Urol Res (2002) 30:240–247
DOI 10.1007/s00240-002-0260-7

ORIGINAL PAPER

E.M.J. Bindels · T.H. van der Kwast · V. Izadifar
D.K. Chopin · W.I. de Boer

Functions of epidermal growth factor-like growth factors during human urothelial reepithelialization in vitro and the role of erbB2

Received: 13 September 2001 / Accepted: 1 May 2002 / Published online: 7 June 2002
© Springer-Verlag 2002

Abstract Transitional epithelium of the urinary bladder can be damaged during, for example, catheterization, overstretching due to obstructed voiding, or partial resection. The subsequent repair process can be stimulated by specific proteins such as epidermal growth factor (EGF) and transforming growth factor-α (TGFα). However, little is known about the role of EGF-like growth factors and their respective receptors in human urothelial repair. In this study, we examined the effects of EGF, TGFα, amphiregulin and heregulin-α (HRGα) on proliferation, wound closure, and the expression of their receptors c-erbB1–c-erbB4 in primary cultures of human urothelial cells in vitro. Under conditions representing intact urothelium, all EGF-like growth factors except HRGα induced proliferation. TGFα induced proliferation up to four times. Amphiregulin increased expression of c-erbB1. Treatment with either TGFα or amphiregulin resulted in higher c-erbB1 activation and c-erbB3 levels. None of the growth factors affected the constitutive expression of c-erbB2 and c-erbB4. In the repair model, both EGF and TGFα stimulated the wound closure most strongly. This was mainly achieved by increased cellular migration. Receptor expression was not affected by the addition of exogenous growth factor. The role of c-erbB2 in wound healing was further investigated with the use of antisense DNA. Wound closure could be delayed up to 50% by antisense c-erbB2 but not by mismatched or sense oligonucleotides. Excessive production (e.g. in bladder tumors) or application of EGF, TGFα or amphiregulin, but not HRGα may lead to either hyperplasia or a faster repair of damaged urothelium in vivo. These effects seem to be mediated not only via c-erbB1 but also via c-erbB2. Our results suggest that modified members of the EGF-EGFR family are potential targets for future therapies for bladder wound healing and malignancy.

Keywords Reepithelialization · Bladder · ErbB-receptor · EGF-like growth factors · Oligonucleotides

E.M.J. Bindels · T.H. van der Kwast
Department of Pathology,
Erasmus University Medical Center,
Rotterdam, The Netherlands

W.I. de Boer (✉)
Department of Pulmonary Medicine,
Erasmus University Medical Center,
Dr. Molewaterplein 50, 3015 GE Rotterdam,
The Netherlands
E-mail: deboer.pim@hetnet.nl
Tel.: +31-10-4087701
Fax: +31-10-4089453

V. Izadifar · D.K. Chopin · W.I. de Boer
Service D'Urologie, Hopital Henri Mondor AP-HP,
EMI INSERM 99-09, Université Paris XII, Créteil, France

Introduction

Normally, the cell turnover of bladder epithelium is very slow [24], but urothelial damage, e.g. by catheterization, outlet obstruction, deposition of urinary crystals or partial resection, results in a very rapid regeneration [14]. Urothelial regeneration is a tightly regulated process involving proliferation, migration, differentiation and extracellular matrix production. Peptide growth factors are involved in all of these steps of wound healing. Several studies have demonstrated that members of the epidermal growth factor (EGF)-family are important mediators of wound healing. The EGF-family consists of a number of related proteins, such as transforming growth factor-α (TGFα), EGF, amphiregulin, epiregulin, heparin-binding EGF-like growth factor and heregulins [2]. Exogenous application of EGF, TGFα or heregulin stimulates epidermal wound healing in vivo [7, 12, 32]. In addition, EGF and TGFα promote the reepithelialization of the injured gastrointestinal tract [29].

The EGF-like growth factors bind to the erythroblastosis virus gene (erbB)-family of tyrosine kinase receptors. The erbB-family is composed of four receptors: erbB-1, also called epidermal growth factor receptor (EGFR) or human EGF receptor 1 (HER1); erbB-2,

also called HER2 or Neu; erbB3 (or HER3) and erbB4 (or HER4). ErbB1 is the primary receptor for EGF, TGFα and amphiregulin, whereas erbB3 and erbB4 are the actual specific receptors for heregulins [8]. Although a specific ligand for erbB2 is still unknown, c-erbB2 plays a major coordinatory role in the erbB-family because it has the ability to form heterodimers with every other erbB-receptor. In addition, the other members of the erbB-family can form homodimers or heterodimers with each other [2]. Dimerized erbB-receptors concomitantly autophosphorylate and thereby become docking sites for proteins bearing SH2 domains, which in turn couple to downstream signaling pathways [22].

The epidermal growth factor-family probably plays an important role in the normal or abnormal growth of bladder epithelium [26]. Expression of EGF, TGFα and amphiregulin was found in normal human urothelium [9, 25] and EGF or TGFα was detected in the urine of healthy individuals [26] or bladder cancer patients [25], respectively. In normal human urothelium, EGF-receptor expression is confined to the basal cell layer, but in bladder carcinomas the expression is seen in all cell layers [26]. In addition, the expression of other members of the EGFR-family is increased in carcinoma of the bladder [23, 30]. Functional studies showed that EGF, TGFα and amphiregulin could stimulate the proliferation and/or migration of normal urothelial cells or bladder carcinoma cells [16, 17, 31]. Although these studies emphasize the importance of the EGF-family in the normal or abnormal growth of the bladder, little is known about the function of EGF-family members and their erbB-receptors in wound healing of the bladder.

