ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL EXPRESSION OF HER2 AND EPIDERMAL GROWTH FACTOR RECEPTOR AS PRACTICAL TOOL TO CHARACTERIZE MUSCLE-INVASIVE UROTHELIAL CARCINOMA OF THE BLADDER

Tiberiu Adelman[2], Ioan Ioiart[1], Anca Maria Cimpean[2], Marius Raica[2,*]

[1]Vasile Goldiș West University, Department of Urology, Arad, Romania
[2]Victor Babes University of Medicine and Pharmacy, Department of Microscopic Morphology, Histology, Angiogenesis Research Center Timișoara, Timișoara, Romania
*Corresponding Author

ABSTRACT

Cancer of the urimary bladder is a frequent malignant condition in human. The worst prognosis is noticed for patients with invasive tumors that are incompletely characterized in terms of the molecular profile. Consecutively, the therapeutic strategies are limited and often, not efficient. Based on these data, we analized 50 patients with muscle-invasive tumors of the urinary bladder. We tested the immunohistochemical expression of HER2 and EGFR, and we found statistic significant correlation with the pathological type and degree of differentiation. HER2 was overexpressed in 26.66% of the cases, and EGFR in 82.22%. Based on the expression of EGFR and overexpression of HER2 we have identified three subgroups of patients: EGFR+/HER2-, EGFR+/HER2+, and EGFR-/HER2+, which may benefit from the specific targeted therapy. To the best of our knowledge, it is the first report on the stratification of patients with bladder cancer based on two molecular targets for therapy.

Key words: bladder, urothelial carcinoma. HER2, epidermal growth factor receptor

INTRODUCTION

Urothelial carcinoma of the urinary bladder is the 4th malignancy in men and the 9th in women, with a specific mortality over 50.000 cases each year only in United States. The majority of bladder tumors are superficial in the moment of diagnosis and survival is over 90% at five years in the case of early detection. On the other hand, 20% of bladder tumors are diagnosed in advanced-stage, tumor cells invading muscularis propria, perivesical tissues and surrounding organs (Grivas et al, 2011). In these cases, the therapeutic strategy includes surgery and chemotherapy, but survival at five years is only 44% (Lae et al, 2010). The prognosis is worst in the metastatic disease and less than 5% of the patients survive after five years, regardless adjuvant therapy. Based on these data, the identification of new molecular targets for therapy becomes a necessity, and for these, we analyzed HER2 and epidermal growth factor receptor (EGFR).

HER2 is the receptor for epidermal growth factor 2, a transmembrane protein with 1225 amino acids (Padhy et al, 1982; Schechter et al, 1984). HER2 is well known in oncology, particularly because of its overexpression in breast cancer. HER2 overexpression induces proliferation, growth and cell survival, and these properties become more evident in tumor cells. Introduction of trastuzumab in the clinical practice had a real impact on survival, and virtually, trastuzumab in one of the first drugs that was addressed to a specific molecular target. In last years it has been shown that HER2 overexpression is not restricted to the breast cancer, but also in a percentage of gastric and urothelial carcinomas. In urothelial carcinoma, HER2 overexpression has been reported particularly in invasive tumors but positive results range between large limits (9 to 81%). Differences could be explained mainly by the criteria of inclusion and methodology of evaluation of the positive reaction. Although the overexpression of HER2 in urothelial carcinoma has been demonstrated 15 years ago, the relationships with prognosis and rate of recurrences is unclear, and an accepted strategy for the therapy with trastuzumab is lacking.

EGFR, also known as HER1, belongs to the family of tyrosinkinase receptors and binds the epidermal growth factor. EGFRs are active in dimeric form, and formation of dimers depends on the concentration of both receptors and ligands (Thazar et al, 1997; Yarden and Sliwkowski, 2001). EGFR activation stimulates proliferation and cell survival, and inhibits or restricts apoptosis (Memon et al, 2011). EGFR shows a different expression in various tumors, including bladder cancer, in comparison with normal tissues. Almost all studies published until now show that aberrant expression of EGFR is related to the unfavorable prognosis (Garcia et al, 2003). Although there are many studies on the EGFR expression in bladder tumors, its clinical significance is still uncertain.

Currently, there are active inhibitors of EGFR on the market (iressa or gefitinib), but recently, a phase II clinical trial showed that gefitinib associated to conventional chemotherapy did not improved

significantly the evolution of advanced-stage and metastatic urothelial carcinoma (Miller et al, 2016). This is why a better characterization of molecular targets and identification of new therapeutic variants are of interest for the practice. Strategies based on the inhibition of all four member of HER family are ongoing, and it has been shown that HER3 and HER4 overexpression protect the patients from the consequences of HER2 and EGFR overexpression (Memon et al, 2006).

The biological resistance to EGFR inhibitors might be generated by mutations and/or changes of some intracellular signaling, and it has been noticed in many tumor types (Carlsson et al, 2015). The therapeutic strategy in invasive bladder cancer does not include EGFR inhibitor, based on the lack of evidences. A clear characterization of EGFR expression at protein level is still needed to evaluate the potential impact on therapy in invasive urothelial carcinoma.

MATERIAL AND METHODS

Patients' data. There were investigated 50 consecutive cases with T2-T4 invasive bladder cancer, and the diagnosis was based on clinical, imagistic, endoscopic and pathological data, according to largely accepted procedures. All patients were treated by radical cystectomy and multiple biopsies were taken from each case. Staging according to TNM system was available in all cases.

Primary processing. Biopsies were washed in buffer saline and then fixed in buffer formalin for 48-72 hours. Paraffin sections 3 μm thick were stained with haematoxylin-eosin and examined for the routine diagnosis in terms of the histological type, the level of invasion, and grade. Additional sections from each case were prepared for immunohistochemistry.

