PhD THESIS

ABSTRACT

EPITHELIAL-MESENCHIMAL TRANSITION ROLE IN BLADDER UROTHELIAL CARCINOMA

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Key words: bladder cancer, epithelial-mesenchimal transition, urinary system, imunohistochemical study, imunohistochemical markers
INTRODUCTION

Bladder cancer is most frequent cancer in urinary tract, after prostate cancer (Boring CC, Squires TS, Tong T, 1995). It has important geographical variations, higher incidence rates is on males living in developing countries. In Europe is the highest rate of bladder cancer around the world. It is five times more frequent in males compared to females. Even is more frequent in males, prognosis is improved compared to females and mortality rate is lower, bladder carcinoma being diagnosed in initial stages (Shariat SF, 2010).

Urothelial carcinogenesis, recurrence, progression and metastasis can involve numerous molecules that will be discovered. On this time there is no diagnosis, prognosis or therapeutics molecular biomarkers for urothelial carcinoma (Liang Cheng, Darrell D. Davison, Julia Adams et al, 2014).

Doctorate thesis is structurated in two major parts:
I Actual know, where in three chapters i have notice aspects regarding histology and histofiziology of urinary sistem, bladder cancer pathology and role of epithelio-mezenchimal transition in bladder cancer carcinogenesis.
II Personal contribution with main objective to found correlations between clinical aspects, imagistic, histological and imunohistochemical and i realized one clinical-epidemiology study, one for mophology and one imunohistochemical study, each with more objectives.

LITERATURE REVIEW

CHAPTER 1
HISTOLOGY AND HISTOFIZIOLOGY OF URINARY TRACT

Urinary system have multiple functions: produce and eliminate urine, facilitating cleaning the body from catabolic products, some highly toxic, maintaining acido-basic balance and hidro-electrolitic, excretion of water and selective resorption for specific subtances and ions, endocrine function including blood pressure regulation by production of hormonal substance (renin), stimulation of red blood cells by production of erytropoetin. All this functions performed by urinary system contribute on body homeostasis.

Urinary system development is very close to genital system development, with mesodermal origins. Intermediate mesoderm is segmented on cervical region, forming nephrotoms (Sadler TW, 2010), from which are developing three main structures: pronephros, mesonephros and metanephros.

Urinary system is formed from kidney, pair located in retroperitoneum, in kidney lodge, both parts of the spine, T11-L3, with secretion role, producing urine from intrarenal urinary (small calics, large calics, urynari pelvis) and extrarenal (ureters, bladder, urethra), from which urine is transported, stocated and eliminated. (Chesbrough RM, Burkhard TK, Martinez AJ, Burks DD, 1989; Crăiţoiu Ş, 2003; Sinescu I, Gluck G, 2008).
CHAPTER 2
BLADDER TUMOR PATHOLOGY

Bladder cancer is on second place between uro-genital cancers, being on the first place on the urinary tract, after prostate cancer (Boring CC, Squires TS, Tong T, 1995). Incidence is around 5% from new diagnosed cancer each year, on the 4th place on males after prostate cancer, lung and colon cancer (Tomescu P, Pănuş A, 2006). Bladder cancer incidence seems to decrease, probably because decreasing exposition to different risk factors like smoking or different chemical agents (Sievert KD, Amend B, Nagele U et al, 2009). Bladder cancer is two times more frequent on white males compared to black males, while on white females appearance rates is with only 44% higher than for black females (Cutler SJ, Young JL, 1975).


CHAPTER 3
ROLE OF EPITHELIAL-MEZENCHIMAL TRANSITION IN BLADDER CARCINOGENESIS

Epithelial-mesenichmal transition process (MET) was observed since embrional development, being an important process for embrional development and forming different tissues and organs (Savagner P, Boyer B, Valles AM et al, 1994). Interest for this process is mainly because of his role in neoplasic progression (Thiery JP, Sleeman JP, 2006). In 1982 MET theory was proposed, that reveals in epithelial cells cultures, they are morphological convert in mesenchimal cells with gel mobility (Greenburg G, Hay ED 1982). MET represent dinamic tranformation of epithelial cells with mesenchimal phenotype (van der Horst G, Bos L, van der Pluijm G, 2012). MET is not specific to neoplasms, with important role in tissue differentiatiation, organs development and wound healing (Peinado H, Olmeda D, Cano A, 2007). MET is reversible, involved cells beeing able to regain epithelial phenotype (Chen J, Han Q, Pei D, 2012). MET have three different subtypes, separated by production pathways and functions completed, one associated with neoplasms. (Chaw SY, Majeed AA, Dalley AJ, 2012; Kalluri R, Weinberg RA, 2009).
PERSONAL CONTRIBUTIONS

