ORIGINAL PAPER

# E-cadherin and p63 immunoexpression in dysplastic lesions and urothelial carcinomas of the bladder

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**Abstract**

In this study, we investigated 30 cases of dysplastic lesions and bladder carcinomas. The lesions presented different degree of differentiation and depth of invasion. The aim of this study was to appreciate the immunoexpression of some proteins, like E-cadherin and p63, which are implicated in the cellular adhesiveness and the normal development of the urothelium, depending on the degree of tumoral differentiation and the depth of invasion. The immunoreactivity of the markers was qualitative and quantitative evaluated. Both markers presented an immunoexpresion diminishing with depth of invasion, the carcinomas with muscularis propria invasiveness having a minimum intensity reaction (0 or 1), and a medium percentage of marked cells by 10–50% for E-cadherin, and 30% for p63. The immunostaining intensity for E-cadherin decreased with the tumoral degree of differentiation, the poorly differentiated carcinomas having a reaction with minimum intensity (0 or 1). The p63-immunoexpression was correlated with the degree of differentiation in superficially cases of chorion invasiveness carcinoma. The immunostaining intensity in cases with muscularis propria invasiveness was diminished as the number of epithelial layers becomes bigger, but there was no variation depending on tumoral differentiation. In conclusion, E-cadherin and p63 are implicated in tumoral progression and may be used as a prognostic markers.

***Keywords*:** bladder urothelial carcinoma, E-cadherin, p63.

## Introduction

Over 90% from bladder primitive tumors are urothelial carcinomas [1]. The estimation is that these tumors accomplish 5th place between malignant neoplasms, every year being diagnosticated around 336 000 new cases worldwide [2–4]. These urothelial lesions represent a unique model for study by endoscopic accessibility, concerning their prognostic and recurrence. At the diagnosis moment, 65% of tumors are in a superficial stage, and have an excellent prognosis [3]. Nevertheless, more than half of these tumors presents relapse in the first year [5, 6].

The identification of the molecular mechanisms that are implicated in tumors appearance, progression and recurrence, represents priorities of the research programs. The alteration in bladder normal urothelium architecture is based on epithelial growth and differentiation impaired.

The purpose of our study was the evaluation of the neoplastic epithelium's intercellular adhesion and its regenerative capacity using E-cadherin and p63-immunoexpression.

## Material and Methods

We accomplished a retrospective study, which has included 30 cases of dysplastic and carcinomatous urothelial lesions. The cases were selected from bladder tumoral casuistry of the Pathology Lab of the Emergency County Hospital of Craiova in 2008. The biological material was represented by cystectomy pieces from Urology Clinic, which were processed by common histopathological technique using 10% formalin-fixation, paraffin embedding and Hematoxylin–Eosin stain. The histopathological diagnosis was done in conformity with criterions established in 2004 by *IARC* nominated work group for urinary tract tumors within *World Health Organization* [7, 8].

The immunohistochemical processing was made on serial sections from each paraffin-embedded block using the Universal Immuno-enzyme Polymer (UIP) NICHIREI method. As antigen retrieval, we used Heat-Induced Epitope Retrieval (HIER) Techniques at microwave for 15 minutes in Tris-EDTA buffer pH 9 for E-cadherin and in citrate buffer pH 6 for p63.

The sections were incubated overnight at $4^0$C with mouse monoclonal primary antibodies: anti-human E-cadherin (clone NCH38, IgG1 kappa, Dako) diluted 1:50 and anti-human p63 (clone 4A4, IgG2a kappa, Santa Cruz Biotechnology) diluted 1:30. After three times washing in fresh PBS, sections was incubated with Universal Immuno-peroxidase Polymer, Anti-Mouse and -Rabbit at room temperature for 30 minutes.

For the visualization of the reaction the diamino-benzidine-tetrahydrochloride (DAB) was used, followed by counterstained with Hematoxylin. Negative external control staining was done by omitting primary anti-bodies and for positive external control, oral mucosa was used.

Immunohistochemical reactions were quantified by means of the intensity of the reaction and the percentage of marked cells, establishing a score for both parameters (Table 1).

**Table 1 –** ***Qualitative and quantitative estimation for used antibodies***

| Score | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| *Intensity* | absence | low | medium | high |
| *Percentage* | absence | <10% | 10–50% | >50% |

Two observers (MC and RC) without knowledge of the studied data of the cases performed the evaluation of the staining.

The acquisition of the images was done with Nikon Eclipse E600 and software program Lucia 5.

