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Simulated physiological stretch-induced proliferation of human bladder smooth muscle cells is regulated by MMPs

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ABSTRACT

Mechanical stimulation is an essential factor for organisms to develop normally. In bladder development matrix metalloproteinases (MMPs) play an important role through structure remodeling and regulating the cell proliferation. In this study, we investigated the simulated physiological stretch induced proliferation of HBSMCs; MMPs/TIMPs expression in stretch and non-stretch groups. HBSMCs were exposed to cyclic stretch with defined parameters (5%, 10% and 15% elongation). The expression of MMPs and TIMPs in each parameter and non-stretch groups was examined at the transcriptional and translational levels respectively. 5-Ethynyl-2′-deoxyuridine (EdU) assay was used to assess cell proliferation. In the presence of the broad spectrum MMPs inhibitor (Batimastat), cells proliferation, MMPs and tissue inhibitors of metalloproteinases (TIMPs) expression were assessed again. Compared with non-stretch group, HBSMCs in stretch groups showed higher proliferation. The expression of MMP-1, 2, 3, 7 was up-regulated in stretch groups, and it remained at the same high level in 10% and 15% stretch groups. TIMP-1, 2 expression only increased under 15% stretch. Stretch resulted in elevated cell proliferation was abolished by Batimastat. In conclusion, the proliferation of HBSMCs induced by stretch was resulted from the stretch-induced MMPs expression and release.

Introduction

Because of the special structure of hollow internal organs such as windpipe, urinary bladder and heart, the inner wall of these organs is constantly subjected to mechanical force during filling and contraction cycles. The cells in these organs are affected by the force in their microenvironment.

Many studies have illuminated that apposite mechanical stimulation is required for the activation of signaling pathways, cell proliferation, differentiation, migration, function maturation and reasonable composition of the extracellular matrix (ECM)[2] [1–6]. Conversely, the abnormal mechanical environment may cause some pathological changes such as histological structure remodeling and proportional changes of ECM [7,8].

Human bladder smooth muscle cells (HBSMCs) are constantly subjected to mechanical stimulation, including hydrodynamic pressure and stretch, during filling and voiding cycles. Previous studies showed that both hydrodynamic pressure and stretch can stimulate HBSMCs proliferation and viability in a certain range, while stress exceeded the limits, these biological behaviors of HBSMCs would be restrained [9–11]. In this mechanical parameters-depended dynamic process, HBSMCs behaviors are influenced by several factors, including activation of signaling pathways and alteration of cellular morphology.

Matrix metalloproteinases (MMPs) constitute a family of structurally related zinc-dependent proteolytic enzymes that conjointly are able to degrade major components of the extracellular matrix. This rapidly growing family contains at least 28 members who, despite significant overlap, display clear substrate specificity [12].

The MMPs family mainly includes the collagenases (MMP-1, MMP-8, MMP-13), which degrade fibrillar interstitial collagens. Gelatinases (MMP-2 and MMP-9) cleave denatured collagen fibrils

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[2] *Abbreviations used:* MMP, matrix metalloproteinase; HBSMCs, human bladder smooth muscle cells; ECM, extracellular matrix; TIMP, tissue inhibitors of metalloproteinase; EdU, 5-ethynyl-2′-deoxyuridine.

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and basement membrane type IV collagen. Stromelysins (MMP-3, MMP-7, MMP-10, MMP-11, MMP-12, MMP-18) are enzymes with broad substrate specificity, and the membrane-type MMPs (MT-MMPs) [13–15].

Some of these proteases can be secreted by HBSMCs and other cells in the bladder, and MMPs have been reported to play a critical role in not only structure remodeling but also cell proliferation [16].

In vitro, the mRNA expression of MMP-7 of HBSMCs exhibited a trend of increasing when hydrostatic pressure in a certain range, while cell proliferation also increased simultaneously [17].

Aitken and colleagues found that gelatinase activity of bladders is increased during mechanical stretch intervention ex vivo, and the increased secreted MMP activity can stimulate bladder cell proliferation through triggering MAPK signaling pathway [18].

Additionally, we recently observed that physiological stretch also increased HBSMCs contractility by examining the percentage of retraction of the collagen gel reaction [11], and this increased contractility was also regulated by MMPs activity [19].

The activity of MMPs is modulated by a group of endogenous proteins called tissue inhibitors of metalloproteinases (TIMPs). A survey of the literature reveals that TIMPs play the role of regulator not by inhibiting the expression of MMPs but only by inhibiting the activity of MMPs [20].

In answer to mechanical force in hollow internal organs, structure remodeling and cell proliferation were related to modifications in activities and levels of the MMPs [8]. Well development of bladder response to stretch in a reasonable range is owing to the balance and harmony of MMPs/TIMPs, but the dysregulation of MMPs caused by excessive mechanical stimulation may lead to fibro-proliferative [16].