Previously, we have shown that EGF can stimulate reepithelialization in an organotypic culture of mouse urothelium [15, 31]. In this study, an analogous in vitro model of human urothelium, which closely mimics the differentiation and multilayering of normal urothelium [16], was used to investigate the role of members of the EGF-family in normal and regenerating urothelium. Results of our study will be beneficial to the field of bladder reconstruction and replacement surgery. Furthermore, knowledge of the function of the EGF/EGFR family in the maintenance and repair of normal urothelium will also be relevant for urothelial tumorigenesis, because growth factors and their receptors can serve as modulators of tumor cell biology, thereby influencing tumor growth rate, local invasion and metastasis.

Materials and methods

Chemicals

Biochemicals were obtained as follows: tissue culture additives and EGF (Sigma, St. Louis, USA); human collagen type IV (Fluka, Buchs, Switzerland); amphiregulin and heregulin-α (HRGα, R and D Systems, Minneapolis, USA) and TGFα (Boehringer Mannheim, Mannheim, Germany). Effective concentrations of these ligands were previously determined by the construction of dose-response curves [17]. Antibodies were purchased as follows: anti c-erbB1 (Oncogene Research, Uniondale, USA); anti phosphorylated c-erbB1 (Transduction Laboratories, Lexington, USA); anti c-erbB2 and secondary antibodies (DAKO, Glostrup, Denmark); and anti c-erbB3 and anti c-erbB4 (Santa Cruz Biotechnology, Santa Cruz, USA). The anti-BrdU antibody was kindly donated by Dr. B. Schutte (University of Maastricht, Maastricht, The Netherlands).

Cell culture

Primary human explant cultures were established as described previously [16]. Briefly, fresh and macroscopically normal appearing ureter specimens were obtained from resection material from patients treated for non-malignant renal diseases. For processing, the urothelium was stripped from the submucosa and processed onto cyclopore membranes (Falcon culture inserts, Becton Dickinson) coated with collagen type IV (25 μg/ml). Primary explant cultures were obtained using DMEM/Ham's F12 medium supplied with 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenite, 5 nM hydrocortisone, 10 mM HEPES, 2 mM glutamine, 100 IU penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated FCS. This medium is referred to as routine medium. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 until 2 weeks post-confluency. At 24 h before the start of an experiment, the remaining explant was removed and culture medium was replaced with routine medium devoid of insulin and FCS, but supplemented with 0.1% bovine serum albumin, 4 μM spermine and spermidine, and 1 μM ethanolamine (referred to as serum-free medium: SF). Experiments were performed in SF.

Confluent culture model mimicking intact urothelium

After reaching confluency, cultures were maintained in routine medium for an additional 2 weeks to ensure proper differentiation of the whole culture. Before treatment with growth factors, cultures were rinsed twice with SF followed by incubation in SF for 24 h. Subsequently, medium was replaced with SF with or without growth factor for another 96 h. Medium was replaced daily during the course of the experiments.

Culture model mimicking reepithelializing urothelium

Injuries were made directly after the 24-h incubation with SF. Four circular imprints were made with a biopsy punch (Stiefel, Offenbach am Main, Germany; internal diameter 4 mm) [6]. The urothelium within the injured areas was scraped away microscopically. After injury, the cultures were washed twice with SF followed by incubation of the cultures with SF with or without the addition of growth factors. At different time-points during cultivation, the wound edge was monitored through a light microscope. From the acquired image, the uncovered area was measured and expressed as a percentage of the initial wound area.

In the experiments, when the de novo c-erbB2-protein production was blocked using an antisense oligonucleotide strategy, 24 h before damage, culture medium was replaced with SF containing 5 nmol/ml oligonucleotides. After 24 h, damage was made as described. During the next 48 h, medium with or without oligonucleotides was replaced twice. Phosphorothioated oligonucleotides were obtained in collaboration with Dr. M. Lemâitre (Eurogentec, Liège, Belgium) based on experiments described by Vaughn et al. [35]. Sequences of the respective c-erbB2 oligonucleotides are: antisense oligonucleotide (AS): 5′-GAGGTACCAC GAGTG-3′; mutated variant of the antisense oligonucleotide MUT): 5′-GAGTGACCACAGGTG-3′; sense oligonucleotide (S): 5′-CTCCATGGTGCTCAC-3′.

Proliferation assay

At 2 h before termination, cultures were incubated with 40 μg/ml bromodeoxyuridine (BrdU). Subsequently, cultures were rinsed with PBS, pH 7.2, and fixed with 70% ethanol followed by

immunohistochemistry. Eight prefixed areas of 0.15 mm^2 per culture were counted. Four areas just outside each wound and another four areas within the wound that were covered by regenerative urothelium were counted. The BrdU-incorporation was expressed as the labeling index: the relative number of BrdU-positive nuclei in four prefixed areas of 0.15 mm^2 per culture.

Immunohistochemistry

The immunostaining of the cultures was performed as described [16]. The expression was visualized using appropriate dilutions of the primary antibodies in a conjugated immunoenzyme assay. Secondary antibodies were either peroxidase or alkaline phosphatase conjugated. As substrates, we used 3,3′-diaminobenzidine tetrahydrochloride or naphtol AS-MX phosphate. The level of c-erbB-receptor expression was determined semi-quantitatively, with 0 = no expression; 1 = low expression; 2 = moderate expression; 3 = high expression; 4 = very high expression [14].

Statistics

Experiments were performed at least in triplicate. A Student's *t*-test was used to determine the statistical significance of the data, which showed a Gaussian distribution. At $P < 0.05$, data were accepted as statistically significant. Data are represented as mean ± SEM.

