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Expression of Growth Factors and Receptors during Specific Phases in Regenerating Urothelium after Acute Injury *in Vivo*

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We investigated the spatio-temporal changes in RNA and protein expression of growth factors and their receptors by* in situ *hybridization and immunocytochemistry during regeneration after acute injury of mouse urothelium* in vivo. *These data were correlated with changes in morphology and proliferation during regeneration. Except for an enhanced muscular transforming growth factor-β_1 (TGF-β_1) and TGF-β type II receptor expression, changes in expression patterns of growth factors or receptors were confined to the urothelium. Increased mucosal RNA expression of insulin-like growth factor-II (IGF-II) and particularly of type I IGF receptor, as well as fibroblast growth factor-1 (FGF-1) but not of FGF-2, coincided with re-epithelialization and urothelial proliferation. Both high levels of urothelial TGF-β_1 RNA and protein expression were associated with re-epithelialization and differentiation. In addition, TGFβ type II receptor protein expression was similarly enhanced in the same urothelial cells. Platelet-derived growth factor-A (PDGF-A) RNA was expressed constitutively in the mucosa but decreased in the reepithelialization phase. The data are consistent with the notion that urothelial regeneration can be achieved by paracrine or autocrine acting, urothelium-derived growth factors. Since analogous growth factor RNA expression patterns in regenerating skin epidermis have been found, a more general growth factor-regulated mechanism for epithelial regeneration may be suggested. (Am J Pathol 1994, 145:1199–1207)

Bladder urothelium consists of a three-layered transitional epithelium. Under normal conditions, this slowly proliferating epithelium has a very low turnover.[1] Upon urothelial injury, eg, by catheterization, endoscopical examination, biopsies, deposition of urinary crystals, acute outlet obstruction, or short exposure to chemicals, an enormous increase in urothelial proliferation is observed resulting in a rapid re-epithelialization.[1–4] Other organs, such as stomach and gall bladder, also show a rapid re-epithelialization of the mucosa layer upon damage.[5,6] Such a fast epithelialization results in a rapid restoration of the functional integrity of these epithelia. Little is known about the factors involved in this process. Studies on skin epithelium suggested that multiple growth factors and growth factor binding proteins are implicated in wound healing of the skin epithelium. These growth factors may be derived from not only, eg, damaged blood platelets, endothelial basement membranes, and activated, infiltrating macrophages, but also from epithelial cells. Among these growth factors are epidermal growth factor (EGF), transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF).[7–14] Recent studies also demonstrated FGF-1 protein expression in regenerating stomach epithelium[15] and an acceleration of gastric ulcer healing by FGF-1 application in vivo.[16]

Direct actions of growth factors on urothelial cells include stimulation of [^{3}H]thymidine uptake by basal urothelial cells in vivo,[17] stimulation of proliferation and migration of murine urothelial cell lines and urothelial cells in primary culture by EGF and FGF-1,[18–21] inhibition of proliferation and induction of terminal differentiation of mouse urothelial cell lines or urothelial cells in primary culture by TGF-β_1.[20,21] Furthermore, cell cycle progression factors, such as IGF-I and IGF-II, are also required for growth of epi-

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thelial cells, including urothelial cells,[20] whereas the IGF-mediated actions may be affected by insulin-like growth factor binding proteins (IGFBPs).[22,23] Little is known about PDGF action on epithelial cells. Few studies report a biological function of PDGF in epithelia. Antoniades et al[11,12] demonstrated that during regeneration porcine skin epithelial cells express both PDGF and PDGF receptors. Expression of PDGF type α receptor was found in rat olfactory epithelium[24] and in three murine urothelial cell lines.[20] PDGF was shown to enhance growth and maturation of lens epithelium in vitro,[25] to stimulate the proliferation of mouse urothelial cell lines,[20] and to induce migration of retinal pigment epithelial cells.[26]