## Results

The study comprised seven cases of medium dysplastic lesions and 23 cases of urothelial carcinoma (Table 2). The carcinomas were well differentiated in eight cases, moderate differentiated in 10 cases, and poorly differentiated in five cases. The carcinomas have invaded the chorion in 13 cases, the rest of them being invasive in muscularis propria (Table 2, Figure 1).

**Table 2 –** ***Histopathological analysis of the investigated cases***

| Urothelial carcinomas | Well differentiated | Moderate differentiated | Poorly differentiated |
|---|---|---|---|
| *Stromal invasion* | 7 | 4 | – |
| *Muscularis propria invasion* | 3 | 4 | 5 |

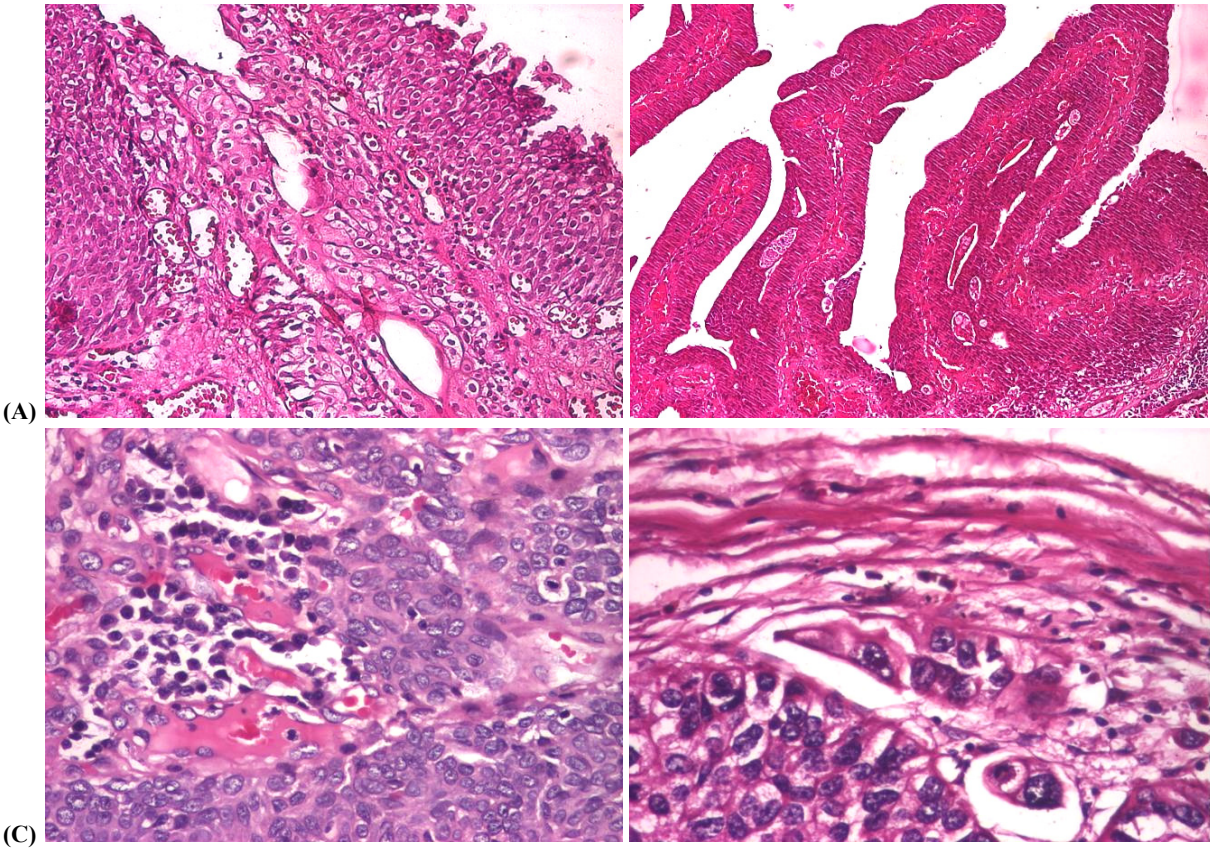


**Figure 1 –** ***(A) Medium urothelial dysplasia (HE stain, ×100); (B) Well differentiated urothelial carcinoma (HE stain, ×100); (C) Moderate differentiated urothelial carcinoma with chorion invasion (HE stain, ×200); (D) Poorly differentiated urothelial carcinoma with muscularis propria invasion (HE stain, ×200).***

The membrane reaction for E-cadherin and nuclear reaction for p63 were present on positive control tissues. The marked cells percentage was 90% for E-cadherin and 85% for p63. A maximum intensity reaction was present in both cases. The immunostaining was absent for negative control tissue.

