Virchows Arch (1997) 430:461–466

ORIGINAL ARTICLE

Nan-Haw Chow · Hsiao-Sheng Liu · Hsiao-Bai Yang
Shih-Huang Chan · Ih-Jen Su

Expression patterns of erbB receptor family in normal urothelium and transitional cell carcinoma

An immunohistochemical study

Received: 10 September 1996 / Accepted: 7 January 1997

Abstract The class I tyrosine kinase growth-factor receptors include epidermal growth factor receptor (EGFR), ErbB2 (c-erbB-2, HER-2/neu), ErbB3 and ErbB4. To elucidate their role in the regulation of homeostasis and carcinogenesis, we examined the expression of the receptors in normal urothelium and in urothelial carcinoma by immunohistochemistry. EGFR was expressed in the basal cells of normal urothelium, while ErbB2, ErbB3 and ErbB4 were present mainly in the superficial layer. A distinct reciprocal distribution was observed between the EGFR and the remaining members of the subclass (P = 0.0001). Both BCL-2 protein and Ki-67 antigen (MIB-1) showed a strong positive association with EGFR (P = 0.002) and an inverse correlation with ErbB2, ErbB3 or ErbB4 (P = 0.0004, 0.0000, and 0.001, respectively). With regard to carcinoma, there was no important relationship between receptor overexpression and tumour grading ($P > 0.1$), while only EGFR overexpression was correlated with muscular invasion (P = 0.02). Coexpression of EGFR-ErbB3 and ErbB3-ErbB4 was more often detected in high-grade tumours and correlated with the extent of tumour invasion. Our data indicate that class I receptors are differentially expressed in normal urothelium in vivo, but an orchestrated expression pattern does not exist during tumorigenesis.

Key words erbB Receptor family · Urothelium · Differentiation · Carcinogenesis

N.-H. Chow (✉) · H.-B. Yang · I.-J. Su
Department of Pathology,
National Cheng Kung University Hospital, 138,
Sheng-Li Road, Tainan, Taiwan 70428, Republic of China
Fax: (886) 6-2766195, E-mail: chownh@mail.ncku.edu.tw

H.-S. Liu
Department of Microbiology, National Cheng Kung University,
Tainan, Taiwan 704, Republic of China

S.-H. Chan
Department of Statistics, National Cheng Kung University,
Tainan, Taiwan 704, Republic of China

Introduction

Urothelium consists of a three-layered transitional epithelium lining the urinary tract from the renal pelvis down to the urethra. Although the cellular turnover in resting state is low, the regenerative potential of urothelium after injury is remarkable [22]. Previous studies suggested that multiple growth factors and their binding receptor proteins are implicated in the regenerative processes [2, 5, 22]. These include insulin-like growth factor, platelet-derived growth factor, transforming growth factor beta, and fibroblast growth factor-1. Epidermal growth factor (EGF) is probably the most important, being present at 20 ng/mg creatinine in human urine [5]. There is no information concerning the heregulin family of growth factors in the urine, although the transcripts for ErbB3 and ErbB4 can be detected in human kidney [11, 18].

EGF receptor (EGFR) is the first member of class I tyrosine kinase receptors. The remaining three subclass members are ErbB2 (c-erbB-2, HER-2/neu), ErbB3, and ErbB4 [21]. They share structural homologies, especially at the intracellular domain, and are normally coexpressed in various combinations in diverse tissues excluding the haematopoietic system. EGFR is only expressed in the basal cells of normal urothelium [14, 28]. ErbB3 is found mainly on the superficial cells [20]. However, there are contradictory reports with regard to ErbB2 [7, 12, 19, 28], and information on ErbB4 is lacking. Given that bladder mucosa is subject to necro-inflammatory reactions, an understanding of the topographic distribution of the receptor proteins in normal urothelium may shed some light on their role in the homeostasis of cell turnover. Expression of EGFR or subclass of receptors may determine the rate of cell proliferation [5, 22].

The aim of this study was designed to analyse the expression patterns of class I receptor family in normal urothelium in vivo, and their correlation with cell growth rate or *bcl-2* gene product (BCL-2) expression. The potential implication of the receptor family in the urothelial carcinogenesis was then evaluated in transitional cell carcinoma (TCC).