CHAPTER 4
CLINICAL-EPIDEMIOLOGY STUDY OF BLADDER UROTHELIAL CARCINOMA

Study was prospective and included 784 hospitalised patients for specific treatment between 2013-2015 in Urology Department, Emergency County Hospital Craiova, diagnosed with bladder tumor. For clinical analysis we followed patients distribution by years, age, gender, patients origin, risk factors and associated symptoms, associated with imaging investigation (Fig. 4.1 - Fig. 4.5). Statistical processing used average values, standard deviations and comparative tests (t Student, Chi pătrat, Pearson), completed with SPSS10 software (SPSS Inc., Chicago, IL, USA). Table 4.1 presents investigated cases by years.

Table 4.1 Cases repartition by years

<table>
<thead>
<tr>
<th>Year</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr. Of Cases</td>
<td>205</td>
<td>262</td>
<td>317</td>
</tr>
<tr>
<td>%</td>
<td>26.1</td>
<td>33.4</td>
<td>40.5</td>
</tr>
</tbody>
</table>

Fig. 4.1 Patients repartition by age

Fig. 4.2 Patients repartition by gender

Fig. 4.3 Patients repartition by risk factors

Fig. 4.4 Patients repartition by presentation symptoms
CHAPTER 5
MORPHOLOGICAL STUDY OF BLADDER UROTHELIAL CARCINOMA

Morphological study included 760 urothelial invasive bladder carcinoma, diagnosed in Pathology Laboratory of Emergency County Hospital Craiova. Histopathological analyses included tumoral differentiation grades (Fig. 5.1), of invasion (Fig. 5.2, Fig. 5.3, Fig. 5.4) and pTNM stadialisation. We also followed tumor histology differences (Fig. 5.5, Fig. 5.6), vascular invasion (Fig. 5.7) and perineural (Fig. 5.8), also histology aspects of tumor front, defined as most profound invasion area of urothelial carcinoma on bladder wall or on resection pieces after transurethral resection of bladder.

Histopathological parameters of urothelial carcinoma are important also for terapeuthics strategies and for recurrence risk and survival period. Integrated analyses of clinical-imaging parameters and histopathological can improve therapeutic management for better prognosis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variable</th>
<th>Nr.cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor number</td>
<td>unique</td>
<td>766</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td>multiple</td>
<td>18</td>
<td>2.3</td>
</tr>
<tr>
<td>Size</td>
<td>&lt;2cm</td>
<td>686</td>
<td>87.5</td>
</tr>
<tr>
<td></td>
<td>2-10cm</td>
<td>82</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>&gt;10cm</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Localisation</td>
<td>trigon</td>
<td>160</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>dome</td>
<td>102</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>anterior</td>
<td>152</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td>posterior</td>
<td>137</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>lateral</td>
<td>215</td>
<td>27.4</td>
</tr>
</tbody>
</table>

Fig. 4.5 Patients repartition by number, size and tumor localisation
Fig. 5.3 Urothelial carcinoma invading lamina propria (pT1), col. HE, x100

Fig. 5.4 Urothelial carcinoma invading entire wall (pT3), col. HE, x100

Fig. 5.5 Urothelial carcinoma, squamous differentiation, col. HE, x100

Fig. 5.6 Urothelial carcinoma, clear cell differentiation, col. HE, x100

Fig. 5.7 Urothelial carcinoma, vascular invasion, col. HE, x200

Fig. 5.8 Urothelial carcinoma, perineural invasion, col. HE, x200
CHAPTER 6
IMUNOHISTOCHEMICAL STUDY OF BLADDER UROTHELIAL CARCINOMA

Imunohystochemical analysis was performed on a selected batch of 65 cases, which included 35 cases in which it was practiced total cystectomy and 30 cases in which it was practiced tumor resection transurethral. All the cases are from the same batch that was clinical and morphological analyzed with the same processing in Urology department and Pathology department from Emergency Hospital Craiova.

