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UNI-AXIAL MECHANICAL STRETCH CHANGES CELL SHAPE WITH ACTIN STRESS FIBER REORGANIZATION AND ACTIVATES C-JUN NH2 TERMINAL KINASE IN HUMAN BLADDER SMOOTH MUSCLE CELLS / INVOLVEMENT OF RHOA AND ROCK.

Hypothesis / aims of study

Bladder outlet obstruction is known to cause structural alterations in the bladder wall, including smooth muscle hypertrophy/hyperplasia. Although the mechanisms responsible for these pathologic changes are still obscure, an excessive mechanical overload may be involved in the pathogenesis. Repetitive stretch and relaxation applied to bladder smooth muscle cells *in vitro* have been used to model the overloaded detrusor smooth muscle under condition of bladder outlet obstruction. Using the above model, we previously showed that mechanical stretch activated c-Jun NH2 terminal kinase (JNK), which is a member of mitogen activated protein kinase (MAPK). And also, it is suggested that JNK activation may contribute to smooth muscle remodelling. It has been demonstrated recently that the expression of RhoA and Rho kinase (ROCK) increased in the obstructed bladder (1). In addition, RhoA is known as regulator of actin cytoskeleton. Thus, the present study was undertaken to investigate whether a RhoA/ROCK pathway is involved in actin stress fiber reorganization as well as in JNK activation in human bladder smooth muscle cells (HBSMCs) subject to mechanical stretch, which would contribute to smooth muscle hypertrophy/hyperplasia.

Study design, materials and methods

In all experiments, we used commercially established HBSMCs purchased from CAMBREX and made verification by staining alfa-smooth muscle actin. To simplify the analysis, we used uni-axial stretch device (STREX) which can stimulate adherent cells to a single direction stretch by controlled motor unit (fig.1). After reaching confluent by usual culture, cells were plated on silicone elastomer-bottomed culture plate that had been coated with 1 microgram/ml fibronectin dissolved in PBS. Further, after 24 hr incubation in static condition, cells were stimulated by 15 % elongated uni-axial stretch at 1 Hz. Activity of JNK was measured by western blotting method using anti-phospho JNK antibody which binds to activate form of JNK. The shape of stimulated HBSMCs was observed by using microscope. Actin stress fiber was labelled by phalloidin conjugated with Alexa-Fluor 594 and observed by using Olympus fluorescence microscopy. To investigate role of RhoA and ROCK for JNK activation and actin stress fiber formation, HBSMCs were pre-incubated with several inhibitors before and during stretch. All experiments were performed at least three times.

Results

1. Stretch stimulated HBSMCs were harvested at each time point and the activities of JNK were measured. The activity of JNK peaked at 30 min after stretch about 3.0 fold (fig.2a), and the activity was partially abrogated by pre-incubation with 5 microgram/ml C3 exoenzoyme (RhoA inhibitor) and 1 microM Y27632 (ROCK inhibitor), respectively (fig.2b).
2. After 4 hr stretch, HBSMCs began to change their shape as elongated in a horizontal direction. And also, actin stress fiber was more strongly formed and became aligned perpendicular to the stretch direction (fig.3b).The cell structural change had continued 12 hr. After pre-treatment with C3 exoenzyme and Y27632 respectively, cell shape change and actin stress fiber reconstruction were suppressed (fig.3c, 3d). 10 microM SP600125 (JNK specific inhibitor) had no effect on changing cell shape and actin stress fiber reconstruction (fig.3e).

Interpretation of results

Uni-axial stretch applied to HBSMCs activates JNK and changes cell shape with actin stress fiber reorganization. The experiments using specific inhibitors indicate that RhoA/ROCK may have signal cross-talk with JNK, though inhibition of JNK itself does not effect the cell structural change. Therefore, it is suggested from these results that the stretch activated RhoA/ROCK pathway has two different roles; one is to contribute to cell structural change and other to proliferative signal cascade including JNK. Considering these phenomena of cell shape change and stress fiber reorganization, HBMCs seem to have the ability of sensing mechanical stress and adapting to overload by making cytoskeletal remodeling. JNK was shown to act as a regulator of activator protein-1 which binds to consensus sequence in the promoters of genes that regulate cell hypertrophy in some cell types (2). Thus, there is a possibility that up-regulated RhoA/ROCK strengthens signal transduction for JNK and contributes to smooth muscle hypertrophy/hyperplasia.

