

# Expression Profiles of ErbB Family Receptors and Prognosis in Primary Transitional Cell Carcinoma of the Urinary Bladder<sup>1</sup>

Nan-Haw Chow,<sup>2</sup> Shih-Huang Chan,  
Tzong-Shin Tzai, Chung-Liang Ho,  
and Hsiao-Sheng Liu

Departments of Pathology [N-H. C., C-L. H.], Urology [T-S. T.], and Microbiology [H-S. L.], College of Medicine, and Department of Statistics [S-H. C.], National Cheng Kung University, Tainan, Taiwan 70428, Republic of China

## ABSTRACT

*In vitro* experiments have demonstrated that epidermal growth factor (EGF)-related peptides activate distinct subsets of ErbB receptors and differ in their biological activities. The implications of cross-talk among ErbB family receptors in human cancer, however, remain to be clarified. This cohort study was performed to examine the expression patterns of ErbB receptors by immunohistochemistry in primary human bladder cancer ( $n = 245$ ) and compared with conventional biological indicators for their prognostic significance. Expression of individual EGF receptor (EGFR) and ErbB2, ErbB3, or ErbB4 receptors was detected in 72.2, 44.5, 56.3, and 29.8% of bladder cancer cases, respectively. Expression of two of the receptors varied from 14.7 to 42.4%, of three of the receptors between 11.0 and 22.0%, and of all four of the ErbB receptors by 8.6%. Important indicators in association with patient survival were tumor staging ( $P = 0.017$ ), ErbB2 ( $P = 0.018$ ), EGFR-ErbB2 ( $P = 0.023$ ), and ErbB2-ErbB3 ( $P = 0.042$ ). In the subset of grade-2 tumors, EGFR-ErbB2-ErbB3 and EGFR-ErbB2 predicted the development of second recurrence ( $P = 0.026$  and  $0.039$ , respectively), and ErbB2-ErbB3 tended to correlate with patient survival ( $P = 0.09$ ). The results indicate that a combination of EGFR, ErbB2, and ErbB3 expression profile may be a better prognostic indicator than any family member alone. Given that ErbB2 is the preferred coexpression partner of ErbB family members, expression of other ErbB receptors may significantly affect the prognostic implication of ErbB2 for bladder cancer patients.

Received 1/19/01; revised 4/9/01; accepted 4/17/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> Supported by Research Grant NCKUH-85-042 from National Cheng Kung University Hospital and NSC-88-2314-B006-075, NSC 89-2314-B-006-027 from the National Science Council, Taiwan, Republic of China.

<sup>2</sup> To whom requests for reprints should be addressed, at Department of Pathology, National Cheng Kung University Hospital, 138 Sheng-Li Road, Tainan 70428, Taiwan. Phone: 886-6-2353535, extension 5288; Fax: 886-6-276-6195; E-mail: chownh@mail.ncku.edu.tw.

## INTRODUCTION

Protein tyrosine kinases play a crucial role in many cell regulatory processes, such as proliferation, migration, adhesion, and potential cellular transformation. Members of the type-1 ErbB family receptors of protein tyrosine kinases include EGFR,<sup>3</sup> ErbB2 (c-erbB-2, HER-2/neu), ErbB3, and ErbB4 (1). They share structural homologies, especially at the intracellular domain, with each other and are normally coexpressed in various combinations in diverse epithelial tissues. We have demonstrated (2) that expression of ErbB family receptors is associated with urothelial differentiation *in vivo*; *e.g.*, EGFR appears on the basal layer (immature cells) only, ErbB2 is predominantly expressed on the superficial (terminally differentiated) cells, and ErbB4 is almost always present on the superficial layer of normal urothelium. Given that human urine contains high concentrations of EGF (20 ng/mg creatinine; Ref. 3) and transforming growth factor- $\alpha$  (0.6 ng/mg creatinine; Ref. 4), interactions of ligands with their cognitive receptors may play a certain role in the homeostasis of bladder mucosa. There is, however, no information regarding the neuregulin family of growth factors in human urine, although the transcripts for ErbB3 and ErbB4 have been detected in the kidneys (5, 6).

Recent mechanistic study (7) implies that signal transduction by ErbB family receptors involves an array of 10 possible homodimeric and heterodimeric combinations diversifying biological responses to ligands of the EGF and neuregulin families. The ability of EGF-family hormones to activate dimeric combinations of receptors adds enormous combinatorial complexity to the EGF hormone/receptor system. This is significant because the receptors are generally expressed in combinations and because each receptor generates unique signals. Overall, heterodimers were found to be more biologically active than homodimers. In fact, several types of heterodimer combinations have been implicated in the neoplastic processes, such as EGFR-ErbB2 (8), ErbB2-ErbB3 (9, 10), and EGFR-ErbB3 (11). Moreover, differential receptor phosphorylation may account for the differences in signaling properties observed for each dimerization partner (12). Despite this, evidence supporting the involvement of coexpression of ErbB family receptors in primary human cancers is relatively limited (2, 13–19), especially for the ErbB4 receptor (2, 15, 18, 19).

