[Frontiers in Bioscience, 2, d592-595, December 1, 1997]

CELLULAR SIGNALING IN THE BLADDER

Laurence S. Baskin[1,3], Simon W. Hayward[1,2], Ronald A. Sutherland[1], Michael S. DiSandro[1], Axel A.Thomson[2] and Gerald R. Cunha[1,2]

Department of Urology[1], Anatomy[2] and Pediatrics[3] University of California, San Francisco San Francisco, California, 94143-0738

TABLE OF CONTENTS

1. ABSTRACT

Embryologically, the urinary bladder is formed from endodermally derived epithelial cells and mesenchymal cells from the urogenital sinus and allantois. Experimentally, we have shown that bladder mesenchyme differentiates into bladder smooth muscle via an unknown signaling mechanism that originates from the urothelium. It is hypothesized that this signaling between the cellular types, occurs via growth factors. Evidence supporting this hypothesis is that a number of known growth factors, such as TGF beta 2 and 3, KGF and TGF alpha, as well as their receptors are regulated as a function of bladder development and are also modulated during experimental bladder outlet obstruction. Furthermore, growth factors most likely affect extracellular matrix degradative proteins which play a role in bladder remodeling during development, as well as in partial outlet obstruction. There is certainly impressive cellular communication that occurs during development and also occurs postnatally; such as during bladder injury. We have recently shown that KGF is directly responsible for the proliferation of urothelium during bladder injury. This normally quiescent cell, which in humans turns over once every six months to a year when injured, has the incredible ability to immediately proliferate covering the exposed areas of bladder muscle and submucosa. This proliferation is due to the direct effects of KGF, a classic paracrine growth factor which is secreted by the stromal compartment of the bladder and acts directly on the urothelium which harbors the receptor. The bladder also has an uncanny ability to regenerate. In a model to study the basic science behind bladder regeneration, a partial cystectomy was performed and an acellular tissue matrix devoid of all cellular elements was sutured to the defect. Within four days, the urothelium completely covered the acellular matrix, and within two weeks native smooth muscle was seen streaming into the acellular matrix in association with a new epithelium. It is hypothesized that cellular interactions between the epithelium and the mesenchyme, as we have shown in bladder differentiation, are encouraging the new growth of smooth muscle.

For the bladder to be a safe and effective storage chamber the ideal cellular lining should be urothelium.Cells from the gastrointestinal are not optimal for this purpose since they either secrete or absorb electrolytes. We believe that the cellular interactions that occur between the urothelium and the foreign intestinal stroma will in time change the phenotype of the urothelium. Newer strategies for bladder replacement which take into account cellular signaling are critical for our young patients with neurogenic bladder disorders.

Received 11/13/97 Accepted 11/25/97

Send Correspondence to: Laurence S. Baskin, M.D., Chief Pediatric Urology, University of California, San Francisco, UCSF Children's Medical Center, San Francisco CA, 94141, Tel: (415)-476-1612, Fax:(415)-476-8849, E-mail: baskinl@itsa.ucsf.edu

2.MESENCHYMAL-EPITHELIAL INTERACTIONS

Embryologically, the urinary bladder is derived from undifferentiated mesenchyme and endoderm of the urogenital sinus and allantois. During development, the undifferentiated mesenchyme differentiates into bladder smooth muscle (1). What are the signaling mechanisms that allow this process to occur? Previously, we have shown that epithelium is necessary for bladder smooth muscle differentiation (figure 1)(2). Using the rat as an experimental model, we first defined the ontogeny of both epithelial and smooth muscle differentiation using a panel of smooth muscle and epithelial proteins (1). The smooth muscle proteins, alpha-actin, myosin, vinculin, desmin, laminin and vimentin, are sequentially expressed as a function of developmental stage. In the embryonic rat bladder at 14 days gestation, none of these markers were detected by immunostaining, whereas at 16 days gestation, when smooth muscle is first noted by histologic criteria, alpha-actin stained cells are detected in the periphery of the bladder. Subsequently, these alpha-actin positive cells sequentially acquire the remaining smooth muscle markers. We hypothesized that epithelial mesenchymal signaling is required for the smooth muscle differentiation to occur (2). To test this hypothesis, 14 day embryonic rat bladders

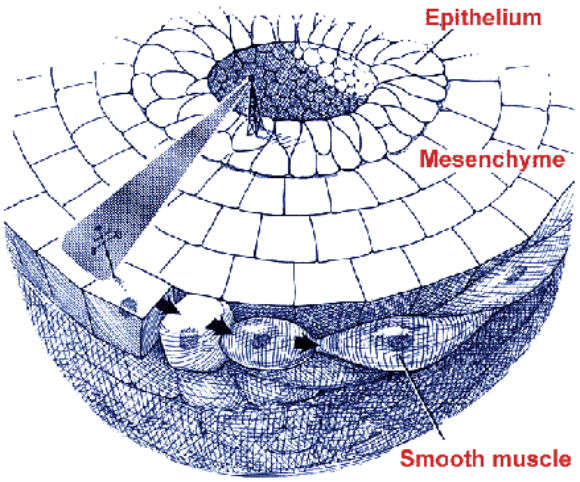

Figure 1. Epithelium is required for bladder mesenchyme to differentiate into bladder smooth muscle. Signaling occurs via an unknown mechanism possibly diffusable growth factors (12) (Used with Permission) .

