Prognostic Value of CD44 and HER2/Neu in Invasive Transitional Cell Carcinoma of the Urinary Bladder

GHADA A. ABD EL-FATTAH, M.D.; RASHA M. EL-SAWI, M.D. and RASHA M. ABD-RABUH, M.D.
The Department of Pathology, Faculty of Medicine, Benha University

Abstract

Purpose: Urinary bladder cancer is a very common worldwide cancer. In Egypt, there was a significant rise in the incidence of TCC in the last years. CD44 is a transmembrane glycoprotein thought to play an important role in cell-cell and cell-matrix interactions. HER-2/neu is an oncogene. Its overexpression has been associated with some different types of human cancers. The present study aimed at evaluating the prognostic value of CD44 and HER2/neu in invasive TCC of urinary bladder.

Patients and Methods: This study includes 6 cases of normal bladder mucosa as a control and 40 cases of TCC (15 cases of grade 2 and 25 cases of grade 3). Cases were collected from Pathology Department, Faculty of Medicine, Benha University and Egyptian National Cancer Institute (NCI) in the period 2009 – 2011. Follow-up of the selected cases was recorded for 2 years. Immunohistochemical staining for CD44 and HER2/neu was performed in all cases and the patterns of expression were analyzed.

Results: The expression of CD44 was higher in control cases than TCC and was significantly decreased in cases with poor patient prognosis ($p < 0.05$). The high tumor grades and the deeply invasive tumors (T3) were significantly associated with low CD44 expression ($p < 0.01$). The expression of HER2/neu increased in tumor cases than control. HER2/neu expression was significantly increased in cases with higher tumor grade ($p < 0.01$).

Conclusion: Low CD44 expression was correlated to higher tumor grade and stage and poor patient’s survival. HER2/neu overexpression was relevant in high grade (grade 3) specimens compared with lower grade of TCC.

Key Words: CD44 – HER2/neu – Transitional cell carcinoma (TCC) – Immunohistochemistry (IHC).

Introduction

URINARY bladder cancer ranks the ninth in the worldwide cancer incidence [1]. According to WHO, transitional cell carcinoma (TCC) accounts for 90% of all bladder tumors [2]. In Egypt, according to NCI registry, urinary bladder carcinoma constituted 12.22% of total malignant tumors. There was a significant rise in the incidence of TCC in Egypt in the last years. In the cancer registry during the years 1985 1989, TCC represented 30.89% of all bladder cancers. This incidence raised to 64.20% during the years 2003 – 2004 [3-5].

Generally, standard histopathological characteristics of urothelial cancers, including tumor grade and stage determine the tumor’s behavior but description of new tumor characteristics may be helpful for the patients’ treatment [6]. Molecular changes in bladder tumors involve three main mechanisms. Chromosomal alteration (the initial event in carcinogenesis), tumor proliferation due to loss of cell cycle regulation, and metastasis aided by processes such as angiogenesis and the loss of cell adhesion [7].

CD44 which is a transmembrane glycoprotein comprises a family of membrane adhesion molecules encoded by a single gene and diversified by alternative splicing and extensive post-translational modifications [8]. CD44 is thought to play an important role in cell-cell and cell-matrix interactions [9]. It is expressed in most adult tissues and in the majority of neoplasms. Loss of expression of these adhesion molecules may contribute to progression and metastasis formation in various human malignancies [10]. Some studies show that there is an inverse correlation between the expression of CD44 and histological grade and tumour stage exists and loss of CD44 variants may provide an additional parameter in identifying patients with urothelial carcinoma at risk for tumour recurrence [11].

HER-2/neu (the Human epidermal growth factor receptor 2) is an oncogene located on chromosome
17q11-21 encoding a type 1 tyrosine kinase growth factor receptor [12]. Activation of the HER2/neu receptor following auto-phosphorylation of the tyrosine kinase residues results in the activation of a cascade of intracellular proteins. Ultimately, the mitotic activity and metastatic potential of the cell increases. Overexpression of HER-2/neu has been associated with some different types of human cancers [13]. The aim of this study is to investigate the relation of the CD44 and HER-2/neu expression to the behavior of TCC using immunohistochemical technique.