Materials and methods

Non-neoplastic urothelium was obtained from 58 patients who underwent surgery with the diagnosis of chronic pyelonephritis (n = 31), chronic ureteritis (n = 4), kidney laceration (n = 7), uretero-pelvic junction stenosis (n = 12), or duplication of ureter (n = 4). All samples were formalin-fixed and paraffin-embedded. There was no history of previous or simultaneous urothelial carcinoma for the control cases. A total of 56 cases of TCC was examined for expression patterns of class I receptors. Tumour grade and clinical staging classification were determined according to the WHO classification (1973) and the American Joint Committee on Cancer (1983), respectively.

Serial sections from appropriate tissues were cut and submitted for deparaffinization. Monoclonal anti-ErbB2 and EGFR antibodies (Triton Diagnostics, Alameda, Calif.) were selected on the basis of their advantage in detecting the gene product in routinely processed tissue [23]. The immunostaining dilution and cross-reactivity have been described previously [12]. Monoclonal anti-ErbB3 (RTJ.2) antibody (Santa Cruz Biotechnology, Santa Cruz, Calif.) was raised against cytoplasmic domain of the human ErbB3. ErbB4 was studied by using rabbit polyclonal antibody (Santa Cruz Biotechnology) raised against a peptide corresponding to amino acids 1291–1308 mapping at the carboxyl terminus of human ErbB4. Optimal dilution for ErbB3 (1:50) and ErbB4 (1:20) was determined using colonic mucosa [20] and human kidney [18] as controls. Monoclonal anti-BCL-2 oncoprotein (Dako, Carpenteria, Calif.) and MIB-1 (Dianova, Hamburg, Germany) antibodies were used for differentiation and cell growth rate analyses. The appropriate dilution for MIB-1 was determined as previously described with slight modification [8], and the BCL-2 was established using human tonsil as control.

Sections were first washed for 5 min with phosphate-buffered saline (pH 7.2) and blocked with 0.1 mol/l HCl for 20 min at room temperature. Then they were covered with 3% normal horse serum for 15 min. Primary antibodies to EGFR (1:2), ErbB2 (1:10), BCL-2 (1:40), ErbB3 and ErbB4 were incubated for 2 h at room temperature. For MIB-1 staining, sections were first treated with the Antigen Retrieval Citra system (BioGenex, San Ramon, Calif.) in a microwave oven for 5 min at 750 W. Then sections were washed three times in a Tris buffer for 3–5 min. The StrAviGen Super Sensitive MultiLink kit (BioGenex) was used to detect the resulting immune complex. The procedures of blocking, linkage, and labelling of binding reaction were carried out as per the instructions. The peroxidase activity was visualized by the 3,3'diamino-benzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, Mo.), except that EGFR and MIB-1 were demonstrated by aminoethyl carbazole substrate kit (Zymed Laboratory, San Francisco, Calif.). Finally, sections was counterstained with haematoxylin.

In evaluation of the expression of receptors, only a membranous reaction was regarded as positive. The MIB-1 index, expressed as the percentage of positive nuclei among a total of 100 counted cells [8], was scored independently by two pathologists. To correlate receptor expression more specifically with MIB-1 index or BCL-2, the assessment was performed for different layers of urothelium. For TCC, those exhibiting full thickness of immunostaining for class I receptors was defined as positive result [12]. A focal reactivity of tumor cells was classified as "+" and diffuse immunostaining, as "++".

Fisher's exact test was used to analyse the correlation of class I receptors with BCL-2 expression, tumour grading, stage, and the interrelationship between subclass members. The association of cell proliferation with the expression of receptor family was examined by Student's t-test. The correlation of receptor expression or MIB-1 index, after logarithmic transformation of (1-MIB-1) for analysis in relation to the compartment of the urothelium, was compared by analysis of variance (ANOVA). Only those variables with a P-value of 0.05 or lower were considered significant.

Results

The distribution of class I receptor family in benign urothelium is shown in Table 1. An orchestrated expression pattern of the class I receptor family was maintained in normal urothelium. The staining for EGFR was faint and restricted to the basal cells of urothelium (Fig. 1) in 42 cases (P = 0.0001). ErbB2 protein product was detected in 56 out of 58 (96.6%) cases of non-neoplastic urothelium, with 41 cases in superficial cells only, and an additional 15 in intermediate cells (P = 0.0001). The immunostaining was rather strong on the basolateral side of cell membrane (Fig. 2). Expression of ErbB3 was mostly localized on the superficial cells of urothelium (P = 0.0001), while in some cases there was full-thickness staining, either focal or patchy in distribution (Fig. 3). ErbB4 was preferentially expressed on the membrane of superficial cells (P = 0.0001). There was more cytoplasmic reaction than in other subclass members (Fig. 4). As shown in Table 1, a distinct reciprocal distri-

