation between the expression of cyclooxygenase-2 and E-cadherin in human bladder transitional cell carcinomas. Virchows Arch., 457: 319-328. DOI: 10.1007/s00428-010-0943-3

Jemal, A., F. Bray, M.M. Center, J. Ferlay and E. Ward *et al*., 2011. Global cancer statistics. CA Cancer J. Clin., 61: 69-90. DOI: 10.3322/caac.20107

Khorrami, M.H., M. Hadi, M.R. Gharaati, M.H. Izadpanahi and A. Javid *et al*., 2012. E-cadherin expression as a prognostic factor in transitional cell carcinoma of the bladder after transurethral resection. Urol. J., 9: 581-585. PMID: 22903481

Lee, Y.C., W.J. Wu, W.M. Li, H.H. Lin and C.N. Huang *et al*., 2013. Prognostic value of p53 protein overexpression in upper tract urothelial carcinomas in Taiwan. Anticancer Res., 33: 1091-1098. PMID: 23482786

Lim, M.G., N.V. Adsay, D.J. Grignon and A.O. Osunkoya, 2011. E-cadherin expression in plasmacytoid, signet ring cell and micropapillary variants of urothelial carcinoma: Comparison with usual-type high-grade urothelial carcinoma. Mod. Pathol., 24: 241-247. DOI: 10.1038/modpathol.2010.187

Priya, T.P., V.K. Kapoor, N. Krishnan, V. Agrawal and S. Agrawal, 2010. Role of E-cadherin gene in gall bladder cancer and its precursor lesions. Virchows Arch., 456: 507-514. DOI: 10.1007/s00428-010-0908-6

Shariat, S.F., C. Bolenz, G. Godoy, Y. Fradet and R. Ashfaq *et al*., 2009. Predictive value of combined immunohistochemical markers in patients with pT1 urothelial carcinoma at radical cystectomy. J. Urol., 182: 78-84. DOI: 10.1016/j.juro.2009.02.125

Tomaskin, R., B. Elias, K. Machalekova, K. Kajo and I. Sopilko *et al*., 2008. Quality of transurethral resection in immediate and high risk primary non-muscle invasive bladder cancer. Endoskopie, 17: 59-63.

Ziaran, S., F.M. Goncalves and J. Breza, 2013. Patients with prostate cancer treated by ADT have significantly higher fibrinogenemia than healthy control. World J. Urol., 31: 289-292. DOI: 10.1007/s00345-012-0926-x

Ziaran, S., F.M. Goncalves ans J. Breza Sn., 2011. Bone mineral density, pathological fractures and bisphosphonate therapy in prostate cancer patients on androgen deprivation therapy. Endocr. Regul., 45: 199-204. DOI: 10.4149/endo_2011_04_199