JBUON 2013; 18(3): 653-659
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

ErbB2, ErbB3 and ErbB4 expression in urothelial tumors of the upper urinary tract and their prognostic significance

I. Galanakis[1], E. Spyropoulos[1], I. Gkialas[2], A. Nomikos[3], E. Souka[4], D. Mitropoulos[5]

[1]*Urology Department, Athens Naval Hospital, Athens;* [2]*Urology Department, Athens General Hospital "G.Genimmatas", Athens;* [3]*Pathology Department, Asklepieio Voulas General Hospital, Athens;* [4]*Pathology Department, "Evaggelismos" General Hospital, Athens;* [5]*Urology Department, Athens General Hospital "Laiko", Athens, Greece*

Summary

Purpose: *ErbB family represents a promising therapeutic target in upper urinary tract urothelial carcinoma (UUTUC). Our study aimed to correlate ErbB2, ErbB3 and ErbB4 expression in UUTUC with other clinicopathological parameters as well as patient outcome.*

Methods: *ErbB2, ErbB3 and ErbB4 were immunohistochemically assessed in 99 consecutive UUTUC specimens.*

Results: *With a median follow-up of 52.5 months (range 1-127) 28 patients (28.3%) died 1-95 months after the first surgical treatment and the mean survival was 18.9±24.2 months. ErbB2, ErbB3 and ErbB4 expression was positive in 64.8, 19.5, and 20.8% of the tumors, respectively. Combined expression of all 3 receptors was found in 7.9% of the tumors, combined expression of 2 receptors in 14.5% and 48.7% expressed at least one ErbB receptor. No ErbB expression was found in 28.9% of the tumors. We found no significant correlation between ErbB2, ErbB3 and ErbB4 expression with tumor stage, grade, recurrence or cancer specific survival apart from the inverse relation between ErbB2 expression and time to recurrence (p=0.027).*

Conclusion: *Of the 3 receptors evaluated, neither ErbB3 nor ErbB4 showed any prognostic significance in the UUTUC. ErbB2, however, was inversely associated with recurrence and needs further evaluation in well-designed, prospective, randomized trials.*

Key words: *ErbB receptors, prognostic factors, targeted therapies, upper urinary tract, urothelial carcinomas*

Introduction

UUTUC is a rare tumor accounting only for 5% of all urothelial carcinomas. Its annual incidence is 1.6-1.9/100,000, although a recent study raises it up to 2.1-2.4/100 000 [1,2]. Of the patients with solitary UUTUC, 20-50% will develop bladder cancer in the future [3]. Contralateral UUTUC will occur in 2-4% of the patients [3]. The standard treatment is nephroureterectomy and bladder cuff resection, while the only established prognostic factors are, mainly, tumor stage and, secondly, grade. However, the aggressive nature of this tumor along with the usually unpredictable clinical course, render the identification of novel therapeutic approaches rather essential.

The ErbB family receptors are a field for research of high interest. It comprises four members: EGFR/HER1, ErbB2/HER2, ErbB3/HER3 and ErbB4/HER4. All members are homologous transmembrane receptors containing an extracellular ligand binding region, a hydrophobic transmembrane region, a cytoplasmic tyrosine kinase domain and cytoplasmic tyrosine residues that serve as sites for receptor phosphorylation [4]. Ligand binding results in receptor dimerization, phosphorylation across receptor dimers on tyrosine residues, recruitment of signaling molecules to the phosphorylated tyrosine residues, and coupling to downstream effectors and biological responses [4]. The signal transduction pathways, therefore, activated by the ErbB family receptors play important roles in various cellular processes, such as cell proliferation, differentiation, adhesion, migration and apoptosis [5]. Trastuzumab and lapatinib are already FDA-approved as target-

Correspondence to: Ioannis Galanakis, MD, FEBU. Department of Urology, Athens Naval Hospital 70, Dinokratous street, Athens 11521, Greece. Tel: +30 6974 663 438, Fax: +30 211 401 9800, E-mail: galanakisioannis@yahoo.gr
Received: 23/01/2013; Accepted: 19/02/2013

ed therapies against metastatic breast and gastric cancer and the research is ongoing [6-8].