The clinical relevance of ErbB family receptors in transitional cell carcinoma of the urinary bladder remains enigmatic. Although EGFR by itself was reported (13, 20–23) to have an apparent relationship to clinical outcome, recent studies could not verify its independent importance in predicting the risk of tumor invasion (24) or patient survival (25–28). Reports about ErbB2 are also contradictory. Earlier studies demonstrated a

<sup>3</sup> The abbreviations used are: EGFR, epidermal growth factor receptor; RR, relative risk.

positive association of ErbB2 with tumor progression (21, 29, 30) or patient survival (27, 31), but other studies (13, 24, 32) do not support its prognostic value. With expanded knowledge of the receptor dimerization and cross-phosphorylation of ErbB receptors *in vitro*, the clinical implications of coexpression patterns in human cancers warrant clarification (15). A recent preliminary study (19) of oral cancer suggested that a combination of EGFR, ErbB2, and ErbB3 may provide stronger prognostic information than any single member of the ErbB receptors. To clarify the clinical implication of this hypothesis, we performed this cohort study to examine in great detail the expression patterns of ErbB family receptors in primary bladder cancer and to compare their prognostic significance with conventional biological indicators.

## MATERIALS AND METHODS

**Patients.** This cohort study included a total of 245 cases of primary transitional cell carcinoma of the urinary bladder from our hospital. There were 80 female patients and 165 male patients with mean ages at 63.3 years. Each tumor was reviewed for histological grading according to the WHO classification (1973). A total of 47 (19.2%) were classified as grade I, 118 (48.2%) as grade II, and 80 (32.7%) as grade III. Clinical staging was determined according to the tumor-node-metastasis staging protocol of the American Joint Committee on Cancer (1983) with a survey of the clinical details, image studies, and pathological data. Of them, 103 (42.0%) were stage pTa, 72 (29.4%) were pT1, 41 (16.7%) were stage pT2, 17 (6.9%) were stage pT3, and 12 (4.9%) were stage pT4. Pertinent clinical data were obtained from medical records. The characteristics of primary tumors included gross appearance (203 papillary tumors, 42 nodular tumors), multiplicity (138 single tumors, 107 multiple tumors), and tumor size (73 <1 cm, 103 between 1 and 3 cm, 69 >3 cm).

The treatment and follow-up of patients were conducted according to the standard strategy described in detail previously (20). Briefly, all of the superficial bladder cancer (pTa and pT1) received transurethral resection and postoperative intravesical chemotherapeutic agent instillation with either thiotepa (30 mg in normal saline 30 ml; 120 patients) or epirubicin (40 mg in normal saline 40 ml; 55 patients) weekly for a consecutive 8 weeks starting 1 week after transurethral resection. They were followed up every 3 months for the first 2 years, then every 6 months for another 2 years, and yearly thereafter. Each recurrence was confirmed by biopsy. Whenever a recurrent tumor was found, they were treated by repeated transurethral operation followed by another course of 8-week intravesical chemotherapeutic instillation therapy or radical/partial cystectomy if disease progression was noted. For those with muscle-invasive tumors ( $n = 70$ ), a radical operation was the standard procedure. Systemic chemotherapy with methotrexate, cisplatin, doxorubicin, and vinblastine was given in 55 patients. The survival status was determined by outpatient clinic record and/or confirmed by interview with patients' families. Clinical follow-up ranged from 24 to 95 months (median, 54 months).

**Immunohistochemical Investigation.** Serial sections from appropriate tissues of original tumor were cut and submitted for deparaffinization. Primary antibodies used in this study

