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# Molecular Mechanisms Related to Parturition-Induced Stress Urinary Incontinence

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## Abstract

**Background—**The molecular mechanisms underlying stress urinary incontinence (SUI) at the tissue level are poorly understood.

**Objective—**To study genetic and molecular alterations in the urethra of animals with experimentally induced SUI.

**Design/Setting/Participants—**Cohort analysis of primiparous 2-month-old female Sprague-Dawley rats with experimentally induced SUI versus those who did not develop SUI in a university research laboratory setting

**Intervention—**Within 24 h of parturition, rats underwent intravaginal balloon dilation and bilateral ovariectomy. Transvesical cystometry was performed 12 wk after parturition. Rats were classified as continent (C) or incontinent (I) according to the results of cystometry.

**Measurements—**The expression of over 22,000 genes in urethral tissue from the two groups was assessed with the use of an oligo microarray. The expression of relevant genes was confirmed by real-time polymerase chain reaction. Protein expression of small mothers against decapentaplegic 2 (Smad2), one of the differentially expressed genes, was extensively studied by immunohistochemistry and Western blot analysis. Regulation of Smad2 activity by transforming growth factor-β (Tgf-β) was assessed in cultured urethral smooth muscle cells (USMCs).

**Results & Limitations—**After intervention, 14 (58.3%) rats remained continent and 10 (41.7%) became incontinent. There were significant differences in the expression of 42 urethral genes between continent and incontinent rats. The expression of genes involved in the TGF cellular signaling pathway (Smad2), collagen breakdown (matrix metalloproteinase 13 [Mmp13]), and smooth muscle inhibition (regulator of G-protein signaling 2 [Rgs2]) was significantly increased in the incontinent group. Smad2 protein expression was significantly upregulated in the incontinent rats. In cultured USMCs, Smad2 phosphorylation and nuclear translocation increased after Tgf-β treatment.

**Conclusions—**Genes important in inflammation, collagen breakdown, and smooth muscle inhibition are upregulated in the urethras of female rats with parturition-associated incontinence.



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**Keywords**

Birth trauma; Stress urinary incontinence; Molecular mechanisms oligo microarray; Sprague-Dawley rat

## 1. Introduction

Stress urinary incontinence (SUI) is a prevalent urological problem that is most common in women. It had been reported that up to 50% of women older than 60 yr have symptoms of stress-induced or urge urinary incontinence [1,2]. Although progress has been made in the treatment of SUI [3], our understanding of the molecular mechanisms underlying the condition is poor.

Chen et al [4] studied gene expression in the periurethral connective tissue of the vagina in human women with SUI; they found that genes related to elastin metabolism were upregulated compared with healthy controls. Chen further demonstrated that expression of matrix metalloproteinase 1 (MMP1) messenger RNA was increased in human female SUI patients, suggesting that increased collagen breakdown may be an etiological factor in SUI via breakdown of pelvic ligamentous tissues [5].

In light of the limited availability of human tissue for study, animal models are an important adjunct in improving our understanding of SUI [6,7]. In 1999, our laboratory developed a rat model of parturition-associated female SUI in which female rats were subjected to intravaginal balloon dilatation (similar to birth trauma) after pregnancy and vaginal delivery of pups, followed by ovariectomy 1 wk later to simulate menopause. These investigations indicated that the final common pathway for SUI seems to be neuronal and muscular (smooth and striated) changes in the urethra and pelvic floor. Our prior studies have included functional, anatomical, and histological assessment of these model animals and have indicated that our model system approximates the human condition of SUI [6,7]. As a follow-up to our initial studies, we investigated molecular changes in the urethra of rats with parturition-associated SUI by means of oligo microarray, real-time polymerase chain reaction (PCR), Western blot analysis, and cell culture techniques.

## 2. Materials and methods

### 2.1. Animals and overview

Twenty-four, 2-month-old, female primiparous pregnant (at gestational day 16) Sprague-Dawley rats weighing approximately 230[en]280 g were obtained from a commercial vendor. They were housed at a constant 16 °C room temperature and 47% humidity, with a 12-h light-dark cycle. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of California at San Francisco.