There is a lack of studies in the literature regarding the ErbB status in UUTUC, probably explained by the rarity of these tumors. Thus, despite several reports in bladder cancer, only a few studies have investigated ErbB expression in the upper urinary tract [9-15]. The aim of this study was to correlate ErbB2, ErbB3 and ErbB4 expression in UUTUC with other clinicopathological parameters as well as patient outcome.

Methods

The study retrospectively included 99 patients with UUTUC who were treated surgically in two study centers between 1997 and 2007. After patient's informed consent and the approval of the Ethics Review Board, their files were reviewed and the following data were collected: gender, age, type of surgery (total nephroureterectomy or partial ureterectomy), tumor stage according to the 2002 TNM classification, tumor grade according to the 1973 WHO classification, type of adjuvant/salvage treatment (chemotherapy, radiotherapy), time to recurrence (local, distant or bladder) and, finally, cancer specific survival. Follow-up consisted of evaluations every 3 months for the first year, every 6 months for the next 2 years and annually thereafter. Follow-up included physical examination, serum biochemical profile, urine cytology, renal sonoghraphy, chest radiography and periodical cystoscopy. Additional radiographic imaging, when indicated, included intravenous urography, computed tomography and/or bone scanning.

Immunohistochemical staining and evaluation

Serial sections from appropriate tissues of original tumor were cut and submitted for deparaffinization. Antigen retrieval involved positioning of 4μm sections of blocks of the above tissues in a pressure cooker at 56oC for 24 h, followed by microwave heat-induced epitope retrieval (595W microwave for 10 min) using the Trilogy system (Cell Marque, Hot Springs, Arkansas, USA) as per the manufacturer's instructions. We then used an Envision/horse raddish peroxidase (HRP) ChemMate TM/TechMate TM detection system (Dako-Cytomation A/S, Glostrup, Denmark), using primary monoclonal mouse antibodies against ErbB2 (monoclonal, clone CB11, Biocare Medical, diluted 1:100), ErbB3 (monoclonal, 2Q320, Santa Cruz Biotechnology, diluted 1:50) and ErbB4 (monoclonal, SPM338, Santa Cruz Biotechnology, diluted 1:40) for 30 min. A 3-min application of 3% H2O2 was followed by Envision TM incubation for 25 min and diaminobenzidine staining for 10 min. The slides were then rinsed with water and counterstained with aqueous haematoxylin. Sections incubated in Tris (hydroxymethyl) methylamine Anal-ar buffer (BDH Laboratory Supplies, Poole, UK), instead of the primary antibody, were used as negative controls.

All immunostained slides were analyzed by one pathologist who had no previous knowledge of the clinical and pathological data. Membranous immunoreactivity was semi-quantitatively assessed. Grades of positivity were determined according to the percentage of tumor cells stained (< 5% of tumor cells stained = 0, 6-50% of tumor cells stained = 1 and 51–100% of tumor cells stained = 2).

Statistics

All analyses were performed using the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA), version 16. The probabilities of progression-free survival and cancer specific survival were calculated using the Kaplan-Meier method. Significant differences were calculated using the log-rank test and a p-value <0.05 was considered significant.

Results

The clinical features of the 99 patients (70.7% males, 29.3% females) are shown in Table 1.

Table 1. Patient and disease characteristics

Characteristics	*N*	%
Gender		
Male	70	70.7
Female	29	29.3
Age, years, mean ± SD	67.5±11.8	
Location		
Pelvis and/or calyces	47	47.5
Ureter	25	25.2
Multiple pelvis/ureter	27	27.3
Grade		
I	6	6.1
II	49	49.5
III	44	44.4
Stage		
T_a	27	27.3
T_1	22	22.2
T_{2-3}	42	42.4
Metastatic	8	8.1
Recurrence	40	40.4
Retroperitoneal space	4	4
Urinary bladder	28	28.3
Other organs	8	8.1
Survival		
Alive	71	71.7
Deceased	28	28.3

Mean age was 67.5 ± 11.8 years, ranging from 34 to 93 years. The location of the disease was: renal pelvis and/or calyces in 47 patients (47.5%), ureter in 25 (25.2%) and multiple sites in pelvis and ureter in 27 (27.3%).

