

## Original Article

# Mechanism of bladder dysfunction in the diabetic rat

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Received April 28, 2016; accepted June 22, 2016; Epub February 1, 2017; Published February 15, 2017

**Abstract:** Objective: The diabetic SD rat model was established to investigate the mechanism of the detrusor contractile dysfunction by analyzing the different expression of functionally regulatory proteins in the detrusor cell. Methods: Streptozotocin was used to establish the diabetic SD rat model. The altered expression of HSP27, CaD (full name), and TM (full name) was measured with fluorescence immunoassay, real-time PCR (RT-PCR), and western blot. Results: Compared with the controls, the protein expression of HSP27 and phosphorylated HSP27 was down-regulated, while the protein expression of CaD and TM was up-regulated in the detrusor cells of the cases. Conclusion: The expression of functionally regulatory proteins dramatically changes in the diabetic SD rat model.

**Keywords:** Diabetic bladder dysfunction, HSP27, caldesmon, tropomyosin

### Introduction

Diabetic bladder dysfunction (DBD), also known as diabetic cystopathy (DCP), is a common complication of diabetes mellitus (DM) [1-3]. The precise pathogenesis of DBD, which is not very clear yet, is predominantly attributed to detrusor dysfunction caused by hyperglycemia and peripheral autonomic neuropathy, and the synergistic effect of the muscle-derived and neurogenic factors leads to the changes of bladder excitability, elasticity and contractility, which results in DM bladder dysfunction [4, 5]. The ultrastructural changes of the detrusor and the structural injury of the contractile proteins lay the structural foundation for detrusor contractile dysfunction [6, 7]. It is clinically significant to effectively preserve the detrusor structure and promote its recovery. In this study, the functionally regulatory proteins such as HSP27, caldesmon (CaD) and tropomyosin (TM) in the detrusor cell were investigated, further revealing the pathogenesis of DBD, providing new perspectives for exploring protective measures of detrusor contractile dysfunction and treatment targets, and laying new basis for prognosis evaluation.

### Materials and methods

#### Animals

Twelve SD rats with six for each gender (Shanghai Slaccas, China) [license: SC-XK (Shanghai) 20082-005] were selected for this study.

#### Main reagents

Streptozotocin (Sigma, USA), HSP27 antibody (mouse, self-provided), specific antibody labels for phosphorylated HSP27 (hsp27-ser15, hsp27-ser78, hsp27-ser82, self-provided), TM antibody (mouse, self-provided), CaD antibody (rabbit, self-provided), second antibody (anti-mouse, anti-rabbit, RD, USA), primers, RT reagents and RT-PCR reagents (Western Biotechnology).

#### Establishment of the DM model

Six SD rats (half for each gender) were injected i.p. with a single dose of 150 mg/kg streptozotocin to build DM models. Six controls were injected with 0.1 M citric acid buffer. All rats were sacrificed 12 weeks after injection to collect target tissues.

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**Table 1.** Primers for QRT-PCR

Target gene	Name	Sequence	Product length	Source
HSP27	rhsp27F	TGGCAAGCACGAAGAAAGG	113 bp	literature [10]
	rhsp27R	GGGACAGGGAAGAGGACACC		
CaD	rCaDF	CTATGGCAACTGCGTAACGG	152 bp	literature [11]
	rCaDR	GGTGCTGGGTGACCTTGA		
TM	Thbd-F	AATACCCGAGATCCTGAGGG	238 bp	literature [12]
	Thbd-R	TCCTGTTGCACATGTACTCG		
β-actin	rat actin f	CCCATCTATGAGGGTTACGC	150 bp	literature [13]
	rat actin r	TTAATGTCACGCACGATTC		

Reactive conditions:  
25°C for 10 min,  
42°C for 50 min,  
85°C for 5 min. QRT-PCR reactive system: 25 µL 2×PCR buffer, 1 µL×2 primers (25 pmol/µL), 0.5 µL Sybr green I (20×), 2 µL template, 20.5 µL DEPC buffer. Reaction conditions: 94°C for 4 min; 94°C

### *Detection of cellular ultrastructure with electron microscopy*

The collected bladder tissues of the cases and controls were stained to observe the detrusor ultrastructure with electron microscopy.