The principal markers investigated in this study were addressed to the main biomolecular mechanisms: epithelio-mesenchymal tumor of bladder epithelial phenotype alteration, respectively, the purchase mezenchimal phenotype, the cadherinic switch, transcription factors (Table 6.1):

Tabel 6.1 Antibodies used for the investigation of the transition epithelial-mesenchymal at the level of the bladder

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clona/ Producer</th>
<th>Dillution</th>
<th>Recuperarea antigenică</th>
<th>Control extern pozitiv</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK7</td>
<td>OV-TL 12/30 Dako</td>
<td>1:100</td>
<td>EDTA, pH 8</td>
<td>Urothelium</td>
</tr>
<tr>
<td>CK20</td>
<td>Ks 20.8/Dako</td>
<td>1:75</td>
<td>EDTA, pH 9</td>
<td>Colon</td>
</tr>
<tr>
<td>Vimentina</td>
<td>SP20 Thermo Scientific</td>
<td>1:150</td>
<td>Citrat, pH 6</td>
<td>Colon</td>
</tr>
<tr>
<td>E-caderina</td>
<td>NCH 38 Dako</td>
<td>1:100</td>
<td>Citrat, pH 6</td>
<td>gland mamara</td>
</tr>
<tr>
<td>N-caderina</td>
<td>6G11/Dako</td>
<td>1:50</td>
<td>Citrat, pH 6</td>
<td>Amigdals</td>
</tr>
<tr>
<td>Twist</td>
<td>Twist 1/LSbio</td>
<td>1:1000</td>
<td>Citrat, pH 6</td>
<td>Colon</td>
</tr>
<tr>
<td>Snail 1</td>
<td>Polyclonal Abcam</td>
<td>1:75</td>
<td>Citrat, pH 6</td>
<td>Placenta</td>
</tr>
</tbody>
</table>

The imunoexpression of the cytokeratin 7 was identified in all cases analyzed with tumoral cells. The marking was variable intensity and located at the cytoplasmic level (fig. 6.1). The imunoexpression of cytokeratin 20 was identified at the level of selected strain of tumor cells in 62.9% of cases, intensity of the reaction was variable (fig. 6.2). The imunoexpression of Vimentinei has been identified at the level of selected strain of tumor cells in 12 cases and mesenchymal elements. The markings have been identified only in carcinomas of high-grade, with his own invasion of muscle/whole wall or adjacent organs (pT2-T4) and in stages II to IV (fig. 6.3, Figure 6.4). The imunoexpression of E-cadherin has been observed at the level of tumor cells membrane. The intensity of reactions both at the front and intratumoral bleeds invasion was variable and differences were observed in the number of cells marked (fig. 6.5 fig. 6.6). The imunoexpression was present at the level of the membrane and selected strain of tumor cells, and stromal elements (fig. 6.2, fig. 6.2) . The immunoreaction Twist has been identified at the kernel level of tumor cells and stromal elements such as fibroblasts, macrophages, endothelial cells, lymphocytes (Fig. 4.4, fig. 6.10) . The immunoreactions Snail 1 were identified at the level of selected strain and nucleus of tumor cells. The reaction was present at the level of lymphocytes and stromal fibroblasts (fig. 6.11, 6.12).
Fig. 6.1 Low grade urothelial, invasion front, CK7, x100
Fig. 6.2 High grade urothelial, front of invasion, CK20, x100

Fig. 6.3 Urothelial carcinoma, invasion front, marked Vimentina, x200
Fig. 6.4 Urothelial carcinoma, invasion, double marked Vimentina (brun)/ CK7 (red), x200

Fig. 6.5 Low grade urothelial carcinoma, invasion front, marked Ecadherin, x100
Fig. 6.6 High grade urothelial carcinoma, intratumor, marked Ecadherin, x100
Fig. 6.7 Low grade urothelial carcinoma, invasion front N-cadherin, x100

Fig. 6.8 High grade urothelial carcinoma, invasion front, N-cadherin, x100

Fig. 6.9 Low grade urothelial carcinoma, intratumor, marked Twist, x100

Fig. 6.10 High grade intratumor urothelial carcinoma, marked Twist, x100

Fig. 6.11 Low grade urothelial carcinoma, invasion front, marked Snail 1 x 100

Fig. 6.12 High grade urothelial carcinoma, intratumoral, marked Snail 1, x100
CONCLUSIONS

Bladder urothelial carcinomas are lesions with increasing incidence, the patient profile including over 60 years of age, male, smoker, with hematuria at presentation.

Hematuria was the most common symptom at presentation (56.2%) patients, and imaging investigations have shown tumor predominence unice (97.7%), with sizes below 2 cm (87.5%) and localization at the level of the lateral wall of the bladder and trigon.

High grade urothelial carcinomas and advanced stage presents the invasion front level ability to purchase of mezenchimal phenotype in epithelial phenotype conservation conditions.

Cadherinic switch is present in MET urothelial tumour and offer informations regarding lesions agressivity.

Markers study involved in urothelial carcinoma MET indicates specific imunophenotipes reported with histopathological prognostic parameters, aspect that can select them as therapeutic targets.

Cadherinic profile and transcription factors reported with histopathological parameters ofurothelial carcinoma, can improve selection criteria of included patients in further trials.

BIBLIOGRAPHY