Patients and Methods

This retrospective studied group included random 40 cases of TCC and 6 cases of normal bladder mucosa (acquired from the margin of excised bilharzial lesion) as a control, selected from histopathologic archive of files of Pathology Department of Faculty of Medicine, Benha University and Egyptian National Cancer Institute (NCI) in the period 2009 – 2011. Cases were selected according the availability of clinical and follow-up data for at least 2 years. Only patients with primary TCC who had not undergone previous irradiation or chemotherapeutic treatment were included in the study.

Paraffin-embedded tissue sections were obtained from archival tissue blocks of the hospital. Hematoxylin and eosin sections were reviewed to confirm diagnosis. Cases were graded according to the WHO three tiered scheme into G1, G2, G3 [5] and were classified according to TNM staging system [14,15]. Two experienced pathologists blindly and independently confirmed the histological diagnosis of each lesion and agreed on the grading and staging. Other sections were mounted on positively-charged slides, immunohistochemically stained with antibodies against CD44 and HER2/neu using the Ultra Vision Detection System (Antipolyvalent, HRP/DAB, ready-to-use, Lab Vision corporation).

Immunohistochemical staining: Steps of staining followed the manufacture instructions. Antigen retrieval was made with microwave treatment in 10mM citrate buffer (Neo-Markers, Cat. # AP-9003), pH 6.0. Sections were incubated with mouse monoclonal antibody against CD44 (Lab Vision, Thermo scientific, USA, Cat. # MS-668-R7, ready to use) & mouse monoclonal HER2/neu (Lab Vision, Thermo scientific, USA, Cat. # MA1-34495, ready to use). Slides were incubated for 60 minutes with each antibody at room temperature. The freshly prepared DAB-substrate-chromogen solution was applied.

For CD44, colonic adenocarcinoma was used as positive control. Sections of the invasive ductal breast carcinoma for HER2/neu were used as a positive control. Negative controls were performed by omitting the primary antibody step.

Interpretation of immunohistochemical staining of CD44:

All specimens were examined under a light microscope. Positive reactions for CD44 appeared as a cytoplasmic/membranous immunostaining for CD44. The fraction of CD44 positive cells was evaluated in four grades of percentages of stained malignant cells: 0 (0-5%), 1 (6-25%), 2 (26-50%), or 3 (51-100%) [16].

Interpretation of immunohistochemical staining of HER2/neu:

Sections were scored as positive only if membrane staining was evident and ranked as 0 (no staining or <10% staining of tumor cells), 1+ (faint staining in >10% of cells; however, only partial staining of the membrane is seen), 2+ (weak-to-moderate, complete membrane staining in >10% of tumor cells), or 3+ (strong, complete membrane staining in >10% of tumor cells) [17,18].

Statistical analysis:

Statistical analysis was performed using the SPSS (version 16.0 for windows) software package according to Sperman’s correlation coefficient. Correlation between several variables was computed using Fisher’s exact test. p-value less than 0.05 (<0.05) was considered significant and <0.01 was highly significant.

Results

The studied cases included 27 males and 13 females with mean age of 59 years. There were 15 cases of grade 2 and 25 cases of grade 3. No cases of grade 1 were available. All collected cases showed muscle invasion and according to TNM staging system were classified into 27 cases of stage T2 and 13 cases of stage T3. No cases were reported as T1 or T4.

A- Immunohistochemical results of CD44 staining:

In control cases, there was a strong membranous positivity to CD44 (grade 3) in 5 cases (83.3%) of bilharzial cystitis. In studied cases of TCC, only 13 cases (32.5%) were strongly positive. There was an inverse statistically significant correlation between CD44 expression and type of lesion (p<0.05) (Table 1).
A high inverse statistically significant correlation was found between CD44 and tumor grade \((p<0.01)\), extent of muscle invasion \((p<0.01)\) and patient’s survival \((p<0.01)\) (Table 1). The expression of CD44 was lower in high tumor grade (Fig. 1), in cases with deeper muscle invasion (Fig. 2) and in cases with poor patient prognosis.

**B- Immunohistochemical results of HER2/neu staining:**

The expression of HER2/neu increased in TCC cases than control. All control cases (100%) were negative to HER2/neu expression, while 20 cases (50%) of TCC were strongly positive with complete membranous staining. This was a high statistically significant correlation \((p<0.01)\) (Table 2).