Results

Confluent cultures representing intact urothelium

As shown in Fig. 1A, treatment with amphiregulin and TGFα, significantly enhanced the proliferation of the urothelial cultures three to four times as visualized by the labeling index ($P < 0.003$). In contrast, treatment with HRGα or EGF did not stimulate urothelial proliferation. The cellularity of the cultures was increased upon treatment with amphiregulin, TGFα or EGF (Fig. 1B). The fact that EGF induced an enhanced cellularity of the cultures must imply that EGF had transiently stimulated proliferation before the addition of BrdU or that EGF could inhibit apoptosis in these urothelial cultures, as has previously been described for other cell cultures [11, 21, 34].

The amphiregulin and TGFα mediated induction of proliferation was associated with an enhanced expression of c-erbB3. The constitutive expression of c-erbB2 and c-erbB4 was not changed upon growth factor treatment. The constitutive expression of c-erbB1 was only induced by amphiregulin, whereas the expression of phosphorylated c-erbB1 was slightly enhanced by both amphiregulin and TGFα (Fig. 1C).

Given the upregulation of both phosphorylated c-erbB1 as well as c-erbB3 by amphiregulin and TGFα, it is tempting to hypothesize that amphiregulin, EGF, and/or TGFα also mediate their effects via c-erbB3. Furthermore, the expression of c-erbB4 in the intact cultures was constitutively high irrespective of the growth factor treatment, and it is known that c-erbB3 has a high affinity for c-erbB4 to form a heterodimer [8]. However, HRGα, which primarily signals through the c-erbB3/c-erbB4 heterodimer, had no proliferative effect on human urothelial cells (Fig. 1A).

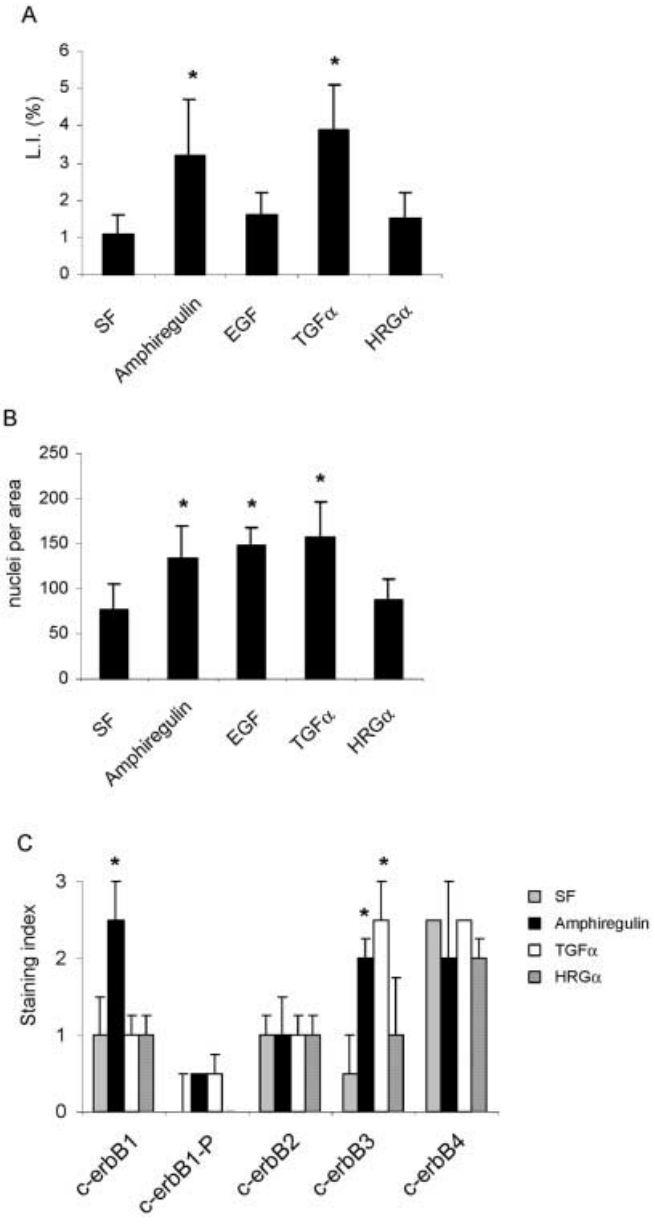

Fig. 1. Effect of EGF-like growth factors on: **A** the labeling index, **B** the number of nuclei and **C** the expression of erbB-family members in confluent primary urothelial cultures. An *asterisk* indicates a significant difference ($P < 0.05$) as compared to SF. *C-erbB1-P* = antibody against phosphorylated c-erbB1

Regenerating urothelial cultures

Under serum-free conditions, approximately 84% of the wound area was covered with new urothelium within 48 h. The reepithelialized area was markedly enlarged upon treatment with TGFα or EGF, and to a lesser extent, by amphiregulin (Fig. 2). In fact, when treated with EGF or TGFα, wounds completely closed within 48 h. Treatment with HRGα had no effect on urothelial regeneration as compared to non-treated cultures.