Few growth factors, such as TGF-β and EGF, normally occur in the urine.[27,28] In addition, normal urothelial cells are reported to express TGF-β RNA *in vivo*.[29,30] Upon injury to skin dermis and epidermis, several growth factors, such as TGFβs, FGFs, IGFs, and PDGFs, are released by both mesenchymal cells (eg, blood platelets, endothelial cells, and activated, infiltrating macrophages) and epithelial cells. In analogy to skin epidermis, it may be hypothesized that these growth factors have distinct functions during urothelial regeneration after injury. Synthesis, expression, and functioning of most growth factors in transitional bladder epithelium has not yet been established during wound healing *in vivo*. In this study we correlated the expression of different growth factors and growth factor receptors at the RNA or the protein level in regenerating urothelium with proliferation, differentiation, and migration of urothelial cells. We used mice in which we shortly overdistended the urine bladder causing acute superficial damage to the urothelium, but little damage to the submucosa and muscle layer. At several intervals after the injury, bladders were dissected for determination of growth factors and growth factor receptor RNA or protein using *in situ* hybridization and immunocytochemical techniques, respectively.

Materials and Methods

Chemicals

RNA polymerases, tRNA, and RNAse inhibitor were obtained from Pharmacia (Woerden, The Netherlands). RNAse-free DNAse was purchased from Promega (Madison, WI), and RNAse T1 from Boehringer (Mannheim, Germany). cDNA probes for mouse IGF-I and mouse IGF-II were kindly provided by Dr. G. I. Bell, the rat type I IGF receptor by Drs. H. Werner and D. LeRoith, and the human PDGF-A and PDGF type α and type β receptor by Dr. C.-H. Heldin. Anti-BrdU antibody IIB5 and anti-TGF-β_1 antibody were kindly donated by Dr. F.C.S. Ramaekers and Dr. W. Boersma. Rabbit polyclonal antibodies against TGFβ type II receptor was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Bladder Injury Experiments

Female BCBA mice 8 to 10 weeks of age were housed in macrolon cages and fed *ad libitum* with water and murine chow food (Hope Farms, Woerden, The Netherlands). The bladder was distended by intravesical injection of 200 µl of 0.15 mol/L NaCl via a catheter. This overstretching was maintained for 5 minutes. Histological examination of the kidneys did not reveal any damage due to reflux of the urine/NaCl. The presence of blood in the urine immediately after the treatment was a qualitative measure for the presence of lesions in the urine bladder. Control mice did not undergo this treatment. After overdistension of the bladder, the mice were kept under the mentioned caging conditions for 1 hour to 10 days. Before mice were sacrificed by CO_2 anesthesia, mice received intraperitoneally BrdU in 0.15 mol/L NaCl (40 mg/kg body weight) during 1 hour. Six mice per interval were sacrificed. Isolated bladders were fixed in 4% phosphate-buffered formalin during 16 hours before embedding in paraffin.

cRNA Probe Generation and Labeling

cDNA fragments specific for the used growth factors or receptors were ligated into pBluescript KS +/– (Stratagene, La Jolla, CA). Labeled RNA transcripts were obtained from linearized plasmid using T7, T3, or SP6 RNA polymerase, respectively, in the presence of 50 µCi [^{35}S]UTP (Amersham, Aylesbury, UK) as described.[31] For the present study we used an *Eco*RI-*Sac*I fragment of PDGF-A cDNA, an Sst II-Pvu II fragment of the PDGF-B cDNA, a *Sac*I-*Kpn*I fragment of the PDGF-α receptor cDNA, a *Pvu*II fragment of the PDGF-β receptor cDNA, a *Bgl*II-*Xho*I fragment of the FGF-1 cDNA, an *Eco*RI-*Acc*I fragment of FGF-2 cDNA, a *Sma*I-*Bam*HI fragment of TGFβ_1 cDNA, an *Eco*RI fragment of mouse IGF-I cDNA, an *Eco*RI-*Sac*I fragment of mouse IGF-II cDNA, and a 265-bp *Eco*RI fragment of the rat IGF type I receptor cDNA. A cDNA probe of rat IGFBP-2 was generated by polymerase chain reaction.[31]