Of the 99 patients, 71 (71.7%) underwent nephroureterectomy, 2 (2%) partial ureterectomy and 26 (26.3%) nephrectomy and ureterectomy without removing the bladder cuff. Pathological stage was pTaN0M0 in 27 cases (27.3%), pT1N0M0 in 22 cases (22.2%), pT2/3N0M0 in 42 cases (42.4%) and metastatic in lymph nodes or other organs in 8 cases (8.1%). Pathologic grade was: grade I in 6 cases (6.1%), grade II in 49 cases (49.5%) and grade III in 44 cases (44.4%). Twelve patients received adjuvant/salvage therapy (chemotherapy and/or radiotherapy) 7 because of locally advanced disease and 5 because of metastasis.

During follow up (median 52.5 months, range 1-127) the disease recurred in 40 (40.4%) patients and the sites of recurrence were: bladder 28 (28.3%) cases, retroperitoneal space 4 (4%) and other organs 8 (8.1%) cases. Time to recurrence ranged from 3 to 122 months (mean 54.8±6.9), and median 40.0 months; 95% CI 11.8-68.2; Figure 1).

Time to recurrence depended on the site of recurrence. Local recurrences developed after 3-17 months (mean 9.8±3.0 months; 95% CI: 3.8-15.7 and median 7.0 months; 95% CI: 0-15.8), distant metastasis to other organs after 3-44 months (mean 10.4±5.6 months, 95% CI: 0-21.5 and median 5.0 months; 95% CI: 2.4-7.6) and bladder recurrence after 3-61 months (mean 16.7±3.3 months; 95% CI: 10.3-23.1 and median 12.0 months; 95% CI: 8.5-15.5), as shown in Figure 2.

Time to recurrence according to tumor stage is shown on Figure 3. In TaN0M0 cases there were only bladder recurrences, in T1N0M0 there were one local and 7 bladder recurrences, in T2/3N0M0 there were 13 bladder and 5 distant recurrences and, finally, in 3 metastatic cases there were two local progressions and one in the liver.

Twenty-eight patients (28.3%) died during follow-up, 1-95 months (median 17) after the first surgical treatment and the mean survival was 18.9±24.2 months (Figure 4). Seventy-one patients were still alive 3-127 months after the operation (mean 54.7±32.4 months).

ErbB2, ErbB3 and ErbB4 expression in UUTUC

Patterns of ErbB family receptor expression in UUTUC are shown in Table 2. In 99 tumors, expression of a single receptor (score 1 and 2) was found in 64.8% of the tumors for ErbB2, in 19.5% for ErbB3 and in 20.8% for ErbB4. Combined expression of all 3 proteins was found in 7.9% of the tumors, combined expression of 2 receptors in 14.5% and 48.7% expressed at least one ErbB receptor. No ErbB expression was found in 28.9% of the tumors.

Table 2. ErbB2, ErbB3, ErbB4 expression (% cases)

Percent of cells with antigen expression	*ErbB2 %*	*ErbB3 %*	*ErbB4 %*
< 5*	35.1	80.5	79.2
5-50†	48.6	16.9	18.2
> 50#	16.2	2.6	2.6

* vs †, p<0.001; * vs #, p<0.001; † vs #, p=0.5773

ErbB2 expression

There was no apparent relationship between ErbB2 expression and tumor stage (p=0.06) or

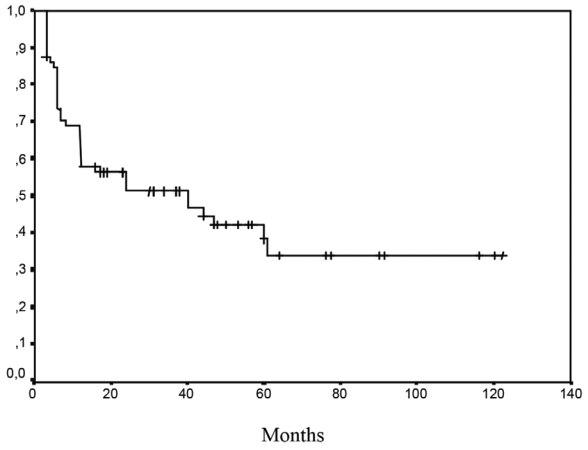

Figure 1. Kaplan-Meier plot of time to recurrence regardless of tumor location.