An immunostaining membrane E-cadherin-positive reaction was noticed in 27 investigated cases, the negative or cytoplasmatic reactions being noticed in carcinomas invading muscularis propria. The positive immunostaining was limited to all cellular but with variable intensity.

The dysplastic lesions had a maximum intensity of the reaction and a medium percentage of marked cells (85%). The urothelial carcinomas with chorion invasion had a high intensity reaction (score 2 or 3) and a medium percentage of marked cells (75%). The carcinomas with muscularis propria invasion had a low intensity reaction (score 0 or 1) and a medium percentage of marked cells (10–50%). The well and moderate differentiated carcinomas had a high intensity for staining reaction (score 2 or 3), the lowest intensity being noticed for poorly differentiated carcinomas. There were not noticed any significant differences between tumoral grade differentiation regarding the percentage of marked cells (Figure 2).

The immunoreaction for p63 at nuclear level was present in 28 cases, the negative staining being

observed in carcinomas with invasion of muscularis propria. The dysplastic lesions and the carcinomas with invasion of chorion had a medium percentage of marked cells (70%). In these cases, we noticed a maximum intensity reaction in the nuclei of basal epithelial cells, which was gradually diminished towards the epithelium surface, in the nuclei of mature cells. The decrease of the intensity reaction was heterogeneous founding mature cells with maximum intensity reaction.

For the muscularis propria invasiveness carcinomas we have noticed an incomplete, granular, nuclear reaction in an average percentage of 30% of the cells. The reaction's intensity was low (score 1 or seldom 2), being the same in all epithelium layers. The basal layer's cells do not have a particular stain. A negative reaction was present in all the cases for the cells that came in direct contact with the bladder lumen.

For the carcinomas with stromal invasiveness the reaction intensity was maximum (score 3) in all well differentiated cases with thin tumoral papillae covered by few cell layers. The immunostaining intensity in cases with muscularis propria invasiveness was diminished as the number of epithelial layers became bigger, but there was no change with regards of tumoral differentiation (Figure 3).