In Situ *Hybridization*

Embedding of bladders, sectioning, and pretreatment procedures were performed as described previously.[31] Sections (5 μ thick) of paraffin-embedded bladders were mounted on slides precoated with 3-amino-propyltrioxy-silane (Sigma Chemical Co., St. Louis, MO). After the pretreatment and subsequent dehydration with an increasing ethanol gradient and drying, the sections were prewarmed to 63 C. Sections were then hybridized with [^{35}S]-labeled RNA probes (2×10^5 cpm/slide) for 16 hours at 63 C in a solution containing 50% formamide, 1× Denhardt's solution, 1 mg/ml tRNA, 10% dextran sulfate, 10 mM dithiothreitol, 0.2 mg/ml herring sperm DNA, and 1× (for the PDGF-A probe) or 4× (for the other probes) SSC. After the hybridization, sections were washed subsequently with 50% formamide/2× SSC at 50 C (two times); 20 mmol/L β-mercaptoethanol/0.1× SSC at 62 C; 1 mmol/L EDTA/2× SSC with 2 U/ml RNAse T1 at 37 C; and 20 mmol/L β-mercaptoethanol/0.1× SSC at 62 C. After dehydration with increasing ethanol gradients containing 0.3 M sodium citrate, the sections were dried and exposed to Kodak AR X-ray film (Eastman Kodak, Rochester, NY) for 1 to 9 days. Finally, sections were exposed to Kodak NTB2 autoradiographic emulsion for 1 to 21 days and developed and stained with nuclear fast red for histochemical analysis. The level of nonspecific binding was determined using RNA sense probes for each growth factor or receptor.

Immunohistochemistry

After removal of paraffin by xylene, sections were hydrated and, before incubation with the anti-BrdU antibody, treated with HCl and Borax buffer as described.[32] Antigen expression was demonstrated with appropriate dilutions of the primary antibody in a conjugated immunoenzyme assay using a secondary peroxidase-conjugated rabbit anti-mouse antibody (DAKO, Etten Leur, The Netherlands). As chromogen served 3,3′-diaminobenzidine tetrahydrochloride (Fluka, Oud-Beijerland, The Netherlands). Finally, sections were counterstained with hematoxylin and mounted in malinol. Phosphate-buffered saline instead of primary antibody was used as a control. As a control for binding of the TGF-β type II receptor antibody, we preincubated this antibody with the TGFβ type II receptor synthetic peptide against which the antibody was raised, as described by the manufacturer. The immunostaining disappeared after this treatment.

Analysis of RNA Expression and Immunohistochemistry

Successive sections of overdistended mouse bladders and untreated mouse bladders were used for hybridization with an antisense probe, immunohistochemical analysis of BrdU expression and morphological analysis, and hybridization with the related sense probe, respectively. The level of RNA expression was determined semi-quantitatively since variations in thickness of the autoradiographic emulsions precluded an exact quantification. The level of RNA expression was compared with a specific binding of the probe to the luminal area of the bladder sections. The sense probes did barely hybridize to the sections. The level of protein expression was also determined semi-quantitatively.

Morphological analysis was done by light microscopy with respect to different parameters. The presence of a single cell layer of flattened, elongated cells in the wound area was considered a parameter for migrating cells. For maturation and differentiation we examined the number of cell layers and the presence of large, flattened, superficially located umbrella cells. Furthermore, we examined the extent of the damage and the repair by the presence of extravascular erythrocytes, edema, and infiltrating leukocytes.

For analysis of the proliferation, the number of BrdU-positive nuclei was counted among 1600 urothelial nuclei at four different, preset sites per bladder. The urothelial proliferation per bladder is expressed as the labeling index (LI):

$$\frac{\text{Number of BrdU-positive nuclei}}{\text{Total number of nuclei}} \times 100\%$$

Both the LI in Table 1 and the RNA or protein expression levels in Figure 3 were calculated as the mean of 6 bladders ± the standard deviation at each time point.