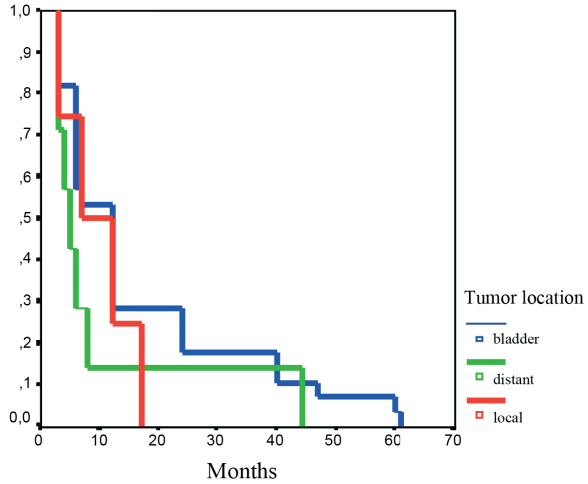

Figure 2. Kaplan-Meier plot of time to recurrence according to tumor location (p=0.235).

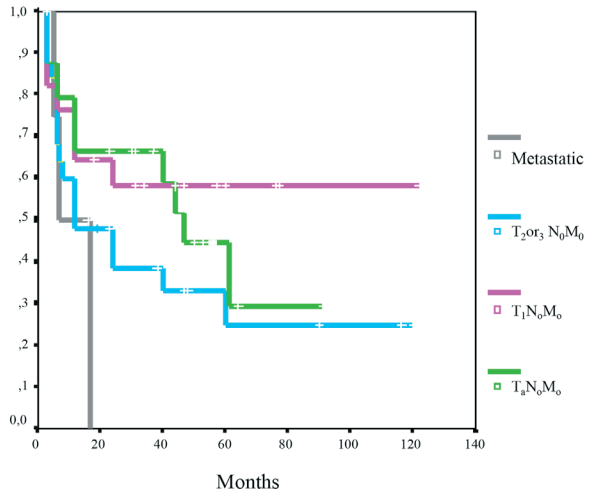

Figure 3. Kaplan-Meier plot of time to recurrence according to tumor stage (p=0.234).

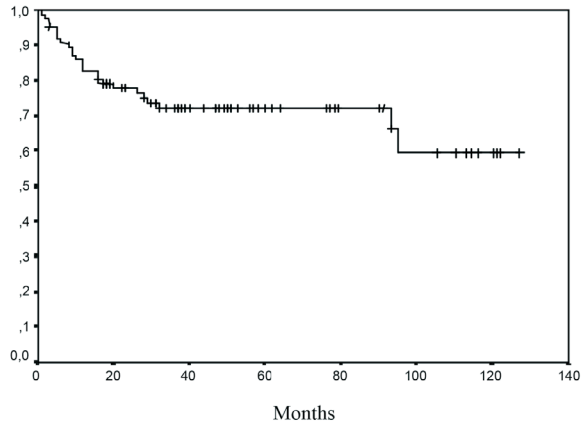

Figure 4. Kaplan-Meier plot of cancer specific survival.

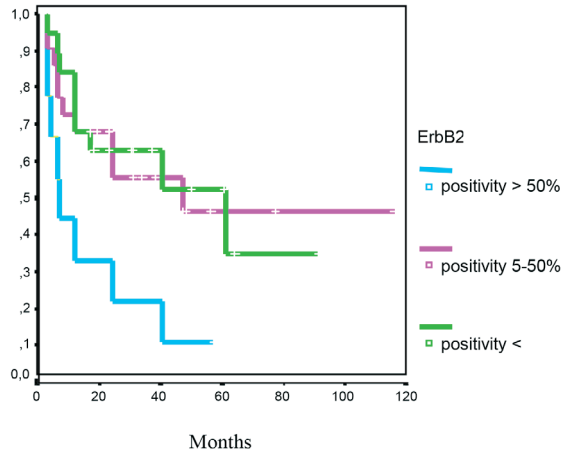

Figure 5. Kaplan-Meier plot of time to recurrence according to ErbB2 expression (p=0.027).

grade (p=0.194). However, time to recurrence was inversely associated with ErbB2 expression, in a statistically significant grade (log rank test=7.18, p=0.027), as shown in Figure 5.