### 2.2. Delivery, balloon dilation and ovariectomy treatment, and transvesical cystometry

Creation of the animal model and transvesical cystometry were performed as previously described [6,7]. Briefly, all rats were anesthetized with ketamine (100 mg/kg) and, within 24 h of pup delivery, underwent intravaginal balloon dilation with a Foley catheter balloon inflated with 3 ml of fluid for 3 h to simulate prolonged labor. One week after balloon dilation, the rats were again anesthetized with ketamine and underwent bilateral ovariectomy.

Twelve weeks after delivery (11 wk after ovariectomy) the animals were sedated with ketamine. A midline laparotomy was made and a 27G butterfly needle was inserted into the

bladder dome. All urine was evacuated. With the use of three-way stopcock, the needle was connected to both a Baxter Uniflow pressure transducer (Baxter Healthcare Corp, Irvine, CA, USA) and a Harvard Model 22 infusion pump (Harvard, Millis, MA, USA). Saline at 37 °C was infused at 0.1 ml/min after calibration of the pressure transducer to zero atmospheres. Data were collected wit the use of a customized Macintosh Quadra 800 (Apple Computer, Inc, Cupertino, CA, USA) and LabView 4.0 software (National Instruments Corp, Austin, TX, USA).

Micturition was considered normal if a cystometric pressure increase was followed by expulsion of saline from the meatus and bladder emptying. Animals were classified as incontinent if intermittent leakage occurred without bladder contraction during bladder filling. Upon completion of the cystometry, the animals were euthanized and samples of the whole urethra were carefully dissected for microarray analysis, real-time PCR, Western blot analysis, and immunohistochemical evaluation.

### 2.3. Microarray analysis

Rat Genome Oligo Set, version 3.0, which contains 26,962 probes representing 22,012 genes, was purchased from Qiagen, Inc (Valencia, CA, USA). The probes/genes were printed on polylysine-coated glass slides by using the standard protocol (http://derisilab.ucsf.edu/core/protocols) on a microarrayer designed in 48 blocks by Galfilemaker 3.0 (http://derisilab.ucsf.edu/core/softwares).

Total RNA from the urethra was isolated with the RNAeasy isolation Kit (Qiagen). All RNA used in this experiment was of high quality as indicated by a ratio of 2:1 for 28S/18S rRNA and a ratio of >1.9 for $OD_{260}/OD_{280}$. According to the policy for the designing of a microarray experiment, we selected the universal RNA method. One microgram of urethral RNA, side by side with 1 μg of universal rat reference RNA (Stratagene, La Jolla, CA, USA), was linearly amplified through two rounds of modified in vitro transcription that had been testified by the technique control [8]. The amplified messenger RNA and coupling were performed according to the method of Hughes et al [9]. The labeled urethral and reference complementary DNA (cRNA) probes were combined and hybridized to a rat oligo microarray slide at 48 °C for 12[en]16 hr. The microarray slides were scanned with Axonimager 4000B and GenePixPro 6 software (Axon Instruments, Union City, CA, USA). GenePix median of ratios was subjected to linear normalization in NOMAD (http://derisilab.ucsf.edu); the normalized data were then analyzed with Cluster 3.0 (http://bonsai.ims.utokyo.ac.jp/~mdehoon/software/cluster/) [10,11]. The resulting cluster data were imported into the Significance Analysis of Microarrays (SAM) software package (http://www-stat.stanford.edu/~tibs/SAM/) [12]. Delta was chosen to limit the output gene list so that less than 1% of predicted false positives would be included.

