Two members of the fibroblast growth factor (FGF) family of polypeptides were identified as positive regulators of urothelial proliferation. FGF-7 and FGF-10 were cloned from human urinary bladder and expressed as biologically active proteins in the cytoplasm of Escherichia coli. Each recombinant (r) protein was engineered to contain a C-terminal histidine hexamer. Both rFGF-7-His and rFGF-10-His bound heparin with affinities similar to those of their wild-type counterparts. Each polypeptide stimulated the incorporation of [3H]-thymidine into the cellular DNA of primary cultures of human urothelial cells in vitro. Stimulation of DNA synthesis was concentration dependent. Analyses of cellular lysates indicated that the 92-kDa FGF-R2IIIb transmembrane receptor kinase is involved for transducing the rFGF-7-His signal but not necessarily the rFGF-10-His signal. Immunoprecipitations of FGF-R2IIIb+rFGF-7/-10-His complexes have revealed the existence of a novel 72-kDa polypeptide from urothelial cells cultured from renal pelvis or from urinary bladder.

Secreted protein acidic and rich in cysteine (SPARC) was identified as a negative regulator of urothelial cell proliferation. Recombinant SPARC inhibited urothelial cell DNA synthesis under the same conditions in which recombinant FGF was mitogenic. Urothelial-derived SPARC was found to be secreted into the abluminal compartment. It is hypothesized that constitutively-expressed levels of SPARC function to maintain the urothelium in a quiescent state in vivo. A proposed mechanism to achieve this quiescence is by abrogating the binding of FGF to its cognate receptor on the basolateral face of the urothelial cell plasma membrane.

ICBR-11

Role of Prostaglandins in Bladder Smooth Muscle Cell Gene Expression

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Bladder smooth muscle cells (SMC) play an important role in the progression of bladder deterioration after outlet obstruction. Altered mechanical environment within the obstructed bladder may be a critical factor in triggering pathological bladder SMC responses. We have previously demonstrated that bladder obstruction stimulated the expression of inducible cyclooxygenase isoform, COX-2, in bladder SMC and that this occurred by increased mechanical stretch. COX-2 regulates a key rate-limiting step in prostaglandin biosynthesis. The exact function of COX-2 gene activation in bladder SMC is not known, but a possible role may be that COX-2-mediated prostaglandins regulate the expression of other downstream genes. Peroxisome proliferator-activated receptor-γ (PPARγ) is a member of a group of nuclear receptors that play a key role in adipocyte differentiation and inflammation. It has been demonstrated recently that a prostaglandin J2 metabolite, 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2), is a potent endogenous ligand for PPARγ.

We investigated whether this paradigm of 15d-PGJ2/PPARγ nuclear regulation exists in bladder SMC in the setting of outlet obstruction. By Western immunoblot analysis, we were able to detect the presence of PPARγ protein in the rat bladder SMC in vivo. Furthermore, with chronic outlet obstruction, there was a specific pattern of alteration in PPARγ protein levels. When the partially obstructed rat bladders were assessed at up to 6 weeks, COX-2 gene activation was seen only during the first 24 hours, whereas PPARγ levels transiently declined between 1 and 3 days of obstruction and gradually increased thereafter. Although the exact functional significance of these changes is not known, such a pattern of PPARγ levels suggests a possible biologic role. We also studied the role of the 15d-PGJ2/PPARγ nuclear regulation pathway in the stretch-stimulated expression of COX-2 in cultured bladder SMC. Primary rat bladder SMC were grown on collagen I–coated silicone membranes and stimulated with cyclical stretch and relaxation (0.1 Hz, 20% elongation; FX-3000, Flexercell Corp., McKeesport, Pa) in serum-deficient media. When the cells were stretched in the presence of 15d-PGJ2, there were dose-specific alterations in the stretch-induced expression of COX-2. At 1 to 5 μmol/L 15d-PGJ2, there was a synergistic superinduction of COX-2 level after stretch stimulation. At 10 μmol/L, however, 15d-PGJ2 significantly attenuated COX-2 expression. This superinduction response by low-dose 15d-PGJ2 was completely suppressed by p38/SAPK inhibitor, SB203580 (10 μmol/L), and was unaffected by ERK/MAPK inhibitor, PD98059 (30 μmol/L). By electrophoretic mobility shift assay, we also demonstrated that stretch-induced activator protein-1/DNA binding was similarly affected by 15d-PGJ2 in a dose-specific manner. A synthetic thiazolidinedione compound known to activate PPARγ, troglitazone (20 μmol/L), suppressed the stretch-stimulated COX-2 expression, whereas a PPARγ-specific ligand, WY-14643 (10-100 μmol/L), had no effect. Interestingly, when the cells were stretched for 24 hours in the presence of COX-2 inhibitor, NS-398 (30 μmol/L), PPARγ protein levels decreased in bladder SMC.

Collectively, these findings suggest that PPARγ may be a novel transcriptional regulator for bladder SMC in the setting of outlet obstruction. Furthermore, 15d-PGJ2/PPARγ pathway may be a potential mechanism by which COX-2-mediated prostaglandins play a role in the nuclear regulation of downstream target genes in bladder SMC.