ErbB2 expression did not correlate with cancer specific survival (log rank test =0.85, p=0.65; Figure 6).

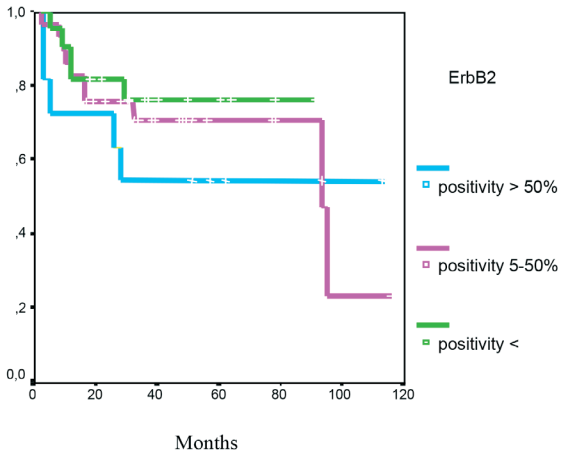

Figure 6. Kaplan-Meier plot of cancer specific survival according to ErbB2 expression (p=0.65).

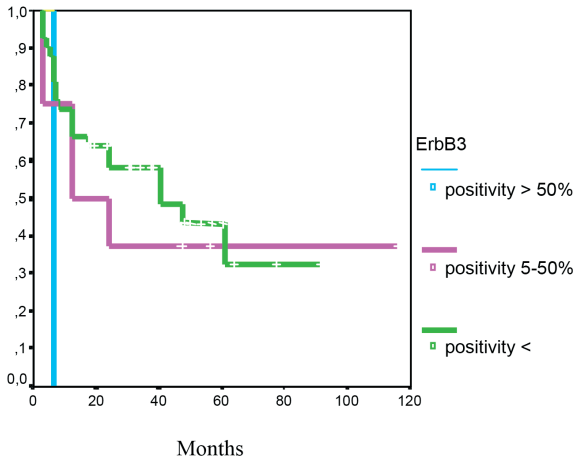

Figure 7. Kaplan-Meier plot of time to recurrence according to ErbB3 expression (p=0.23).

ErbB3 expression

ErbB3 expression did not correlate neither with stage nor with grade (p=0.83, p=0.64, respectively). ErbB3 expression did not also correlate with time to recurrence (log rank test =2.95, p=0.23), as shown in Figure 7. However, there was one case with >50% ErbB3 expression which recurred after 6 months.

ErbB3 expression did not correlate with cancer specific survival (log rank test=0.4, p=0.82), (Figure 8). However, there was one case with >50% ErbB3 expression still alive 62 months after the operation.

ErbB4 expression

ErbB4 expression did not correlate neither with stage nor with grade (p=0.97 and p=0.4, re-

spectively). Accordingly, there was no strong association between ErbB4 expression and time to recurrence (log rank test =5.05, p=0.08), as shown in Figure 9. However, there was one case with >50% ErbB4 expression which recurred after 6 months, while time to recurrence was lower, on average, in patients with <5% expression than in those with >5%.

ErbB4 expression did not correlate with cancer specific survival (log rank test=0.54, p=0.765; Figure 10). However, there was one case with >50% ErbB4 expression still alive 62 months after the operation.

Discussion

The development of the monoclonal anti-c-erbB-2 directed antibody trastuzumab for the treatment of patients with metastatic breast cancer has raised expectations regarding the possible usefulness of this antibody (or similar) even in other types of cancer [16]. ErbB overexpression has already been widely reported in various tumors as well as in urothelial carcinomas of the bladder [17-20]. In UUTUC, in contrast, the literature is very limited, probably because of their rarity but, also, because of their nature. It is believed that urothelial carcinomas share common oncogenetic mechanisms, representing a field change phenomenon, having the possibility of synchronous or metachronous appearance in any site of the urinary tract covered by transitional epithelium. Of the patients with UUTUC, 20-50% will have a bladder recurrence after nephroureterectomy, but only 0.5-2% with primary bladder cancer will develop UUTUC in the future [3]. A possible explanation could be the activation of genetic changes caused by the accumulation of carcinogens and/or growth factors gathered in the bladder urine. High caution is needed, therefore, while interpreting the studies.