### *Detection of the expression and distribution of HSP27, CaD and TM*

The literature [8] was referred to for the method. The bladder samples were fixed with paraformaldehyde, dehydrated, cleared, embedded and subjected to heating induced epitope retrieval (HIER) and sectioning. After PBS washing, 6% normal goat serum was used for one-hour blocking. The first antibodies (HSP27 antibody, CaD antibody and TM antibody) were added at 1:200, and then incubated at 4°C overnight. The second antibodies (anti-mouse, anti-rabbit) were added at 1:1000 and incubated at 37°C for 1 h. Then the nuclei were stained with DAPI after 15-minute incubation in darkness. The samples were observed and photographed under the confocal laser scanning microscope, and the data were documented.

### *Genetic detection of HSP27, CaD and TM with QRT-PCR*

The literature [9] was referred to for the method. Three rats were selected from each group for RNA extraction and two repetitions were set for each rat. TRIZOL, chloroform, isopropanol and ethanol were used to extract the total RNA from the models and controls. Then random primers were used for reverse transcription to prepare cDNA. QRT-PCR was performed with the specific primers of HSP27, CaD and TM shown in **Table 1** and sybr green I fluorochrome, with mouse β-actin as the internal reference. RT-PCR reactive system (20 µL): 10 µL 2×RT buffer, 1 µL 6N random primers (100 pmol/µL), 1 µL RT mix, 5 µL template, 3 µL DEPC buffer.

for 20 s, 60°C for 30 s, 72°C for 30 s, 35 circles; signal detection at 72°C.

The relative copy number of each test item was calculated according to the standard curve. The corrected value of mRNA content for each test item was calculated by the ratio of the relative copy number of each test item to relative copy number of the internal reference. The expressive difference between the cases and the controls was defined by the corrected values. T-test was adopted to determine statistical significance.

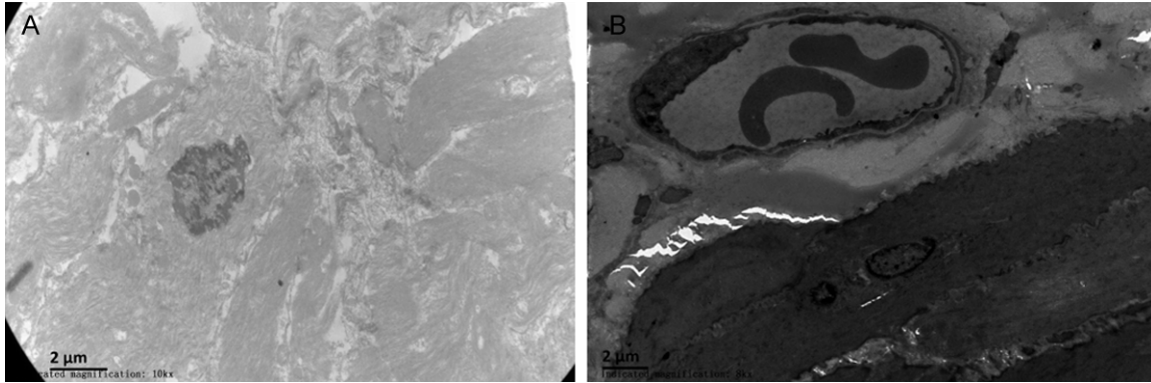
### *Detection of the expression of HSP27, phosphorylated HSP27, CaD and TM with WB*

The literature [14] was referred to for the method. Three rats were selected from each group and two repetitions were set for each rat. The total protein extracted from the bladder tissues was subjected to 4% SDS-PAGE gel electrophoresis, membrane-transfer, one-hour blocking with TBST buffer containing 5% skim milk powder. Then the first antibodies (HSP27, hsp27-ser15, hsp27-ser78, hsp27-ser82, CaD, TM) diluted at 1:1000 were added at 1:3000 with the internal references (glyceraldehyde-3-phosphate dehydrogenase, GAPDH). The incubation at 4°C continued overnight. After washing, the second antibodies labelled with horseradish peroxidase (diluted at 1:5000) were added for 1.5-hour incubation. The enhanced chemiluminescence (ECL) system (Amersham Biosciences) was employed to detect the protein content.