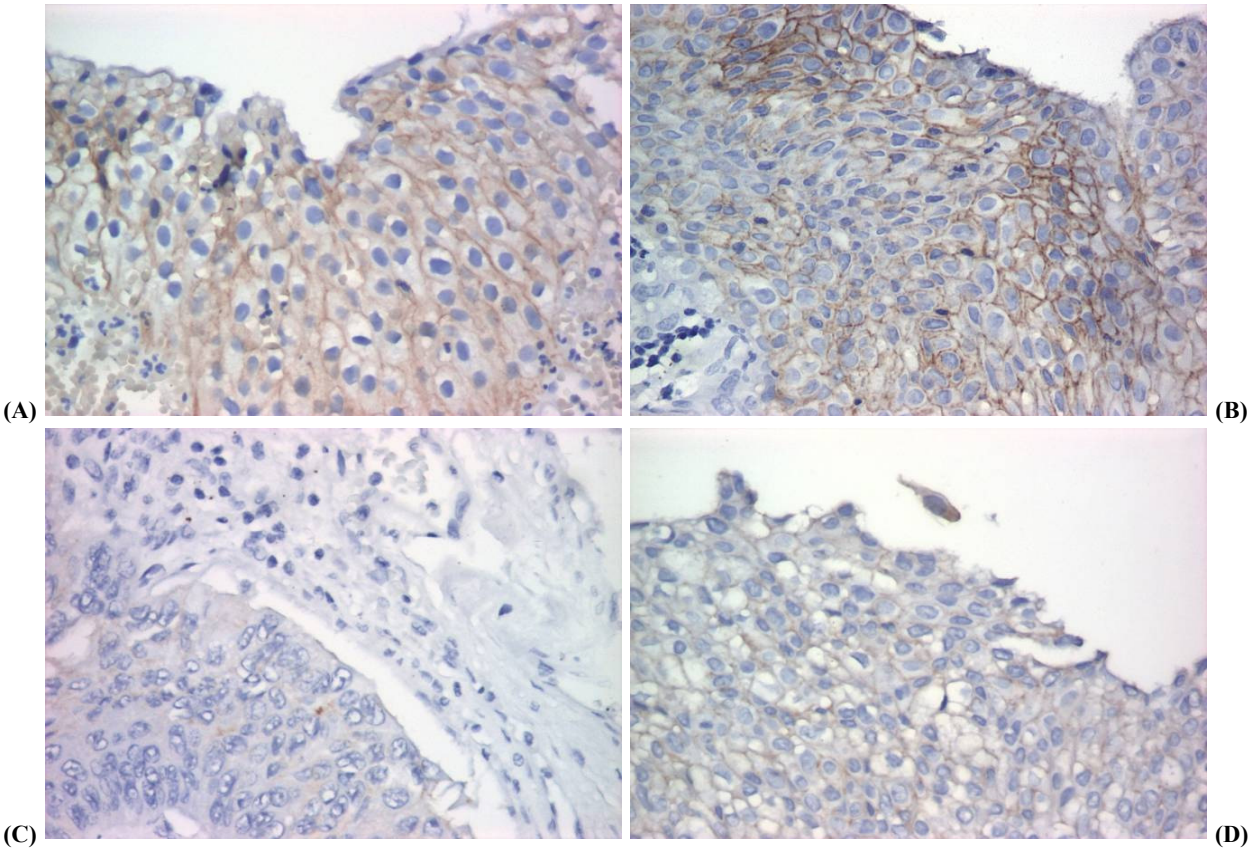



**Figure 2 –** ***(A) Medium urothelial dysplasia, E-cadherin immunostaining, ×200; (B) Well differentiated urothelial carcinoma with chorion invasion, E-cadherin immunostaining, ×200; (C) Moderate urothelial carcinoma with muscularis propria invasion, E-cadherin immunostaining, ×200; (D) Moderate urothelial carcinoma with chorion invasion, E-cadherin immunostaining, ×200.***

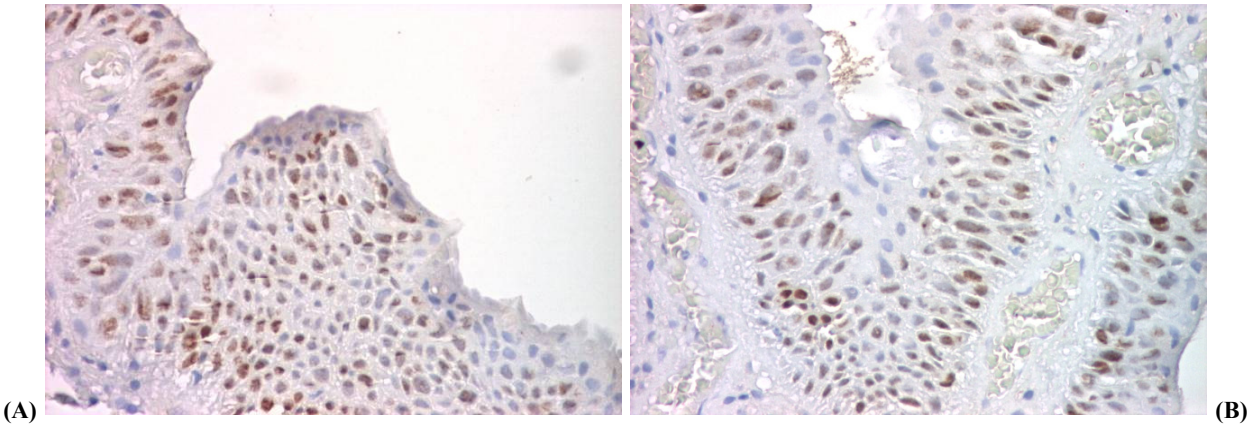



**Figure 3 –** ***(A) Moderate dysplasia of the bladder, p63 immunostaining, ×100; (B) Well differentiated carcinoma with chorion invasion, p63 immunostaining, ×100.***

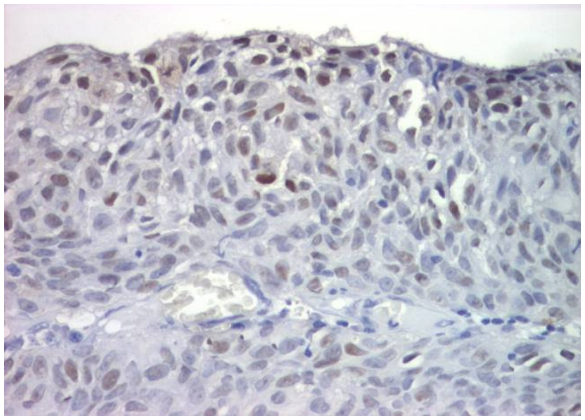
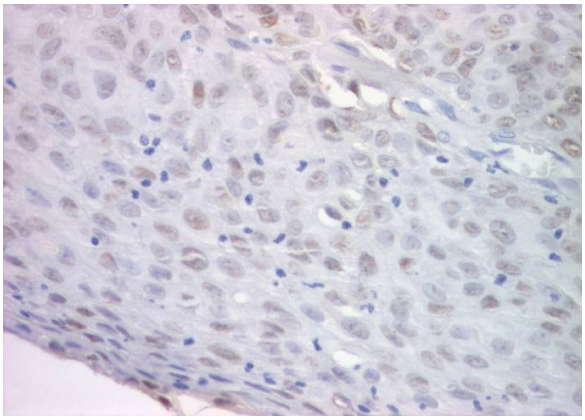
(C)   (D)