Results

First we examined the morphology and proliferation of regenerating urothelia at different intervals after injury. As presented in Table 1, the urothelia of untreated mice are stratified in three cell layers with normal differentiation into superficial umbrella cells. The overall proliferation is very low to none. One hour after the injury, the damage to the urothelium was multifocal, frequently with a complete loss of urothelium. The overall proliferation was already increased at 8 hours after the injury and was maximal at 16 hours. As early

Table 1. *Changes in Morphology and Proliferation of the Regenerating Urothelium**

	Control	Time after Wounding (hours)						
		1	4	8	16	24	72	110	240
LI	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 1.9	31.2 ± 9.3	21.9 ± 8.5	3.5 ± 1.3	2.1 ± 2.1	0.2 ± 0.2
Cell layers	3	0–3	0–3	0–3	0–3	1–3	3–6	3–6	3–4
Terminal differentiation	+	–	–	–	–	–	–/+	–/+	+

* Morphological and immunohistochemical analysis of regenerating bladder urothelium. The proliferation is given as the mean LI of urothelia from six bladders ± the standard deviation (*see Materials and Methods*). The urothelial maturation and differentiation status is given by the minimal and maximal number of mucosal cell layers in six bladders (cell layers) and the presence of superficial cells with the typical features of umbrella cells within the regenerating area (terminal differentiation). +, umbrella cells present; –, umbrella cells absent; –/+, superficial cells different from intermediate cells.

as 4 hours after the injury, a re-epithelialization was seen in wounds where the urothelium had completely detached. This process started adjacent to intact mucosa and was completed after 24 hours. After 3 days, the LI was decreased and the urothelia were hyperplastic. Terminally differentiated umbrella cells were noted to line hyperplastic urothelia at day 5. The proliferation was normalized after 5 to 10 days of repair and at day 10 the urothelia showed the differentiated stratification of normal, intact urothelium.

The *in situ* hybridization and immunocytochemistry experiments visualized the cells expressing growth factor and growth factor receptor RNA and protein, respectively. The cellular RNA localization in mouse bladders is given in Figure 1, the protein expression in Figure 2. A semi-quantification of the RNA expression levels in the urothelium during regeneration is presented in Figure 3. In untreated mouse urothelium, the expression of FGF-1, FGF-2, IGF-I, type I IGF receptor, and PDGF-B chain RNA was low to undetectable, whereas IGF-II, TGF-β_1, and PDGF-A-chain RNAs were expressed constitutively (Figures 1 and 3). In bladders of control mice, TGF-β_1 RNA localized in particular to the muscle cells. Strikingly, the TGF-β_1 RNA expression in urothelial cells was strongly enhanced within 1 hour after the injury, especially in the

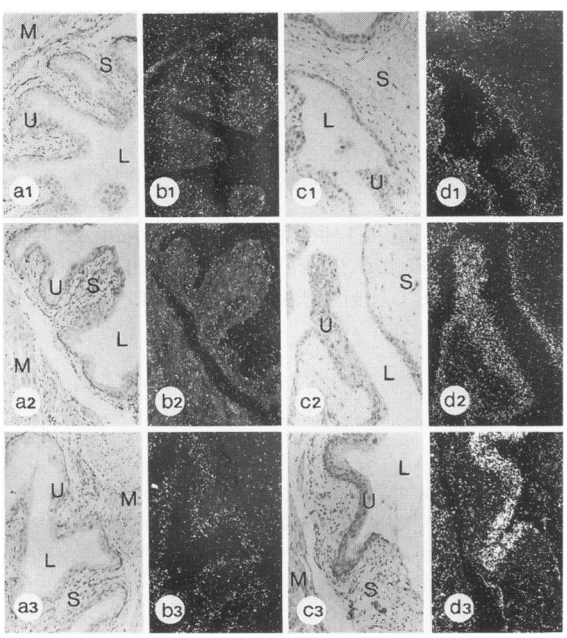

Figure 1. *Localization of RNA expression in the normal, intact mouse bladder* (**a** *and* **b**) *and the regenerating mouse bladder 24 hours after injury* (**c** *and* **d**). *Micrographs* (**a**) *and* (**c**) *are bright field photos, and* (**b**) *and* (**d**) *show the corresponding dark field images. RNA localization for FGF-1, type I IGF receptor, and TGF-*β_1 *is given in micrographs* (**1**) *to* (**3**), *respectively. Sections were stained with nuclear fast red. L, lumen of the bladder; U, urothelium; S, submucosa; M, muscle. Magnification ×200.*