Concluding message

In this study, we presented the roles of RhoA and ROCK in HBSMCs stimulated by uni-axial mechanical stretch. It seems likely that they act as key molecules for bladder wall remodelling process.

References

(1) Am J Physiol Renal Physiol (2003) 285(5); F990-997
(2) FASEB J (1996) 10; 631-636

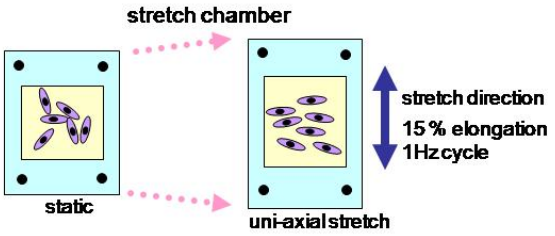

Fig 1. schema of uni-axial stretch device

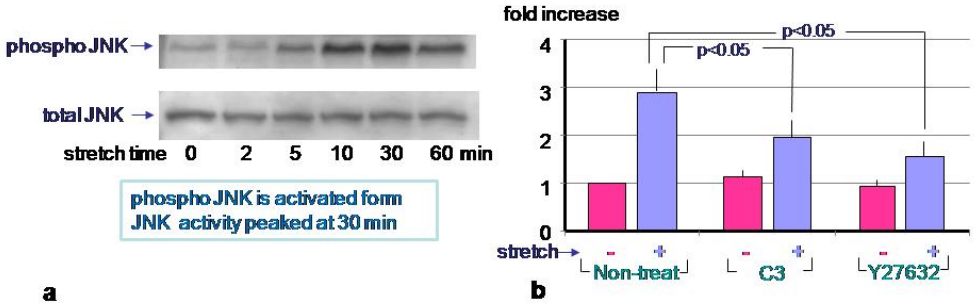

Fig 2. a : time course of JNK activity by uni-axial stretch

b : JNK activity by pre-treatment C3 (RhoA inhibitor) and Y27632 (ROCK inhibitor)

- activity of JNK was measured at 30 min after stretch
- each activity compared with non-treatment stretch (-)

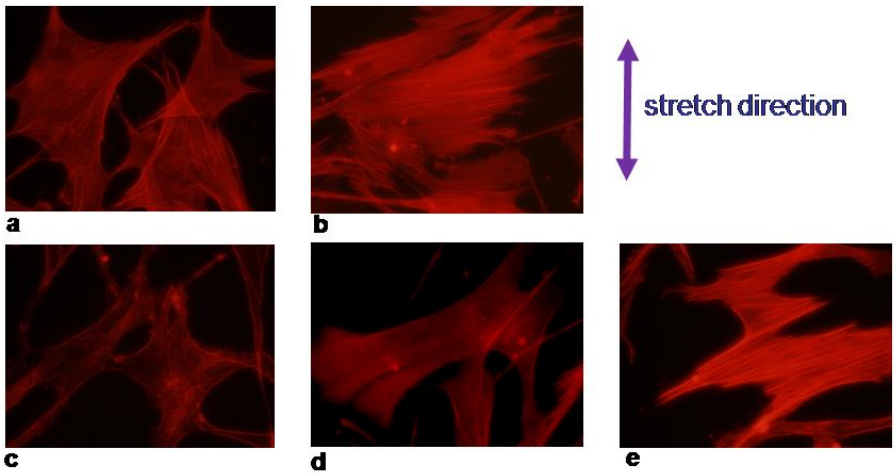

Fig 3. a : non-stretch b : after 4 hr uni-axial stretch

c : pretreatment with C3 and after 4 hr uni-axial stretch

d : pretreatment withY27632 and after 4 hr uni-axial stretch

e : pretreatment with SP600125 (JNK inhibitor) and after 4 hr uni-axial stretch

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