Immunohistochemitry. Slides were stained for immunohistochemical methods using Bond Max (Leica Biosystems Newcastle Upon Tyne NE12 8EW, UK) and the full procedure was automated based on a standard technique. Briefly, dewaxing was performed with Bond Max Dewax solution. Antigen retrieval has been performed with Bond Epitope Retrieval 1 and 2, solution pH6 for EGFR, and pH9 for HER2 (Leica Biosystems Newcastle Upon Tyne NE12 8EW, UK). Endogeneous peroxidase was blocked with 3% hydrogen peroxide for 5 minutes and incubation with primary antibodies last for 29 minutes. Slides were then incubated with the secondary antibody for 8 minutes, and with the tertiary for 8 minutes. The final product of reaction was visualized with diamino-benzidine dihydrochloride for 10 minutes, and finally, nuclei were stained with haematoxylin. After each step a thorugh washing with washing solution and distillated water has been performed. Primary antibodies used in this study were c-erb-2 oncoprotein, clone CB11, monoclonal, prediluted, and anti-EGFR, clone EGFR 25, monoclonal, prediluted, both from Novocastra, Leica Biosystems Newcastle Upon Tyne NE12 8EW, UK. The visualization system was the biotin-free Bond Polymer Refine Detection DS 9800 (Leica Biosystems Newcastle Upon Tyne NE12 8EW, UK). Stained slides were dehydrated with 1900% ethanol, dried and mounted with automatic Leica CV 5030 (Leica Biosystems Newcastle Upon Tyne NE12 8EW, UK).

Microscopic evaluation.

Evaluation of EGFR expression. Because of many controversial data on the immunohistochemical expression of EGFR, we propose a score that was applied to all cases included in the present study. The score is based on the percent of positive tumor cells and intensity of the final product of reaction (Table 1). The final result was represented by the sum between the two criteria, therefore the minimal score was 0 and the maximal 6. Cases with score 3 or less were considered negative, and cases with final score between 4 and 6 were considered positive.

Score	% of positive cells	Intensity of reaction
0	0	-
+1	<10%	Weak
+2	10 to 30%	Moderate
+3	>30%	Strong

Table 1.
EGFR scoring system

Evaluation of HER2 expression was performed on the score largely applied for breast cancer, as follows (Table 2).

Score	Overexpression	Staining pattern
0	Negative	No staining, or weak staining in less than 10% of tumor cells
+1	Negative	Weak staining of the membrane in more than 10% of tumor cells, but discontinuous
+2	Weakly positive	Weak to moderate complete membrane staining in more than 10% of tumor cells
+3	Strong positive	Strong and complete membrane staining in more than 10% of tumor cells

Table 2. Interpretation test of HER2 reaction

RESULTS

The normal urothelium did not express HER2. The final product of reaction was found only in tumor cells in positive cases. In cases with overexpression, the final product of reaction had a membrane pattern, continuous, with strong intensity. On occasion, we noticed a weak cytoplasmic reaction that could be the result of the defective receptor (p95) that lacks extracellular domain.

field (fig.2a and b). Notably, dominant positive tumor cells were located mainly at the front of proliferation and invasion.

Five cases with bladder cancer were excluded from the statistics, based on the inappropriate primary processing of specimens. Fron 45 cases included in this part of the study, 33 were negative, and 12 showed overexpression (26.66%).

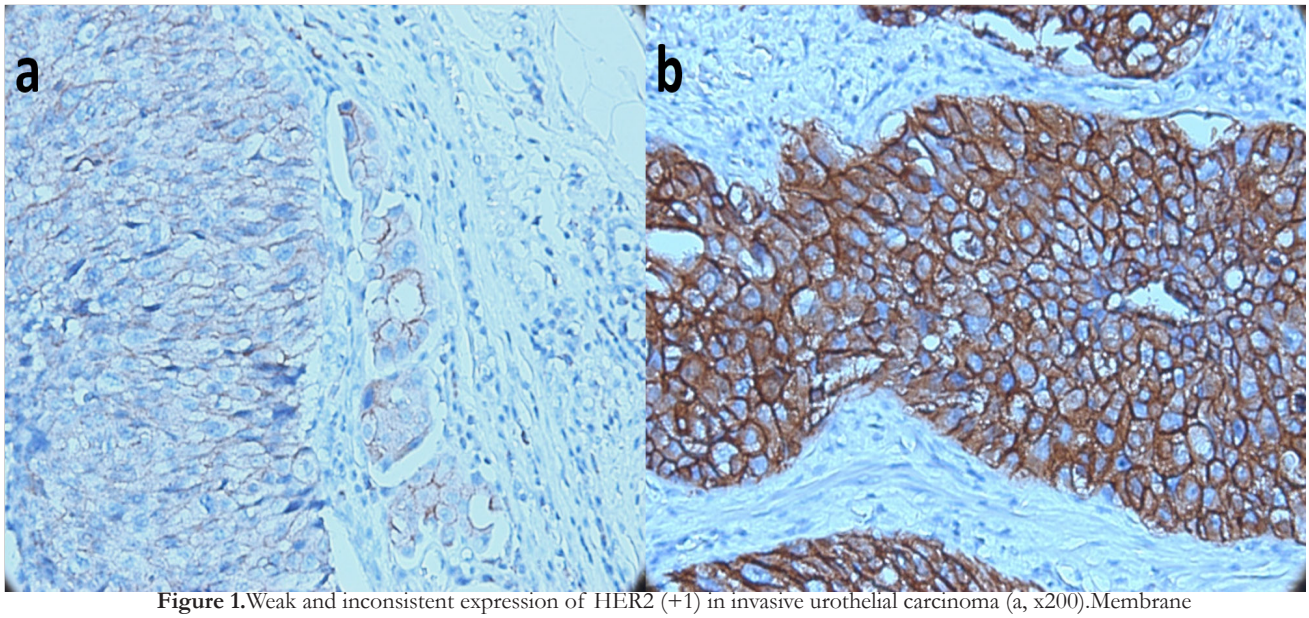


Figure 1. Weak and inconsistent expression of HER2 (+1) in invasive urothelial carcinoma (a, x200).Membrane continuous staining (+3) in invasive urothelial carcinoma of the urinary bladder (b, x200). Immunoreaction for HER2.