### 2.4. TaqMan real-time RT-PCR

Cellular RNA (2.5 μg) was annealed to 0.4 μg of oligo-dT primer in a volume of 12 μl. Four microliters of 5X buffer, 2 μl of 0.1 mol/l dithiothreitol, 1 μl of 10 mmol/l deoxynucleotide triphosphates, and 1 μl of SuperScript reverse transcriptase (Invitrogen, La Jolla, CA, USA) were then added to bring the final reaction volume to 20 μl. After 1 h of incubation at 42 °C, the reverse transcriptase (RT) mixture was incubated at 70 °C for 10 min to inactivate the RT. Eighty microliters of TE buffer was then added to make a 5X diluted cDNA library, from which 4 μl was used in the following TaqMan PCR. All reagents for TaqMan PCR, including the primers for rat small mothers against decapentaplegic 2 (Smad2), matrix metalloproteinase 13 (MMP-13), polo-like kinase 1 (PLK1), regulator of G-protein signaling 2 (RGS2), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), were purchased from Applied Biosystems (Foster City, CA, USA). The reactions were run in Applied

Biosystems' PRISM 7900HT sequence detection system by using the 96-well plate format. The cycling conditions included an initial phase at 95 °C for 3 min, 40 cycles at 95 °C for 15 s, and 55 °C for 60 s. The real-time PCR results were analyzed by Applied Biosystems' SDS 7000 software to determine the expression levels of genes of interest relative to that of GAPDH.

### 2.5. Western blot analysis

Urethral tissue protein samples were prepared by homogenization of cells in a lysis buffer containing 1% IGEPAL CA-630, 0.5% sodium deoxycholate, 0.1% sodium docecyl sulfate, aprotinin (10 μg/ml), leupeptin (10 μg/ml), and phosphate-buffered saline (PBS). Cell lysates containing 20μg of protein were electrophoresed in sodium docecyl sulfate polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride membrane (Millipore Corp, Bedford, MA, USA). The membrane was stained with Ponceau S to verify the integrity of the transferred proteins and to monitor the unbiased transfer of all protein samples. Detection of Smad2 and β-actin on the membranes were performed with an electrochemiluminescence kit (Amersham Life Sciences Inc, Arlington Heights, IL, USA) with the use of anti-SMAD2 (BD Biosciences, San Jose, CA, USA), and anti-β-actin antibody (Santa Cruz Biotechnologies, Santa Cruz, CA, USA) (at 1:500 and 1:3000, respectively). The resulting images were analyzed with ChemiImager 4000 (Alpha Innotech Corporation, San Leandro, CA, USA) to determine the integrated density value of each protein band.

### 2.6. Isolation and culture of urethral smooth muscle cells

Urethral smooth muscle cells (USMCs) were isolated from excised urethral specimens and cultured as previously described [13]. Indirect immunofluorescence staining for α[en]smooth muscle actin was used to confirm that the cells obtained were smooth muscle cells. Cells at the fourth passage were used in this study.

### 2.7. TGF- β treatment for Smad2 translocation and immunofluorescence staining

USMCs were treated with TGF- $\beta$ (10 ng/ml) for 0, 30, and 120 min. Cellular protein was isolated and assayed as described previously for Western blot analysis. For immunofluorescence staining, the cells were fixed with ice-cold methanol for 8 min, permeabilized with 0.05% Triton X-100 for 5 min, and blocked with 5% normal horse serum in PBS for 1 h at room temperature. The cells were then incubated with the primary antibody (anti[en]phospho-SMAD2, 1:500) for 1 hr at room temperature. After being washed with PBS three times, the cells were incubated with fluorescein isothiocyanate[en]conjugated secondary antibody for 1 hr at room temperature. After another three washes with PBS, the cells were further stained with 40,6-diamidino- 2-phenylindole (DAPI; for nuclear staining) for 5 min and viewed under fluorescence microscopy.

### 2.8. Immunohistochemistry and image analysis

Freshly dissected tissue was fixed with cold 2% formaldehyde and 0.2% picric acid in 0.1 mol/l phosphate buffer followed by immersion in buffer containing 30% sucrose. The fixed tissues were then frozen in optimal cutting temperature compound. Sections were cut at 6 microns and treated with hydrogen peroxide/methanol to quench endogenous peroxidase activity. After being rinsed, sections were washed twice in PBS for 5 min, followed by 30 min of incubation with 3% horse serum/PBS/0.3% triton X-100. After excess fluid was drained, sections were incubated overnight at 4 °C with mouse anti-SMAD2 (Santa Cruz Biotechnologies) or mouse anti[en]α-smooth muscle actin (Sigma-Aldrich, St Louis, MO, USA). After being washed, sections were immunostained by the avidin-biotin-peroxidase

method (Elite ABC; Vector Labs, Burlingame, CA, USA), with diaminobenzidine as chromagen, followed by counterstaining with hematoxylin. For image analysis, five randomly selected fields per animal for each treatment group were photographed and recorded with the use of a Retiga Q Image digital still camera and ACT-1 software (Nikon Instruments Inc, Melville, NY, USA).