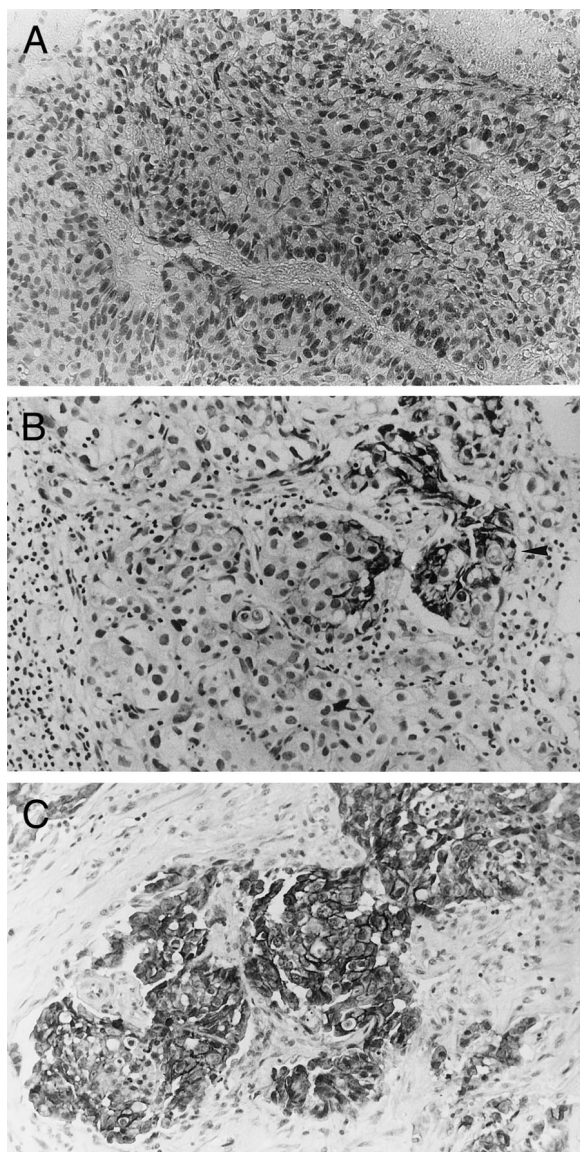
included monoclonal EGFR antibody (Triton, Alameda, CA) and anti-ErbB2 antibody (BioGenex Laboratories, San Ramon, CA), which were validated by correlation with differential PCR results (33). The optimal dilution (1:200 for EGFR and 1:400 for ErbB2) was determined by using a J82 human bladder cancer cell line as a control (34). The immunohistochemical staining for anti-ErbB3 (RTJ.2) and anti-ErbB4 antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was performed as described in detail previously (2). Briefly, sections were first washed with PBS (pH 7.2) and blocked with 3% hydrogen peroxide in ethanol for 5 min at room temperature. The sections were then covered with 3% normal horse serum for 15 min. Primary antibodies were incubated for 2 h at room temperature, respectively. The StrAviGen Super Sensitive MultiLink kit (BioGenex Laboratories) was used to detect the resulting immune complex. The procedures of blocking, linking, and labeling of binding reaction were carried out according to the manufacturer's instructions. The peroxidase activity was visualized by the 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO). Finally, the sections were counterstained with hematoxylin. Negative control was performed by incubation of nonimmune mouse IgG in substitution for the primary antibody.

When evaluating the expression of ErbB receptors (by N-H. Chow and T-S. Tzai), only membranous reaction was considered positive (35). Because the intensity of immunostaining did not vary obviously, expression of ErbB receptor family was graded according to the percentage of tumor cells stained, as suggested previously (33). Tissue sections showing immunostaining in less than 5% of tumor cells or lack of any immunoreactivity were classified as negative expression (2). Those with a staining reaction between 5 and 50% of tumor cells were defined as low levels of protein expression, whereas those with immunostaining in greater than 50% were defined as high levels of protein expression, as shown in Fig. 1 (33).

**Statistics.** The correlation of expression patterns of ErbB family receptors with clinicopathological factors of bladder cancer was examined, where suitable, by Fisher's exact test or  $\chi^2$  test. The relationship between biological indicators or patterns of receptor expression and clinical outcome was analyzed by multiple logistic regression. The RR in relation to patient prognosis was assessed by a proportional hazards model after adjustment for clinicopathological parameters. Only those variables with a  $P \leq 0.05$  were considered significant.

## RESULTS

Patterns of ErbB family receptor expression in bladder cancer are shown in Table 1. Expression of a single receptor was found in 72.2% of tumors for EGFR, 44.5% for ErbB2, 56.3% for ErbB3, and 29.8% for ErbB4. Combined expression of two of the subclass members varied from 14.7 to 42.4%, of three of the subclass members from 11.0 to 22.0%, and of all four of the receptor proteins in 8.6% (21 cases). The expression patterns were correlated with patients' clinicopathological factors and are summarized in Table 2. A positive association of receptor expression with biological indicators was as follows: EGFR with tumor size; ErbB2 with histological grading; and ErbB3 with size and number of tumors and with histological grading



**Fig. 1** Panels of immunohistochemical expression of ErbB2 in human bladder cancer cells. **A**, tumor cells with no immunostaining were classified as negative expression. **B**, the positively stained tumor cells were between 5 and 50%, representing an example of the low level of ErbB2 expression. **C**, greater than 50% of the cancer cells showing strong membranous reaction were defined as having high levels of ErbB2 expression (Original magnification,  $\times 150$ ).

( $P < 0.05$ ). There was no apparent relationship between ErbB4 expression and clinicopathological factors ( $P > 0.1$ ). A significant association between expression patterns ( $P < 0.05$ ) and at least one of the biological indicators was observed in EGFR-ErbB2 (expression of both EGFR and ErbB2), EGFR-ErbB3, ErbB2-ErbB3, and EGFR-ErbB2-ErbB3. Expression of all four of the receptor proteins did not correlate with any parameter ( $P > 0.1$ ).

