

Differential gene expression in bladder hypertrophy following bladder outlet obstruction in mouse

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OBJECTIVES

Bladder outlet obstruction (BOO) leads to compensatory bladder hypertrophy resulting in bladder dysfunction. However, the molecular mechanisms of bladder hypertrophy remain poorly understood. Although transcriptional profiling of the urinary bladder has been investigated in some pathological conditions, there are no published studies on differential gene expression in mouse obstructed urinary bladder.

The aim of this study was to determine the altered gene expression profile after BOO in mice using Taqman Low Density Array (TLDA) technology, a valuable new tool for rapid, sensitive and quantitative gene expression profiling (1).

METHODS

BOO was induced in female mice by partial ligation of the urethra for 5 weeks (BOO group, n=10; no ligation sham group, n=6). Total RNA was extracted from whole urinary bladder and reverse transcription performed.

TLDA technology allows the simultaneous measurement of expression of up to 384 genes in a single sample. Each array has eight separate loading ports that feed into 48 separate wells. Each 1 µL well contained specific, user-defined primers and probes, capable of detecting a single gene.

For this study, sixteen different cDNA samples were charged in 2 cards, containing 48 wells for 48 different genes (in triplicate).

Each set of 48 genes contained specific probes for cytokines, chemokines, growth factors, apoptosis markers, oxidative stress markers, tissue-specific markers (Table 1) as well as 3 house-keeping genes (gusb, hprt, ppia).

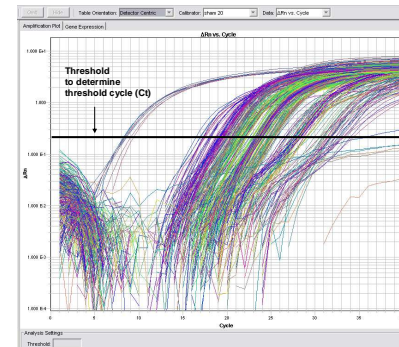
A solution (100 µL) containing cDNA (200 ng), Taq polymerase and deoxynucleotide triphosphates were distributed to the wells of a 384-well plate via microfluidic channels in the card.

Using mean values, the changes in gene expression between BOO vs sham mice were calculated using the comparative cycle threshold method (Figure 1) and expressed as fold change relative to sham group. Difference between BOO and sham group were analyzed by a Student t-test.

Table 1: List of the 48 genes studied.

Gene Name	Function
Plasminogen activator inhibitor 1	serine protease inhibitor
Bcl-2 associated X protein	apoptotic protein
Caspase 8	apoptotic protein
Natriuretic peptide precursor type A	Cardiac hormone
Natriuretic peptide precursor type B	Cardiac hormone
Vimentin	Cytoskeletal protein
Chemokine (C-C motif) ligand 2	Cytokine
Interleukin 6	Cytokine
Tumor necrosis factor (Tnf)	Cytokine
ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	Energy metabolism
Calmodulin 1	Energy metabolism
Fibronectin 1	extracellular matrix protein
Procollagen, Type I, alpha 1	extracellular matrix protein
Procollagen, Type III, alpha 1	extracellular matrix protein
Fibroblast growth factor 2	Growth factor
Insulin-like growth factor 1, transcript variant 2	Growth factor
Transforming growth factor beta	Growth factor
Vascular endothelial growth factor A, transcript variant 3	Growth factor
Rho-associated, coiled-coil forming protein kinase p160 ROKC-1	Kinase protein
Thymoma viral proto-oncogene 1	Kinase protein
Matrix metalloproteinase 2	metalloproteinase
Matrix metalloproteinase 3	metalloproteinase
Matrix metalloproteinase 9	metalloproteinase
Tissue inhibitor of metalloproteinase 3	metalloproteinase inhibitor
Actin, alpha 1, skeletal muscle	Muscle protein
Actin, alpha 2, smooth muscle, aorta	Muscle protein
Myosin, heavy polypeptide 6, cardiac muscle, alpha	Muscle protein
Myosin, heavy polypeptide 7, cardiac muscle, beta	Muscle protein
5-Hydroxytryptamine (serotonin) receptor 2A	Neurotransmitter receptor
5-Hydroxytryptamine (serotonin) receptor 2B	Neurotransmitter receptor
Nitric oxide synthase 3, endothelial cell	NO metabolism
Catalase	Oxidative stress
Glutathione peroxidase 1	Oxidative stress
Heme oxygenase (biliverdin)	Oxidative stress
Hypoxia inducible factor 1, alpha	Oxidative stress
Manganese oxidase A	Oxidative stress
Manganese oxidase B	Oxidative stress
Superoxide dismutase 2, mitochondrial	Oxidative stress
Xanthine oxidase, xanthine dehydrogenase	Oxidative stress
Solute carrier family 6 (transporter sodium), member 4	sodium metabolism
A disintegrin and metalloproteinase domain 17	TNFα metabolism
DATA binding protein 4	Transcription factor