### 2.9. Statistics

Statistical analysis was performed according to the *Primer of Biostatistics*, 3rd edition (Glantz SA, McGraw-Hill, Inc, New York, NY, USA). Data were expressed as means ± standard deviation. The Student-Newman-Keuls test was used to determine significance ($p < 0.05$).

## 3. Results

### 3.1. Rates of incontinence in subject rats

On the basis of cystometric criteria, there were a total of 14 (58.3%) continent rats and 10 (41.7%) stress incontinent rats. None of the rats manifested non-voiding contractions suggestive of bladder overactivity. Eleven urethras (6 from normal and 5 from incontinent animals) were randomly selected for protein isolation and Western blot analysis. The other 13 urethras (8 from normal and 5 from incontinent animals) were used for microarray, real-time PCR, and immunohistochemistry analyses.

### 3.2. Long-term, age-related, labor-induced changes in urethral gene expression

Gene expression profiles of urethral tissues from the continent and incontinent rats were compared. Approximately 4000 of the 26,962 rat genes surveyed were found to have greater than a 2-fold difference in expression levels compared with their corresponding genes in the universal rat reference RNA. Hierarchical clustering of these ~4000 genes revealed a variable but ordered expression pattern in urethral tissues from both continent and incontinent animals, with genes having similar expression patterns clustered in adjacent rows. To identify genes that were expressed at significantly different levels between continent and incontinent urethras, we further analyzed the data for clustered genes using Significance Analysis of Microarrays (SAM) software (Fig 1), which limits predicted false-positive genes to less than 1% in the output gene list. By these criteria, 23 genes were overexpressed and 19 genes were underexpressed in urethral tissues from the incontinent rats compared with continent rats (Table 1). These genes belonged to 9 gene superfamilies, including (1) apoptosis, (2) neuron related, (3) Rho A/Rho kinase (ROK) pathway related, (4) smooth muscle related, (5) TGF signaling pathway related, (6) wnt/Frizzled signaling pathway related, (7) cellular adhesion, (8) cellular metabolism, and (9) transcriptional regulation.

### 3.3. Confirmation of differential expression of selected genes by real-time RT-PCR analysis

Changes of gene expression observed by microarray analysis were confirmed with a small set of known genes by TaqMan real-time RT-PCR. Expression of Smad2, Plk1, and Rgs2 was significantly higher in incontinent rat urethras compared with continent rat urethras as assessed by real-time PCR ($p < 0.05$). Matrix metalloproteinase 13 (Mmp13) was expressed at a higher level in the incontinent urethra versus gene chip as assessed by Taqman, but the difference was statistically insignificant because of the large standard deviation ($p > 0.05$; Fig. 2). When gene expression profiles for Smad2, Plk1, Mmp13, and Rgs2 obtained by either microarray analysis or RT-PCR were compared, the patterns of expression were very similar with regard to the direction and degree of differences in expression (sub-table in Fig 2).

### 3.4. Identification and localization of upregulated Smad2 protein expression in urethra by Western blot and immunohistochemistry

The expression level of Smad2 was normalized to β-actin. Smad2 expression was significantly enhanced in urethras from incontinent rats relative to urethras from continent rats (*$p < 0.001$, Fig. 3 A and B). Immunohistochemistry confirmed that Smad2 expression was higher in the urethras of incontinent rats relative to continent rats. Smad2 staining in incontinent urethras was strongest in the nuclei of smooth muscle cells in the muscularis mucosae (Fig. 3 C).