Table 1 Distribution of erbB receptor family and BCL-2 expression in relation to differentiation of normal urothelium

Factors	Layering of urothelium (n=58)			P-value
	Basal	Intermediate	Superficial	
EGFR	42[a]	0	0	0.0001
ErbB2	0	15	56	0.0001
ErbB3	7	7	47	0.0001
ErbB4	0	0	39	0.0001
BCL-2	32	14	3	0.0001

[a] The numbers indicate cases positive for immunostaining

Fig. 1 Weak membranous staining of EGFR is present in the basal layer of human urothelium only. Biotin–streptavidin stain/aminoethyl carbazole chromogen/Mayer's haematoxylin, original magnification ×300

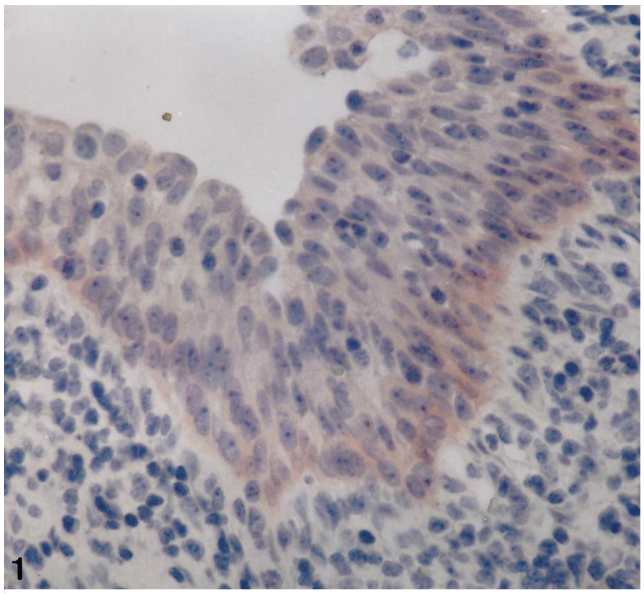
Fig. 2 ErbB2 immunostaining is revealed at the cell membrane, especially the basolateral portion, of superficial cells of human urothelium. Biotin–streptavidin stain/diamino-benzidine chromogen/Mayer's haematoxylin, original magnification ×300

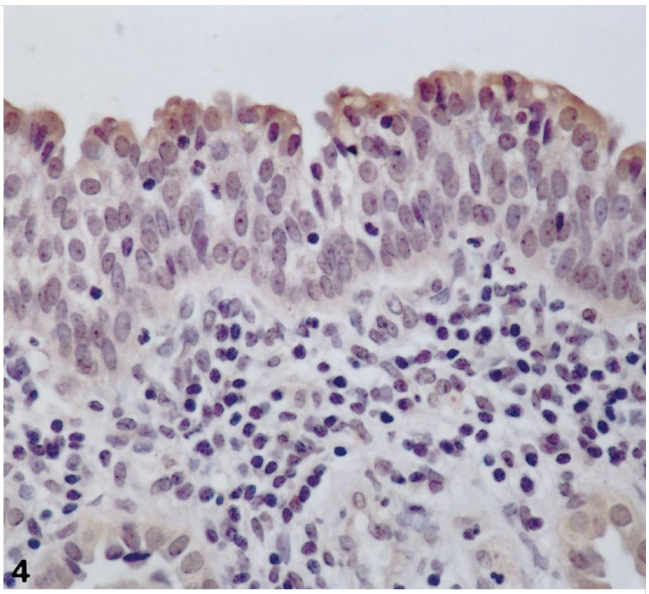
Fig. 3 The ErbB3 is distributed throughout the whole layer of urothelium at the left side, whereas the right half shows predominant staining on the superficial layer. Biotin–streptavidin stain/diamino-benzidine chromogen/Mayer's haematoxylin, original magnification ×300