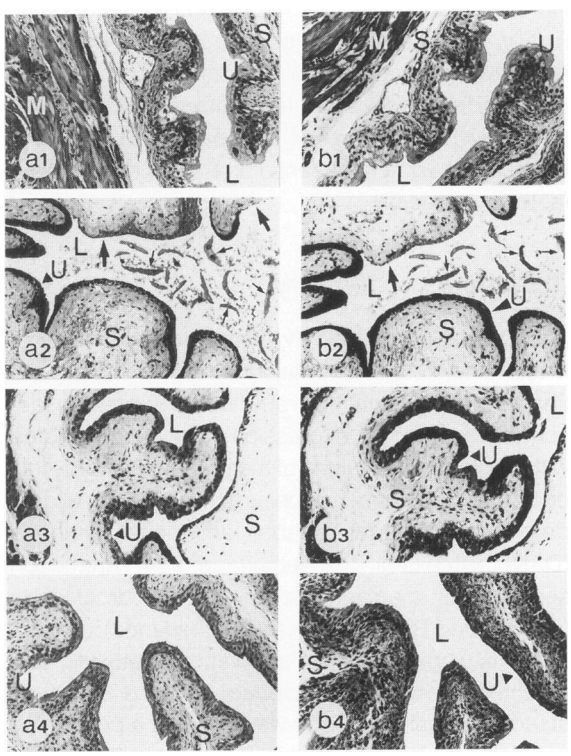

Figure 2. *Immunocytochemical peroxidase-staining of TGF-*β_1 (**a**) *and TGF-β type II receptor* (**b**) *during mouse urothelial regeneration* in vivo. *The micrographs show: intact bladder mucosa from control mice* (**1**), *and damaged mucosa 1 hour, 24 hours, and 5 days after the injury* (**2**, **3**, *and* **4**, *respectively*). *Sections were counterstained with hematoxylin. L, lumen of the bladder; U, urothelium; S, submucosa; M, muscle. The large arrows indicate denuded bladder submucosa. Note the detached umbrella cells in* (**a2**) *and* (**b2**) (*small arrows*). *Magnification ×200.*