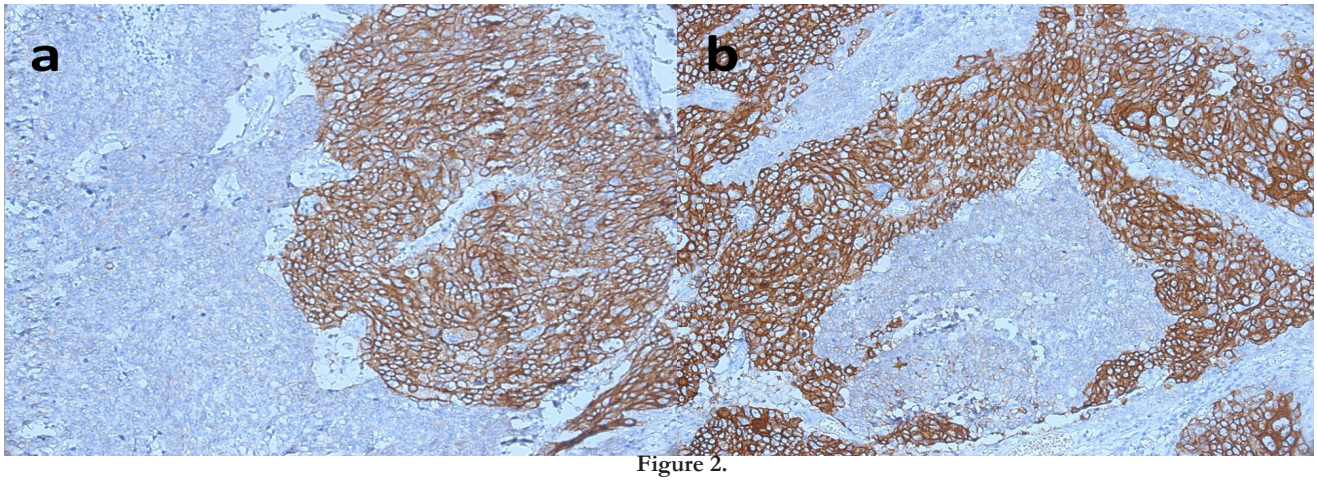


Figure 2.

Homogeneous (a) and heterogeneous (b) HER2 overexpression in urothelial carcinoma. Magnification x100. .

In cases scored as 0 (n=20) any staining was found or a weak and inconsistent reaction in less than 10% of tumor cells. In cases scored as +1 (n=13) there were stained over 10% of tumor cells, but with discontinuous membrane pattern and low intensity. We found 5 cases scored as +2 (n=5), characterized by enhanced membrane staining, associated with weak or moderate intensity of reaction (fig.1a). finally, +3 scored cases showed a strong continuous membrane reaction in almost all tumor cells (fig.1b). Our findings support the heterogeneous character of HER2 overexpression. Large area of positive tumor cells alternate with negative, and such aspects are evident even in the same microscopic

Sarcomatoid urothelial carcinoma, squamous cell carcinoma, clear cell carcinomoa and adenocarcinoma were negative. We found a significant correlation between the grading and HER2 overexpression ($p<0.0001$). The distribution of positive cases and the relationship between overexpression and grading is shown in Table 3.

EGFR. Normal urothelium closed to the tumor showed strong reaction with homogeneous pattern. Dysplastic urothelium and carcinoma in situ showed a heterogeneous positive reaction with intensity decreasing from basal to superficial cells. In muscle-invasive urothelial carcinoma we identified three pattern of distribution of the final product of reaction: strong cytoplasmic

(found in the majority of positive cases), cytoplasmic with membrane enhancement or membrane only. In most of positive cases almost all tumor cells were intensely stained. A statistic significant correlation was found between the expression of EGFR and grading.

Some histological types of urothelial carcinoma, as clear cel land sarcomatoid, ipically negative with HER2, were intensely stained for EGFR. Squamous cell carcinoma were intensely staine, but not adenocarcinoma. Particular features of the immunohistochemical reaction for EGFR are shown in fig.4.

G	0	+1	+2	+3	Total
1	2	0	1	0	3
2	4	6	1	0	11
3	14	7	3	7	31
Total	20	13	5	7	45

Table 3. Distribution of cases based on HER2 overexpression and grading (G). HER2 detected by immunohistochemistry