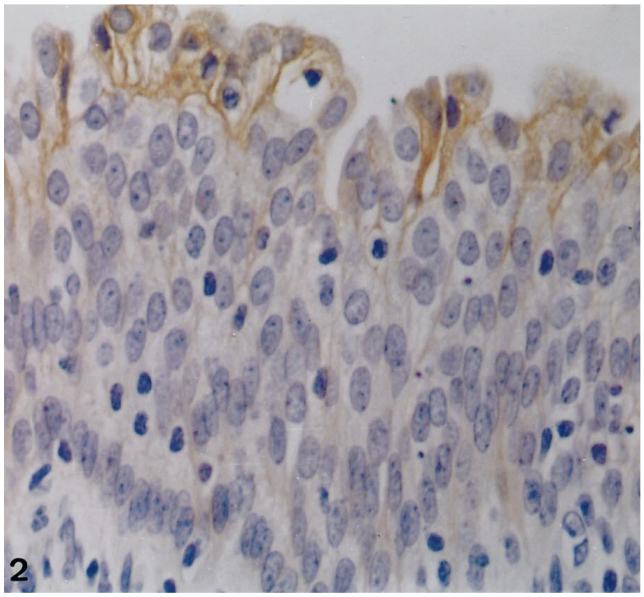
Fig. 4 The ErbB4 is weakly stained on the superficial layer of urothelium with some cytoplasmic reaction. Biotin–streptavidin stain/diamino-benzidine chromogen/Mayer's haematoxylin, original magnification ×300

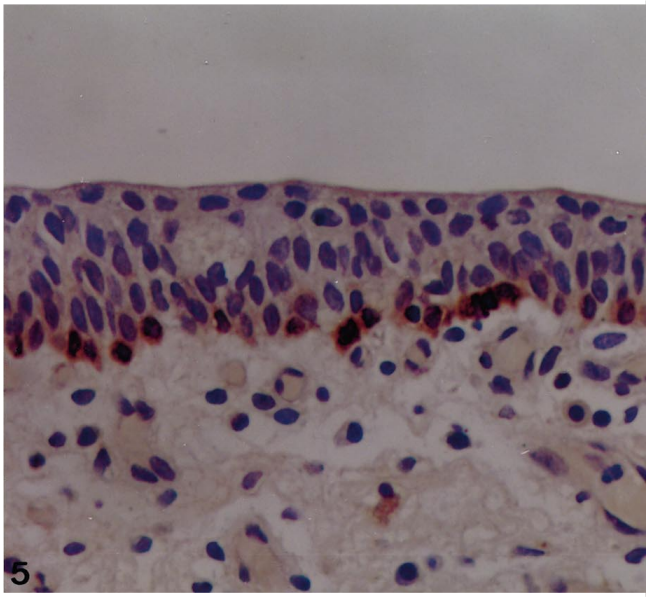
Fig. 5 The BCL-2 is restricted to the basal layer of urothelium with strong cytoplasmic reaction. Biotin–streptavidin stain/diamino-benzidine chromogen/Mayer's haematoxylin, original magnification ×300)

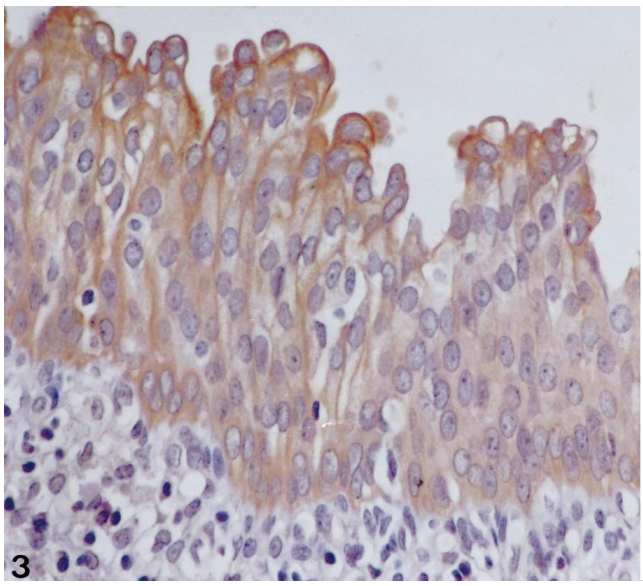
Fig. 6 The MIB-1 is predominatly stained on the basal and some intermediate cells of urothelium. Biotin–streptavidin stain/aminoethyl carbazole chromogen/Mayer's haematoxylin, original magnification ×300


