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MYOSTATIN INHIBITS GROWTH OF SATELLITE CELLS IN HUMAN URETHRAL RHABDOSPHINCTER VIA PHOSPHOLATION OF SMAD-2

Hypothesis / aims of study

Myostatin (MST), which is a member of the transforming growth factor-β (TGF-β) super family, is a negative regulator of myogenesis of skeletal muscle (1). Decrease of human urethral rhabdosphincter (RS) with aging is reported and suggested a cause of urinary incontinence in the elderly (2). To develop a new strategy for the regeneration of human RS, the effect of MST on the growth of human RS satellite cells (muscle stem cells).

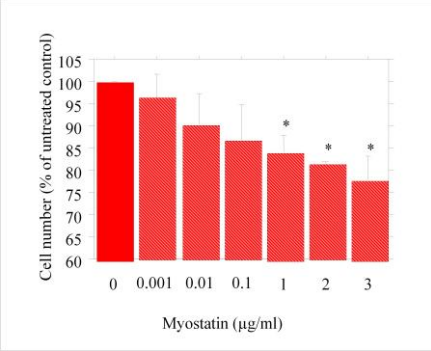
Study design, materials and methods

Human RS was obtained from the patients undergoing radical cystectomy for bladder cancer. Human RS satellite cells were selectively cultured by magnetic affinity sorting method (MACS) using anti-neural cell adhesion molecule (NCAM) antibody (3). Primary RS satellite cells were transfected with SV40 large T antigen to elongate the life, and used for the following experiments. The effects of MST on the growth of RS satellite cells were investigated under serum-free medium. In order to study the autocrine actions of endogenous MST, the expression of MST was examined by immunocytochemistry and RT-PCR. Moreover, immunoneutralization test was performed using MST antibody. The intracellular signal transductions of MST were examined by immunoblot assay.

Results

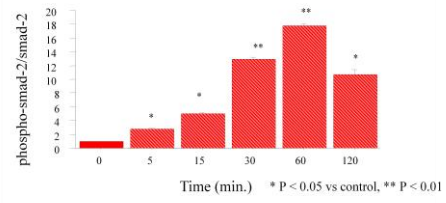
1. Inhibitory effect of MST on the proliferation of human RS satellite cells

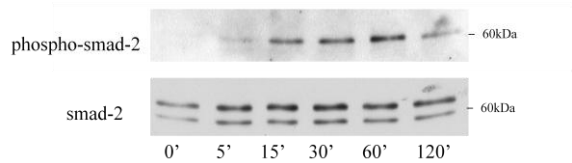
The addition of 1.0 μg/ml or more of MST decreased the cell number to 84% compared with the control.



2. Signal transduction of MST in human RS satellite cells

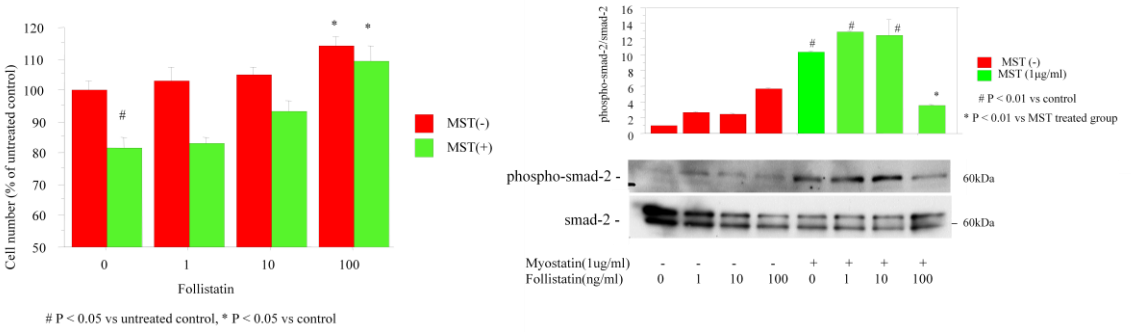
Phosphorylation of smad-2 occurred within 5 minutes and peaked 60min after MST stimulation.





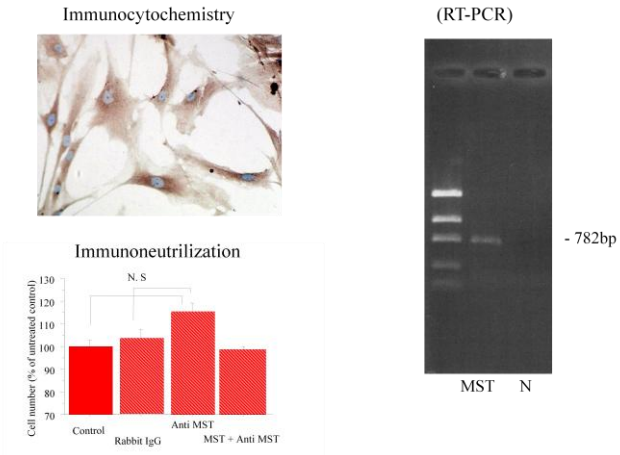
3. Effects of follistatin, a MST inhibitor, on the signal transduction and the proliferation of human RS satellite cells

A MST inhibitor, follistatin (FST), suppressed the phosphorylation of smad-2 and enhanced the growth of RS satellite cells at the concentrations on 100ng/ml.



4. Autocrine secretion of MST from human RS satellite cells

RT-PCR demonstrated that HRS satellite cells expressed detectable levels of mRNAs of MST. Immunocytochemistry revealed the protein expression of MST in human RS satellite cells. In immunoneutralization tests, anti-MST antibodies tended to enhance the proliferation of human RS satellite cells to about 116% of the control antibodies or serum-free medium alone. However, there was no difference among each group.



Interpretation of results

MST inhibited the proliferation of human RS satellite cells. On the contrary, FST promoted the proliferation of human RS satellite cells by inhibiting the phospholation of smad-2 induced by MST. On the other hand, current results could not indicate that human RS satellite cells had an autocrine action of MST for the growth inhibition by itself.

Concluding message

Inhibition of MST function may be useful in the development of a novel technique for the regeneration of human RS to treat urinary incontinence.

References

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