ICBR-12

Uropathogenic Escherichia coli: Interactions with Bladder Epithelium

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Uropathogenic strains of Escherichia coli are the primary causative agents of cystitis and upper urinary tract infections. Filamentous surface-adhesive organelles called type 1 pili are encoded by virtually all strains of uropathogenic E. coli and are assembled via the chaperone/usher pathway. The periplasmic chaperone comprises 2 immunoglobulin(Ig)-like domains. It forms chaperone-subunit complexes that are targeted to the outer membrane usher, where the subunits are assembled into the pilus.

Using high-resolution electron microscopy, we have shown that the adhesive tips of type 1 pili, which contain the adhesin molecule FimH, can interact directly with host receptors on the luminal surface of the bladder epithelium. FimH is composed of a pilin domain and a receptor-binding domain based on x-ray crystallography. The pilin domain of FimH and the single domain of the pilus subunits have Ig folds that lack the seventh (C-terminal) β-strand present in canonical Ig folds. The absence of this strand produces a deep groove along the surface of the pilin domain. In the chaperone-subunit complexes, the chaperone contributes its G1 β-strand to complete the Ig fold of the subunit by occupying the groove, an interaction termed “donor strand complementation.” During pilus
ion channel, recently discovered by Caterina et al. (Nature 389: 816, 1997), known as vanilloid receptor subtype 1 (VR1). This receptor is a nonselective cation channel and is activated by small myelinated A-δ fibers. C-fiber afferents, which are small and unmyelinated, have very high mechanical thresholds and do not respond to even high levels of intravesical pressure. C-fibers are activated by noxious chemical irritation or by cold. In the irritated state, these fibers become responsive to low-pressure bladder distension–like mechanoreceptive A-δ fibers. C-fibers, therefore, are normally “silent” and appear to have a specific function, i.e., signaling of inflammatory or noxious events in the bladder (Chancellor and de Groat, J Urol 162: 3–11, 1999). The vanilloids, capsaicin and resiniferatoxin, activate nociceptive sensory nerve fibers through an ion channel, recently discovered by Caterina et al. (Nature 389: 816, 1997), known as vanilloid receptor subtype 1 (VR1). This receptor is a nonselective cation channel and is activated by increases in temperature to within the noxious range and by protons, suggesting that it functions as a transducer of painful thermal stimuli and acidity in vivo. When activated, the channel opens, allowing an influx of calcium and sodium ions that depolarize the nociceptive afferent terminals, initiating a nerve impulse that travels through the dorsal root ganglion (DRG) into the central nervous system. Noxious temperature uses the same elements, which explains why the mouth feels hot when eating chili peppers (Clapham, Nature 389: 783, 1997). Previously called the capsaicin receptor, VR1 has been localized in the spinal cord, DRG, and visceral organs, including the bladder, urethra, and colon. Activation of VR1 results in spike-like currents (Liu and Simon, J Neurophysiol 75: 1503, 1996), and selectively excites and subsequently desensitizes C-fibers. Capsaicin desensitization is defined as long-lasting, reversible suppression of sensory neuron activity (Craft et al., Pain 61: 317, 1995).

Lazzeri et al. (J Urol 163: 60A, 2000) recently presented prospective, randomized results of intravesical RTX in interstitial cystitis (IC) patients from Italy. In 18 patients with bladder hypersensitivity, there was a significant improvement in urinary frequency per 24 hours, nocturia, and pain scale recording in RTX (10 nmol/L) compared with placebo (saline) treatment patients after 30 days, and there was partial persistent effect after 90 days. We feel it would be a priority to study intravesical RTX in IC patients in a US Food and Drug Administration–approved protocol in the United States.

We believe that the field of urology, along with all specialties of medicine, is on the brink of a revolution called molecular medicine. Whereas traditional medicine treats symptoms, gene therapy addresses the deficiency that causes the symptoms. With improved understanding of the human genome and evolving techniques to construct gene therapy vectors that manipulate our genetics, the way we practice medicine will be forever changed.

We propose a revolutionary concept in the treatment of IC and visceral bladder pain that is independent of etiology. We hypothesize that targeted and localized expression of enkephalin in the nerves that innervate the bladder by gene transfer can treat bladder pain. β-Galactosidase staining was used to detect lacZ expression in female Sprague-Dawley (250 to 300 g) rat bladder and L6 DRG after bladder injection with SHZ virus (herpes simplex virus [HSV]–1 with lacZ insert, 5 × 10⁸ plaque-forming units [pfu]) (Figure). At 7, 14, and 30 days after bladder inoculation with SHPE (HSV-1 with preproenkephalin [PPE] cDNA insert, 5 × 10⁴ pfu), bladder tissue and DRG (L4, L6, S1) transgene levels were quantified with polymerase chain reaction (PCR) techniques using primers specific for human PPE gene. L4 was chosen for the minimal afferent innervation from the bladder. Preliminary CMG experiments were performed in untreated Sprague-Dawley rats after administration of intrathecal met-enkephalin and intrathecal naloxone. Cystometric studies under urethane anesthesia were also done 1 week after injection with SHPE (n = 10) or SHZ as control (n = 10). Continuous intravesical capsaicin (15 μM) infusion was used as a bladder irritant, whereas intramuscular naloxone (0.5 mg/kg) was used as an opioid antagonist. β-Galactosidase staining was observed in bladder and L6 DRG 1 week after bladder injection with SHZ. Small-