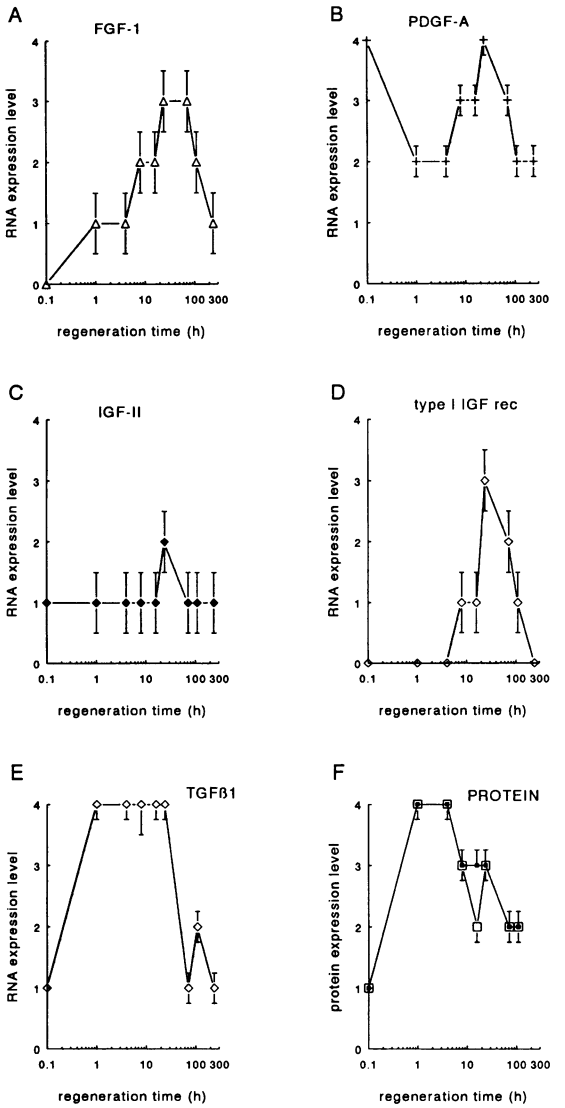

Figure 3. *Semi-quantification of RNA expression levels during regeneration of the urothelium. The regeneration time after injury is given in hours. RNA expression levels of FGF-1, PDGF-A, IGF-II, type I IGF receptor, and TGF-β_1 are given in* (A), (B), (C), (D), *and* (E), *respectively. The TGF-β_1* (□) *and TGF-β type II receptor* (■) *protein expression levels are given in* F. *RNA and protein expression levels were examined in the wounded area of the urothelium and calculated as the mean expression ± standard deviation (n = 6). 0, no detectable expression; 1, low expression; 2, moderate expression; 3, high expression; 4, very high expression.*

epithelial cells next to the site of injury (Figures 1 and 3). This expression pattern changed to an overall high expression throughout the urothelium at 8 hours after the injury and was decreased after 3 days in hyperplastic urothelium (Figure 3 and Table 1). At day 5, when the urothelium started to show a fully differentiated stratification with superficial umbrella cells, the TGF-β_1 RNA expression was increased again (Figure 3). The TGF-β_1 protein expression during urothelial regeneration closely parallelled its RNA expression (Figures 2 and 3). In control bladders, a low level of TGF-β_1 protein was noted in basal urothelial cells, whereas during wound healing both basal and intermediate cells were stained more intensively compared with control bladders. After 5 days, mainly the superficial cells stained for TGF-β_1, coinciding with terminal differentiation of the superficial cells into umbrella cells. In addition, TGF-β type II receptors were expressed by the same urothelial cells and its temporal expression pattern followed that of TGF-β_1 proteins (Figures 2 and 3).

The urothelial RNA expression of PDGF-A chain and, to a lesser extent, PDGF-B chain, as well as FGF-1, but not FGF-2, was increased maximally after 24 hours (Figures 1 and 3), coinciding with the re-epithelialization and enhanced urothelial proliferation (Table 1). Furthermore, the expression of IGF-II and type I IGF receptor was maximal after 24 hours (Figures 1 and 3), also coinciding with re-epithelialization and a maximal urothelial proliferation. In addition, we did not observe alterations in the constitutive expression of IGFBP-2, which was mainly confined to the basal urothelial cells. Expression of IGFBP-1 and IGFBP-3 to -6 was not detectable in the bladder (data not shown). The expression of PDGF type α and type β receptor, as well as IGF-I RNA were undetectably low in the urothelium.

Overstretching the bladder often caused edema of the submucosa and occasionally some hemorrhage in the muscle layer. The edema diminished within 5 days. Only a slight increase in the number of BrdU-positive cells was noted in both the submucosa and the muscle layer between 24 hours and 5 days after the injury. At day 10, the proliferation and morphology of the submucosa and muscle layer was normalized (data not shown). In muscle cells from control bladders, TGF-β_1, TGF-β type II receptor, IGF-II, and, to a lesser extent, IGF-I were highly expressed, while PDGF-B RNA was not detected. The expression of TGF-β_1 RNA and protein in the submucosa, as well as FGF-1 and PDGF-A in both muscle and stromal layers, was low in control bladders. Only the muscular TGF-β_1 RNA and protein and TGF-β type II receptor protein expression levels were slightly increased during the first 3 days; the RNA and protein expression levels of other growth factors and receptors did not change in submucosal or muscle cells during the regeneration process.