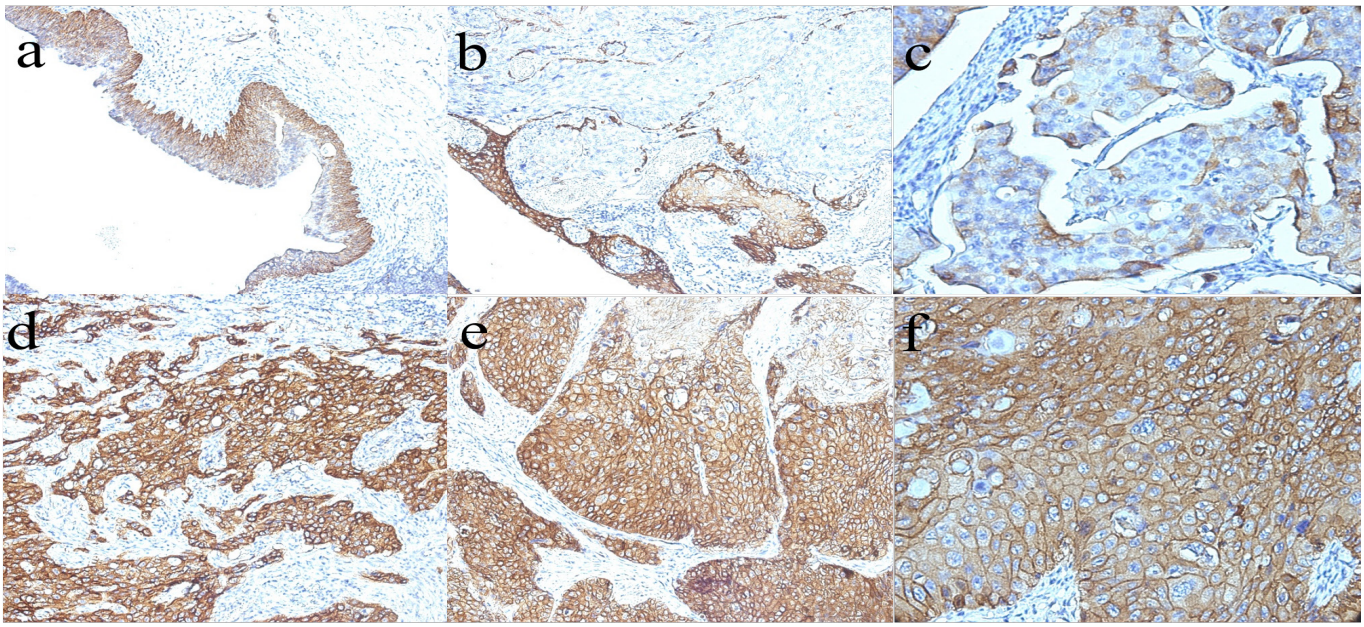


Figure 3. Dysplastic urothelium (a, x200). Remnants of urothelium closet to a negative carcinoma (b, x200). EGFR positive in less than 10% of tumor cells (c, x400). Strong reaction in all tumor cells (d, x400). Strong reaction at the front of invasion (e, x200). Strong reaction with membrane pattern (f, x400). Immunohistochemical reaction for EGFR.

Patterns of distribution of the immunohistochemical reaction for EGFR in urothelial carcinoma are shown in fig.3 (a-f).

All 45 cases were evaluated for the expression of EGFR based on the score given in Material and methods. From 45 cases, 37 had score between 4 and 6, therefore 82,22% were positive. The relationship between EGFR expression, overexpression of HER2 and grading is shwon in Graph 1.

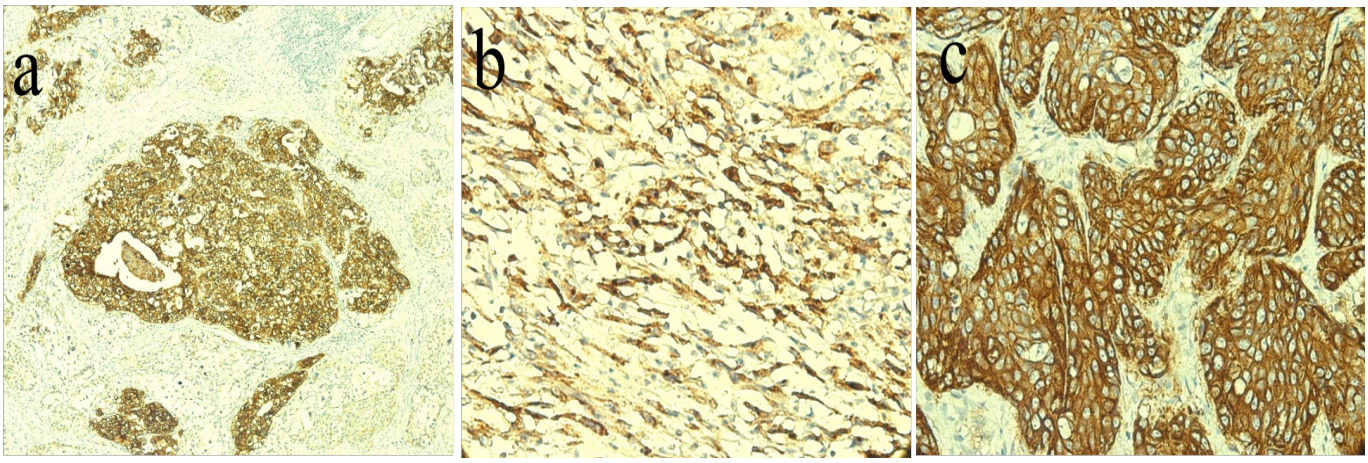


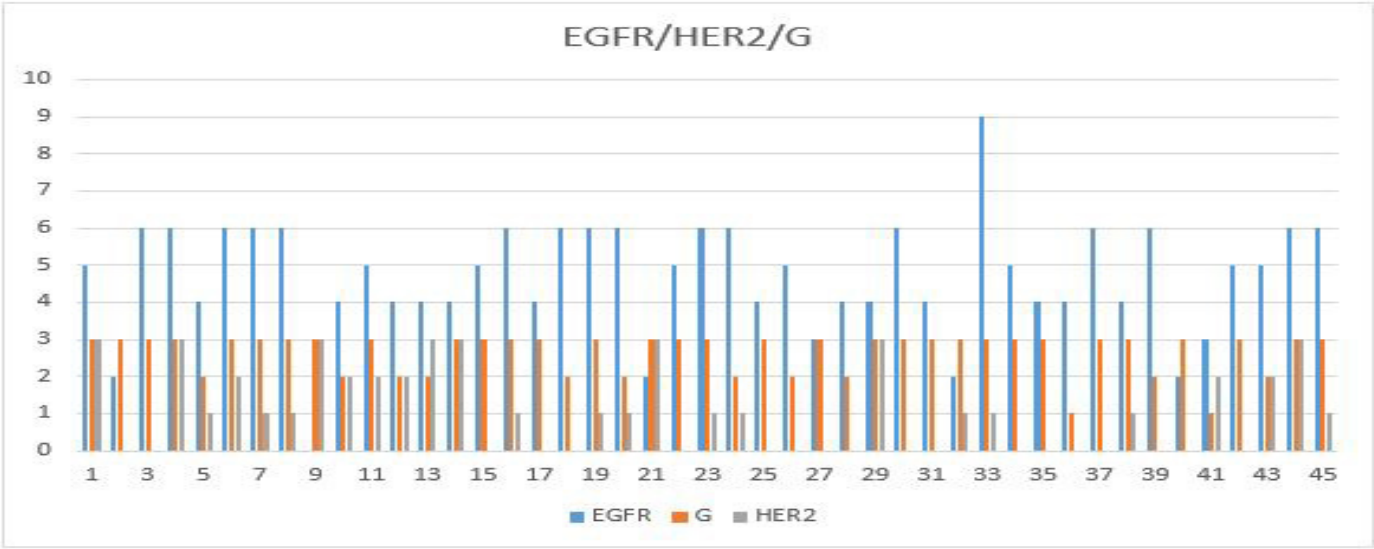
Fig.4. Clear cell urothelial carcinoma (a, x100). Sarcomatoid carcinoma (b, x200). Squamous cell carcinoma (c, x400). Imunoreaction for EGFR.