1


4


2


5


3

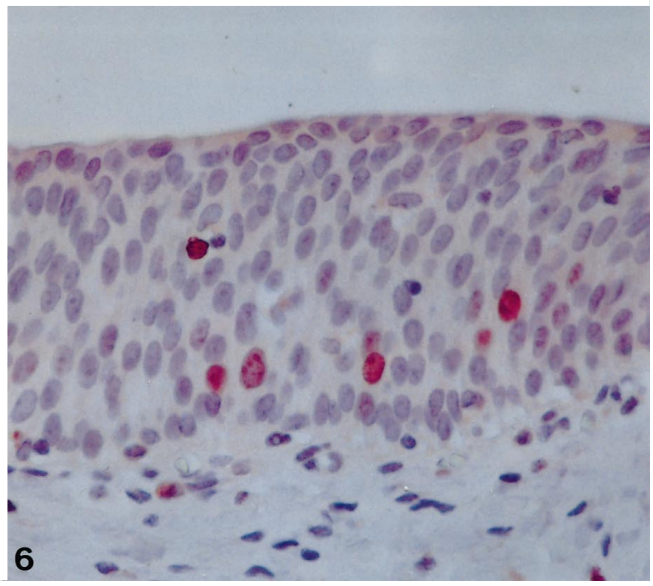

6

Table 2 Correlation of erbB receptor family expression with cell growth rate in normal urothelium (*n*=58)

erbB Receptors	MIB-1 Index[a]			Overall	*P*-value[b]
	Basal	Intermediate	Superficial		
EGFR (-)	2.7±4.5 (*n*=14)	1.8±3.3 (*n*=50)	0.6±1.3 (*n*=50)	1.4±2.9 (*n*=114)	0.002
(+)	2.8±4.8 (*n*=35)	– (*n*=0)	– (*n*=0)	2.8±4.8 (*n*=35)	
ErbB2 (-)	2.8±4.7 (*n*=49)	1.5±2.7 (*n*=38)	0 (*n*=1)	2.2±4.0 (*n*=88)	0.0004
(+)	– (*n*=0)	2.6±4.6 (*n*=12)	0.6±1.3 (*n*=49)	1.0±2.4 (*n*=61)	
ErbB3 (-)	3.0±4.8 (*n*=45)	1.9±3.4 (*n*=45)	1.1±1.9 (*n*=10)	2.3±4.0 (*n*=100)	0.0000
(+)	0.8±1.2 (*n*=5)	0.2±0.2 (*n*=5)	0.5±1.1 (*n*=40)	0.5±1.1 (*n*=50)	
ErbB4 (-)	2.8±4.6 (*n*=50)	1.8±3.3 (*n*=50)	0.5±1.1 (*n*=14)	2.0±3.8 (*n*=114)	0.001
(+)	– (*n*=0)	– (*n*=0)	0.7±1.4 (*n*=36)	0.7±1.4 (*n*=36)	
Mean	2.8±4.6	1.7±3.3	0.6±1.3		0.0001[c]

[a] Mean ± standard deviation
[b] By Student's *t*-test
[c] ANOVA was used

Table 3 Correlation of erbB receptor family expression with clinicopathologic properties of urothelial carcinoma (*n*=56)

Parameters		Tumour grading		*P* value	Tumour stage			*P* value
		1	2 & 3		Ta-T1	T2-T3	T4	
EGFR	(-)	6[a]	37	0.79	25	13	5	0.02
	(+)	0	7		0	6	1	
	(++)	0	6		3	3	0	
ErbB2	(-)	6	40	0.70	23	18	5	0.41
	(+)	0	7		2	4	1	
	(++)	0	3		3	0	0	
ErbB3	(-)	4	29	1.00	19	10	4	0.24
	(+)	2	18		9	9	2	
	(++)	0	3		0	3	0	
ErbB4	(-)	6	44	1.00	25	20	5	0.41
	(+)	0	5		3	1	1	
	(++)	0	1		0	1	0	

[a] The numbers indicate cases positive for immunostaining

bution was observed between EGFR and ErbB2, ErbB3 and ErbB4 (P = 0.0001 respectively); while, among the ErbB2, ErbB3, or ErbB4, a positive correlation was demonstrable between two of them (P = 0.0001).

BCL-2 protein was expressed predominantly in basal cells of urothelium as seen in Fig. 5 (P = 0.0001). The relationship between BCL-2 expression and class I receptors was examined specifically in different layer of urothelium. There was a positive relationship between BCL-2 and EGFR and inverse relationships of BCL-2 with ErbB2, ErbB3 or ErbB4 (P = 0.0001).

MIB-1 was more often expressed in the basal, and occasionally in the intermediate, cells of normal urothelium (Fig. 6), with mean values at 2.8%, 1.7% and 0.6% for basal, intermediate and superficial layers, respectively (Table 2). The MIB-1 index showed a stepwise decrement accompanying cellular differentiation (P = 0.0001 by ANOVA). Pairwise comparisons confirmed a difference between each two compartments (P < 0.005 respectively). There was a strong, positive association of growth rate with the expression of EGFR (P = 0.0001), whereas an overall negative correlation with ErbB2, ErbB3, or ErbB4 (P = 0.0001, respectively). In addition, pairwise comparisons revealed a decreased growth rate in association with ErbB2 expression (P = 0.0004). A comparable reduction of MIB-1 index was also observed for ErbB3 and ErbB4 (P = 0.00001 and 0.001, respectively).