Discussion

Here we described several aspects of urothelial regeneration *in vivo*: differentiation and maturation by morphological analysis, proliferation by immunohis-

tochemistry, and localization of several growth factors and their receptors by *in situ* hybridization and immunocytochemistry. We demonstrated that re-epithelialization of mouse urothelium *in vivo* started within 4 hours after the damage and that this re-epithelialization by one cohesive sheet of flattened cells was completed within approximately 24 hours. This early onset and rapid regeneration has also been described for damaged gastric mucosa in rats after exposure to ethanol.[6] During the early phase of regeneration, urothelial cells lining the wound, and adjacent to intact mucosa, were flattened and elongated representing the features of migrating cells. Proliferation activity was particularly enhanced near the wound edges of eroded areas. These observations support the idea that urothelial re-epithelialization is brought about by both an enhanced migration and proliferation analogous to skin wound repair.[33] The second phase in urothelial regeneration seems to be reflected by the stratification into multiple cell layers resulting in hyperplasia at days 3 to 5. In the third phase the hyperplastic urothelium returns to the original state of a structurally normal urothelium within 5 to 10 days after the damage.

In vivo studies located the expression of several of these growth factors and/or their receptors within the epidermis during the process of skin wound healing.[7,11,14,34] Several studies demonstrated that exogenous application of EGF, TGF-β, IGF, PDGF, and FGF to skin wounds enhanced epithelial regeneration *in vivo*.[9,10,13] *In vitro* studies pointed out that these growth factors directly affected proliferation, differentiation, migration, and synthesis of extracellular matrix not only in mesenchymal cells, but also in keratinocytes.[35–39] These data suggest that growth factors can regulate the epithelial regeneration not only by paracrine stroma-epithelium interactions, but also by direct interaction of growth factors with their cognate receptors within the epidermis.

In the present model for acute urothelial injury, the lesions were predominantly confined to the mucosa, whereas inflammatory infiltrates were absent. Except for TGF-β_1 and TGF-β type II receptor, changes in RNA or protein expression of growth factors or receptors were exclusively detected in the urothelium. This implies that the urothelial regeneration in this model does not require a paracrine action of stroma-derived growth factors. Such an autonomous stroma-independent mechanism for epithelial regeneration has also been suggested for acute injured skin epithelium during which both growth factors and their receptors were expressed within the epithelial cells.[11,12,34]

High levels of TGF-β_1 RNA were found during the re-epithelialization and the differentiation phases of wound healing, both in regenerating skin epithelium[40,41] and in regenerating bladder epithelium (this study). This phenomenon agrees with the biological effects of these growth factors on epithelial cells *in vitro*. *In vitro* studies showed that TGF-β_1 indeed induced differentiation of keratinocytes, bronchiocytes, and urothelial cells in primary culture.[21,38,42] On the other hand, both TGF-β_1 and the TGF-β type II receptor were expressed *in vivo* in migrating keratinocytes as well as urothelial cells adjacent to the site of injury[34,40] (this study). This is in concordance with *in vitro* studies, which demonstrated that TGF-β stimulated the migration of undifferentiated keratinocytes only.[8,35] As with IGFs and FGFs, the TGF-β-dependent epithelial migration process is probably mediated by growth factor-induced synthesis and secretion of the extracellular matrix proteins fibronectin and collagen type I and type IV in both mesenchymal and epithelial cells,[35,43] and a concomitant expression of specific receptors for fibronectin, $\alpha 5\beta 1$ integrin, and for collagen, $\alpha 2\beta 1$-integrin.[43,44]

The mucosal RNA expression of FGF-1, but not FGF-2, coincided with the migration and the proliferation phases during urothelial regeneration. A similar spatio-temporal expression pattern was seen during epidermal regeneration.[12] Previous studies demonstrated that FGF-1 could induce the proliferation of several murine urothelial cell lines[18,20] and that FGF-1, but not FGF-2, stimulated the migration of a murine urothelial cell line.[19] In addition, studies on primary cultures of urothelial cells pointed out that FGF-1, but not FGF-2, could induce proliferation and migration of these cells, but not differentiation.[21] These *in vitro* data are in line with the present *in vivo* observation, suggesting that FGF-1 is a more relevant physiological factor than FGF-2 in stimulating migration and proliferation of urothelial cells *in vivo*. *In vitro* studies on keratinocytes showed that FGF-1 could induce both their proliferation and migration.[37,39,45] Furthermore, Hannson and Norstrom[15] demonstrated an enhanced expression of FGF-1 in regenerating stomach epithelium *in vivo*. Apparently, the direct interaction of FGFs with epithelial cells to stimulate migration and proliferation represents a common mechanism in a variety of epithelia. Expression of other growth factors, such as FGF-4, which affects urothelial cells *in vitro*,[46] and FGF-7,[14] were not investigated by us but may also be important for the wound healing process in the bladder.