In the present study, we evaluated the expression of ErbB2, ErbB3 and ErbB4 simultaneously in 99 UUTUCs and linked the results to patient outcome. We used immunohistochemistry because it is a routine screening procedure, well documented, with high sensitivity and a high predictive value in detecting loss of protein expression in urothelial carcinomas [21]. We did not find any significant correlation between ErbB2, ErbB3 and ErbB4 expression with tumor stage, grade, recurrence or cancer specific survival apart from the inverse association between ErbB2 expression and time to recurrence (p=0.027).

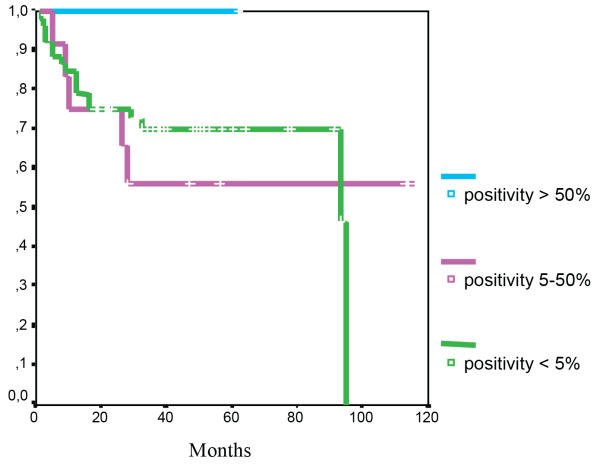

Figure 8. Kaplan-Meier plot of cancer specific survival according to ErbB3 expression (p=0.23).

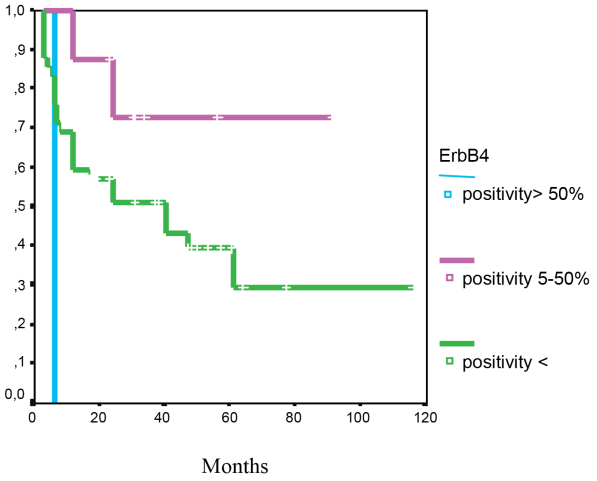

Figure 9. Kaplan-Meier plot of time to recurrence according to ErbB4 expression (p=0.08).

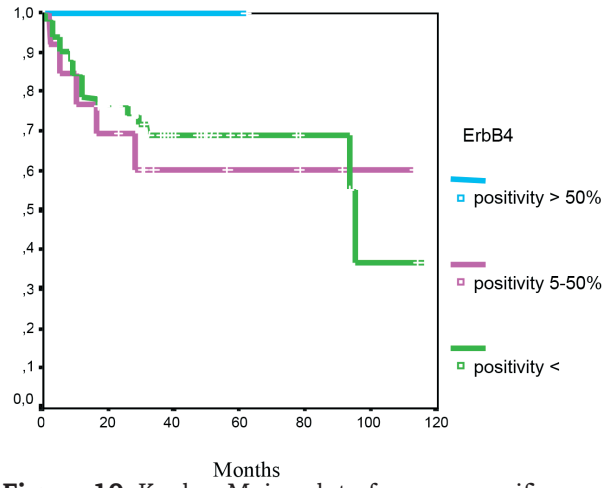

Figure 10. Kaplan-Meier plot of cancer specific survival according to ErbB4 expression (p=0.765).