### 3.5. Smad2 was activated by TGF- β in urethral smooth muscle cells in vitro

Phosphorylation of Smad2 peaked after 30 min of exposure to 10 ng/ml TGF-β and remained high at 120 min after initial exposure (Fig. 4 A and B). Before TGF-β treatment, Smad2 was evenly distributed between the cytoplasm and nucleus. Smad2 accumulated in the nucleus 30 min after TGF-β treatment; this accumulation was sustained at 120 min (Fig. 4 C).

## 4. Discussion

Genes related to the metabolism of collagen, such as MMP2 and tissue inhibitor of metalloproteinase, are expressed differently in vaginal tissue from women with SUI compared with normal women [4,5]. However, assessment of gene expression in vaginal and periurethral tissues may not be representative of gene expression in the urethra. In light of the paucity of human urethral tissue available for analysis, our animal model of labor-induced stress incontinence represents a reasonable proxy for the study of the urethral effects of pregnancy, prolonged labor, and menopause, all of which have been linked to SUI.

Our previous studies ascertained that neuromuscular damage to the urethra was the principal cause of labor-induced SUI. Studies by other researchers have suggested that ischemia-reperfusion injury to the nerves and muscles of bladder and urethra may also be involved in the pathogenesis of incontinence in this model [14,15]. Gene and protein expression analysis to clarify the molecular mechanisms for these observations is the logical next step to further explore SUI in this model system.

In this current study, nine gene superfamilies were found to be expressed differently between continent and incontinent animals subjected to simulated birth trauma. Although all of these gene families may someday serve as potential therapeutic targets, upregulation of Mmp13 (also known as collagenase 3), Rgs2 (a negative regulator of Rho A), and Smad2 (a downstream effector of TGF-β) are of particular interest in for the pathogenesis of SUI

In the urethra, increased activity of Mmp13 would lead to increased collagen breakdown and loss of periurethral support. This could in turn lead to a decrease in the closure pressure of the urethra and an increase in the risk of stress incontinence.

The Rho A/ROK[en]signaling pathway is an important cellular signaling pathway in the regulation of relaxation/contraction of smooth muscle cells by modulating the phosphorylation status of the myosin light chain (MLC). Smooth muscle contraction is initiated by phosphorylation of the (MLC) by MLC kinase (MLCK). When the phosphate moieties are removed by MLC phosphatase (MLCP), the actin fibers slide back to a state of relaxation. Rho A is a critical regulator of MLCP by activation of ROK by membrane-anchored RhoA-guanosine triphosphate. Active ROK has been shown to phosphorylate myosin-binding subunit at certain amino acid residues, which leads to inhibition of MLCP and subsequent increase in MLC phosphorylation, resulting in increased force of muscular contraction [16]. RSG2 is the negative regulator of Rho A in the cell [17]. The increase in

Rgs2 activity in incontinent rats would imply decreased activity of Rho A relative to that found in continent rats, which would lead to smooth muscle relaxation and decreased urethral pressure in incontinent rats.

SMAD2 is a known downstream mediator of TGF-β, which plays an important role in tissue inflammation and other disorders [18]. Upregulation of Smad2 implies that inflammation accompanies the changes of SUI in our rat model system. Modulation of this activity may have future utility in the prevention of SUI.

Limitations of our study include a relatively small sample size. Urodynamic studies were conducted under anesthesia. Cystometric data obtained in conscious rats might have resulted in different classification of continence status, although we suspect that the urethral continence mechanism in these animals should be active regardless of consciousness. Fortunately, none of our subjects manifested evidence of bladder instability, implying that overactive bladder was not present and giving credence to our interpretation of fluid expulsion in the absence of bladder pressure increase as evidence of SUI. Although studies of rat tissues may not be entirely representative of the situation in human women, the lack of available human tissue for this type of study makes the use of this animal model the only economically and ethically viable option for this type of study. The use of gene chip analysis permits exhaustive assessment of genome expression in given tissues, although it is not the most precise means to assess gene expression. We attempted to compensate for this by performing PCR for a few select genes as well as Western blot analysis and immunohistochemical assessment of Smad2 expression.