In general, growth factor treatment had no stimulating effect on urothelial proliferation during the first

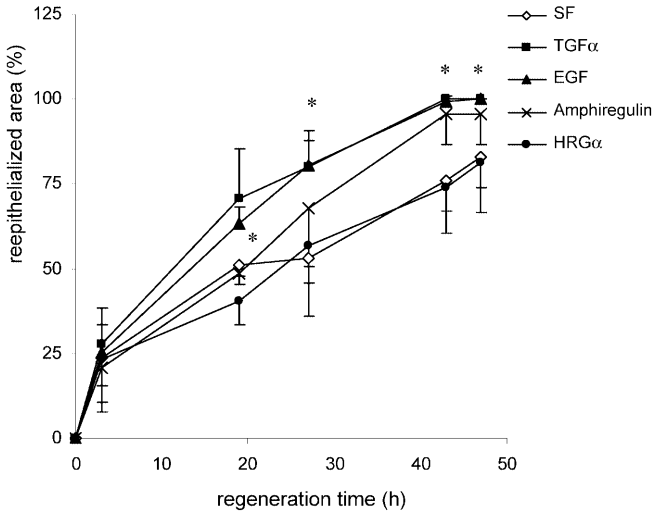

Fig. 2. Influence of EGF-like growth factors on reepithelialization of injured urothelial cultures. An *asterisk* indicates a significant difference ($P < 0.05$) as compared to SF

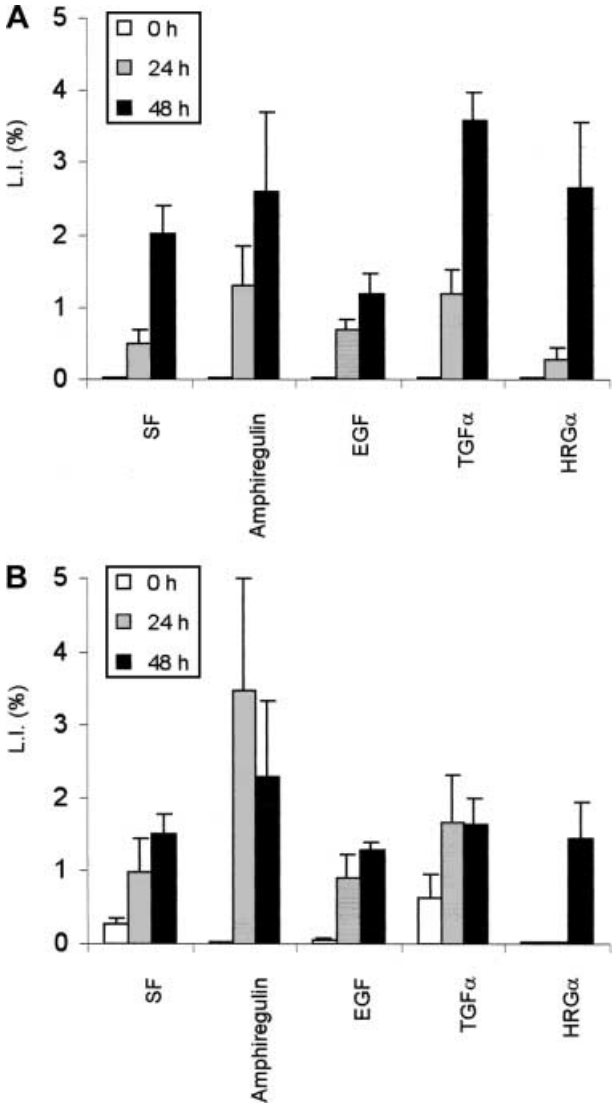

Fig. 3A, B. Effect of EGF-like growth factors on the proliferation of injured urothelial cultures. BrdU incorporation was determined in prefixed regions **A** just outside the injury and **B** within the reepithelialized wound-area. No significant differences were seen as compared to the comparable SF cultures

24 h of wound healing (Fig. 3A, B). During the next 24 h, a gradual increase in proliferation was noted. Nevertheless, labeling indices remained relatively low. Therefore, the observed stimulation of reepithelialization after EGF, TGFα or amphiregulin treatment could not be explained by a significant induction of proliferation. This suggests that the induced reepithelialization could only be explained by an enhanced migration of cells into the denuded area.

The stimulated wound closure may be accompanied by an enhanced expression of specific growth factor receptors. According to other functional studies, EGF-like factors mediate their effects mainly via c-erbB1, c-erbB2, and c-erbB4. Therefore, we quantitated the immunohistochemical expression of c-erbB1, c-erbB2 and c-erbB4. The c-erbB1 expression was low and was not significantly affected by any of the investigated growth factors at any point during the repair-process (data not shown). The c-erbB2 expression tended to be higher only in cells near the edge of the damaged area upon treatment with amphiregulin, TGFα or EGF (Fig. 4A, B). The c-erbB4 expression showed no significant differences during wound healing (data not shown).

Our data point to a function not only of c-erbB1, but also of c-erbB2 in epithelial regeneration. To investigate whether c-erbB2 is necessary for the reepithelialization, we treated cultures with antisense oligonucleotides for c-erbB2 and examined the regeneration during this treatment.

Treatment of injured cultures with c-erbB2 antisense DNA

Treatment of damaged cultures with c-erbB2 AS significantly inhibited reepithelialization by up to 50% (Figs. 5, 6A). The S and the MUT variants of the AS did not affect regeneration (Figs. 5, 6B, C). This indicates that the wound healing was specifically perturbed by the c-erbB2 antisense only. The proliferation was similar in all damaged cultures (Fig. 7A, B) suggesting that this delay in reepithelialization could not be explained by a diminished proliferative capacity of the antisense treated cultures. Also, none of the control oligonucleotides had a significant effect on proliferation after 24 or 48 h of treatment.