On the basis of these findings, the role of class I receptors in urothelial carcinogenesis was investigated in 56 cases of TCC (Table 3). Basically, receptor expression did not correlate with histological grading (P > 0.5). In terms of tumour staging, EGFR was positively correlated with extent of tumour invasion (P = 0.02), as opposed to ErbB2, ErbB3, and ErbB4 (P > 0.1).

With regard to coexpression patterns, the interrelationship between subclass member and tumour grading or stage classification is shown in Tables 4 and 5. For

Table 4 Correlation of erbB receptor family expression in high grade (grades 2 and 3) urothelial carcinoma (n=50)

erbB Receptors	EGFR	ErbB2	ErbB3
ErbB2	0.33		
ErbB3	0.0005	0.86	
ErbB4	0.12	1.00	0.001

Table 5 Correlation of erbB receptor family expression in muscle-invasive (stage T2-T3) urothelial carcinoma (n=22)

erbB Receptors	EGFR	ErbB2	ErbB3
ErbB2	0.20		
ErbB3	0.01	0.61	
ErbB4	0.16	1.00	0.01

high-grade TCCs (grades 2 and 3), EGFR was significantly linked to ErbB3 (P = 0.0005), while ErbB3 was often coupled to ErbB4 (P = 0.001). There was no substantial correlation with grade 1 TCC (data not shown). An identical coexpression pattern was also revealed in muscle-invasive tumours (P = 0.01), except that ErbB3 and ErbB4 was also coexpressed in Ta-T1 tumours (P = 0.03; data not shown). There was no important relationship between the expression of subclass member and occurrence of metastasis (P > 0.3; data not shown).

Discussion

We have demonstrated the appearance of class I receptors in normal urothelium in vivo. Although sequential expression of the receptor family is difficult to elucidate by in situ techniques, our correlative data suggest an orchestrated expression of class I receptors in normal urothelium. EGFR appears in the basal layer only; ErbB2 is expressed on the superficial and some of the intermediate cells; ErbB3 tends to be distributed on the superficial cells, although some is noted in the whole of the urothelium; and ErbB4 is present predominantly in the superficial layer. The conclusion that expression of class I receptors in vivo is associated with cell differentiation agrees with previous reports with regard to EGFR and ErbB2 expression in normal human tissues [27–30].

Expression of class I receptors along the sequence of urothelial regeneration/proliferation can be envisioned as follows: the immature urothelial cells expressing EGFR and some ErbB3 have the capacity to proliferate in response to mucosal injury. When urothelial cells maturate, there will be up-regulation of ErbB2, ErbB3 and ErbB4 with down-regulation of EGFR. In support of these observations, in vitro experiments show evidence of cross-phosphorylation of ErbB3 by EGFR [10]; cooperation of ErbB3 with ErbB2 to provide a high-affinity receptor for heregulin with tyrosine kinase capability [1, 25], and induction of phenotypic differentiation when specific ligand interacts with ErbB4 in the absence of activation of ErbB2 [18]. A plausible explanation for the tendency toward partition of the receptors into opposite compartment of human urothelium seems to act as a counterbalance in tissue homeostasis. An elaborate model study with a defined expression system is mandatory to confirm our hypothesis.

Proto-oncogene *bcl-2* encodes a 25 kDa protein that localizes to the mitochondrial membrane, nuclear envelope, and endoplasmic reticulum [24]. Previous studies have shown that BCL-2 is selectively expressed in the less differentiated (basal) cells of normal mucosa [24]. Thus, the localization of BCL-2 in immature cells of urothelium is consistent with the current concept of gene activity in association with cell differentiation. As a result, the reciprocal distribution of BCL-2 in relation to ErbB2, ErbB3 and ErbB4 provides additional proof for a terminally differentiated attribute of these receptors, though a direct causal relationship between receptor activation and the activity of bcl-2 is lacking.