To our knowledge, this study is the first to specifically assess urethral tissue gene expression in a model of SUI. Our results offer interesting clues to the pathogenesis of SUI and suggest several avenues for novel research and potential new therapies.

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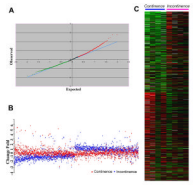
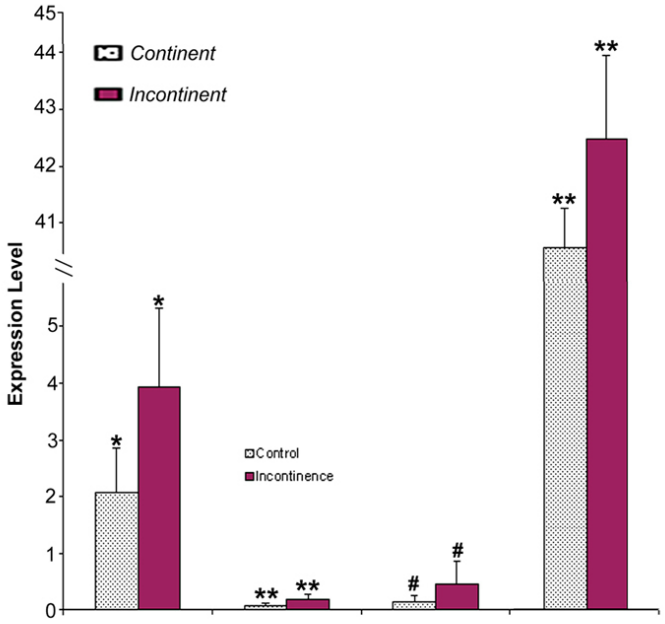


**Fig. 1.**

[en] Differential genes analyzed with Significance Analysis of Microarrays (SAM) software package. (A) The SAM plot shows the up-regulated genes (red) and down regulated genes (green) in the incontinence groups compared with normal control. (B) The differential genes changed fold compared with universal RNA control. Red: continence; blue: incontinence. (C) The distribution of significantly differential genes compared with universal RNA in continence and incontinence groups. Red: upregulated genes; green: downregulated genes.




| Change Fold | SMAD2 | PLK1 | MMP13 | RGS2 |
|---|---|---|---|---|
| Q-PCR | 1.89 | 2.45 | 3.59 | 2.29 |
| Microarray | 2.50 | 8.06 | 3.78 | 3.12 |

**Fig. 2.**

[en] Differential messenger RNA expression of selected genes. Levels of Smad2, Plki1, Mmp13, and Rgs2 were quantitated by TaqMan real-time polymerase chain reaction, with expression of glyceraldehyde 3-phosphate dehydrogenase as internal control (X ± SD). The sub-table indicates the covalence of gene expression between the quantitative polymerase chain reaction and microarray. *The difference in expression of Smad2, Plk1, and Rgs2 was significant between normal ($n = 8$) and incontinent ($n = 5$) rats ($p < 0.01$). #The difference in expression of Mmp13 between continent and incontinent rats was not significant ($p > 0.05$). Each bar represents the average of samples.

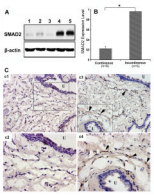


**Fig. 3.**

[en] Urethral expression of Smad2. (A) The Smad2 proteins in urethras were checked by Western blot analysis. Lanes 1[en]3 represent continent urethras; lanes 4 and 5 represented incontinent urethras. (B) The expression level of Smad2 was normalized to β-actin. Smad2 expression was significantly enhanced in incontinent urethras (*$p < 0.001$). (C) Smad2 expression (brown stain) in incontinent urethras was most intense in the muscularis mucosae (arrows in c3); most of the Smad2 was localized to the nucleus (arrows in c4). c1: Continent urethra (original magnification: ×200); c2: continent urethra (original magnification: ×400); c3: incontinent urethra (original magnification: ×200); c4: incontinent urethra (original magnification: ×400).