Based on these studies, we realize that MMPs play a vital role in stretch-induced HBSMCs behaviors, but these studies, results are still not comprehensive. MMPs activity is proved to be related to mechanical stretch, but most of the researches were conducted under pathologic conditions.

The most notable problem is that distinct MMPs expression and release under physiological stretch remained undefined. On top of that, it is still unclear how TIMPs dynamically regulates MMPs function under different stretch parameters. Solving these problems are important for understanding how physiological stretch affects bladder cells' microenvironment and further influences their behaviors, and deeper understanding of these mechanisms will contribute to the construction of tissue engineering of bladder in vitro.

The aim of the present study was to depict the total expression picture and interactions of various MMPs and TIMPs under physiological stretch in different orders of magnitude. Lastly, to determine whether stretch-induced HBSMCs proliferation is MMPs-dependent in vitro.

The results indicated that the mechanical stretch can promote the proliferation of HBSMCs under specific stretch parameters. HBSMCs proliferation was induced by simulated physiological stretch in a MMPs-dependent manner.

Materials and methods

Cell culture

HBSMCs (ScienCell, USA, Cat. No. 4310) were cultured in Dulbecco's modification of Eagle's medium (DMEM, Low Glucose, HyClone, USA) supplemented with 10% fetal bovine serum (FBS, HyClone, USA), penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37 °C in a humidified atmosphere of 95% air/5% CO_2. All experiments were done using cells at passages 3 to 7. The broad spectrum MMPs inhibitor, Batimastat (Sigma–Aldrich Co., St Louis, MO, USA), was used (10 μM concentration) for experiments.

Physiological stretch procedures

We chose definite stretch parameter (5%, 10% and 15% elongation) to mimic relatively low intravesical strain, and the loading procedures were designed to mimic the 24 h filling–voiding cycles of bladder. HBSMCs were seeded on silicone membranes. The cells were grown to 90% confluence and exposed to simulated physiological stretch in BioDynamic mechanical stretch unit (Bose, Eden Prairie, MN, USA). Stretch patterns were implemented as follows: 4 h/cycle, stretch elongation range were from 0% (no stretch) to 2.5% in the first 3 h, then 5%, 10% or 15% in the next 1 h. The bladder emptying was then mimicked by decreasing very quickly from 5%, 10% or 15% to 0%, in a few seconds. This 4 h/cycle was repeated for 4 times in 24 h, and for the remaining 8 h silicone membranes were maintained in relaxed sate (mimicking bladder wall tension at night) (Fig. 1).

RNA isolation and real-time PCR

Total RNAs were isolated from each group by using RNA prep Pure Cell/Bacteria Kit (TIANGEN BIOTECH, BEIJING) according to the manufacturer's instruction.

The extracted RNA was dissolved in nuclease-free water and then quantified by measuring the absorbance at 260 nm, and then cDNA was synthesized using the iScriptcDNA Synthesis Kit

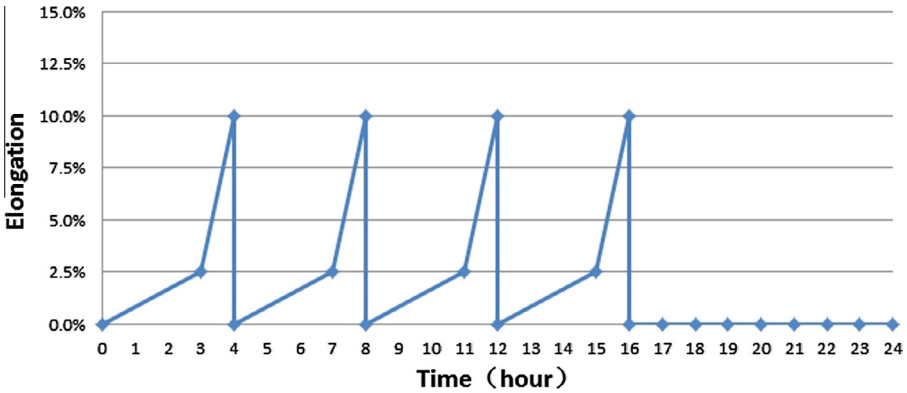

Fig. 1. The application of simulated physiological stretch: elongation was applied from 0% to 2.5% during first 3 h, followed by a progressive increase up to 5%, 10% or 15% during the last hour (completing a 4 h cycle) according to experiment designed. The bladder emptying was then mimicked by decreasing very quickly from 5%, 10% or 15% to 0%, in a few seconds. Silicone membranes were maintained in relaxed sate for the remaining 8 h (mimicking bladder wall tension at night).