There was a highly statistically significant correlation between HER2/neu expression and tumor grade \((p<0.01)\). The higher TCC grades were associated with higher HER2/neu expression (Table 2) (Fig. 3). An insignificant statistical correlation was found between HER2/neu expression and extent of muscle invasion \((p>0.05)\) and patient’s survival \((p>0.05)\) (Table 2).

### Table (1): Correlation between CD44 expression and clinicopathological data in examined cases.

<table>
<thead>
<tr>
<th>Clinicopathological variable</th>
<th>Total</th>
<th>CD44 expression</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Type of tissue examined:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TCC</td>
<td>40</td>
<td>10 (25%)</td>
<td>10 (25%)</td>
</tr>
<tr>
<td><strong>Tumor grade:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Grade 3</td>
<td>25</td>
<td>10 (40%)</td>
<td>10 (40%)</td>
</tr>
<tr>
<td><strong>Extent of muscle invasion:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>20</td>
<td>4 (20%)</td>
<td>0</td>
</tr>
<tr>
<td>T3</td>
<td>20</td>
<td>5 (25%)</td>
<td>10 (50%)</td>
</tr>
<tr>
<td><strong>Patient’s survival:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>20</td>
<td>6 (30%)</td>
<td>0</td>
</tr>
<tr>
<td>Recurrent</td>
<td>15</td>
<td>5 (33.3%)</td>
<td>10 (66.7%)</td>
</tr>
<tr>
<td>Dead</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

TCC: Transitional cell carcinoma. \(^*\): Significant. \(^{**}\): Highly significant.

### Table (2): Correlation between HER-2/neu expression and clinicopathological data in examined cases.

<table>
<thead>
<tr>
<th>Clinicopathological variable</th>
<th>Total</th>
<th>HER2/neu expression</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Type of tissue examined:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>6 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>TCC</td>
<td>40</td>
<td>6 (15%)</td>
<td>7 (17.5%)</td>
</tr>
<tr>
<td><strong>Tumor grade:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>15</td>
<td>6 (40%)</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td>Grade 3</td>
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<td>2 (8%)</td>
</tr>
<tr>
<td><strong>Extent of muscle invasion:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>20</td>
<td>5 (25%)</td>
<td>0</td>
</tr>
<tr>
<td>T3</td>
<td>20</td>
<td>1 (5%)</td>
<td>7 (35%)</td>
</tr>
<tr>
<td><strong>Patient’s survival:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>20</td>
<td>3 (15%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Recurrent</td>
<td>15</td>
<td>2 (13.3%)</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td>Dead</td>
<td>5</td>
<td>1 (20%)</td>
<td>2 (40%)</td>
</tr>
</tbody>
</table>

TCC: Transitional cell carcinoma. \(^{**}\): Highly significant.
Discussion

The adhesion molecule CD44 is a member of a large family of cell adhesion molecules that is responsible for mediating communication and adhesion between adjacent cells and between cells and the extracellular matrix (ECM) [19].

A highly significant inverse statistical correlation was found between CD44 and tumor grade ($p$-value $<0.01$). This current finding is in agreement with Omran and Ata [20]. In contrast, in another study there was no correlation between CD44 variant expression and clinicopathologic criteria such as stage, grade and survival [21].
The exact reasons for these contradictory results may be explained by differences in the number of cases, or in methods used in staining evaluation.

In this study, a high statistically significant inverse correlation was found between CD44 expression and extent of muscle invasion ($p$-value $<0.01$). This is in agreement with Gadalla et al., [22] in their series of bladder carcinomas. This can be explained by thinking that the decreased CD44 expression leads to disruption of intercellular adhesion between tumor cells which can result in their dissociation, invasion and metastasis.

Concerning patient’s survival, a significant correlation was found between it and CD44 expression ($p$-value $<0.01$) as (70%) of cases with disease free survival showed grade 3 CD44 expression while (66.7%) of cases who were associated with disease recurrence showed grade 1 positivity for CD44 expression. All died cases (100%) showed weak to moderate CD44 immunoreaction. This may be explained in part by the fact that disorders in the intercellular adhesions are closely related to the progression and aggressiveness of malignant tumors. This in accordance with Shitivelman et al., [23], Fujita et al., [24] and Kallakury [25] who reported down-regulation of CD44s expression with poor prognosis in other solid cancers including prostate, endometrium, and neuroblastoma. In contrast Jalkanen, et al., [26], Joensuu, et al., [27] and Woodman et al., [28] reported that increased expression of CD44 has been postulated to be associated with aggressive tumor behavior as demonstrated in human carcinomas of breast, stomach, and non-Hodgkin lymphoma.