Based on the distribution of cases, three subgroups of patients could be identified: expressing EGFR only (n=24), co-express EGFR and HER2 (n=3), and the third that shows only HER2 overexpression (n=1).

DISCUSSIONS

HER2 oncoprotein belongs to the erbB receptors family and it is involved in cell proliferation trough tyrosinkinase activity. The gene that encodes HER2 protein, c-erbB2, is located on the chromosome 17, and also encodes a tyrosinkinase defective receptor, homologue to the receptor for epidermal growth factor (Coussens et al, 1985), and forms heterodimers with EGFR.

Overexpression correlates with aggressivity of the tumor, supported by the frequence of lympho-vascular invasion (Lammers and Witjes, 2011). On the other hand, there were reported some discordances between the primary bladder tumor, lymph node and distant metastases in terms of HER2 overexpression. There were reported HER2 negative primary tumors with positive lymph node metastases (45%, Grivas et al, 2011), but also the reversal (Grandmark et al, 2005). Hansel et al (2008) reported 88% concordance between primary tumor and metastases in a study conducted on 53 patients. Discordances could be explained by HER2 overexpression heterogeneity (Jimenez et al, 2001), as it has been also shown by our observations.



Graph 1. Expression od EGFR, HER2, and grading (G)

Co-expression of HER2 and EGFR is a frequent finding, noticed in over 50% from urothelial tumor cells (Carlsson et al, 2015).

In normal conditions HER2 is involved in differentiation and proliferation of epithelial cells, but low levels restrict its detection by immunohistochemistry as we showed for the normal urthelium.

Overexpression of HER2 has been reported in some human tumors, including urothelial carcinoma. HER2 stimulate proliferation of tumors cells and enhances the metastatic potential (Wallerand et al, 2008). HER2 overexpression was also reported in urothelial carcinoma of the upper urinary tract in 74% of the cases, and the rate of the positive reaction significantly increases with stage and grading (Ehsani and Osunkoya, 2014). Although it could be an anatomic site-specific molecular profile, the incidence reported by this study is over of all other tumors. Until now, these results were not re-confirmed. In the present study on invasive tumors of the bladder we found 26.66% positive cases that is in accord with other findings, and similar with data reported for other organs, like breast cancer.

HER2 overexpression in urothelial carcinoma of the urinary bladder has predictive role on recurrences, which is important particularly in pT1G3 tumors treated by transurethral resection (Janane et al, 2011).

The prognostic value of HER2 overexpression was also analized in patients with urothelial carcinoma treated by adjuvant chemotherapy. Tsai et al (2007) shown the limited prognostic value in these cases, but overall survival was 33 months in HER2 positive compared with 50 months for patients with HER negative tumors (Grivas et al, 2011). In a relatively recent metaanalysis including nine representative studies, it has been noticed that the rate of overexpression ranges between 9.2 and 85.2% (Zhao et al, 2015), and significantly correlates with grading, lymph node metastases, and survival. Such differences can be explained by the selection of cases, working system, reagents from different producers and last but not liest, the scoring system applied by author. In invasive tumors of the urinary bladder, the incidence of overexpression is higher with immunohistochemistry than fluorescent in situ hybridization (Wulfing et al, 2005; Hammam et al, 2015).

The best-known inhibitor of HER2 is trastuzumab, a humanized monoclonal antibody that binds and blocks the extracellular domain of the receptor. Currently, data on the effects of transtuzumab as single agent in patients with invasive bladder cancer are not available. Addition of trastuzumab to chemotherapy signioficantly increases the rate of response and survival (Hussain et al, 2007). Similar results were noticed using Lapatinib, a specific inhibitor of

HER1 and HER2, in a phase II clinical trial (Wulfing et al, 2009). Currently, advanced-phase clinical trial based on trastuzumab and chemotherapy are in progress in patients with bladder cancer. The most significant reports on HER2 overexpression in bladder cancer are shown in Table 4.

Older studies suggest that HER2 abnormalities occur before the invasion of muscularis propria. Latif et al (2003) showed the increase in the number of HER2 copies in patients treated for bladder tumors, comparing preinvasive and postinvasive status. In the same study it was reported HER2 overexpressionmore frequently in superficial tumors than in invasive, which is in contradiction with the aggressivity of the bladder cancer. In the same category fall a study that reported more HER2 positive in stage III than in stage IV (92 versus 62%) (Gandour-Edwards et al, 2002). Grading was not taken into account and the result is most probably the consequence of a methodology older than 10 years. Recently, Tschui et al (2015) identified in patients with bladder cancer and HER2 amplification high incidence of micropapillary carcinoma, proliferation of tumor cells like nests, and abundant inflammatory infiltrate.