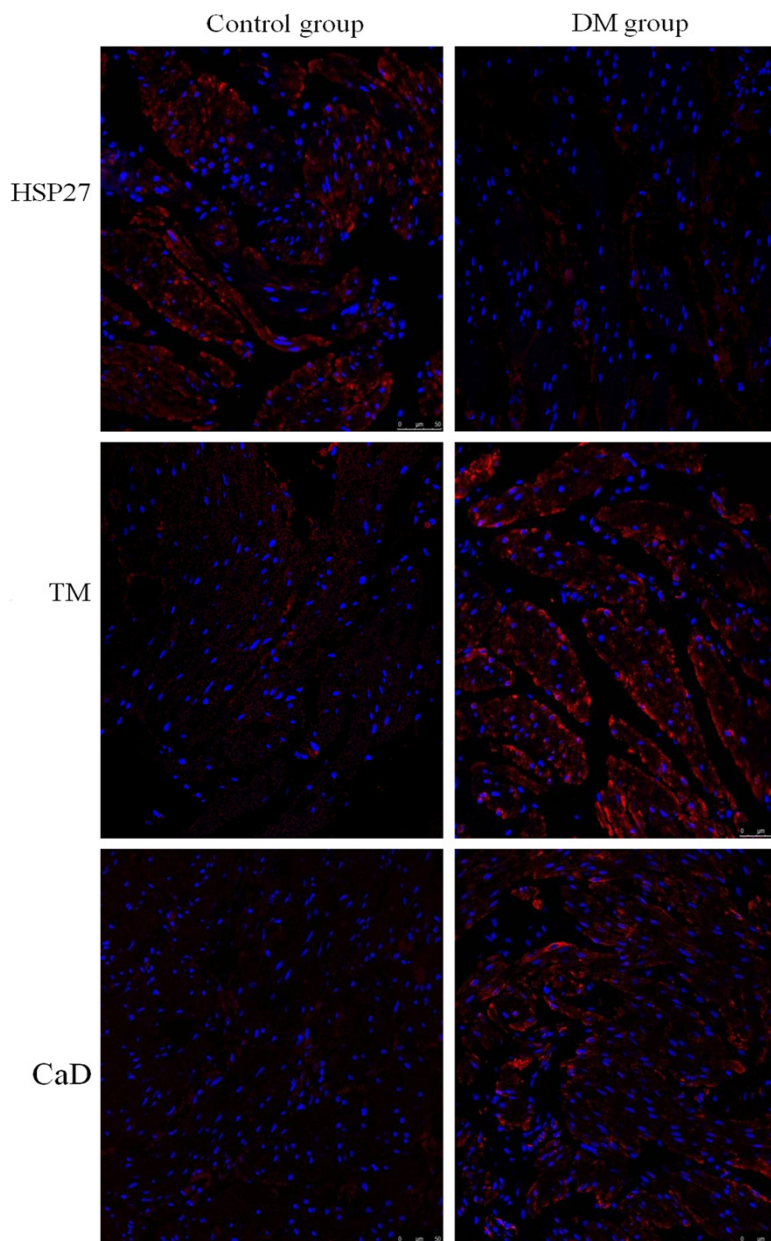
## Results

### *Detection of the detrusor ultrastructure with electron microscopy*

Compared with the controls, the detrusor cells of the models were hypertrophic with intracel-



**Figure 1.** Detection of the detrusor ultrastructure of the controls (A) and DM models (B) with electron microscopy.



**Figure 2.** Immunofluorescence staining of detection of HSP27, CaD, and TM protein.

ular myofilaments decreased and atrophic (**Figure 1**).

*Detection of the expression and distribution of HSP27, CaD and TM with immunofluorescent staining*

The immunofluorescent result showed that (See **Figure 2**) in the detrusor cells of both the DM models and controls, the protein expression of CaD and TM was up-regulated to some extent, while the expression of HSP27 was down-regulated. All of the three proteins were expressed in cytoplasm and gathered on cytomembrane.

*Genetic detection of HSP27, CaD and TM with QRT-PCR*

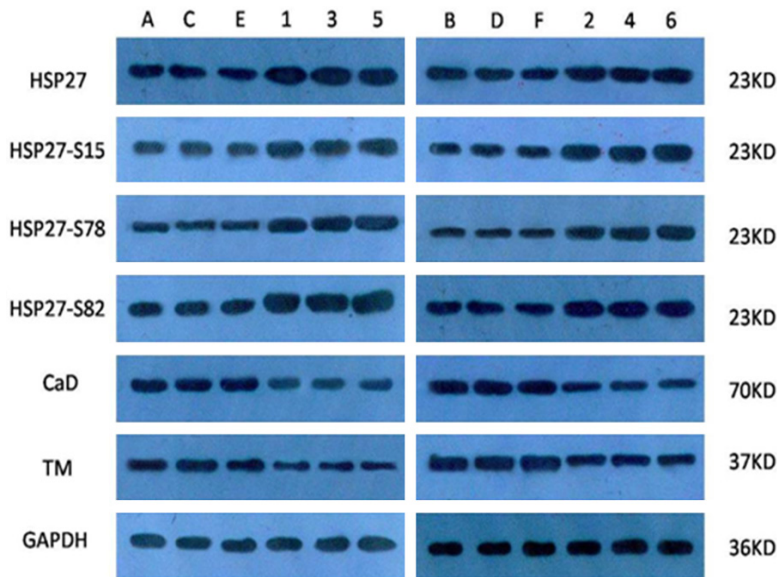
The relative expression levels of HSP27 mRNA of the models and controls were 2.35 and 5.13 respectively. In the model group, the mRNA expression levels of CaD and TM were  $0.35 \pm 0.12E+07$  and  $0.63 \pm 0.22E+07$  respectively, while in the control group, they were  $0.16 \pm 0.08 E+07$  and  $0.29 \pm$