**Figure 3 –** ***(C) Moderate differentiated carcinoma with chorion invasion, p63 immunostaining, ×200; (D) Poorly differentiated carcinoma with muscularis invasion, p63 immunostaining, ×200.***

## Discussion

The loss of cells adhesion represents a mechanism, which provides the tumoral progression. E-cadherin represents a calcium-depending transmembranar glycoprotein, which has a strong relationship with cells cytoskeleton via cathenins [9]. E-cadherin is the principal component of the intercellular adhesion system, its alteration being essential to tumoral progression and metastases [9–11].

Ours results are similar to other studies, which have demonstrated that the diminishing of E-cadherin expression correlates with tumoral aggressiveness [12–14]. Popov Z *et al.* [9] performed a study, in 2000, for E-cadherin immunoexpression investigation in 111 bladder tumors cases, establishing its correlation with tumoral stage and differentiation degree ($p<0.0001$, respectively $p<0.001$). The analysis of the recurrence, progression and 36 months survival rate has indicated a strong correlation between E-cadherin expression and the patients' prognosis. In another study published in 2004, Sun W. *et al.* [11], it was showed that the E-cadherin expression is maintained in dysplastic lesions and carcinoma *in situ*, diminishing in proportion with the tumoral nest's dimension and the depth of the invasion. The predictive value of the marker was contested in some studies, when immunoreaction at tumor level was similar to normal urothelium. Clairotte A *et al.* (2006) [12] sustained in these cases, the absence of correlation between E-cadherin and patients prognosis especially when tumors are superficially. The explanation was that E-cadherin immunoexpression and normal function are not always in concordance. The function of E-cadherin may be altered because of the damage of other components of the intercellular adhesion systems, like cathenins, although E-cadherin immunoexpression may be maintained.

P63 is a recent discovered molecule, which is part of p53 family and plays an essential role in the normal development and stratification of many epithelia (oral, gastric and cervix mucosae), including urothelium [16–18].

The results of our study are similar with other studies, which tried to elucidate the role of p63 in urothelial carcinogenesis. Urist MJ *et al.* [19] showed in 2002 that p63 is not indispensable for urothelium development but is necessary for its differentiation and stratification. The conclusion of this study was that the p63-expression diminished in high-grade invasiveness carcinomas. Koga F *et al.* (2003) [17] reached the same conclusion in a similar study, but also indicated that the p63-immunoexpression diminishing is synchronically with the $\beta$-catenins one. In a study from 2004, which tried to analyze the morphofunctional complexity of p63, Westfall MD *et al.* [20] presumed that this protein plays an important role in maintaining stem cells population and the epithelia regenerative potential [21]. Many studies suggest that p63 is an additional useful biomarker, while others consider it as crucial for tumorigenesis [16–21]. The diminishing p63 expression at the urothelium surface is an evidence for a normal maturation of the epithelium. In our experience correlated with other studies from literature, the p63-expression is homogenous in all layers of the neoplastic epithelium [17].

## Conclusions

This study proved the decreasing of E-cadherin and p63-immunoexpression with the depth of invasion, the percentage of marked cells and intensity of the immunoreaction being minimum or absent in carcinomas with muscularis propria invasion.

The E-cadherin immunostaining intensity diminished with the degree of differentiation. The p63-expression was correlated with the degree of differentiation in the superficially lesions and with the number of cell layers, which covered the tumoral papillae in muscularis propria invasiveness carcinomas.

Therefore, both E-cadherin and p63 have in bladder urothelial carcinomas a similar immunoexpression variation regarding the tumoral degree of differentiation and the tumoral depth of invasion. These results recommend the utilization of E-cadherin and p63 as prognostic markers in both bladder dysplastic lesions and urothelial carcinomas.

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*Received: February 25th, 2009*

*Accepted: June 15th, 2009*