EGFR is a glycoprotein with 170 kD, with extracellular, transmembrane, and cytoplasmic domains, characterized by tyrosinkinase activity. Expression of EGFR in normal tissues of epithelial, mesenchymal and nervous

Authors	Cases	pT	G	Overexpression	Significance
Gandour-Edwards et al 2002	39	2-3	NS	28/39 (71%)	Reduce mortality in patients treated by chemotherapy
Kruger et al 2002	138	2-3	I-III	57/138 (41%)	Correlation with G, no correlation with stage and N
Latif et al 2003	25	2-3	NS	13/25 (52%)	HER2 abnormalities precede invasion
Latif et al 2004	75	2	III	42/75 (57%)	NS
Wulfing et al 2005	127	2-3	II-III	95/127 (74.8%)	Relation with N and M No relation with S
Grandmark et al 2005	90	2-4	I-III	71/90 (79%)	N, M concordance 76%
Hauser-Kronberger et al 2006	62	NS	NS	37/62 (58%)	Potential use of trastuzumab
Tsai et al 2007	114	2-3	II-III	74/114 (60.7%)	Correlation with progression and survival
Caner et al 2008	36	2-3	III	22/36 (61.1%)	NS
Skagias et al 2009	80	NS	NS	41/80 (51.25%)	Correlation with survival
Lae et al 2010	1005	2-3	II-III	115/1005 (11.4%)	Targeted therapy
Janane et al 2011	40	1	II	14/40 (35%)	Predicts recurrence
	44	1	III	30/44 (68.2%)	
Olsson et al 2012	201	1	NS	25/201 (12.4%)	Not useful for prognosis
Chow et al 1997	178	a/1	III	16/178 (9%)	Predicts recurrence and progression
Hammam et al 2015	33	a/3	I-III	20/33 (60%)	Correlation with T and G
Yan et al 2015	475	NS	NS	59/475 (12.4%)	NS
Millis et al 2015	441	NS	NS	45/441 (10.2%)	Associated with advanced stage and metastases
Present study	45	1-3	II-III	12/45 (26.6%)	Correlation with G, but not with T

Table 4. Overexpression of HER2 in invasive urothelial carcinoma of the bladder (IHC)
Legend: IHC immunohistochemistry, N lymph node metastases, M distant metastases, S survival. NS, not shown.

is critical for cell proliferation and tissue differentiation (Simon, 2000). Dimer formation activates the cytoplasmic domain, which justified to take into account the cytoplasmic pattern into the final score.

In the normal urothelium EGFR is expressed only in the basal cells. Binding of epidermal growth factor to EGFR is restricted by the superficial layers of the urothelium. Damages of this natural barrier allow the binding of EGF to EGFR, and thus the complex could have tumorigenic potential (Chow et al, 1997), as we noticed in dysplastic urothelium and carcinoma in situ.

We found expression of EGFR in 82.22% of the cases, a little bit higher than previous studies (Mooso et al, 2015), and this can be explained by the selection only of invasive tumors. Existing data showed that reaction for EGFR is not significantly different between papillary tumors and normal urothelium, but becomes stronger in invasive tumors, including clear cell and sarcomatoid urothelial carcinoma. In the current work we described three patterns of EGFR immunohistochemical reaction, although some authors do not take into account the cytoplasmic distribution (Carlsson et al, 2015). Our results showed that in some cases the strong reaction obscures the membrane enhancement, and in some cases may lead to a subevaluation. This is the main reason because we purposed a new scoring system for EGFR reaction, based on intensity and percent of positive tumor cells.

The expression of EGFR in bladder tumors is high enough (range between 40 and 100%) to represent an attractive therapeutic target. On the other hand, EGFR is expressed by some normal human cells. In this condition, it is important to find out an EGFR variant able to selectively block the receptor located on tumor cells. Association between EGFR and HER2 is already demonstrated, and our observations support it. Blocking both receptors in tumor cells of the bladder cancer could work better than inhibition of each. This is particularly important in patients with advanced stage and metastatic bladder cancer.

The potential role of anti-EGFR in the therapy of urothelial carcinoma is also supported by experimental evidences. It was found that gefitinib induces apoptosis on urothelial carcinoma cell lines in combination with radiotherapy (Maddieni et al, 2005). Inhibiting EGFR and vascular endothelial growth factor with vandetanib induced high sensitivity of urothealial carcinoma cell to cisplatin, dose-dependent (Flaig et al, 2009). The team of Dinney (Blehm et al, 2006) imagined an experimental model for urothelial carcinoma implanting 253J malignant cell line in the bladder wall of the nude mouse. Induction of EGFR overexpression induced increased metastatic growth, which support the contribution of EGFR to tumor progression. The growth has been limited by administration of cetuximab, which determined reduction of both primary tumor and its metastases. Taken together all these data, the field of growth fators and their specific antibodies show a lot of diagnostic, prognostic, and therapeutic possibilities in bladder cancer.

CONCLUSION

We have investigated the immunohistochemical expression of EGFR and HER2 in specimens from 50 patients with muscle invasive bladder cancer. The expression of EGFR was noticed in tumor cells in 82.22% of the cases. There were positive most of the cases with urothelial carcinoma and scuamocelular carcinoma. Negative reaction was observed in adenocarcinoma and low-grade urothelial carcinoma. Overexpression of HER2 was found in 26.66% of the cases. Based on the expression of EGFR and overexpression of HER2 we identified three subgroups of patients: EGFR+/HER2-, EGFR+/HER2+, and EGFR-/HER2+, which may benefit from the specific targeted therapy.

ACKNOWLEDGEMENTS.

Authors thank to Assoc Prof Raluca Amalia Ceausu for her contribution to the immunohistochemical processing, and to Patricia Berzava and Ciprian Oniga for their excellent technical assistance.

REFERENCES

1. Grivas PD, Day M, Hussain M. Urothelial carcinomas: a focus on human epidermal receptors signaling. Am J Transl Res, 2011, 3: 362-373.

2. Lae M, Couturier J, Oudard S, Radvanyi F, Beuzeboc P, Vieillefond A. Assessing HER2 gene amplification as a potential target for therapy in invasive urothelial bladder cancer with a standardized methodology: results in 1005 patients. Ann Oncol, 2010, 21: 815–819.

3. Padhy LC, Shih C, Cowing D, Finkelstein R, Weinberg RA. Identification of a phosphoprotein specifically induced by the transforming DNA of rat neuroblastomas. Cell, 1982, 28, 4: 865–871.

4. Schechter AL, Stern DF, Vaidyanathan L et al. The neu oncogene: an erb-B-related gene encoding a 185,000-M tumour antigen. Nature, 1984, 312, 5994: 513–516.