To determine their prognostic significance, expression of ErbB family receptors was correlated with the development of tumor recurrence or survival rate and compared with conven-

**Table 1** Expression profiles of ErbB receptor family in human bladder cancer

Expression patterns	Negative No. (%)	Low levels No. (%)	High levels No. (%)
EGFR	68 (27.8)	110 (44.9)	67 (27.3)
ErbB2	136 (55.5)	88 (35.9)	21 (8.6)
ErbB3	107 (43.7)	110 (44.9)	28 (11.4)
ErbB4	172 (70.2)	59 (24.1)	14 (5.7)
EGFR-ErbB2 <sup>a</sup>		83 (33.9) <sup>b</sup>	
EGFR-ErbB3		104 (42.4)	
EGFR-ErbB4		61 (24.9)	
ErbB2-ErbB3		67 (27.3)	
ErbB2-ErbB4		36 (14.7)	
ErbB3-ErbB4		54 (22.0)	
EGFR-ErbB2-ErbB3		54 (22.0)	
EGFR-ErbB2-ErbB4		30 (12.2)	
EGFR-ErbB3-ErbB4		42 (17.1)	
ErbB2-ErbB3-ErbB4		27 (11.0)	
EGFR-ErbB2-ErbB3-ErbB4		21 (8.6)	

<sup>a</sup> This and the following 10 hyphenated abbreviations represent expression of the subclass members of ErbB family receptors in the same tumor.

<sup>b</sup> Both low and high levels of receptor expression were considered positive in the coexpression pattern analysis.

tional biological indicators (Table 3). Factors that predicted first tumor recurrence were multiple tumors ( $P = 0.009$ ), ErbB3 ( $P = 0.036$ ), or ErbB2 ( $P = 0.038$ ), whereas tumors showing expression of EGFR-ErbB2-ErbB3 or overexpression of ErbB2 alone had a significantly higher risk of developing second tumor recurrence ( $P = 0.028$  and  $0.030$ , respectively). Important factors associated with poor long-time survival were tumor staging, ErbB2, EGFR-ErbB2, and ErbB2-ErbB3 ( $P = 0.017$ ,  $0.018$ ,  $0.023$ , and  $0.042$ , respectively).

To examine the potential implication of coexpression patterns in grade-2 bladder cancer, conventional biological indicators and expression of ErbB family receptors were correlated to patient outcome. None of the conventional biological indicators predicted clinical outcome in a multivariate statistical model, except that multiple tumors had a 2.46-fold higher risk of developing first tumor recurrence (data not shown). In contrast, patterns of EGFR-ErbB2-ErbB3 and EGFR-ErbB2 expression were significantly associated with second tumor recurrence ( $P < 0.05$ ), with RR estimated at 2.58 and 2.35, respectively (Table 4). ErbB2-ErbB3 tended to correlate with poor patient survival, but without statistical significance ( $P = 0.09$ ).

## DISCUSSION

In this study, we found that tumor staging at diagnosis remains the most important factor in predicting clinical outcome for patients with bladder cancer. In addition, ErbB2, whether by itself or coexpressed with EGFR or ErbB3, was also an important predictor of patient survival. Important predictors of first tumor recurrence were multiple tumors and expression of ErbB2 or ErbB3, and important predictors of second tumor recurrence were ErbB2 or EGFR-ErbB2-ErbB3. The data support that expressing multiple ErbB receptors plays an important role in the tumorigenesis of human bladder, and that ErbB2 serves as a critical component of ErbB interactions *in vivo*, especially with

Table 2 Correlation of ErbB receptor family expression with clinicopathological parameters of bladder cancer

Expression patterns	Grade	Stage <sup>a</sup>	Size <sup>b</sup>	No.	Shape
EGFR	0.145	0.291	0.026 <sup>c</sup>	0.134	0.813
ErbB2	0.006 <sup>c</sup>	0.123	0.055	0.428	0.228
ErbB3	0.013 <sup>c</sup>	0.084	0.048 <sup>c</sup>	0.043 <sup>c</sup>	0.105
ErbB4	0.452	0.854	0.769	0.324	0.247
EGFR-ErbB2	0.036 <sup>c</sup>	0.029 <sup>c</sup>	0.001 <sup>c</sup>	0.087	0.101
EGFR-ErbB3	0.015 <sup>c</sup>	0.076	0.001 <sup>c</sup>	0.438	0.752
EGFR-ErbB4	0.385	0.789	0.426	0.452	0.987
ErbB2-ErbB3	0.158	0.133	0.001 <sup>c</sup>	0.591	0.246
ErbB2-ErbB4	0.890	0.700	0.320	0.866	0.299
ErbB3-ErbB4	0.375	0.694	0.379	0.164	0.443
EGFR-ErbB2-ErbB3	0.227	0.023 <sup>c</sup>	0.001 <sup>c</sup>	0.418	0.194
EGFR-ErbB2-ErbB4	0.903	0.708	0.101	0.939	0.285
EGFR-ErbB3-ErbB4	0.347	0.840	0.177	0.465	0.707
ErbB2-ErbB3-ErbB4	0.714	0.938	0.538	0.535	0.860
EGFR-ErbB2-ErbB3-ErbB4	0.691	0.929	0.231	0.695	0.841

<sup>a</sup> Analysis was performed on stage pTa, stages pT1–3, and stage pT4, respectively.

