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# Resveratrol improves smooth muscle carcinogenesis in the progression of chronic prostatitis via the downregulation of c-kit/SCF by activating Sirt1

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ABSTRACT

*Purpose:* Bladder smooth muscle cell death accompanied by hyperplasia and hypertrophy, as induced by inflammation, is the primary cause for poor bladder function. There are emerging evidences on the role of chronic inflammation as a factor involved in carcinogenesis and progression. We aim to determine the bladder smooth muscle pathological changes and dysfunction in chronic prostatitis (CP), to investigate whether resveratrol can improve the urinary dysfunction and the role of c-kit/SCF pathway, that has been associated with the smooth muscle carcinogenesis.

*Method:* Rat model of CP was established via subcutaneous injections of DPT vaccine and subsequently treated with resveratrol. H & E staining was performed to identify the histopathological changes in prostates and bladders. Western blotting and immunohistochemical staining examined the expression level of C-kit, stem cell factor (SCF), Sirt1, apoptosis associated proteins.

*Results:* the model group exhibited severe diffuse chronic inflammation, characterized by leukocyte infiltration and papillary frond protrusion into the gland cavities, and a notable increase in prostatic epithelial height. Meanwhile, bladder muscle arranged in disorder with fracture, and cells appeared atypia. The activity of C-kit/SCF was up-regulated, the carcinogenesis associated proteins are dysregulated significantly in CP rats. Resveratrol treatment significantly improved these factors by Sirt1 activation.

*Conclusions:* activated c-kit/SCF and bladder muscle carcinogenesis were involved in the pathological processes of CP, which was improved after resveratrol treatment via the downregulation of c-kit/SCF by activating Sirt1.

## 1. Introduction

Chronic prostatitis (CP) is a common disease of urology, voiding dysfunction is the main clinical manifestations [1], and also an important factor that affects the quality of life of CP patients. It has been reported that the presence of urinary tract obstruction was revealed in patients with CP by urodynamic examination. As reported, this urinary tract obstruction mainly includes unstable bladder, sphincter spasm and detrusor-external sphincter dyssynergia [2,3].

As reported, [4,5], chronic inflammation could induce smooth muscle expansion, and promote bladder smooth muscle hyperplasia and hypertrophy which is the primary cause for poor bladder function. On the other hand, collective evidence from molecular, experimental and clinical data suggests that inflammation can contribute or promote urothelial bladder carcinogenesis [6], but the effect of chronic inflammation on bladder muscle carcinogenesis has not been investigated. Therefore we aim to study whether and how chronic inflammatory involved bladder dysfunction and smooth muscle carcinogenesis during CP progression.

As an important member of tyrosine kinase family, c-kit receptor causes specific expression of certain genes, regulates inflammation or tumor-related diseases [7–9], and plays a key role in cellular functions through activating the downstream signaling molecules following interaction with stem cell factor (SCF) [10]. Targeting SCF/c-kit signaling pathway is closely related to the therapy for some certain inflammation diseases and cancer [11,12]. And their role in smooth muscle carcinogenesis also has been indicated [13,14]. However, there is no report on the role of c-kit/SCF in the bladder muscle carcingenesis of CP.

Resveratrol [15] (trans-3,4,5-trihydroxy stilbene) is widely found in many plants and is well known as Sirt1 activator. The reliable and extensive anti-inflammatory effects of resveratrol arouse the attention of researchers [16]. Interestingly, resveratrol produced an improvement

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effect on muscle dysfunction [17]. And also, resveratrol has gained enormous attention in other smooth muscle cell research, including differentiation, apoptosis and proliferation [18,19].

Therefore, the present study was performed to evaluate the role of SCF/c-KIT and bladder muscle carcinogenesis in the development and progression of CP in rats, and to investigate the improve effect of resveratrol. The obtained findings will contribute to elucidating the molecular mechanisms underpinning the association between chronic prostatitis and bladder dysfunction, and represent a valuable information to define safety and efficacy of resveratrol-based therapies against CP.

## 2. Materials and methods

### 2.1. Chemicals

Resveratrol of > 99% purity is purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). DPT vaccine is obtained from Wuhan Institute of Biological Products Co., Ltd. (Wuhan, China). All other chemicals used in this study are analytical grade and commercially available.

### 2.2. Animals and ethics statement

Male Sprague-Dawley (SD) rats (180 ± 20 g) are purchased from the Experimental Animal Center of Dalian Medical University (Dalian, China). Rats are allowed access to water and food ad libitum, but fast overnight with water available before surgery. All animal experiments are approved by the ethics committee of Dalian Medical University and performed in accordance with the institutional guidelines.