5. Tzahar E, Pinkas-Kramarski R, Moyer JD, Klapper LN, Alroy I, Levkowitz G, Shelly M, Henis S, Eisenstein M, Ratzkin BJ, Sela M, Andrews GC, Yarden Y. Bivalence of EGF-like ligands drives the ErbB signaling network. EMBO J, 1997, 16: 4938– 4950.

6. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol, 2001, 2: 127–137.

7. Memon AA, Munk M, Nexo E, Sorensen BS. Calcium-induced apoptosis is delazed by HER1 receptor signaling through the Akt and PLCy pathways in bladder cancer cells. Scand J Clin Lab Invest, 2011, 71: 45-51.

8. Garcia I, Vizoso F, Martin A, Sanz L, Abdel-Lah O, Raigoso P, Garcia-Muniz JL. Clinical significance of the epidermal growth factor receptor and HER2 receptor in resectable gastric cancer. Ann Surg Oncol, 2003, 10: 234–241.

9. Miller K, Morant R, Stenzl A, Zuna I, Wirth M. A phase II studz of the Central European Societz of anticancer-drug research (CEZAR group): results of an open-label-study of gemcitabine plus cisplatin with or without concomitant or sequential Gefitinib in patients with advanced or metastatic transitional cell carcinoma of the urothelium. Urol Int, 2016, 96: 5-13.

10. Memon AA, Sorensen BS, Meldgaard P, Fokdal L, Thykjaer T, Nexo E. The relation between survival and expression of HER1 and HER2 depends on the expression of HER3 and HER4: a study in bladder cancer patients. Br J Cancer, 2006, 94: 1703–1709.

11. Carlsson J, Wester K, De La Torre M, Malmström P-U, Gårdmark T. EGFR-expression in primary urinary bladder cancer and corresponding metastases and the relation to HER2-expression. On the possibility to target these receptors with radionuclides. Radiol Oncol, 2015, 49(1): 50-58.

12. Chakravarti A, Winter K, Wu CL, Kaufman D, Hammond E, Parliament M, Tester W, Hagan M, Grignon D, Heney N, Pollack A, Sandler H, Shipley W. Expression of the epidermal growth factor receptor and Her-2 are predictors of favorable outcome and reduced complete response rates, respectively, in patients with muscle-invading bladder cancers treated by concurrent radiation and cisplatin-based chemotherapy: a report from the Radiation Therapy Oncology Group. Int J Radiat Oncol, Biol Phys, 2005, 62: 309–317.

13. Popov Z, Gil-Diez-De-Medina S, Ravery V, Hoznek A, Bastuji-Garin S, Lefrere-Belda MA, Abbou CC, Chopin DK. Prognostic value of EGF receptor and tumor cell proliferation in bladder cancer: therapeutic implications. Urol Oncol, 2004, 22: 93– 101.

14. Ravery V, Grignon D, Angulo J, Pontes E, Montie J, Crissman J, Chopin D. Evaluation of epidermal growth factor receptor, transforming growth factor alpha, epidermal growth factor and c-erbB2 in the progression of invasive bladder cancer. Urol Res, 1997, 25: 9–17.

15. Kramer C, Klasmeyer K, Bojar H, Schulz WA, Ackermann R, Grimm MO. Heparin-binding epidermal growth factor-like growth factor isoforms and epidermal growth factor receptor/ErbB1 expression in bladder cancer and their relation to clinical outcome. Cancer, 2007. 109: 2016-2024.

16. Coussens L, Yang-Feng TL, Liao YC, Chen E, Gray A, McGrath J, Seeburg PH, Libermann TA, Schlessinger J, Franke U. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene, Science, 1985, 230:1132–1139.

17. Wallerand H, Robert G, Bernhard JC, Ravaud A, Ferrière JM. Targeted therapy for locally advanced and/or metastatic bladder cancer. Prog Urol. 2008, 18(7):407-417.

18. Ehsani L, Osunkoya AO. Human epidermal growth factor receptor 2 expression in urothelial carcinoma of the renal pelvis: correlation with clinicopathologic parameters. Int J Clin Exp Pathol 2014, 7(5): 2544-2550.

19. Janane A , Hajji F, Ismail TO, Elondo JC, Ghadouane M, Ameur A, Abbar M, Bouzidi A. Evaluation of HER2 protein overexpression in non-muscle-invasive bladder cancer with emphasis on tumour grade and recurrence. Actas Urol Esp. 2011, 35(4):18994.

20. Lammers RJ, Witjes JA. Discussion on the influence of HER2 status on the clinical outcome of bladder cancer continues. Expert Rev Anticancer Ther, 2011, 11(6):853-858.

21. Grandmark T, Wester K, De La Torre M, Carlsson J, Malmstrom P-U. Analysis of HER2 expression in primary urinary bladder carcinoma and corresponding metastases. BJU Intern, 2005, 95: 982–986.

22. Hansel DE, Swain E, Dreicer R, Tubbs RR. HER2 overexpression and amplification in urothelial carcinoma of the bladder is associated with MYC coamplification in a subset of cases. Am J Clin Pathol, 2008; 130: 274–281.

23. Jimenez RE, Hussain M, Bianco FJ Jr et al. Her-2/neu overexpression in muscleinvasive

24. urothelial carcinoma of the bladder: prognostic significance and comparative analysis in primary and metastatic tumors. Clin Cancer Res, 2001, 7: 2440–2447.

25. Tsai YS, Tzai TS, Chow NH. Does HER2 immunoreactivity provide prognostic information in locally advanced urothelial carcinoma patients receiving adjuvant MVEC chemotherapy? Urol Int. 2007;79 (3):210-216.

26. Zhao J , Xu W, Zhang Z, Song R, Zeng S, Sun Y, Xu C. Prognostic role of HER2 expression in bladder cancer: a systematic review and meta-analysis. Int Urol Nephrol, 2015, 47(1):87-94.

27. Wülfing C, von Struensee D, Bierer S, Bögemann M, Hertle L, Eltze E. Expression of Her2/neu in locally advanced bladder cancer: implication for a molecular targeted therapy. Aktuelle Urol. 2005, 36(5):4239.