<sup>b</sup> Analyses were compared for tumors  $\leq 1$  cm,  $> 1$  cm, but  $\leq 3$  cm, and  $> 3$  cm.

<sup>c</sup>  $P < 0.05$ .

Table 3 Significance of biological indicators and expression patterns of ErbB receptor family in relation to clinical outcome (multiple logistic regression)<sup>a</sup>

Factors analyzed	First recurrence	Second recurrence	Survival
Conventional biological indicators			
Stage <sup>b</sup>	0.123	0.121	0.017 <sup>b</sup>
Size <sup>c</sup>	0.285	0.543	0.971
Number	0.009 <sup>b</sup>	0.237	0.067
Shape	0.560	0.912	0.811
ErbB Family Receptors <sup>c</sup>			
EGFR	0.150		
ErbB2	0.038 <sup>b</sup>	0.030 <sup>b</sup>	0.018 <sup>b</sup>
ErbB3	0.036 <sup>b</sup>		
EGFR-ErbB2	0.069		0.023 <sup>b</sup>
ErbB2-ErbB3	0.311	0.053	0.042 <sup>b</sup>
ErbB2-ErbB4	0.132	0.057	
EGFR-ErbB2-ErbB3	0.256	0.028 <sup>b</sup>	0.084
EGFR-ErbB2-ErbB4			0.069

<sup>a</sup> The  $P$ s of conventional biological indicators in relation to patient survival were derived from coanalysis with EGFR and the value for tumor recurrence coanalysis with ErbB2.

<sup>b</sup>  $P < 0.05$ .

<sup>c</sup> Each coexpression variable was chosen because of their significance in the univariate model.

EGFR and/or ErbB3. Although the results are not in full agreement with an earlier pilot study (2), the conclusions basically concur with the current hypothesis that collaboration of ErbB receptors may enhance the deregulation of cellular proliferation associated with tumor progression (36).

It is, however, intriguing to note that ErbB2 is an orphan receptor and does not bind any EGF family hormone when expressed by itself. The predictive value of EGFR-ErbB2 and ErbB2-ErbB3 suggests that expression of ErbB2 may increase the response of cancer cells to urinary growth factors. This interpretation essentially agrees with experiments *in vitro* showing a stronger biological effect for ErbB2-containing heterodimers than the respective homodimers (37–40). ErbB2 was shown to sensitize tumor cells to the mitogenic effects of het-

Table 4 Significance of ErbB receptor expression patterns in grade 2 bladder cancer (by proportional hazards model)<sup>a</sup>

Factors analyzed	Second recurrence		Survival	
	RR	$P$	RR	$P$
ErbB2	ND <sup>b</sup>	0.069	ND	0.138
EGFR-ErbB2	2.345	0.039 <sup>c</sup>	ND	0.127
ErbB2-ErbB3	ND	0.082	ND	0.090
ErbB2-ErbB4	ND	0.065	ND	0.174
EGFR-ErbB2-ErbB3	2.578	0.026 <sup>c</sup>	ND	0.140
EGFR-ErbB2-ErbB4	ND	0.057	ND	0.164

<sup>a</sup> Each coexpression variable was chosen because of its significance in the univariate model.

<sup>b</sup> ND, not done.

<sup>c</sup>  $P < 0.05$ .

erologous growth factors by retarding degradation of liganded EGFR heterodimers (39). Expression of EGFR and ErbB2 also increases cell migration, an important step in metastasis formation (41).

Moreover, although unable to form tumors when expressed alone, ErbB2 became tumorigenic in animals when expressed with EGFR or ErbB3 but not with ErbB4 (8). Of all of the expression complexes analyzed, cells expressing EGFR and ErbB2 were found to be the most aggressive (8). On the other hand, coexpression of ErbB2 and ErbB3 may contribute to the progression of breast cancer through EGF and/or betacellulin stimulation (10). The results appear to suggest that expression of ErbB2 alone is insufficient to determine the cellular response to ligand stimulation (42). Taken together with the prognostic importance of ErbB expression profiles, other ErbB receptors should be taken into account for future evaluations of the consequence of ErbB2 expression in human cancer, as has been demonstrated *in vitro* (12, 41).