Discussion

Earlier studies on the functional effects of EGF-like growth factors on urothelium are conflicting. We have previously shown that the growth and regeneration of

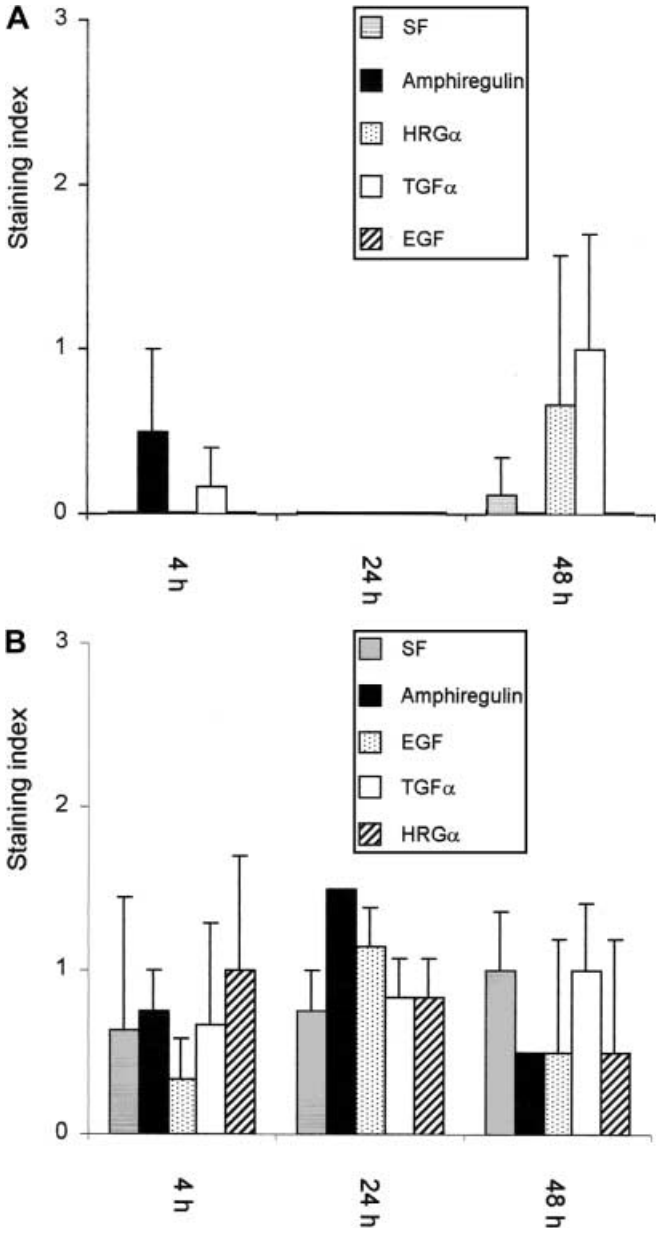

Fig. 4A, B. Expression of c-erbB2 in EGF-like growth factor treated injured urothelial cultures. C-erB2-expression was determined at various time-points in regions **A** just outside the injury and **B** within the reepithelialized wound area

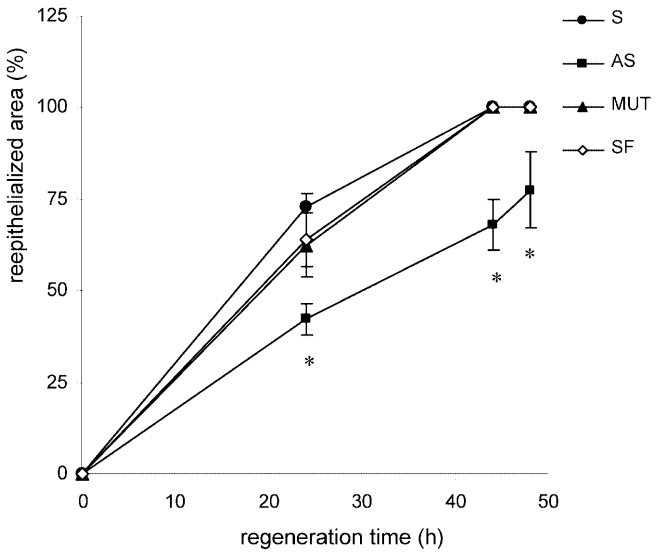

Fig. 5. Influence of c-erbB2 oligonucleotides on the reepithelialization of injured urothelial cultures. An *asterisk* indicates a significant difference ($P < 0.05$) as compared to SF. *S* = sense oligonucleotide c-erbB2, *AS* = antisense oligonucleotide c-erbB2, *SF* = serum free medium, *MUT* = mutant oligonucleotide c-erbB2

murine urothelium in vitro was stimulated by TGFα or EGF [6, 15, 31]. Furthermore, in vivo studies indicated that EGF induced urothelial proliferation and hyperplasia in rats and pigs [26, 37]. However, others have claimed that despite the presence of EGF-receptors on human urothelium [16, 27], EGF was not essential for the growth of normal human urothelial cells in vitro [9, 20, 27, 33].

In this study, using a previously described in vitro organotypic model for human urothelium, amphiregulin, TGFα and EGF stimulated the proliferation of intact human urothelium. No functional effects of amphiregulin on the growth of human urothelium have been reported previously. But Cilento et al. showed that human urothelial cells in culture can produce high levels of amphiregulin mRNA, suggesting that amphiregulin is involved in the autocrine growth regulation of urothelium [9].

We demonstrated that urothelial reepithelialization is enhanced by the EGF-like factors TGFα, EGF and amphiregulin but not by HRGα. The mode of action of TGFα/EGF/amphiregulin may be mainly through interaction with EGFR which either transduces the signal by homodimerization or by heterodimerization with c-erbB2. Treatment of the regenerating urothelial cultures with c-erbB2 antisense DNA confirmed that c-erbB2 is involved in urothelial regeneration. The role of c-erbB3 during the EGF/TGFα/amphiregulin stimulated reepithelialization is not yet clear as these factors do not bind to c-erbB3, although heterodimerization of c-erbB1 or c-erbB2 with c-erbB3 may contribute to the observed effect [1, 28]. An alternative role for c-erbB3 is that endogenously expressed HRGα may stimulate the proliferation in a c-erbB3-dependent way. Furthermore, the constitutive, high expression of c-erbB4 during urothelial reepithelialization is suggestive for a role of c-erbB4 in urothelial maintenance and/or regeneration. Future functional in vitro studies may clarify possible functions of these respective EGFR family members in urothelial regeneration.