(prior to the differentiation of smooth muscle) were isolated and the epithelium was separated from the mesenchyme following tryptic digestion which degrades the epithelial basement membrane (3). The isolated bladder mesenchyme, bladder mesenchyme recombined with urothelium and intact embryonic bladders were then grown underneath the kidney capsule of syngeneic hosts. The intact 14 day embryonic bladders underwent normal differentiation, defined by the expression of smooth muscle proteins. In contrast, when isolated bladder mesenchyme was grown alone underneath the kidney capsule, the tissue survived, but the mesenchyme did not differentiate into smooth muscle. However, when bladder mesenchyme was recombined with bladder epithelium, smooth muscle differentiation occurred, although this was less organized than if the epithelium was left intact in its natural state.

The same experiments were repeated *in vitro*, where intact embryonic bladders prior to smooth muscle differentiation were grown on top of filter paper nourished by standard tissue culture medium. Compared to bladder mesenchyme cultured alone, there was impressive growth of the intact organs. When epithelium was recombined with bladder mesenchyme, again the growth was greatly improved compared to the mesenchyme alone. Labeling of the organ culture with ^{3}H-thymidine showed extensive cellular proliferation in the tissue recombinants composed of bladder mesenchyme plus bladder epithelium and in intact bladders, whereas in the mesenchyme alone, little growth occurred. Histologically, tissue recombinants of bladder mesenchyme plus epithelium structures revealed healthy cellular histology where as the bladder mesenchyme alone showed evidence of cellular degeneration.

A final experiment determined whether epithelium from a different species could induce bladder smooth muscle differentiation. This hypothesis was tested by performing a unilateral nephrectomy in nude mice (2). Undifferentiated, 14 day rat bladder mesenchyme with the epithelium removed was then surgically attached to the severed mouse ureter. The bladder mesenchyme and mouse ureter were allowed to grow for one month *in situ*, with the idea that the ureteral epithelium would induce smooth muscle differentiation of the rat bladder mesenchyme. As expected, the mouse urothelium induced smooth muscle differentiation in the rat bladder mesenchyme resulting in the expression of smooth muscle differentiation markers. Species specific probes confirmed that the epithelium was of mouse origin and the smooth muscle was from the rat. These experiments strongly support the concept that cellular signaling between the bladder epithelium and undifferentiated bladder mesenchyme, is required for the induction of bladder smooth muscle.

A number of questions remain unanswered. Why does bladder smooth muscle initially form in the periphery of the bladder at the greatest distance from the signaling epithelium? Does signaling occur by diffusable factors, (a hypothesis that we have subsequently been testing) or does it occur by cellular contact of mesenchymal cells with the epithelial basement membrane followed by signaling peripherally through the undifferentiated mesenchyme to the sub-serosal zone? These questions are the basis for further research.

.

3. GROWTH FACTORS

If diffusable growth factors play a role in smooth muscle differentiation, one would expect to see differences in their expression during development. We therefore utilized RNAse protection assays for a panel of growth factors at strategic times during bladder development and smooth muscle differentiation (4). Specifically, we examined bladders at the embryonic stage before smooth muscle development, (14 days gestation), at the time of first smooth muscle differentiation, (16 days gestation), and then at subsequent time points, 18 days gestation, newborn, 20 days postnatally, and in adulthood. We quantified growth factors that have known differentiation properties in other organ systems. Specifically, we assessed transcripts for KGF and the KGF receptor, TGF alpha and EGF receptor, and the TGF beta family. We found that TGF beta 2 and 3 are regulated as a function of development, being expressed at a high level in early gestation and then decreasing as a function of time. TGF alpha and KGF were expressed in opposite fashion, being minimally expressed in the embryonic period with a greater expression in the mature bladder.

We then created a model of bladder outlet obstruction in the rodent in order to study the effect of obstruction on smooth muscle remodeling (5). This resulted in a six-fold increase in bladder volume and a five-fold increase in bladder weight, as well as a doubling of smooth muscle cell diameter. The mRNA for the growth factor TGF beta 2 increased two-fold, TGF beta 3 increased five-fold. The mRNA for TGF alpha was elevated ten-fold. In contrast, the transcripts for KGF and the receptors for KGF and EGF did not exhibit any changes. TGF betas are known to affect extracellular matrix synthesis, and it may be that the changes in the bladder wall and increased matrix deposition resulted from the up-regulation of these growth factors.