It is well known that grade-2 bladder cancer is heterogeneous in biological potential, with 30% chance of stage progression. The mean interval to the development of muscle-invasive tumors has been estimated at 49 months (43). Thus, identification of biological marker(s) that can further distinguish the risk

of patient prognosis is very important in clinical practice. The significance of ErbB expression profiles in predicting the tumor recurrence, and possibly the clinical outcome, supports the paradigm of receptor transmodulation in the bladder carcinogenesis *in vivo*. But the biological responses and molecular signaling properties emanating from coexpression of ErbB2 with EGFR and/or ErbB3 in bladder cancer remain to be elucidated.

In fact, coexpression of ErbB receptors has been observed in papillary thyroid carcinoma (15), bladder cancer (2), uterine cervical cancer (18), and oral squamous cell carcinoma (19). The findings seem to support a general importance of ErbB receptor interactions in the progression of epithelial carcinogenesis. A recent clinical study (44) showed that natural immunity to all four of the ErbB receptors, either single receptor protein or multiple (two or three) family members, was present in approximately half the sera from patients with different types of epithelial cancer. In addition, humoral antibody against ErbB2 was among the most frequently detected ErbB-specific immune responses, supporting the potential of receptor protein as an excellent target for developing anticancer therapy. Confirmation of this hypothesis will stimulate additional experiments to clarify the benefits of combined therapies against ErbB receptors and/or signal transduction mediators (19).

In summary, the results of our study indicate that evaluation of the expression profiles of EGFR, ErbB2, and ErbB3 is of great help in selecting bladder cancer patients for more aggressive therapy protocols compared with that of any individual receptor member. Given that ErbB2 is the preferred coexpression partner of all of the other ErbB receptors, the contribution of other ErbB receptors should be taken into account for future evaluations of ErbB2 as a target for tumor therapy.