In the same model of urothelial regeneration, Daher et al. recently showed that reepithelialization could also be inhibited by a functional blocking EGFR antibody or by the EGFR-tyrosine kinase inhibitor tyrphostin AG1478 [10]. In their study, these EGFR inhibitors affected both proliferation and migration in urothelial wound repair. Our combined data indicate that in

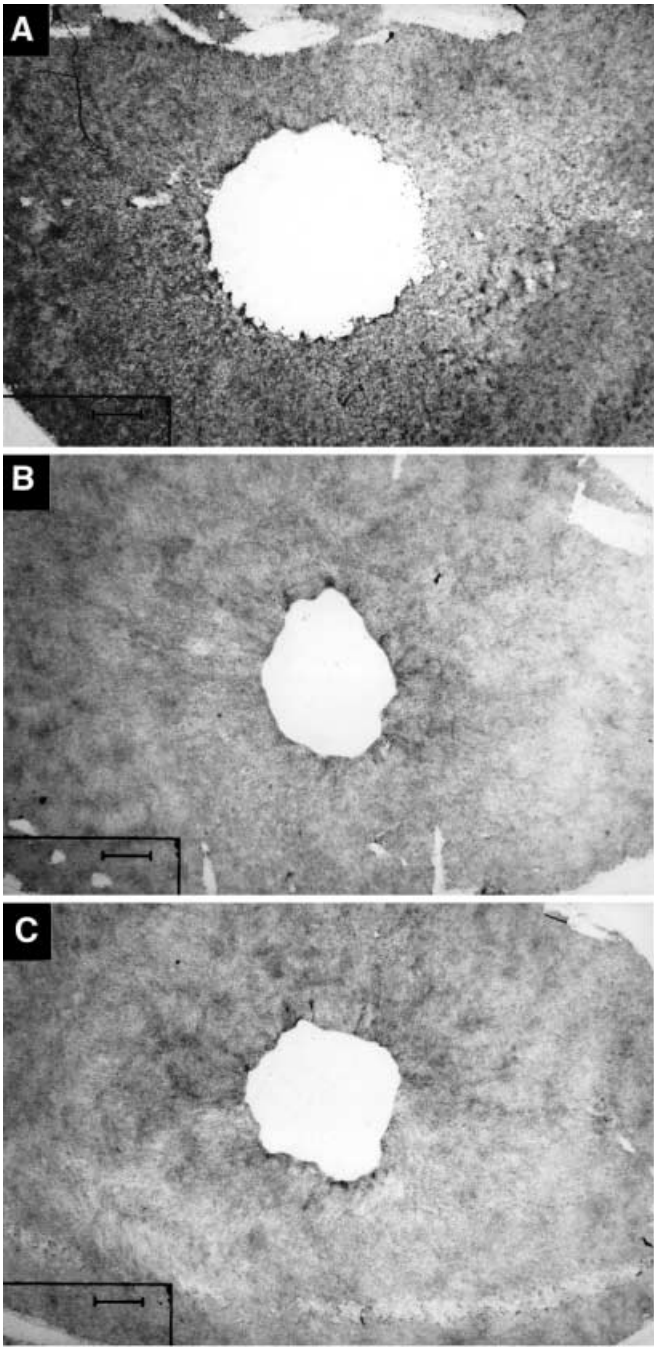

Fig. 6A–C. Topview of standardized injuries made in primary cultures of human urothelium. Effect on reepithelialization after treatment for 24 h with c-erbB2 oligonucleotides: **A** antisense DNA, **B** sense DNA and **C** mutated variant of antisense DNA

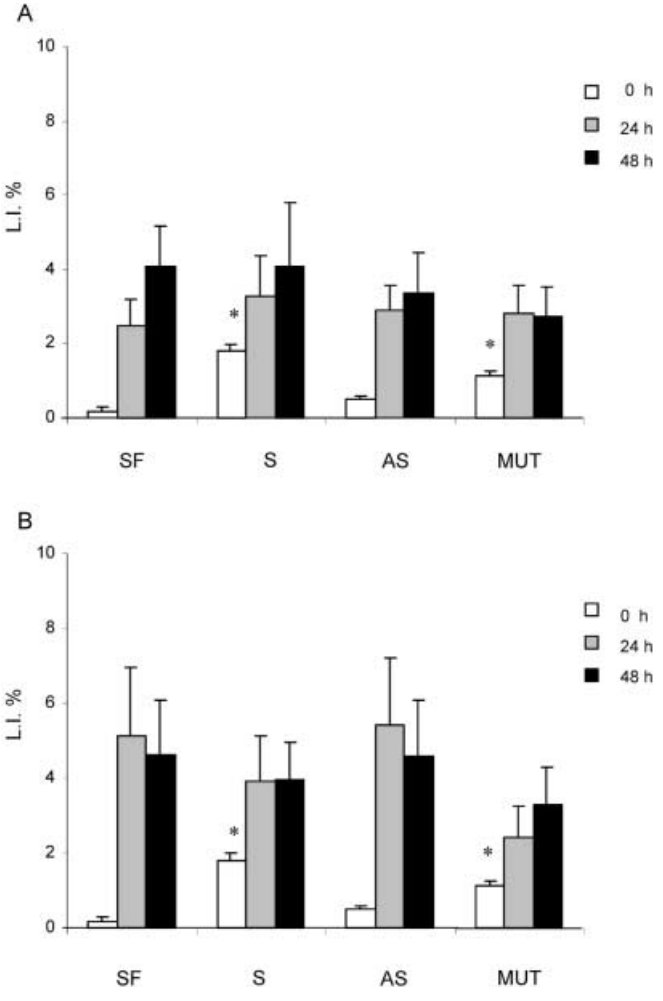

Fig. 7A, B. Effects of c-erbB2 oligonucleotides on the BrdU incorporation, determined **A** within the reepithelialized area, and **B** just outside the damaged areas. An *asterisk* indicates a significant difference ($P < 0.05$) as compared to SF. S = sense oligonucleotide c-erbB2, AS = antisense oligonucleotide c-erbB2, SF = serum free medium, MUT = mutant oligonucleotide c-erbB2