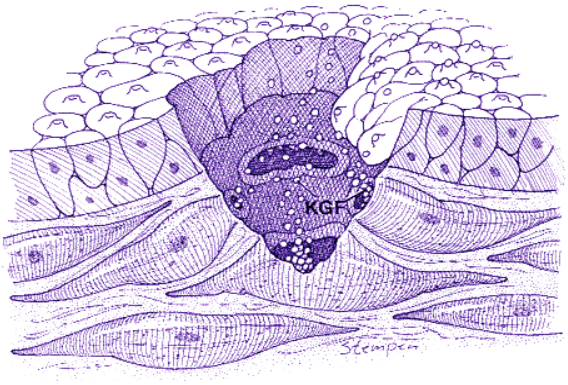

Figure 2. Keratinocyte Growth Factor is released from bladder stroma cells during bladder injury and directly acts through its urothelial located receptor to cause proliferation and regrowth of urothelial cells mending the bladder injury. (12) (Used with Permission) .

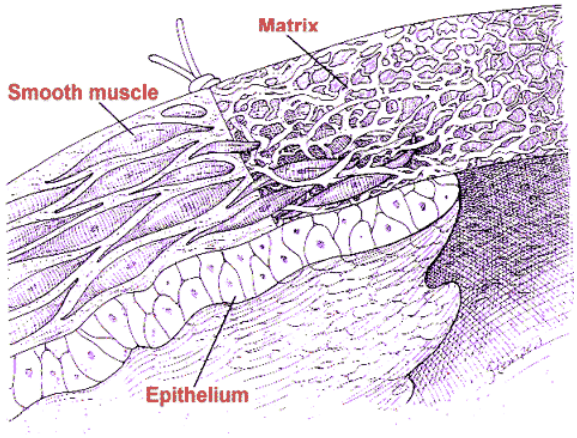

Figure 3. A model of bladder regeneration with an acellular tissue matrix shows that urothelium, smooth muscle and nerves can grow into the tissue matrix most likely under the influence of stromal-epithelial cellular communication. (12) (Used with Permission).

4. BLADDER WOUND HEALING

Further evidence for cellular signaling via growth factors has been shown with our model of bladder wound healing (6). Again, using the rodent as a model, an incisional wound was created in the bladder and then sutured with primary closure. Histologic analysis revealed that the bladder epithelium regrew over the defect within 24 to 48 hours. RNase protection assays of growth factors showed an eightfold increase in the expression of KGF as compared to sham-operated and control animals (6). Interestingly, this increased expression occurred in the first 12 to 24 hours corresponding to the period of intense epithelial proliferation noted histologically. At 5 and 7 days, when the epithelium had completely regrown, the expression of KGF returned to normal.

Another interesting finding is that when the bladder wall was sampled at a distance from the injury, there was still an up-regulation compared to sham and non-operated animals, suggesting that global signaling occurs throughout the bladder in response to injury which facilitates epithelial repair (figure 2). The proliferative activity of the urothelial cell is impressive as it is normallyquiescent with turnover every six months to a year (7). However, following bladder epithelial injury, KGF released by the stroma elicits receptors on the epithelial surface to cause the urothelium to proliferate (8). Further support for the direct effect of KGF in the bladder was tested by injecting human recombinant KGF subcutaneously into neonatal mice. In comparison to saline controls, urothelial proliferation was greatly enhanced as judged by ^{3}H thymidine labeling 6. A similar finding has been reported for the monkey bladder by Ye, *et al* (9).

One of the most clinically relevant areas of bladder signaling may occur at the border between native bladder and augmented intestinal segments (10). Here, the bladder urothelium is placed in an environment where it receives signals from the stroma of both the native bladder as well as the stroma of the gastrointestinal graft. Although the incidence of tumors in augmented bladders is low, tumors tend to occur exactly at this surgical juncture. We hypothesize that the abnormal signaling at this site leads to aberrant communication leading to cellular atypia. Further work is underway using tissue recombinations to assess the effects of intestinal stroma on bladder urothelium and vice versa.