Bjerkehagen et al. were the first to publish a series of 20 patients with UUTUC where they observed total absence of ErbB2 expression [9]. Imai

et al. studied 30 UUTUCs and found a significant correlation between ErbB2 overexpression (36.7%) and number of recurrences (p<0.01), but not with stage or grade [10]. Our results, in a larger number of patients, are in accordance with these findings, with the exception of overexpression (64.8% to 36.7% of Imai et al. [10]). The authors, however, considered >25% positive cells as overexpression. Another difference was the percentage of recurrence (40% vs 28.3% in our study) and the shorter time to bladder recurrence (306 days vs mean value of 16.7±3.3 months in our study). The authors suggested that the immunohistochemical detection of ErbB2 in UUTUC might be a useful method for determining the recurrence potential in the urinary bladder.

Fontana et al., in a study of 61 patients, found no prognostic value for ErbB2 [11]. Lagner et al. concluded that, although ErbB2 overexpression was infrequent in their series of 53 patients, it had an independent prognostic significance with respect to metastasis free survival (p = 0.03) [12]. Similarly, Tsai et al. published their results in a large series with 94 UUTUC, showing that ErbB2 expression (13.8%) was an independent predictor of progression-free, disease-free, and overall survival. ErbB3 expression (27.7%) on the contrary, had no such significance [13].

Alexa et al. concluded, in their 59 UUTUC cases, that ErbB2 overexpression was significantly correlated with grade (p<0.05) but not with stage [14]. There was no comment on recurrence or survival. Finally, Izquierdo et al. found that ErbB2 overexpression was present in 10% of 100 UUTUCs and this was correlated not only with tumor stage but also with grade and positive lymph nodes [15]. In contrast, they did not find ErbB2 overexpression to be an independent factor of either tumor progression or cancer-specific survival.

In summary: i) our data showed that ErbB2, ErbB3 and ErbB4 were expressed in 64.8, 19.5 and 20.8%, respectively, while published studies show 0-52.8% for ErbB2 and 27.7% for ErbB3; ii) there is no study for ErbB4 and it was of no prognostic value in our study; iii) similarly, ErbB3 had no prognostic value, as was also suggested by Tsai et al; iv) published data for ErbB2 vary and the only significant correlation we found was with time to recurrence; and v) there are many discrepancies regarding the ErbB expression and their prognostic significance which could be attributed to different selection criteria, differences in the surveillance period, different receptor evaluation (overexpression vs amplification), different techniques (immunohistochemistry, fluorescence in situ hybridization or polymerase chain reaction), positivity characterization or even small size of the patient series.

In other types of cancer, such as breast or gastric cancer, the overexpression of ErbB2 is the criterion to initiate targeted therapy with trastuzumab, alone or in combination with cisplatin [6-8,22]. In UUTUC there is no study so far correlating trastuzumab efficacy in ErbB2 overexpression. However, treatment of superficial bladder cancer with BCG reduces the expression of ErbB2 [23]. The fact that there are some UUTUCs overexpressing ErbB2 may warrant clinical trials evaluating the efficacy of treatment with the anti-c-erbB-2 directed antibody trastuzumab.

Limitations in our study include: retrospective nature, small series, technical limitations of immunohistochemical procedures, antigenic loss in older cases.

Conclusions

The research on the prognostic significance of the ErbB family has already led to the development of targeted therapies against specific types of cancer. In the urothelial tumors of the upper urinary tract, however, the results are contradictory. The present study showed no prognostic significance for ErbB2, ErbB3 and ErbB4 apart from the inverse association between ErbB2 expression and time to recurrence. This is, undoubtedly, an observation that needs to be evaluated carefully, yet it is not strong enough to initiate anti-ErbB2 therapy in everyday clinical practice.

