Survivin (*BIRC5*) regulates bladder fibrosis in a rat model of partial bladder outlet obstruction

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To the Editor: Fibrosis is a long duration wound healing process triggered by complex cellular and molecular responses that contributes to tissue or organ reconstruction.[1] Bladder fibrosis is initiated by pathological pressure such as hydrostatic pressure, stretching force, and fluid shear stress. Increased pressure leads to a bladder inflammatory microenvironment, smooth muscle hypertrophy, and fibrosis. To date, two common etiological factors of bladder fibrosis are mechanical obstruction (partial bladder outlet obstruction [pBOO]) and chronic cystitis.[2]

In our study, we generated a pBOO rat model and analyzed the genome data through a microarray. We performed differentially expressed gene (DEG) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, gene ontology (GO) enrichment analysis, protein-protein interaction (PPI) network construction, and hub gene identification. Furthermore, one hub gene, positively correlated with bladder fibrosis, was validated by Western blotting (WB) and immunohistochemistry (IHC). The workflow of our study is shown in Supplementary Figure 1A, http://links.lww.com/CM9/A958.

This animal research was approved by the Medical Ethics Committee of West China Hospital, Sichuan University, China (No. 2021227A). Female rats were assigned to the pBOO group ($n = 3$) or the sham group ($n = 3$). After anesthesia, the midline of the lower abdominal wall was incised to find the bladder and proximal urethra. We introduced a urethral catheter (outer diameter = 1 mm) into the bladder. Then, the proximal urethra was ligated with 40 silk. Meanwhile, the sham group underwent the same procedure without urethral ligation. Ampicillin (150 mg/kg) was given for 3 days after the surgery. The bladders were collected after 3 weeks.

Then, microarray analysis was conducted by Kangchen Biotech (Shanghai, China) with a Whole Rat Genome Oligo Microarray (Agilent Technologies, Santa Clara, CA, USA). After the microarray data of the pBOO and sham rats were obtained, we transformed the probe identification numbers into gene symbols. Moreover, we normalized the gene expression values with the Affy package, and log2 transformation and normalization were applied in the Robust Multichip Average signal intensity analysis.

We identified significant DEGs with $|\log2 \text{ fold change}| \geq 3$. The adjusted P value was <0.05. Initially, we uploaded the list of DEGs to the Search Tool for the Retrieval of Interacting Genes (STRING) website and mapped it. A PPI requires a composite score >0.4. Moreover, we analyzed the PPI network file with Cytoscape under JAVA1.8.0 software (Oracle: 500 Oracle Parkway, Redwood Shores, CA94065, USA). Then, we selected the top 20 hub genes ranked by the Matthews correlation coefficient (MCC) method via CytoHubba plug-in in Cytoscape.

GO analyses including biological process, cellular component, and biological function reveal the characteristics of gene products through their annotations. Moreover, KEGG analysis illuminates a known biological signaling pathway. To fully understand the function and signaling pathway of the DEGs, we performed GO and KEGG analyses by Metascape (http://metascape.org), and $P < 0.05$ was considered significant.

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Based on the functional and signaling pathway analysis, we clustered hub genes that correlated with cell proliferation, extracellular matrix (ECM) deposition, and inflammation [Supplementary Table 1, http://links.lww.com/CM9/A958].

Immunoblotting analysis, IHC, and hematoxylin-eosin staining (HE staining) were applied to validate outcomes. Standard protocols were used. The background was quantified by subtracting the band intensity from the immunoblotting using ImageJ software 1.6.0 (National Institutes of Health: 9000 Rockville Pike Bethesda, Maryland 20892, USA). The positive cell counting method was applied to analyze the bladder smooth muscle staining. Positive cells were counted by light microscopy. The mean integral optical density was evaluated by ImagePro Plus 6.0 software (Media Cybernetics: 4202 Bear Lodge Court, Houston, TX 77084, USA).

We analyzed the bladder tissue 3 weeks after radical cystectomy. HE staining revealed that both smooth muscle and collagen fiber proliferated much more in the pBOO group [Supplementary Figure 2, http://links.lww.com/CM9/A958].