5. BLADDER REPLACEMENT

One of the most exciting areas of bladder research is development of new methods of bladder augmentation which avoid the use of intestinal segments. We have created a model of bladder augmentation in which we use an acellular tissue matrix to elicit regeneration of bladder tissue (figure 3) (11). In this model, the dome of the bladder is excised (again, this was first performed in the rodent model) and acellular tissue matrix made from either the bladder or the stomach is sutured to the surgical defect. The acellular matrix is prepared by incubating the bladders or stomachs of adult rats in distilled water for one hour to lyses the cells and to release intracellular contents. Tissues are then suspended in 40 ml of 40% sodium deoxycholate (Sigma Chemical Company, St. Louis, MI), for 2 to 4 hours, followed by treatment with 2,000 Kunitz units of Deoxyribonuclease I (Sigma Chemical Company) in 1 M sodium chloride solution for 1 to 2 hours. This process is repeated 1 to 3 times to extract all cellular material. Histologic confirmation shows an absence of cells. The preparation of the acellular matrix is designed to avoid complete removal of potentially important growth factors, as well as other extracellular matrix ligands. After suturing the acellular matrix patch in place, animals were sacrificed at serial time points to specifically study the interface between the native bladder and the acellular matrix tissue. Complete re-epithelization with urothelial cells occurred by 4 days and smooth muscle regenerated into the acellular matrix by 2 weeks post-grafting. Interestingly, the smooth muscle grew in juxta-position to the epithelial surface, which over time matured into normal size bundles by 26 weeks post-grafting.

Neovascularization was seen by 2 weeks and neural elements from the native bladder formed around the developing bundles of smooth muscle as early as 4 weeks. These animals were able to void and function normally. Careful histologic analysis showed that the acellular tissue matrix did not regenerate into completely normal bladder, but over time smooth muscle bundles did organize into their appropriate position in the periphery of the bladder, and the urothelium itself covered this matrix, presumably under the influence of KGF from tissue injury (6). This model has been very useful for studying the cellular signaling that occurs in the native bladder, especially in relation to potential bladder augmentation and bladder replacement.

6. CONCLUSION

In conclusion, cellular signaling plays an integral part in bladder development and its response to injury, such as obstruction and wound healing. A better understanding of these processes should allow for the strategic design of new therapies, especially in relation to bladder augmentation and bladder replacement.

7. ACKNOWLEDGEMENT

Supported by NIH Grants K08 DK02397-01, CA 59831, DK51101 and DK 47517

8. REFERENCES

1. L. S. Baskin, S. W. Hayward, P. Young and G. R. Cunha: Ontogeny of the rat bladder: Smooth muscle and epithelial differentiation. *Acta Anatomica* 155: 163-171 (1996)

2. L. S. Baskin, S. Hayward, P. Young and G. Cunha: Role of mesenchymal-epithelial interactions in bladder development. *J. Urol* 156: 1820-1827 (1996)

3. L. S. Baskin, S. W. Hayward, R. A. Sutherland, M. J. DiSandro, A. A. Thomson, J. Goodman and G. R. Cunha: Mesenchymal-epithelial interactions in the bladder. *World J Urol* 14(5): 301-9 (1996)

4. L. S. Baskin, R. S. Sutherland, A. A.Thomson, S. W. Hayward and P. D. Gerald R. Cunha: Growth Factors and Receptors in Bladder Development and Obstruction. *Lab Invest* 75: 157-166 (1996)

5. R. S. Sutherland, L. B. Baskin, F. Elfman and G. R. Cunha: The role of type IV collagenases in rat bladder development and obstruction. *Ped Res* 41(3): 430-434 (1997)

6. L. Baskin, R. Sutherland, A. Thomson, H. Nguyen, D. Morgan, S. Hayward, Y. Hom, M. DiSandro and G. Cunha: Growth Factors in Bladder Wound Healing. *J. Urol* 157(6): 2388-2395 (1997)

7. R. M. Hicks: The mammalian urinary bladder: an accommodating organ. *Biol Rev Camb Philos Soc* 50(2): 215-46 (1975)

8. S. A. Aaronson, D. P. Bottaro, T. Miki, D. Ron, P. W. Finch, T. P. Fleming, J. Ahn, W. G. Taylor and J. S. Rubin: Keratinocyte growth factor: A fibroblast growth factor family member with unusual target cell specificity. *Ann. New York Acad. Sci*. 638: 62-77 (1991)

9. E. S. Yi, A. S. Shabaik, D. L. Lacey, A. A. Bedoya, S. Yin, R. M. Housley, D. M. Danilenko, W. Benson, A. M. Cohen, G. F. Pierce and et al.: Keratinocyte growth factor causes proliferation of urothelium in vivo. *J Urol* 154(4): 1566-70 (1995)

10. J. Barrington, S. Fulford, D. Griffiths and T. Stephenson: Tumors in Bladder remnant after augmentation enterocystoplasty. *J. Urol* 157: 482-486 (1997)

11. R. S. Sutherland, L. B. Baskin, S. W. Hayward and G. R. Cunha: Regeneration of Bladder Urothelium, Smooth Muscle, Blood Vessels and Nerves Into an Acellular Tissue Matrix. *J. Urol* 156(2): 571-577 (1996)

12. L. Baskin: The fate of the bladder in patients with congenital infravesical obstruction. *Curr. Opin. Urol*. 7: 325-330 (1997)