References

1. Munoz JJ, Ellison LM. Upper tract urothelial neoplasms: incidence and survival during the last 2 decades. J Urol 2000;164:1523-1525.
2. Cauberg EC, Salomons MA, Kümmerlin IP et al. Trends in epidemiology and treatment of upper urinary tract tumours in the Netherlands 1995-2005: an analysis of PALGA, the Dutch national histopathology registry. BJU Int 2010;105:922-927.
3. Kirkali Z, Tuzel E. Transitional cell carcinoma of the ureter and renal pelvis. Crit Rev Oncol Hematol 2003;47:155-169 (Review).
4. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001;2:127-137 (Review).
5. Bianco R, Gelardi T, Damiano V, Ciardiello F, Tortora G. Rational bases for the development of EGFR inhibitors for cancer treatment. Int J Biochem Cell Biol 2007;39:1416-1431 (Review).
6. Trastuzumab FDA Approval Letter. 25-09-1998.
7. Trastuzumab FDA Approval Letter. 20-10-2010.
8. Lapatinib FDA Approval Letter. 13-03-2007.
9. Bjerkehagen B, Fosså SD, Raabe N, Holm R, Nesland JM. Transitional cell carcinoma of the renal pelvis and its expression of p53 protein, c-erbB-2 protein, neuron-specific enolase, Phe 5, chromogranin, laminin and collagen type IV. Eur Urol 1994;26:334-339.
10. Imai T, Kimura M, Takeda M, Tomita Y. Significance of epidermal growth factor receptor and c-erbB-2 protein expression in transitional cell cancer of the upper urinary tract for tumor recurrence at the urinary bladder. Br J Cancer 1995;71:69-72.
11. Fontana LO, García García F, Arcas Martínez Salas I et al. The expression of p53 and c-erb-2 in transitional cell carcinoma of the kidney pelvis and ureter and its relation to tumor progression and survival. Arch Esp Urol 2002;55:792-796.
12. Langner C, Gross C, Rehak P, Ratschek M, Rüschoff J, Zigeuner R. HER2 protein overexpression and gene amplification in upper urinary tract transitional cell carcinoma: systematic analysis applying tissue microarray technique. Urology 2005;65:176-180.
13. Tsai YS, Tzai TS, Chow NH, Wu CL. Frequency and clinicopathologic correlates of ErbB1, ErbB2, and ErbB3 immunoreactivity in urothelial tumors of upper urinary tract. Urology 2005;66:1197-202.
14. Alexa A, Baderca F, Zăhoi DE, Lighezan R, Izvernariu D, Raica M. Clinical significance of Her2/neu overexpression in urothelial carcinomas. Rom J Morphol Embryol 2010;51:277-282.
15. Izquierdo L, Truan D, Mengual L, Mallofré C, Alcaraz A. HER-2/AKT expression in upper urinary tract urothelial carcinoma: prognostic implications. Anticancer Res 2010;30:2439-2445.
16. Pegram MD, Konecny G, Slamon DJ. The molecular and cellular biology of HER2/neu gene amplification/overexpression and the clinical development of herceptin (trastuzumab) therapy for breast cancer. Cancer Treat Res 2000;103:57-75 (Review).
17. Bolenz C, Shariat SF, Karakiewicz PI et al. Human epidermal growth factor receptor 2 expression status provides independent prognostic information in patients with urothelial carcinoma of the urinary bladder. BJU Int 2010;106:1216-1222.
18. Chow NH, Chan SH, Tzai TS, Ho CL, Liu HS. Expression profiles of ErbB family receptors and prognosis in primary transitional cell carcinoma of the urinary bladder. Clin Cancer Res 2001;7:1957-1962.
19. Kassouf W, Black PC, Tuziak T et al. Distinctive expression pattern of ErbB family receptors signifies an aggressive variant of bladder cancer. J Urol 2008;179:353-358.
20. Røtterud R, Nesland JM, Berner A, Fosså SD. Expression of the epidermal growth factor receptor family in normal and malignant urothelium. BJU Int 2005;95:1344-1350.
21. Fromont G, Rouprêt M, Amira N et al. Tissue microarray analysis of the prognostic value of E-cadherin, Ki67, p53, p27, survivin and MSH2 expression in upper urinary tract transitional cell carcinoma. Eur Urol 2005;48:764-770.
22. Pegram MD, Slamon DJ. Combination therapy with trastuzumab (Herceptin) and cisplatin for chemoresistant metastatic breast cancer: evidence for receptor-enhanced chemosensitivity. Semin Oncol 1999; 26 (4 Suppl 12):89–95.
23. Morgan BE, Salup R, Morgan MB. Differential C-erbB-2 and VEGF expression following BCG immunotherapy in superficial papillary transitional cell carcinoma of the bladder. Urol Oncol 2002; 7:67–72.