Of particular interest is the expression of ErbB2 in mature cells of urothelium and its association with decreased growth rate. This observation is recent [12, 19, 28]. Currently, we have no rationale to relate our observation to earlier experimental data showing an association of ErbB2 with enhanced cell proliferation [6, 9, 13], and some previous reports indicating a negative expression of ErbB2 in normal urothelium. However, a study in renal cell carcinoma found an inverse relationship between the expression of EGFR and ErbB2 mRNA [30]. Micrometastatic tumour cells overexpressing ErbB2 were found to be in a dormant state of cell growth [16]. The evidence described above appears to imply that expression of ErbB2 may confer only limited proliferative capability as suggested in transfection of erbB2 in immortalized human epithelium [17].

Despite the inability to find a specific ligand for ErbB2, recent studies indicate that receptor dimerization and cross-phosphorylation may play an important role in the growth factor signalling cascade [4, 18, 25]. The contradictory biological responses of ErbB2 in previous studies could thus be partly explained by transregulation by other subclass members [26]. This interpretation, however, needs to be verified by a specific gene transfer experiment.

In contrast, expression patterns of class I receptors in urothelial carcinoma appears to be more diverse than in normal urothelium. The finding that overexpression of EGFR was significantly associated with muscular invasion agrees with earlier reports [15]. In addition, coexpression of EGFR and ErbB3 in high grade and/or invasive TCCs is consistent with their interaction observed in vitro [3, 10, 26], despite of the fact that ErbB3 lacks an intrinsic tyrosine kinase activity [3]. Although the preferential localization of ErbB4 in superficial cells supports its linkage with differentiated property as revealed by in vitro experiment [18], we have no explanation to account for the linkage of ErbB4 and ErbB3 with poorly differentiated and/or invasive tumours. To clarify the biological relevance of coexpression of class I receptors in urothelial carcinoma, an additional study is ongoing.

Acknowledgements This study was supported in part by research grant NCKUH-85-042 from National Cheng Kung University Hospital, grant NSC-85-2331-B-006-027 from the National Science Council, and grant DOH 86-HR-617 from the National Health Research Institute, Taiwan, Republic of China.