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**Table 2.** Genetic detection of the DM models and controls

	HSP27		CaD		TM		$\beta$ -actin
	A	B	A	B	A	B	A
Controls	4.92±1.64	5.13	0.16±0.08	0.17	0.29±0.15	0.30	0.96±0.49
DM models	2.82±0.96*	2.35	0.35±0.12*	0.29	0.63±0.22*	0.53	1.20±0.41

Note: \*refers to  $P < 0.05$  compared with the controls. A: Average relative copy number ( $\times 10^7$ ); B: Relative ratio.



**Figure 3.** WB detection of specific phosphorylated HSP27, HSP27, CaD and TM proteins (A-F represent DM group; 1, 2, 3, 4, 5, 6 represent the control group).

0.15E+07 respectively. The result showed that (See **Table 2**) compared with the controls, the expression of CaD and TM was up-regulated, while the HSP27 expression was down-regulated, with significant difference ( $P < 0.05$ ).

### Detection of the expression of HSP27, specific phosphorylated HSP27, CaD and TM with WB

The SDS-PAGE result is shown in **Figure 3**. Compared with the controls, the bands of HSP27 and activated HSP27 (hsp27-ser15, hsp27-ser78 and hsp27-ser82) in the DM model group were light-colored, while the bands of CaD and TM were dark-colored. The gray value of each band was obtained via the gel processing system, and then the ratio of each test item to the internal reference was calculated. The result showed that compared with the controls, in the DM model group, the expression levels of CaD and TM proteins were significantly up-regulated, while the expression levels of both HSP27 and phosphorylated HSP27 were down-regulated (**Figure 3**).

**Figure 3** Detection of the expression of HSP27, specific phosphorylated HSP27, CaD and TM with WB (ABCDEF: DM models; 123456: controls).

### Discussion

DBD is a common complication of DM, accounting for 40-100% among DM patients. The DBD morbidity reaches 25% even among patients with stably controlled blood glucose [15], seriously threatening life quality. The pathogenesis of DBD has not been thoroughly unveiled by far.

Studies show that [16-18] HSP27, as an important molecular chaperone, plays a

pivotal role in the regulation on the contraction of smooth muscles. The expression and activation of HSP27 at the stress state can show a compensatory increase, which contributes to the maintenance and reestablishment of muscle cell contraction. HSP27 has three phosphorylation sites [19]: Ser15, Ser78 and Ser82. Phosphorylated HSP27 can stabilize the cytoskeleton of smooth muscle cells and enhance cellular contraction via inhibiting the depolymerization of F-actin and promoting polymerization of G-actin [20]. Proteins related to thin filament contraction, represented by CaD and TM, undertake a critical role in regulating smooth muscle contraction [21].

A recent research showed that [22] in the rabbit DBD model, the expression of multiple proteins related to thin filament contraction, such as CaD and TM, was up-regulated in the bladder detrusor, with decreased contraction of the detrusor. Likewise, this study also showed an up-regulated expression of CaD and TM and a down-regulated expression of HSP27. A study

shows that [23] the combination of HSP27 and phosphorylated CaD can lead to the dissociation of CaD and TM, which regulates the contraction of colon smooth muscles. This mechanism has not been reported in DBD cases. And the role of HSP27 in the contraction of bladder smooth muscles has also not been reported so far. The functional regulation of the bladder detrusor caused by the altered expression of HSP27, CaD and TM remains to be further studied. Our study is the first to report the down-regulated HSP27 expression in the DM rat model, laying the foundation for further investigating the mechanism of functional regulation of the bladder detrusor, revealing the pathogenesis of DBD and exploring protective measures as well as therapeutic targets for detrusor contractive dysfunction.

### Acknowledgements

The study was supported by the National Natural Sciences Foundation of China (No. 81270845).

### Disclosure of conflict of interest

None.

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