Whereas the elevation in IGF-II RNA expression was limited, type I IGF receptor RNA expression in the urothelium was strongly enhanced within 24 hours

after injury. In contrast, Antoniades et al[12] failed to observe such a change in IGF or IGF receptor expression in skin epithelium during regeneration. Specific IGFBPs have been identified that can inhibit IGF-mediated effects by preventing the IGFs to bind to their receptor.[22] Albiston et al[47] noted that during regeneration of damaged ileum epithelium, the RNA level of IGF-I did not change, whereas IGFBP-3 RNA levels decreased, suggesting a net increase in the availability of IGF-I for its receptor. Other studies demonstrated an enhanced IGF-I RNA and protein expression in regenerating, undifferentiated epithelial cells of the rat ear and the rat kidney.[7,48] In our model for acute urothelial damage, IGFBP-2 RNA was expressed constitutively and predominantly in basal urothelial cells. This expression did not change during regeneration. Thus, in our model the alterations in type I IGF receptor rather than IGF or IGFBP expression seem to play a regulatory role. The *in vivo* function of IGFs during epithelial regeneration is not clear yet. Both IGF-I and IGF-II can act as progression factors in the cell cycle.[49] IGF-I also induces the proliferation, migration and differentiation of keratinocytes *in vitro*.[36,50] Both the expression of IGF-II and the enhanced type I IGF receptor RNA expression in regenerating urothelium in particular during the migration and proliferation phases may be in line with an autocrine effect of IGF-II on urothelial migration and the progression through the cell cycle needed for proliferation of urothelial cells. Moreover, the growth-promoting actions of IGF-II may be potentiated by the urothelium-derived IGFBP-2.[22]

A high constitutive expression of PDGF-A RNA was observed in mouse urothelium, whereas a small increase in PDGF-B RNA was noted after injury. Although we detected a low PDGF-β receptor RNA expression in mouse urothelium, this was only noted during one hybridization. PDGF-α receptor RNA was not detected. Hence, we are now unable to predict any function of PDGFs during wound healing in the bladder *in vivo*. A role for PDGF in epidermal regeneration has been suggested by several studies.[9–11]

Antoniades et al[12] showed that both epidermal TGF-α and EGF receptor RNA expression were enhanced after skin damage, indicating an autocrine role for TGF-α in epidermal repair. Although EGF in the serous salivary glands of mice was stained with a specific antibody against mouse EGF, we could not detect EGF expression in the bladder (data not shown). However, it cannot be excluded that EGF/TGF-α are also involved in urothelial regeneration analogous to skin epithelium. Indirect evidence for a paracrine role for EGF is provided by the observation that EGF has been shown to enhance proliferation and migration of mouse urothelial cells in primary culture,[21] and that EGF stimulates the uptake of tritiated thymidine by basal rat urothelial cells *in vivo*.[17]

In conclusion, in this model for regeneration of acute injured urothelium the alterations in growth factor and receptor expression (RNA and protein) were mainly confined to the urothelium, indicating that urothelial repair is predominantly driven by the urothelium itself. The growth factor and receptor expression in regenerating transitional epithelium correlated with specific phases in the repair process and with the effects of these growth factors on urothelial cells in primary culture. Our data show a great similarity with expression patterns of growth factors and receptors during epidermal wound healing *in vivo*, suggesting a more general mechanism for epithelial regeneration. The present data may also provide a basis to explain biological consequences of an aberrational growth factor or its receptor expression in transitional cell carcinomas.

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