damaged urothelium both EGFR and c-erbB2 are involved in the process of reepithelialization.

Our experiments with the antisense c-erbB2 oligonucleotides suggest that c-erbB2 is an important mediator of migration during wound healing. Few studies have reported on the role of the c-erbB2 protein in cell migration. Overexpression of c-erbB2 in breast or ovarian carcinoma cells or fibroblasts resulted in an increased cell migration [18, 36] or invasion [13]. Furthermore, cytoplasmic expression of p185-neu in human astrocytoma cells is associated with a high degree of migratory activity [5]. The exact mechanisms of c-erbB2-enhanced migration are only poorly understood. A direct mechanism could be via signaling through the Ras-MAP kinase pathway [3], ultimately leading to the transcription of genes involved in cell migration. Indirectly, it may be caused by the downregulation of E-cadherin or α2-integrin [19] or by activation of the PEA3 transcription factor that in turn activates genes which encode enzymes required for cell migration [4].

In conclusion, the EGF-EGFR pathway is involved in the normal growth and regeneration of human urothelium. Amphiregulin, EGF and TGFα might be involved in the (autocrine) growth regulation of urothelium. Enhanced expression of some of these growth factors and their receptors could contribute to the deregulated growth of bladder tumors, making them suitable as targets for future developments in cancer

therapy. On the other hand, EGF and TGFα are important mediators of reepithelialization, suggesting that concise use of EGF-family members could aid in the regeneration of damaged urothelium, and improve bladder reconstruction and replacement surgery.

Acknowledgements D.C. is supported by the Université Paris XII Association Claude Bernard Delegation recherche clinique AP-HP. We thank Dr. M. Lemaitre (Eurogentec, Liège, Belgium) for his help in designing the oligonucleotides.