## REFERENCES

- Pusztai, L., Lewis, C. E., Lorenzen, J., and McGee, J. Growth factors: regulation of normal and neoplastic growth. *J. Pathol.*, *169*: 191–201, 1993.
- Chow, N. H., Liu, H. S., Yang, H. B., Chan, S. H., and Su, I. J. Expression patterns of ErbB receptor family in normal urothelium and transitional cell carcinoma. An immunohistochemical study. *Virchows Arch.*, *430*: 461–466, 1997.
- Mattila, A. L., Perheentupa, J., Pesonen, K., and Viinikka, L. Epidermal growth factor in human urine from birth to puberty. *J. Clin. Endocrinol. Metab.*, *61*: 997–1000, 1985.
- Stromberg, K., Duffy, M., Fritsch, C., Hudgins, W. R., Sharp, E. S., Murphy, L. D., Lippman, M. E., and Bates, S. E. Comparison of urinary transforming growth factor- $\alpha$  in women with disseminated breast cancer and healthy control women. *Cancer Detect. Prev.*, *15*: 277–283, 1991.
- Kraus, M. H., Issing, W., Miki, T., Popescu, N. C., and Aaronson, S. A. Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors. *Proc. Natl. Acad. Sci. USA*, *86*: 9193–9197, 1989.
- Plowman, G. D., Culouscou, J. M., Whitne, G. S., Green, J. M., Carlton, G. W., Foy, L., Neubauer, M. G., and Shoyab, M. Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. *Proc. Natl. Acad. Sci. USA*, *90*: 1746–1750, 1993.
- Burden, S., and Yarden, Y. Neuregulins and their receptors: a versatile signaling module in organogenesis and oncogenesis. *Neuron*, *18*: 847–855, 1997.
- Cohen, B. D., Kiener, P. A., Green, J. M., Foy, L., Fell, H. P., and Zhang, K. The relationship between human epidermal growth-like factor receptor expression and cellular transformation in NIH3T3 cells. *J. Biol. Chem.*, *271*: 30897–30903, 1996.
- Alimandi, M., Romano, A., Curia, M. C., Muraro, R., Fedi, P., Aaronson, S. A., Di Fiore, P. P., and Kraus, M. H. Cooperative signaling of ErbB3 and ErbB2 in neoplastic transformation and human mammary carcinomas. *Oncogene*, *10*: 1813–1821, 1995.
- Alimandi, M., Wang, L. M., Bottaro, D., Lee, C. C., Kuo, A., Frankel, M., Fedi, P., Tang, C., Lippman, M., and Pierce, J. H. Epidermal growth factor and betacellulin mediate signal transduction through co-expressed ErbB2 and ErbB3 receptors. *EMBO J.*, *16*: 5608–5617, 1997.
- Kim, H. H., Sierke, S. L., and Koland, J. G. Epidermal growth factor-dependent association of phosphatidylinositol 3-kinase with the erbB3 gene product. *J. Biol. Chem.*, *269*: 24747–24755, 1994.
- Olayioye, M. A., Graus-Porta, D., Beerli, R. R., Rohrer, J., Gay, B., and Hynes, N. E. ErbB-1 and ErbB-2 acquire distinct signaling properties dependent upon their dimerization partner. *Mol. Cell. Biol.*, *18*: 5042–5051, 1998.
- Rajkumar, T., Stamp, G. W., Pandha, H. S., Waxman, J., and Gullick, W. J. Expression of the type 1 tyrosine kinase growth factor receptors EGF receptor, c-erbB2 and c-erbB3 in bladder cancer. *J. Pathol.*, *179*: 381–385, 1996.
- Shintani, S., Funayama, T., Yoshihama, Y., Alcalde, R. E., Ootsuki, K., Terakado, N., and Matsumura, T. Expression of c-erbB family gene products in adenoid cystic carcinoma of salivary glands: an immunohistochemical study. *Anticancer Res.*, *15*: 2623–2626, 1995.
- Haugen, D. R., Akslen, L. A., Varhaug, J. E., and Lillehaug, J. R. Expression of c-erbB-3 and c-erbB-4 proteins in papillary thyroid carcinomas. *Cancer Res.*, *56*: 1184–1188, 1996.
- Naidu, R., Yadav, M., Nair, S., and Kutty, M. K. Expression of c-erbB3 protein in primary breast carcinomas. *Br. J. Cancer*, *78*: 1385–1390, 1998.
- Maurer, C. A., Friess, H., Kretschmann, B., Zimmermann, A., Stauffer, A., Baer, H. U., Korc, M., and Buchler, M. W. Increased expression of erbB3 in colorectal cancer is associated with concomitant increase in the level of erbB2. *Hum. Pathol.*, *29*: 771–777, 1998.
- Chang, J. L., Tsao, Y. P., Liu, D. W., Han, C. P., Lee, W. H., and Chen, S. L. The expression of type I growth factor receptors in the squamous neoplastic changes of uterine cervix. *Gynecol. Oncol.*, *73*: 62–71, 1999.
- Xia, W., Lau, Y. K., Zhang, H. Z., Xiao, F. Y., Johnston, D. A., Liu, A. R., Li, L., Katz, R. L., and Hung, M. C. Combination of EGFR, HER-2/neu, and HER-3 is a stronger predictor for the outcome of oral squamous cell carcinoma than any individual family members. *Clin. Cancer Res.*, *5*: 4164–4174, 1999.
- Neal, D. E., Sharples, L., Smith, K., Fennelly, J., Hall, R. R., and Harris, A. L. The epidermal growth factor receptor and the prognosis of bladder cancer. *Cancer (Phila.)*, *65*: 1619–1625, 1990.
- Wright, C., Mellon, K., Johnston, P., Lane, D. P., Harris, A. L., Horne, C. H., and Neal, D. E. Expression of mutant p53, c-erbB-2 and the epidermal growth factor receptor in transitional cell carcinoma of the human urinary bladder. *Br. J. Cancer*, *63*: 967–970, 1991.
- Lipponen, P., and Eskelinen, M. Expression of epidermal growth factor receptor in bladder cancer as related to established prognostic factors, oncoprotein (c-erbB-2, p53) expression and long-term prognosis. *Br. J. Cancer*, *69*: 1120–1125, 1994.
- Mellon, K., Wright, C., Kelly, P., Horne, C. H., and Neal, D. E. Long-term outcome related to epidermal growth factor receptor status in bladder cancer. *J. Urol.*, *153*: 919–925, 1995.
- Vollmer, R. T., Humphrey, P. A., Swanson, P. E., Wick, M. R., and Hudson, M. L. Invasion of the bladder by transitional cell carcinoma: its relation to histologic grade and expression of p53, MIB-1, c-erb B-2, epidermal growth factor receptor, and bcl-2. *Cancer (Phila.)*, *82*: 715–723, 1998.
- Ravery, V., Colombel, M., Popov, Z., Bastuji, S., Patard, J. J., Bellot, J., Abbou, C. C., Fradet, Y., and Chopin, D. K. Prognostic value