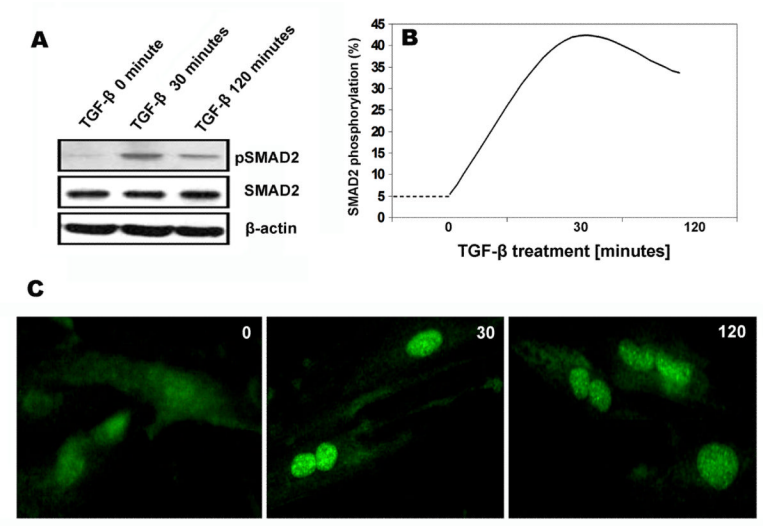



**Fig. 4.**

[en] Smad2 phosphorylation and translocation in the urethral smooth muscle cells. (A) The urethral smooth muscle cells were treated with 10 ng/ml TGF-β for 0, 30, and 120 min in vitro. The phosphorylations of Smad2 were enhanced from 30 min and retained for 120 min. The Smad2 phosphorylation curve is shown in (B). (C) The translocation of Smad2 was checked by the immunofluorescence staining. Before the TGF-β treatment, most of the cytoplasm and nucleus. The Smad2 was translocated by the TGF-β treatment at 30 min and sustained for 120 min.

## Table 1

Genes with expression patterns that were significantly different between continent and incontinent urethras

| | Name | Fold change |
|---|---|---|
| | **Apoptosis related** | |
| **U10188** | SERINE/THREONINE-PROTEIN KINASE PLK (EC 2.7.1.-) (PLK-1). | 8.06622 |
| **L16764** | HEAT SHOCK 70 KDA PROTEIN 1/2 (HSP70.1/2). | 3.51211 |
| **AF522937** | HEAT SHOCK FACTOR BINDING PROTEIN 1. | 2.21360 |
| | **Neuron related** | |
| **AF067769** | OPIOID RECEPTOR, SIGMA 1. | 5.55627 |
| **Y17048** | CALCIUM-BINDING PROTEIN CABP1 (CALDENDRIN). | 2.36242 |
| **AB031014** | MIC2 LIKE 1; SINGLE-PASS TRANSMEMBRANE PROTEIN; RHOMBEX-40. | 2.09523 |
| | CYTOMATRIX PROTEIN P110. | −0.85 |
| **Y08883** | LAMININ CHAIN (FRAGMENT). | −0.72 |
| | **Rho A related** | |
| **AF279918** | REGULATOR OF G-PROTEIN SIGNALING 2 (RGS2). | 3.12335 |
| **AF271235** | DIFFERENTATION-ASSOCIATED NA-DEPENDENT INORGANIC PHOSPHATE COTRANSPORTER; | −0.99 |
| **M17526** | GUANINE NUCLEOTIDE-BINDING PROTEIN G(O), ALPHA SUBUNIT 1. | −0.77 |
| **AY034823** | RHO GUANINE NUCLEOTIDE EXCHANGE FACTOR 7 (PAK-INTERACTING EXCHANGE FACTOR BETA) | −0.59 |
| | CALCIUM/CALMODULIN-DEPENDENT 3′,5′-CYCLIC NUCLEOTIDE PHOSPHODIESTERASE 1B (CAM-PDE 1B) | −0.58 |
| | **Smooth muscle related** | |
| | SEROTRANSFERRIN PRECURSOR (TRANSFERRIN) (SIDEROPHILIN) | 3.38482 |
| **J02780** | TROPOMYOSIN ALPHA 4 CHAIN (TROPOMYOSIN 4) | 2.86376 |
| | CLIP-ASSOCIATING PROTEIN 2. | 2.05104 |
| **AY115564** | FOUR AND A HALF LIM DOMAINS 1; FOUR AND A HALF LIM DOMAINS 1 (SKELETAL MUSCLE LIM-PROTEIN 1). | −0.086 |
| **M75148** | KINESIN LIGHT CHAIN 1 (KLC 1). | −0.65 |
| **AF464909** | MYOCARDIN. | −0.63 |
| **M15883** | CLATHRIN LIGHT CHAIN B (LCB). | −0.59 |
| | VOLTAGE-GATED CA CHANNEL. | −0.58 |
| **X62894** | CHLORIDE CHANNEL PROTEIN, SKELETAL MUSCLE (CHLORIDE CHANNEL PROTEIN 1) | −0.6 |
| | **TGF related** | |
| **M60616** | COLLAGENASE 3 PRECURSOR (EC 3.4.24.-) (MATRIX METALLOPROTEINASE-13) (MMP-13) | 3.78 |
| **AF305813** | TGF-BETA RESISTANCE-ASSOCIATED PROTEIN. | −0.661 |
| **AB010147** | MOTHERS AGAINST DECAPENTAPLEGIC HOMOLOG 2 (SMAD 2) | 2.5 |
| | **Wnt related** | |
| **AF272158** | INHIBITOR OF THE DVL AND AXIN COMPLEX; DVL-BINDING PROTEIN IDAX | 3.13374 |

