# Differential Expression of Class I Small Leucine-Rich Proteoglycans in an Animal Model of Partial Bladder Outlet Obstruction

Conrad C. Maclejewski,* Dariush Honardoust, Edward E. Tredget† and Peter D. Metcalfe‡

*From the Division of Urology (CCM, PDM) and Division of Plastic Surgery (DH, EET), Department of Surgery, University of Alberta, Edmonton, Alberta, Canada*

**Purpose:** Partial bladder outlet obstruction has been shown in a rat model to progress from inflammation to hypertrophy to fibrosis. Small leucine-rich proteoglycans are extracellular matrix components associated with collagen fibrillogenesis and resultant scar formation. Two such critical small leucine-rich proteoglycans are decorin and biglycan. We hypothesized that in keeping with other scar models, decorin would be down-regulated and biglycan would be up-regulated with the onset of fibrosis compared to sham.

**Materials and Methods:** We challenged our hypothesis with female Fisher rats that underwent ligation of the bladder neck or sham surgery. Animals were sacrificed at 4, 8 and 12 weeks, and bladders were harvested. Frozen sections were stained for immunofluorescence for decorin and biglycan. mRNA expression for decorin and biglycan was analyzed using quantitative reverse transcriptase polymerase chain reaction.

**Results:** All rats survived to specified experimental end points in good health. Immunofluorescent stains showed progressive down-regulation of decorin and up-regulation of biglycan during the 12-week course by 0.36 and 1.82-fold, respectively ($p = 0.02$ and $p = 0.02$), compared to shams. Quantitative real-time reverse transcriptase polymerase chain reaction confirmed these findings in 12-week specimens, showing a down-regulation of decorin by a factor of 0.45 ($p = 0.02$) and up-regulation of biglycan by a factor of 2.04-fold ($p = 0.08$).

**Conclusions:** We present the first identification to our knowledge of small leucine-rich proteoglycans in normal and abnormal bladder tissue, and their differential expression in the process of bladder fibrosis, consistent with experimental findings in other anatomical sites. Further investigation into small leucine-rich proteoglycan expression and regulation may allow for the development of new antifibrotic therapeutics.

**Key Words:** urinary bladder neck obstruction, fibrosis, inflammation, proteoglycans

**Abbreviations and Acronyms**

GAPDH = glyceraldehyde 3-phosphate dehydrogenase

pBOO = partial bladder outlet obstruction

PBS = phosphate buffered saline

PCR = polymerase chain reaction

RT-qPCR = reverse transcriptase quantitative polymerase chain reaction

SLRP = small leucine-rich proteoglycan

Supported by the Canadian Institutes of Health Research, the Alberta Heritage Foundation for Medical Research, the Firefighters Burn Trust Fund of the University of Alberta and the Northern Alberta Urological Foundation.

* Correspondence: Department of Surgery, Division of Urology, University of Alberta, Suite 400 Hys Centre, 11010-101 St., Edmonton, Alberta, Canada T5H 4B9 (e-mail: conradm@ualberta.ca).

† Financial interest and/or other relationship with Organogenesis, Exciton and British Canadian Biosciences.

‡ Financial interest and/or other relationship with Eli Lilly Canada Inc.

PARTIAL bladder outlet obstruction is a significant and common problem in urology, resulting in sequelae seen in adult and pediatric patients. Recent experimental work has demonstrated the progressive deterioration of the bladder in an animal pBOO model through a process of initial inflammation, subsequent hypertrophy and ultimately fibrosis.[1] The end stage of the obstructive pathology involves fibrosis from deposition of collagen in the

0022-5347/12/1884-1543/0
THE JOURNAL OF UROLOGY®
© 2012 by AMERICAN UROLOGICAL ASSOCIATION EDUCATION AND RESEARCH, INC.
http://dx.doi.org/10.1016/j.juro.2012.03.045
Vol. 188, 1543-1548, October 2012
Printed in U.S.A.

bladder wall.[2] How this process occurs and how it is regulated remain to be elucidated.

Small leucine-rich proteoglycans are a series of structurally and functionally related extracellular matrix molecules that are present in connective tissue and regulate key steps in cell adhesion, gene expression and growth factor activity.[3] The process of collagen fibrillogenesis is thought to be regulated by a tightly controlled and orchestrated sequence of SLRPs binding to collagen fibrils, resulting in a correctly synthesized matrix with specifically determined physical properties. SLRPs are divided into 4 classes based on their temporal expression.[3] Prior work in models of pulmonary fibrosis,[4] liver cirrhosis[5] and hypertrophic scar[6] has shown that the expression and synthesis of 2 specific SLRPs are pathologically altered. Decorin tends to be down-regulated and biglycan tends to be up-regulated.