- of epidermal growth factor-receptor, T138 and T43 expression in bladder cancer. *Br. J. Cancer*, *71*: 196–200, 1995.
26. Sriplakich, S., Jahnsen, S., and Karlsson, M. G. Epidermal growth factor receptor expression: predictive value for the outcome after cystectomy for bladder cancer? *B. J. U. Int.*, *83*: 498–503, 1999.
27. Liukkonen, T., Rajala, P., Raitanen, M., Rintala, E., Kaasinen, E., and Lipponen, P. Prognostic value of MIB-1 score, p53, EGFR, mitotic index and papillary status in primary superficial (Stage pTa/T1) bladder cancer: a prospective comparative study. The Finnbladder Group. *Eur. Urol.*, *36*: 393–400, 1999.
28. Thøgersen, V. B., Jørgensen, P. E., Sørensen, B. S., Bross, P., Orntoft, T., Wolf, H., and Nexø, E. Expression of transforming growth factor- $\alpha$  and epidermal growth factor receptor in human bladder cancer. *Scand. J. Clin. Lab. Investig.*, *59*: 267–277, 1999.
29. Sato, K., Moriyama, M., Mori, S., Saito, M., Watanuki, T., Terada, K., Okuhara, E., Akiyama, T., Toyoshima, K., Yamamoto, T., and Kato, T. An immunohistologic evaluation of *C-erbB-2* gene product in patients with urinary bladder carcinoma. *Cancer (Phila.)*, *70*: 2493–2498, 1992.
30. Lipponen, P. Expression of c-erbB-2 oncoprotein in transitional cell bladder cancer. *Eur. J. Cancer*, *29*: 749–753, 1993.
31. Miyamoto, H., Kubota, Y., Noguchi, S., Takase, K., Matsuzaki, J., Moriyama, M., Takebayashi, S., Kitamura, H., and Hosaka, M. *C-ERBB-2* gene amplification as a prognostic marker in human bladder cancer. *Urology*, *55*: 679–683, 2000.
32. Ravery, V., Grignon, D., Angulo, J., Pontes, E., Montie, J., Crissman, J., and 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.*, *25*: 9–17, 1997.
33. Thor, A. D., Berry, D. A., Budman, D. R., Muss, H. B., Kute, T., Henderson, I. C., Barcos, M., Cirrincione, C., Edgerton, S., Allred, C., Norton, L., and Liu, E. T. erbB-2, p53, and efficacy of adjuvant therapy in lymph node-positive breast cancer. *J. Natl. Cancer Inst. (Bethesda)*, *90*: 1346–1360, 1998.
34. Sarosdy, M. F., Hutzler, D. H., Yee, D., and von Hoff, D. D. *In vitro* sensitivity testing of human bladder cancers and cell lines to TP-40, a hybrid protein with selective targeting and cytotoxicity. *J. Urol.*, *150*: 1950–1955, 1993.
35. van Diest, P. J., van Dam, P., Henzen-Logmans, S. C., Berns, E., van der Burg, M. E., Green, J., and Vergote, I. A scoring system for immunohistochemical staining: consensus report of the task force for basic research of the EORTC-GCCG. European Organization for Research and Treatment of Cancer-Gynaecological Cancer Cooperative Group. *J. Clin. Pathol. (Lond.)*, *50*: 801–804, 1997.
36. Olayioye, M. A., Neve, R. M., Lane, H. A., and Hynes, N. E. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J.*, *19*: 3159–3167, 2000.
37. Lewis, G. D., Lofgren, J. A., McMurtrey, A. E., Nuijens, A., Fendly, B. M., Bauer, K. D., and Sliwkowski, M. X. Growth regulation of human breast and ovarian tumor cells by heregulin: evidence for the requirement of ErbB2 as a critical component in mediating heregulin responsiveness. *Cancer Res.*, *56*: 1457–1465, 1996.
38. Tzahar, E., Waterman, H., Chen, X., Levkowitz, G., Karunakaran, D., Lavi, S., Ratzkin, B. J., and Yarden, Y. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. *Mol. Cell. Biol.*, *16*: 5276–5287, 1996.
39. Kokai, Y., Myers, J. N., Wada, T., Brown, V. I., LeVea, C. M., Davis, J. G., Dobashi, K., and Greene, M. I. Synergistic interaction of p185c-neu and the EGF receptor leads to transformation of rodent fibroblasts. *Cell*, *58*: 287–292, 1989.
40. Karunakaran, D., Tzahar, E., Beerli, R. R., Chen, X., Graus-Porta, D., Ratzkin, B. J., Seger, R., Hynes, N. E., and Yarden, Y. ErbB-2 is a common auxiliary subunit of NDF and EGF receptors: implications for breast cancer. *EMBO J.*, *15*: 254–264, 1996.
41. Kallioniemi, O. P., Holli, K., Visakorpi, T., Koivula, T., Helin, H. H., and Isola, J. J. Association of c-erbB-2 protein overexpression with high rate of cell proliferation, increased risk of visceral metastasis and poor long-term survival in breast cancer. *Int. J. Cancer*, *49*: 650–655, 1991.
42. Lane, H. A., Beuvink, I., Motoyama, A. B., Daly, J. M., Neve, R. M., and Hynes, N. E. ErbB2 potentiates breast tumor proliferation through modulation of p27(Kip1)-Cdk2 complex formation: receptor overexpression does not determine growth dependency. *Mol. Cell. Biol.*, *20*: 3210–3223, 2000.
43. Chen, S. S., Chen, K. K., Lin, A. T., Chang, Y. H., Wu, H. H., Hsu, T. H., and Chang, L. S. The significance of tumour grade in predicting disease progression in stage Ta transitional cell carcinoma of the urinary bladder. *Br. J. Urol.*, *78*: 209–212, 1996.
44. Bei, R., Masuelli, L., Moriconi, E., Visco, V., Moretti, A., Kraus, M. H., and Muraro, R. Immune responses to all ErbB family receptors detectable in serum of cancer patients. *Oncogene*, *18*: 1267–1275, 1999.