This in part could be explained by thinking that tumor invasion and metastasis may be enhanced by increased cell-matrix interaction caused by increased CD44 expression [19].

The assessment of HER2/neu overexpression in urothelial carcinomas is studied by many authors, because it has been shown that this protein is involved in the pathogenesis of these tumors, to an extent nearly as important as in breast cancer [29,30]. In malignant tumors, the protein overexpression is the direct result of gene amplification [31].

HER2/neu overexpression was investigated in a large number of malignant tumors: In breast, ovarian, prostate, pancreatic and liver, HER2/neu overexpression is associated with bad prognosis [32-34].

In the case of the urothelial carcinomas of the urinary bladder. HER2/neu expression is variable, with an incidence between 2 and 74%, and still has a controversial prognostic significance [35].

In this study, all control cases (100%) were negative to HER2/neu expression, while 85% of patients with transitional cell carcinoma were positive, from which 50% were strongly positive (score 3). There was highly significant correlation ($p<0.01$).

There were similarities between the results of this study and Coogan and colleagues [13] study who found HER-2/neu overexpression in 26% of the TCC cases. In another study, 36% of cases were positive for HER-2/neu using the immunohistochemistry method [36]. In study of Jimenez et al., [37], they have identified HER2/neu overexpression in 28% of primary urothelial carcinomas.

The difference in HER-2/neu overexpression rate between these studies can be due to the small volume and selected nature of study samples.

The current study showed a statistically significant difference in HER2/neu protein overexpression in grade 3 TCC compared with grade 2 ($p<0.01$), with overexpression more prevalent in the higher grade. The expression intensity was significantly correlated with the differentiation grade. Lower expression was most likely present in tumors with lower differentiation grade.

This is in consistent with the findings of several reports [37-41] who assert that expression of HER-2/neu oncogene has a direct relationship with the grade and stage of the bladder TCC.

About patient survival; a statistically insignificant correlation between HER2/neu expression and patient’s survival was found in this work ($p>0.05$). This agrees with other studies which show that HER-2/neu overexpression has no prognostic significance with regard to survival [37]. Underwood et al., [42] and Mellon et al., [43], have found no prognostic significance association, and others. On the other hand, Nguyen et al., [35], Korkolopoulou et al., [44] and Vollmer et al., [48] have linked it to a better clinical outcome. Others suggested that the overexpression of HER-2 was an independent variable in determining patient survival [46]. Bongiovanni et al., [18] study showed that HER-2 expression does not represent a prognostic marker of recurrence/prog ression of disease in high-grade T1 bladder cancer.
However, several studies reported that HER-2 could have a role as prognostic factor in bladder cancer, correlating its overexpression with poor prognosis for patients. Jimenez et al., [37]. Kruger et al., [39], Wolff et al., [47] and Kolla et al., [48] have correlated HER2/neu overexpression with a more aggressive clinical behavior.

These variations in results are due to the heterogeneity of studies with respect to kits and type of antibodies used for IHC analysis, protocols, stage of the disease studied (non muscle-invasive vs muscle-invasive), definition of HER-2 positivity and the material studied (fresh/formalin fixed). Thus, discordant results reported in the literature highlight a need for standardized laboratory methods.

In conclusions, using IHC, low CD44 expression was correlated to higher tumor grade and stage and poor patient’s survival. HER-2/neu overexpression was relevant in high grade (grade 3) specimens compared with lower grade of TCC.

Comprehensive research with longer follow-up period and a larger sample size can determine the probable role of HER-2/neu and CD44 expression as prognostic factors in the TCC of bladder.

References

4. ZAGHLoul M.S., NOUH M.A. and MONEER M.: Time-period and a larger sample size can determine the probable role of HER-2/neu and CD44 expression with a more aggressive clinical behavior.


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