All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

### 2.3. Rat model of CP

Purification of rat prostatic protein: After male SD rats (240 − 300 g) are sacrificed, prostate tissue is removed under sterile conditions and washed with saline solution. Then, prostate tissue is put into a physiological saline solution containing 0. 5% Triton X-100 and homogenized in an ice-water bath with a glass homogenizer. Subsequently, the homogenized liquids are centrifuged (10 000 × *g*) for 10 min, and protein is diluted to 15 mg/ml with PBS buffer (0. 1 mol/L and pH7. 2).

Rats are subcutaneously injected DPT vaccine (0.5 ml/kg) and intradermally multipoint injected the mixture (1.0 ml) of purification of rat prostatic protein and Freund's Complete Adjuvant (FCA) (1: 1) at 0, 15 and 30 d. Rat model of CP is completed at 45 d. Then Rats are randomly divided into three groups (8 rats in each group; n = 8):

1. Control group: Normal rats are orally administered saline for 10 d.
2. CP group: CP rats are orally administered saline for 10 d.
3. Resveratrol group: CP rats are orally administered resveratrol (10 mg/kg) for 10 d.

### 2.4. Bladder pressure and volume test in rats

Rats are anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg) before surgery. Then, the rats are fixed on the operating frame, and the upper edge of the pubic symphysis skin is bladed. The bladder is exposed and placed in the incision to avoid affecting the abdominal pressure on the detrusor pressure. Two 24G tubes are inserted in bladder and fixed, and then bladder is irrigated by saline (0.4 ml/min) via one 24G tube. Moreover, another tube is connected to MedLab biological signal acquisition system via pressure transducer. The maximum capacity of the bladder, residual urine volume and maximum voiding pressure are measured.

### 2.5. Morphological changes

After the end of the bladder pressure and volume test, the rats are sacrificed. The bladder of rats are removed, and fixed in 10% (v/v) neutral formalin processed by standard histological techniques, then stained with haematoxylin and eosin (H & E), and then they are examined for morphological changes of bladder. Inflammation was characterized by assessing the density of infiltrated inflammatory cells and prostatic epithelial height. [20,21]. The samples are used to determinate expression of protein of C-kit (CD-117), SCF, Sirt1 apoptosis associated protein and survivin by western blot analysis or immunohistochemical staining.

### 2.6. Western blot analysis

According to the manufacturer's instructions, proteins were extracted from rat bladder with protein extraction kit (KeyGen Biotech, Nanjing, China). Protein was measured according to the procedure of bicinchoninic acid (BCA) (Solarbio, Beijing, China), with bovine serum albumin as the standard. Proteins (20 μg) were resuspended in electrophoresis sample buffer containing β-mercaptoethanol and separated by electrophoresis on a pre-cast 10% SDS-polyacrylamide gel (Bio-Rad, Hercules, CA), followed by electrotransfer to a PVDF membrane (Millipore, Bedford, MA). Membranes were blocked using 5% non-fat milk in Tris-buffered saline with 0.1% Tween-20 (TBST) for 2 h at 37 °C. β-Actin served as loading control. Membranes were incubated overnight at 4 °C with a 1:1000 dilution of polyclonal antibody for c-Kit, SCF, Sirt1, Caspase-3, Bax, BCL2 and p53, respectively (Cruz Biotechnology, Santa Cruz, USA), and with a 1:1500 dilution of monoclonal antibody for β-actin (Beyotime, China). After subsequent washing with TBST, the blots were then incubated with secondary antibodies. After extensive washing with TBST, membranes were exposed to the enhanced chemiluminescence-plus reagents (ECL) from Beyotime Institute of Biotechnology (Haimen, China) according to the manufacturer's protocol. Emitted light was documented with a BioSpectrum-410 multispectral imaging system with a Chemi HR camera 410(Bio-Rad, Hercules, CA, USA). Protein bands were visualized and photographed under transmitted ultraviolet light. The image was used for semiquantitative measurements based on band densitometry.

### 2.7. Immunohistochemical staining

Histological sections of rat bladder (4 μm thick) were mounted on poly-L-lysine-coated slides. Slides were deparaffinized in xylene and rehydrated in graded alcohols. Sections were pretreated with citrate buffer (0.01 mol/L citric acid, pH 6.0) for 20 min at 95 °C. Then, at room temperature they were immersed in PBS containing 3% $H_2O_2$ for 10 min. After treatment with exposing them to 10% normal goat serum in PBS for 30 min at room temperature, the tissue sections were incubated at 4 °C overnight with rabbit polyclonal anti- survivin antibodies (1:400 dilution) (AS792. Beyotime, China). Then sections were rinsed with PBS, incubated with biotinylated goat anti-rabbit IgG for 20 min at room temperature and treated with 3,30-diaminobenzidine chromogen for 5 min at room temperature. Finally, sections were counterstained with hematoxylin for 6 min. The sections are finally measured using a quantitative digital image analysis system (Image-Pro Plus 6.0) [22], for the semi-quantitative analysis of the protein expression levels of surivivn.

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