28. Hammam O, Nour HH, Mosaad M, Akl M, Khalil H, al Ganzory H, Hindawi A. The clinical significance of HER2 protein amplification/ expression in urinary bladder lesion. Arab J Urol, 2015, 13: 146–152.

29. Yan M, Schwaederle M, Arguello D, Millis SZ, Gatalica Z, Kurzrock R. HER2 expression status in diverse cancers: review of results from 37,992 patients. Cancer Metastasis Rev, 2015, 34:157–164.

30. Hussain MH, MacVicar GR, Petrylak DP et al. Trastuzumab, paclitaxel, carboplatin, and gemcitabine in advanced human epidermal growth factor receptor-2/neu-positive urothelial carcinoma: results of a multicenter phase II. National Cancer Institute trial. J Clin Oncol, 2007, 25: 2218–2224.

31. Wulfing C, Machiels JP, Richel DJ et al. A single-arm, multicenter, open-label phase 2 study of lapatinib as the second-line treatment of patients with locally advanced or metastatic transitional cell carcinoma. Cancer, 2009; 115: 2881–2890.

32. Gandour-Edwards R, Lara PN, Folkins AK, LaSalle JM, Beckett L, Li Y, Meyers FJ, DeVere-White R. Does HER2/neu expression provide prognostic information in patients with advanced urothelial carcinoma? Cancer, 2002, 95:1009–1015.

33. Krüger S , Weitsch G, Büttner H, Matthiensen A, Böhmer T, Marquardt T, Sayk F, Feller AC, Böhle A. HER2 overexpression in muscle-invasive urothelial carcinoma of the bladder: prognostic implications. Int J Cancer. 2002, 102(5): 514-518.

34. Latif Z, Watters AD, Dunn I, Grigor KM, Underwood MA, Bartlett JMS. HER2/neu overexpression in the development of muscle-invasive transitional cell carcinoma of the bladder. Br J Cancer, 2003, 89: 1305–1309.

35. Latif Z, Watters AD, Dunn I, Grigor K, Underwood MA, Bartlett JM. HER2/neu gene amplification and protein overexpression in G3 pT2 transitional cell carcinoma of the bladder: a role for anti-HER2 therapy? Eur J Cancer. 2004, 40(1):56-63.

36. Hauser-Kronberger C, Peham K, Grall J, Rausch W, Hutarew G, Dietze O. Novel approach of human epidermal growth factor receptor 2 detection in noninvasive and invasive transitional cell carcinoma of the bladder. J Urol. 2006, 175(3 Pt 1):875-880.

18. Ehsani L, Osunkoya AO. Human epidermal growth factor receptor

37. Caner V, Turk NS, Duzcan F, Tufan NL, Kelten EC, Zencir S, Dodurga Y, Bagci H, Duzcan SE. No strong association between HER2/neu protein overexpression and gene amplification in high-grade invasive urothelial carcinomas. Pathol Oncol Res, 2008, 14(3):2616.

38. Skagias L, Politi E, Karameris A, Sambaziotis D, Archondakis A, Vasou O, Ntinis A, Michalopoulou F, Moreas I, Koutselini H, Patsouris E. Prognostic impact of HER2/neu protein in urothelial bladder cancer. Survival analysis of 80 cases and an overview of almost 20 years' research. J BUON, 2009, 14(3):457-462.

39. Olsson H, Fyhr IM, Hultman P, Jahnson S. HER2 status in primary stage T1 urothelial cell carcinoma of the urinary bladder. Scand J Urol Nephrol. 2012, 46(2):102-107.

40. Chow NH, Liu HS, Lee EI, Chang CJ, Chan SH, Cheng HL, Tzai TS, Lin JS. Significance of urinary epidermal growth factor and its receptor expression in human bladder cancer. Anticancer Res, 1997, 17: 1293-1296.

41. Millis SZ, Bryant D, Basu G, Bender R, Vranic S, Gatalica Z, Vogelzang NJ. Molecular profiling of infiltrating urothelial carcinoma of bladder and nonbladder origin. Clin Genitourinary Cancer, 2015, e37-e43.

42. Tschui J, Vassella E, Bandi N, Baumgartner U, Genitsch V, Rotzer D, Seiler R, Thalmann GN, Fleischmann A. Morphological and molecular characteristics of HER2 amplified urothelial bladder cancer. Virchows Arch, 2015, 466:703–710.

42. Tschui J, Vassella E, Bandi N, Baumgartner U, Genitsch V, Rotzer D, Seiler R, Thalmann GN, Fleischmann A. Morphological and molecular characteristics of HER2 amplified urothelial bladder cancer. Virchows Arch, 2015, 466:703–710.

43. Simon MA. Receptor tyrosine kinases: specific outcomes from general signals. Cell, 2000, 103: 13-15.

44. Mooso BA, Vinall RL, Mudryj M, Yap SA, de Vere White R, Ghosh PM. The role of EGFR family inhibitors in muscle invasive bladder cancer: a review of clinical data and molecular evidence. J Urol, 2015, 193(1): 19-29.

45. Maddineni SB, Sangar VK, Hendry JH, Margison GP, Clarke NW. Differential radiosensitisation by ZD1839 (Iressa), a highly selective epidermal growth factor receptor tyrosine kinase inhibitor in two related bladder cancer cell lines. Br J Cancer 2005; 92: 125-130.

46. Flaig TW, Su LJ, McCoach C, Li Y, Raben D, Varella-Garcia M, Bemis LT. Dual epidermal growth factor receptor and vascular endothelial growth factor receptor inhibition with vandetanib sensitizes bladder cancer cells to cisplatin in a dose- and sequence-dependent manner. BJU Int, 2009, 103(12): 1729-1737.

Corresponding Author
Marius Raica
Victor Babes University of Medicine and Pharmacy
Department of Microscopic Morphology/Histology
Angiogenesis Research Center Timisoara
Pta Eftimie Murgu 2, 300041 Timisoara, Romania
raica@umft.ro, www.umft.ro