| | Name | Fold change |
|---|---|---|
| | (INHIBITION OF THE DVL AND AXIN COMPLEX). | |
| | **ECM related** | |
| **Y09111** | A DISINTEGRIN AND METALLOPROTEINASE DOMAIN 6. | 2.23498 |
| **X68041** | EXTRACELLULAR SUPEROXIDE DISMUTASE [CU-ZN] PRECURSOR (EC 1.15.1.1) (EC-SOD) | −0.74 |
| | **Cell growth and Metabolic related** | |
| **D14015** | G1/S-SPECIFIC CYCLIN E1. | 10.37283 |
| **X84349** | CARBONYL REDUCTASE [NADPH] 1 (EC 1.1.1.184) (NADPH-DEPENDENT CARBONYL REDUCTASE 1). | 5.03441 |
| | 60S ACIDIC RIBOSOMAL PROTEIN P2. | 13.71968 |
| **X57569** | METABOTROPIC GLUTAMATE RECEPTOR 1 PRECURSOR (MGLUR1). | 4.73186 |
| **Y18208** | PROTEIN PHOSPHATASE 1 (GL-SUBUNIT). | 3.39006 |
| **J04022** | SARCOPLASMIC/ENDOPLASMIC RETICULUM CALCIUM ATPASE 2 (EC 3.6.3.8) (CALCIUM PUMP 2) | 2.85852 |
| | SOLUTE CARRIER FAMILY 12 MEMBER 1 (BUMETANIDE-SENSITIVE SODIUM-(POTASSIUM)-CHLORIDE COTRANSPORTER 2) | 2.52346 |
| **D63149** | OLIGOPEPTIDE TRANSPORTER, KIDNEY ISOFORM (PEPTIDE TRANSPORTER 2) | 2.16958 |
| **AY017337** | EXPRESSED IN NON-METASTATIC CELLS 3, PROTEIN (NUCLEOSIDE DIPHOSP. | −0.86 |
| | **Transcriptional factor** | |
| **AY043393** | MUSASHI HOMOLOG 1. | −0.97 |
| **U31880** | TRANSLATION INITIATION FACTOR EIF-2B BETA SUBUNIT (EIF-2B GDP-GTP EXCHANGE FACTOR). | −0.94 |
| **AF468681** | INHIBITOR OF DNA BINDING 4. | −0.81 |
| | ZINC FINGER PROTEIN 15 (FRAGMENT). | −0.69 |
| **AF521008** | CCCH-TYPE ZINC FINGER ANTIVIRAL PROTEIN (ZAP). | 2.81219 |