A total of 1018 DEGs were eligible, including 420 upregulated genes and 598 downregulated genes as shown in the volcano plot [Supplementary Figure 1B, http://links.lww.com/CM9/A958] and heatmap [Supplementary Figure 3, http://links.lww.com/CM9/A958].

All DEGs were uploaded to STRING (https://cn.string-db.org) for the PPI network, including 883 nodes and 5046 edges [Supplementary Figure 4, http://links.lww.com/CM9/A958]. Then, the top 20 DEGs were selected out via the MCC method in Cytoscape [Supplementary Figure 1C, http://links.lww.com/CM9/A958]. Next, we classified the 20 hub genes into three clusters (proliferation, inflammation, and ECM deposition) associated with bladder fibrosis [Supplementary Table 1, http://links.lww.com/CM9/A958]. Finally, *BIRC5* (survivin) was selected as the critical gene in bladder fibrosis.

We uploaded the DEG file online (METASCAPE) to perform GO and KEGG pathway analyses. $P < 0.05$ was considered significant. As shown in Supplementary Figure 5, http://links.lww.com/CM9/A958, the upregulated DEGs were significantly enriched in the cell cycle and inflammation process. The downregulated DEGs were enriched in cell structure, muscle contraction, and receptor binding process [Supplementary Figure 5A and 5B, http://links.lww.com/CM9/A958]. In KEGG pathway analysis, the upregulated DEGs were enriched in inflammation and cell cycle processes, while the downregulated DEGs were enriched in vascular muscle contraction and