References

1. Alimandi M, Wang LM, Bottaro D, Lee CC, Kuo A, Frankel M, Fedi P, Tang C, Lippman M, Pierce JH (1997) Epidermal growth factor and betacellulin mediate signal transduction through co-expressed ErbB2 and ErbB3 receptors. EMBO J 16: 5608
2. Alroy I, Yarden Y (1997) The erbB signalling network in embryogenesis and oncogenesis: signal diversification through combinatorial ligand-receptor interactions. FEBS Lett 410: 83
3. Ben-Levy R, Paterson HF, Marshall CJ, Yarden Y (1994) A single autophosphorylation site confers oncogenicity to the Neu/ErbB-2 receptor and enables coupling to the MAP kinase pathway. EMBO J 13: 3302
4. Benz CC, O'Hagen RC, Richter B, Scott GK, Chang CH, Xiong X, Chew K, Ljung BM, Edgerton S, Thor A, Hassell JA (1997). Her2/Neu and the Ets transcription activator PEA3 are coordinately upregulated in human breast cancer. Oncogene 15: 1513
5. Bernstein JJ, Goldberg WJ, Laws ER (1993) Migration of fresh human malignant astrocytoma cells into hydrated gel wafers in vitro. J Neurooncol 18: 151
6. Bindels EMJ, Vermey M, Rebel JMJ, Zwarthoff EC, van der Kwast TH (1997) In vitro modulation of implantation and intraepithelial expansion of bladder tumor cells by epidermal growth factor. Exp Cell Res 235: 355
7. Brown GL, Nanney LB, Griffen J, Cramer AB, Yancey JM, Curtsinger LJ, Holtzin GS, Schultz GS, Jurkiewicz MJ, Lynch JB (1989) Enhancement of wound healing by topical treatment with epidermal growth factor. N Engl J Med 321: 76
8. Carraway KL, Cantley LC (1994) A Neu acquaintance for erbB3 and erbB4: a role for receptor heterodimerization in growth signalling. Cell 78: 5
9. Cilento BG, Freeman MR, Schneck FX, Retik AB, Atala A (1994) Phenotypic and cytogenetic characterization of human bladder expanded in vitro. J Urol 152: 665
10. Daher A, El-Marjou A, de Boer WI, Abbou CC, Thiery J-P, van der Kwast T, Radvanyi F, Chopin D (1999) Functional effect of epidermal growth factor receptor in urothelial regeneration. Eur Urol 35 (Suppl 2): a321
11. Dangles V, Femenia F, Laine V, Berthelemy M, Le Rhun D, Poupon MF, Levy D, Schwartz-Cornil I (1997) Two- and three-dimensional cell structures govern epidermal growth factor survival function in human bladder carcinoma cell lines. Cancer Res 57: 3360
12. Danilenko DM, Ring BD, Lu JZ, Tarpley JE, Chang D, Liu N, Wen D, Pierce GF (1995) Neu differentiation factor upregulates epidermal migration and integrin expression in excisional wounds. J Clin Invest 95: 842
13. Davies BR, Auersperg N, Worsley SD, Ponder BAJ (1998) Transfection of rat ovarian surface epithelium with erbB2/neu induces transformed phenotypes in vitro and the tumorigenic phenotype in vivo. Am J Pathol 152: 297
14. De Boer WI, Schuller AGP, Vermey M, van der Kwast TH (1994) Expression of growth factors and receptors during specific phases in regenerating urothelium after acute injury in vivo. Am J Pathol 145: 1199
15. De Boer WI, Rebel JMJ, Vermey M, de Jong AAW, van der Kwast TH (1994) Characterization of distinct functions for growth factors in murine transitional epithelial cells in primary organotypic cultures. Exp Cell Res 214: 510
16. De Boer WI, Vermey M, Gil Diez S, Bindels E, Radvanyi F, van der Kwast T, Chopin D (1996) Functions of fibroblast and transforming growth factors in primary organoid-like cultures of normal human urothelium. Lab Invest 75: 147
17. De Boer WI, Houtsmuller AB, Izadifar V, Muscatelli-Groux B, van der Kwast T, Chopin DK (1997) Expression and functions of EGF, FGF and TGFβ-growth-factor family members and their receptors in invasive human transitional-cell-carcinoma cells. Int J Cancer 71: 284
18. De Corte V, De Potter C, Vandenberghe D, van Laerebeke N, Azam M, Roels H, Mareel M, Vanderkerckhove J (1994) A 50-kDa protein present in conditioned medium of COLO-16 cells stimulates cell spreading and motility and activates tyrosine phosphorylation of neu/HER-2 in human SK-BR-3 mammary cancer cells. J Cell Sci 107: 405
19. D'Souza B, Taylor-Papadimitrou J (1994) Overexpression of the erbB2 gene in human mammary epithelial cells signals inhibition of transcription of the E-cadherin gene. Proc Natl Acad Sci U S A 15: 7202
20. Dubeau L, Jones PA (1987) Growth of normal and neoplastic urothelium and response to epidermal growth factor in a defined serum-free medium. Cancer Res. 47: 2107
21. Gibson S, Tu S, Oyer R, Anderson SM, Johnson GL (1999) Epidermal growth factor protects epithelial cells against Fas-induced apoptosis. Requirement for Akt activation. J Cell Biol 274: 17612
22. Heldin C-H (1995) Dimerization of cell surface receptors in signal transduction. Cell 80: 213
23. Imai T, Kimura M, Takeda M, Tomita Y (1995) 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 71: 69
24. Jost SP (1989) Cell cycle of normal bladder urothelium in developing and adult mice. Virchows Archiv B Cell Pathol 57: 27
25. Kimball ES, Bohn WH, Cockley KD, Warren TC, Sherwin SA (1984) Distinct high-performance-liquid-chromatography pattern of transforming-growth-factor activity in urine of cancer patients as compared with that of normal individuals. Cancer Res 44: 3616
26. Messing EM, Hanson P, Ulrich P, Erturk E (1987) Epidermal growth factor-interactions with normal and malignant urothelium: in vivo and in situ studies. J Urol 138: 1329
27. Messing EM, Reznikoff CA (1987) Normal and malignant human urothelium: in vitro effects of epidermal growth factor. Cancer Res 47: 2230
28. Pinkas-Kramarski R, Lenferink AE, Bacus SS, Lyass L, van de Poll ML, Klapper LN, Tzahar E, Sela M, van Zoelen EJ, Yarden Y (1998) The oncogenic erbB-2/ErbB-3 heterodimer is a surrogate receptor of the epidermal growth factor and betacellulin. Oncogene 16: 1249
29. Podolski DK (1997) Healing the epithelium: solving the problem from two sides. J Gastroenterol 32**:** 122
30. Rajkumar T, Stamp GWH, Pandha HS, Waxman J, Gullick WJ (1996) Expression of the type 1 tyrosine kinase growth factor receptors EGF receptor, c-erbB2 and c-erbB3 in bladder cancer. J Pathol 179: 381
31. Rebel JMJ, De Boer WI, Thijssen CDEM, Vermey M, Zwarthoff EC, van der Kwast TH (1994) An in vitro model of urothelial regeneration: effects of growth factors and extracellular matrix proteins. J Pathol 175: 283
32. Schultz GS, White M, Mitchell R, Brown G, Lynch J, Twardzik DR, Todaro GJ (1987) Epithelial wound healing enhanced by transforming growth factor-α and vaccinia growth factor. Science 235: 350
33. Southgate J, Hutton KAR, Thomas DFM, Trejdosiewicz LK (1994) Normal human urothelial cells in vitro: proliferation and induction of stratification. Lab Invest 71: 583

34. Thompson JS (1999) Epidermal growth factor inhibits somatostatin-induced apoptosis. J Surg Res 81: 95
35. Vaughn JP, Iglehart JD, Demirdji S, Davis P, Babiss LE, Caruthers MH, Marks JR (1995) Antisense DNA downregulation of the ERBB2 oncogene measured by a flow cytometric assay. Proc Natl Acad Sci U S A 92: 8338
36. Verbeek BS, Adriaansen-Slot SS, Vroom TM, Beckers T, Rijksen G (1998) Overexpression of EGFR and c-erbB2 causes enhanced cell migration in human cancer cells and NIH3T3 fibroblasts. FEBS Lett 425: 145
37. Vinter-Jensen L, Juhl CO, Djurhuus JC, Poulsen SS, Dajani EZ, Brown KD, Ørntoft TF, Teglbjaerg PS, Nexø E (1995) Chronic systemic treatment with epidermal growth factor in pigs causes pronounced urothelial growth with accumulation of glycoconjugates. Am J Pathol 147: 1330