Decorin and biglycan are class I SLRPs. Decorin has multiple high affinity binding sites to collagen, and has been shown to regulate the diameter of collagen fibrils so that resultant helices are thinner and more regular in diameter.[7] Biglycan has 2 separate glycosaminoglycan chains, both of which are thought to be critical in its binding mechanism to the forming collagen helix. Biglycan has been shown to be important in regulating collagen cross linking without having an effect on fibril diameter.[8]

SLRPs have not been described in fibrotic bladder. Prior work has demonstrated that the pathology behind the process of pBOO is heavily dependent on immune system activation.[9] We hypothesized that the scarring process in this model should provide a SLRP profile similar to that of other previously studied scar models in that pBOO would show a progressive decrease in decorin with an increase in biglycan.

## MATERIALS AND METHODS

### Animal Use and Surgical pBOO

Full approval from the University of Alberta Animal Care and Use Committee was obtained before commencing research. Adult female Fisher rats underwent a pBOO procedure as previously described.[10] Anesthesia was induced with isoflurane and the bladder was exposed through a lower midline incision. An 18 gauge angiocatheter was introduced into the bladder through a superior cystotomy and advanced to the urethra. Urodynamics were performed at an infusion rate of 0.1 cc per minute with pressure measurements obtained by a transducer at 15-second intervals. Infusion was halted once leakage was visually detected at the urethral meatus.

Following the conclusion of urodynamic measurements, the catheter was advanced antegrade into the urethra and dissection of the bladder neck was performed with a right angle clamp. The clamp was passed around the urethra immediately below the level of the ureters, and a 2-zero silk suture was passed around and gently tied, using the angiocatheter as a calibration stent. The catheter was then removed and the cystotomy closed. The abdomen was closed in 2 layers.

Rats were sacrificed at 4, 8 and 12 weeks along with a sham group at 11 weeks. There were 3 animals included in each point, resulting in a total of 12 animals. In the sham group the surgical procedure was identical with the exception of the passing of a silk suture around the urethra. Animal weight was obtained before surgery and was followed during the course of the experiment as a measure of animal health.

At sacrifice, urodynamics were performed and organs harvested. Harvested tissue was snap frozen in liquid nitrogen for subsequent protein and nucleic acid analysis or fixed in 4% paraformaldehyde as well as Shandon Cryomatrix™. Paraffin and Cryomatrix blocks were mounted and sectioned.

### Reverse Transcriptase PCR

Total RNA was extracted from snap frozen tissue specimens. Tissue was deep frozen in liquid nitrogen, then homogenized using a micro-dismembrator (B. Braun Biotech Inc., Allentown, Pennsylvania). The powder was reconstituted in Trizol® solution. Total RNA was extracted using RNeasy® spin columns. Contamination from genomic DNA was removed by DNAse digestion for 60 minutes. First strand cDNA was synthesized using random primers in a first strand synthesis kit (Sigma, Oakville, Ontario, Canada). Real-time RT-PCR was conducted using Power SYBR® Green PCR Master Mix in a 25 $\mu$l tube for a total reaction volume of 25 $\mu$l, containing 1 $\mu$l first strand product, and 0.2 $\mu$M gene specific upstream and downstream primers for decorin and biglycan. For control reactions GAPDH primers were used. Amplification and analysis were performed using an ABI 7300 real-time system (Applied Biosystems, Foster City, California). Cycling conditions were an initial denaturation at 95C for 3 minutes, followed by 40 cycles consisting of a 15-second denaturation interval at 95C, and a 30-second interval for annealing and primer extension at 60C. GAPDH amplification was used for standardization of the amplification curves. Primer sequences for biglycan were GATGGCCTGAAGCTCAA for the forward sequence and GGTTGTTGAAGAGGCTG for the reverse. Primer sequences for decorin were TGGCAGTCTGGCT for the forward sequence and ACTCACGGCAGTG for the reverse. Primer sequences for GAPDH were CCTGGAGAAACCT-GCCAAGTAT for the forward sequence and CTCGGC-CGCCTGC for the reverse.

### Immunofluorescent Staining

Using Cryomatrix blocks, sections at 8 $\mu$m were mounted and initially fixed in acetone for 10 minutes, then permeabilized using Triton™ X-100 solution at 0.5% for 10 minutes. Slides were then washed with PBS and blocked using 5% bovine serum albumin for 1 hour at room temperature. Incubation with primary antibodies was done at 4C overnight at 1:200 dilution in a solution of PBS containing 1% bovine serum albumin.

Primary antibodies used were sourced from Santa Cruz Biotechnology (Santa Cruz, California), and included mouse anti-human decorin (SC-73896) and rabbit anti-human biglycan (SC-33788). Secondary antibody incuba-

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