Figure 1: Amplification curves of the genes studied. ΔRn=baseline-corrected normalized fluorescence.



RESULTS

Bladder weight increased 1.7 fold in BOO mice (sham mice = 0.09 ± 0.01 % vs BOO = 0.16 ± 0.02 % of body weight, p<0.05).

Among the 48 genes studied, 6 genes were differentially regulated (Table 2). The fold change was between 1.14 and 2.12.

- Up-regulated genes included fibroblast growth factor 2 (bFGF), fibronectin, hypoxia-inducible factor 1α (Hif1α) and one serotonin receptor subtype (5-HT_{2B}).
- Down-regulated genes are reported to be involved in extracellular matrix constituents (collagen I) and TNF metabolism (ADAM17).

Figure 2: Hypothesis for the Hypoxia-Hypertrophy Loop

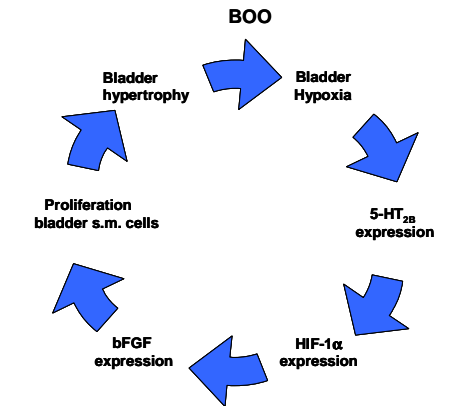


Table 2: Genes up- and down-regulated in female mice urinary bladder following 5 weeks of partial BOO.

Differential regulation	Symbol	Gene Name	Function	Fold change vs sham (mean±s.e.m.)	P level
genes up-regulated	Htr2b	5-hydroxytryptamine receptor 2B	Neurotransmitters receptors	2.12±0.35	< 0.01
	Fn1	Fibronectin 1	Extracellular matrix protein	1.53±0.09	< 0.01
	Hif1α	Hypoxia inducible factor 1, alpha	Oxidative stress	1.39±0.07	< 0.01
	Fgf2	Fibroblast growth factor 2	Growth factor	1.36±0.11	< 0.05
genes down-regulated	Col1α1	Procollagen, Type I, alpha 1	Extracellular matrix protein	1.61±0.05	< 0.05
	Adam17	A disintegrin and metalloproteinase domain 17	TNFα metabolism	1.14±0.03	0.01

CONCLUSIONS

The increase in fibronectin expression may be indicative of fibrosis and correlates with structural changes previously reported in obstructed mice (2). Hypoxia is the key to the increased expression of 5-HT_{2B} receptors (3), HIF-1α and growth factors (4) in cascade. Therefore, the up-regulation of HIF-1α in the current study supports the role of hypoxia in impairment of bladder function following BOO and confirms previous findings in rat bladder (5). Moreover, a great increase of HIF-1α staining in the urinary bladder from obstructed BPH patients vs controls was recently demonstrated (6). Overexpression of bFGF can stimulate the proliferation of bladder smooth muscle cells, and therefore contribute to the hypertrophic remodeling of the smooth muscle layer (Figure 2), as previously reported in the rat bladder *in vitro* (7). Further studies are needed to confirm the physiological importance of these genes in mouse and human urinary bladder hypertrophy.

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