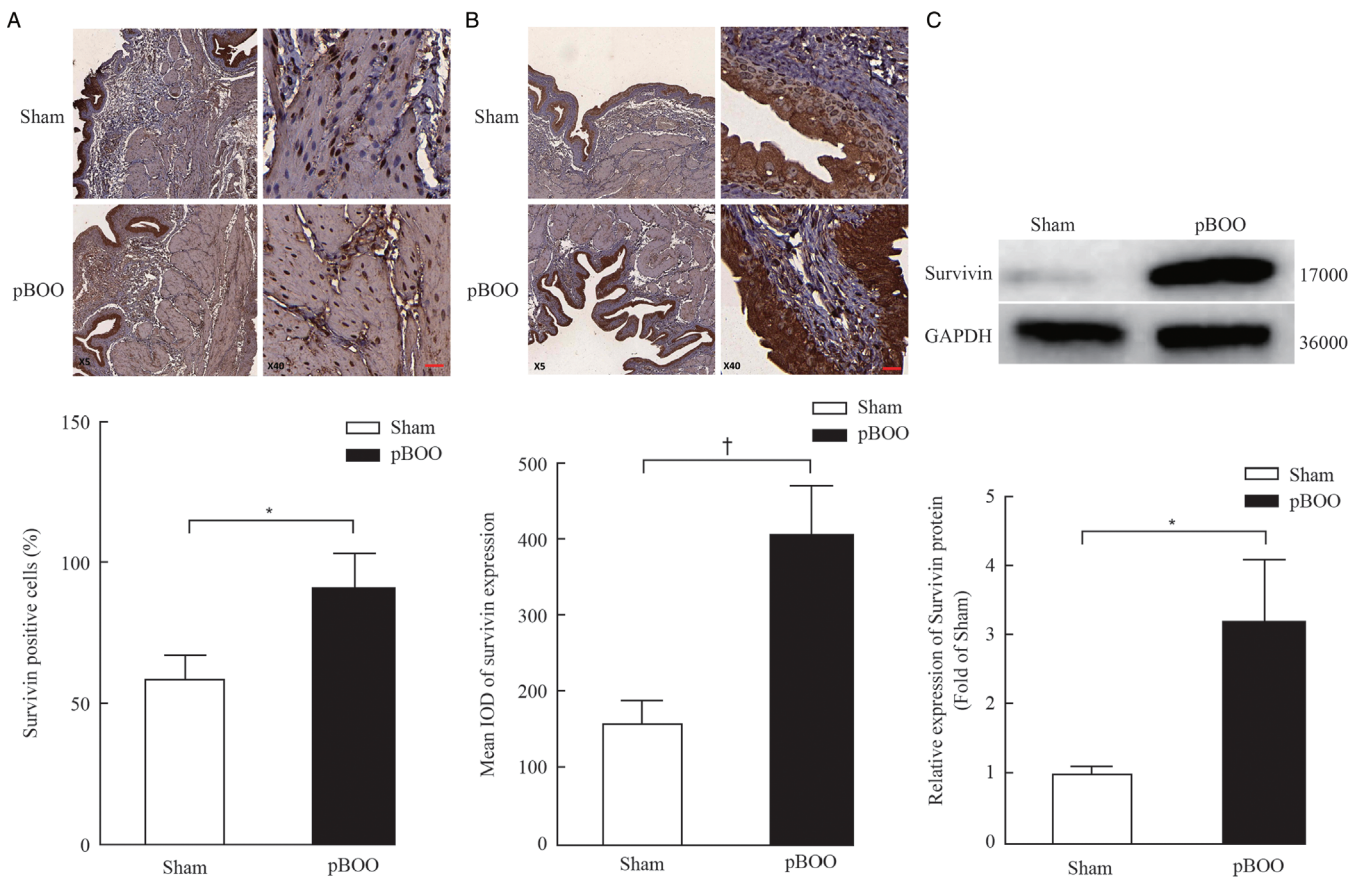

Figure 1: Expression level of survivin in pBOO rat model. (A) Expression of survivin in bladder smooth muscle; (B) Expression of survivin in bladder epithelial layer; (C) Expression of survivin examined by immunoblotting analysis. Values represent mean ± SD ($n = 3$). Pvalue was determined by a two-tailed Student's t test. $^{*}P < 0.05$, $^{\dagger}P < 0.01$, scale bar = 200 μm. pBOO: Partial bladder outlet obstruction; SD: Standard deviation.

hypertrophy [Supplementary Figure 6A and 6B, http://links.lww.com/CM9/A958].

WB and IHC were applied to validate the expression level in the pBOO rat model. As shown in Figure 1, the expression in both smooth muscle [Figure 1A] and the epithelial layer increases to some extent ($P < 0.05$) [Figure 1B]. Moreover, we validated our results through immunoblotting, and we found similar results, as shown in Figure 1C ($P < 0.05$).

The purpose of our study was to identify genetic biomarkers and pathways involved in the development process of bladder fibrosis. We first constructed the pBOO rat model and performed microarray analysis. Then, DEG identification was carried out. Furthermore, GO and KEGG analyses were conducted to identify the functions of the DEGs. From the GO analyses, we found that functions were enriched in the cell cycle, muscle structure development, etc. At the same time, a PPI network was constructed and one hub gene highly related to bladder fibrosis was selected. Finally, survivin expression was validated in the pBOO rat model, and we found that survivin promoted proliferation and ECM deposition in bladder fibrosis.

pBOO can cause an overactive bladder, leading to a sudden and uncomfortable need to urinate with or without urinary leakage. Increased ECM deposition, bladder cell hypertrophy, and hyperproliferation were also induced by pBOO.[3] Survivin is an evolutionarily conserved eukaryotic protein necessary for the inhibition of programmed cell death, migration, angiogenesis, and stemness. However, the molecular mechanism of survivin regulation is not yet fully understood. Our study provides a novel approach to understanding survivin induced bladder fibrosis, and we validated the potential role of survivin.

To our knowledge, several studies have revealed that survivin is engaged in tissue damage and fibrosis processes. Wang *et al*[4] found that tacrolimus can reduce the proliferation of fibroblasts by inhibiting the expression of survivin. Moreover, researchers demonstrated that survivin played a critical role during the transformation of oral submucous fibrosis (OSF) and it might be a useful biomarker for early diagnosis of OSF.[5]

To conclude, survivin was chosen via comprehensive bioinformatic analyses and it mainly regulated proliferation and ECM deposition functions and might be essential in fibrosis. However, more basic research is required to verify the role and mechanism of survivin in fibrosis.

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Conflicts of interest

None.

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