References

1. Alimandi M, Romano A, Curia MC, Muraro R, Fedi P, Aaronson SA, Di Fore PP, Kraus MH (1995) Cooperative signaling of ErbB3 and ErbB2 in neoplastic transformation and human mammary carcinomas. Oncogene 10:1813–1821
2. Boer WI de, Schuller AGP, Vermey M, Kwast TH van der (1994) Expression of growth factors and receptors during specific phases in regenerating urothelium after acute injury in vivo. Am J Pathol 145:1199–1207
3. Carraway KL III, Cantley LC (1994) A new acquaintance for ErbB3 and ErbB4: a role for receptor heterodimerization in growth signaling. Cell 78:5–8
4. Carraway KL, Sliwkowski MX, Akita R, Platko JV, Guy PM, Nuijens A, Diamonti AJ, Vandlen RL, Cantley LC, Cerione RA (1994) The erbB3 gene product is a receptor for heregulin. J Biol Chem 269:14303–14306
5. Chow NH, Tzai TS, Cheng PE, Chang CJ, Lin SN, Tang MJ (1994) An assessment of immunoreactive epidermal growth factor in urine of patients with urological diseases. Urol Res 22:221–225
6. Gasparini G, Pozza F, Meli S, Reitano M, Santini G, Bevilaxqua P (1991) Breast cancer cell kinetics: immunocytochemical determination of growth fractions by nomoclonal antibody Ki-67 and correlation with flow cytometric S-phase and with some features of tumor aggressiveness. Anticancer Res 11:2015–2021
7. Gullick WJ, Berger MS, Bennett PLP, Rothbard JB, Waterfield MD (1987) Expression of the c-erbB-2 protein in normal and transformed cells. Int J Cancer 40:246–254
8. Jochum W, Schroder S, Al-Taha R, August C, Gross AJ, Berger J, Padberg BC (1996) Prognostic significance of nuclear DNA content and proliferative activity in renal cell carcinomas. A clinicopathologic study of 58 patients using mitotic count, MIB-1 staining, and DNA cytophotometry. Cancer 77:514–521
9. Kallioniemi OP, Holli K, Visakorpi T, Koivula T, Helin HH, Isola JJ (1991) 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
10. Kim HH, Sierke SL, Koland JG (1994) Epidermal growth factor-dependent association of phosphatidylinositol 3-kinase with the erbB3 gene product. J Biol Chem 269:24747–24755
11. Kraus MH, Issing W, Miki T, Popescu NC, Aaronson SA (1989) 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
12. Lee SE, Chow NH, Chi YC, Tzai TS, Yang WH, Lin SN (1994) Expression of c-erbB-2 protein in normal and neoplastic urothelium: lack of adverse prognostic effect in human urinary bladder cancer. Anticancer Res 14:1317–1324
13. Lipponen P (1993) Expression of c-erbB-2 oncoprotein in transitional cell bladder cancer. Eur J Cancer [A] 29:749–753
14. Messing EM (1990) Clinical implications of the expression of epidermal growth factor receptors in human transitional cell carcinoma. Cancer Res 50:2530–2537
15. Neal DE, Sharples, Smith K, Fennelly J, Hall RR, Harris AL (1990) The epidermal growth factor receptor and the prognosis of bladder cancer. Cancer 65:1619–1625
16. Pantel K, Schlimok G, Braun S, Kutter D, Lindemann F, Schaller G, Funke I, Izbick JR, Riethmuller G (1993) Differential expression of proliferation-associated molecules in individual micrometastatic carcinoma cells. J Natl Cancer Inst 85: 1419–1424
17. Pierce JH, Arnstein P, DiMarco E, Artrip J, Kraus MH, Lonardo F, Di Fiore PP, Aaronson SA (1991) Oncogenic potential of erbB-2 in human mammary epithelial cells. Oncogene 6: 1189–1194
18. Plowman GD, Culouscou JM, Whitney GS, Green JM, Carlton GW, Foy L, Neubauer MG, Shoyab M (1993) Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. Proc Natl Acad Sci USA 90:1746–1750
19. Press MF, Cordon-Cardo C, Slamon DJ (1990) Expression of the HER-2/neu proto-oncogene in normal human adult and fetal tissues. Oncogene 5:953–962
20. Prigent SA, Lemoine NR, Hughes CM, Plowman GD, Selden C, Gullick WJ (1992) Expression of the c-erbB-3 protein in normal human adult and fetal tissues. Oncogene 7:1273–1278
21. Pusztai L, Lewis CE, Lorenzen J, McGee J (1993) Growth factors: regulation of normal and neoplastic growth. J Pathol (Lond) 169:191–201
22. Rebel JM, Boer WI de, Thijssen CD, Vermey M, Zwarthoff EC, Kwast TH van der (1994) An in vitro model of urothelial regeneration: effects of growth factors and extracellular matrix proteins. J Pathol (Lond) 173:283–291
23. Singleton TP, Niehans GA, Gu F, Litz CE, Hagen K, Qiu Q, Kiang DT, Strickler JG (1992) Detection of c-erbB-2 activation in paraffin-embedded tissue by immunohistochemistry. Hum Pathol 23:1141–1150
24. Sinicrope FA, Ruan SB, Cleary KR, Stephens LC, Lee JJ, Levin R (1995) bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer Res 55:2237–2241
25. Sliwkowsk MX, Schaefer G, Akita RW, Lofgren JA, Fitzpatrick VD, Nuijens A, Fendly BM, Cerione RA, Vandlen RL, Carraway KL (1994) Coexpression of *erb*B2 and *erb*B3 proteins reconstitutes a high affinity receptor for heregulin. J Biol Chem 269:14661–14665
26. Soltoff SP, Carraway KL, Prigent SA, Gullick WG, Cantley LC (1994) ErbB3 is involved in activation of phosphatidylinositol 3-kinase by epidermal growth factor. Mol Cell Biol 14: 3550–3558
27. Tsutsumi Y, Naber SP, De Lellis RA, Wolfe HJ, Marks PJ, McKenzie SJ, Yin S (1990) Neu oncogene protein and epidermal growth factor receptor are independently expressed in benign and malignant breast tissues. Hum Pathol 21:750–758
28. Wagner U, Sauter G, Moch H, Novotna H, Epper R, Mihatsch MJ, Waldman FM (1995) Patterns of p53, erbB-2, EGF-r expression in premalignant lesions of the urinary bladder. Hum Pathol 26:970–978
29. Wang DP Jr, Fujii S, Konishi I, Nanbu Y, Iwai T, Nonogaki H, Mori T (1992) Expression of c-erbB-2 protein and epidermal growth factor receptor in normal tissues of the female genital tract and in the placenta. Virchows Arch [A] 420:385–393
30. Weidner U, Peters S, Strohmeyer T, Hussnaetter R, Ackerman R, Sies H (1990) Inverse relationship of epidermal growth factor receptor and HER-2/neu gene expression in human renal cell carcinoma. Cancer Res 50:4504–4509