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TUMOR NECROSIS FACTOR-A INHIBITS THE DIFFERENTIATION OF MYOGENIC CELLS IN HUMAN URETHRAL RHABDOSPHINCTER.

Hypothesis / aims of study

The external urethral sphincter known as the urethral rhabdosphincter (RS) is mainly composed of striated muscle, which may be a cause of urinary incontinence in the elderly people. Meanwhile, it has been considered that chronic low grade systemic inflammation is widely considered to be a cause of age-related loss of muscle mass. Tumor necrosis factor-α (TNF-α), an inflammatory cytokine, increases systemically with increasing age, and induces apoptosis in murine striated muscle. In the present study, we examined the inhibitory effect of TNF-α for myogenic differentiation in human RS myogenic cells.

Study design, materials and methods

Human urethral RS samples were obtained from patients who underwent total cystectomy. Human RS myogenic cells were immortalized on the protocol; mutated cyclin-dependent kinase4, cyclinD1 and teromerase (Shiomi et al.2011) to expand their life span. Consecutively, we examined differential potential of human RS myogenic cells. These differentiated myotubes were treated by recombinant human TNF-α and/or TNF-α antagonist, etanercept, at different concentrations. Molecular biological analyses were evaluated by western blot, immunostaining, and real-time RT-PCR. To gain further insight into the mechanism by etanercept, we monitored NFκB, Akt and p38 protein levels by western blot.

Results

We could selectively culture human RS progenitor cells for at least 40 passage. Molecular analysis confirmed that the expression of myosin heavy chain (MHC), which was one of differentiated muscle-specific markers, significantly increased after differentiation induction. Although TNF-α treatment reduced the expression of MHC in concentration dependency, etanercept inhibited their suppression (Fig. 1. 2). Regarding signal transduction, TNF-α suppressed phosphorylation of NF-κB, Akt and p38, meanwhile pretreatment by etanercept promoted these phosphorylation and MHC expression in concentration dependency (Fig.3).

Interpretation of results

TNF-α inhibited the differentiation of human urethral RS myogenic cells in part via NF-κB, p-38 MAPK and PI3 pathways. On the other hand, etanercept promoted the differentiation of these cells by suppression of TNF-α effect.

Concluding message

TNF-α may be involved in age related decreases in the number of human urethral RS cells and be a causative factor for urinary incontinence in the elderly population.

Fig.1

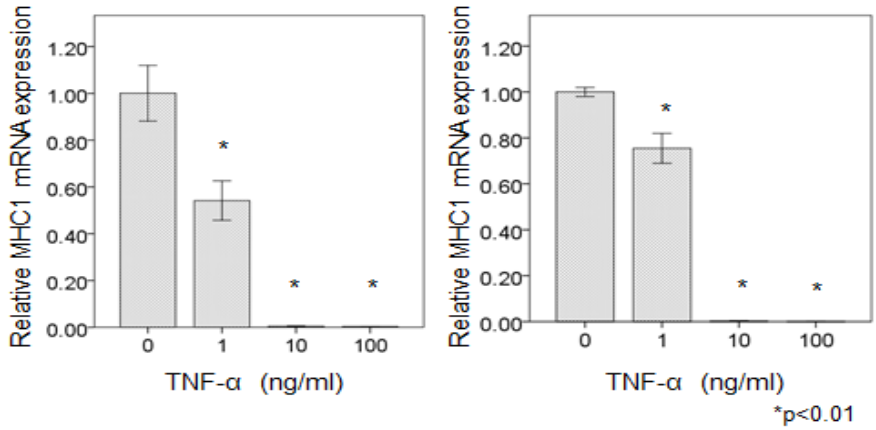


Fig. 2

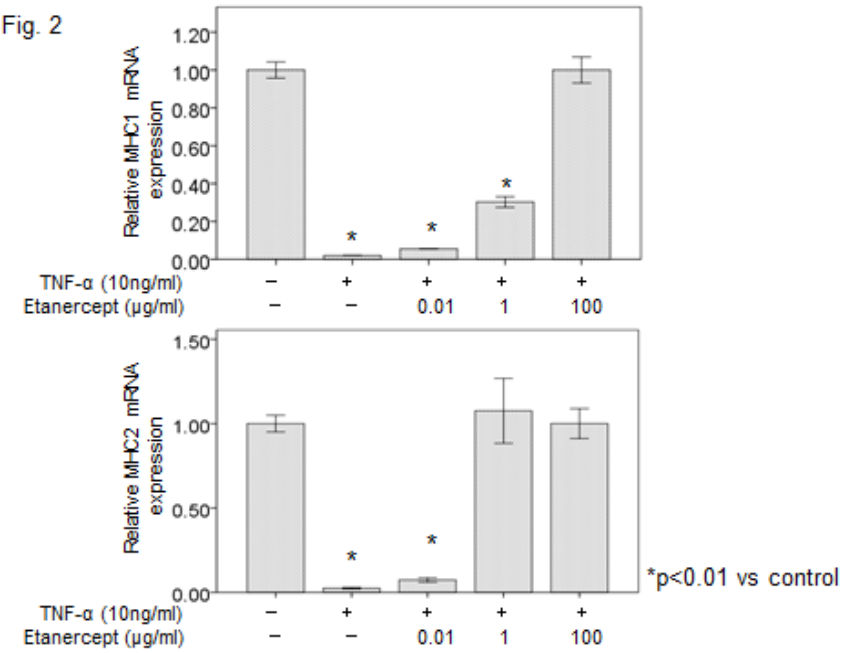
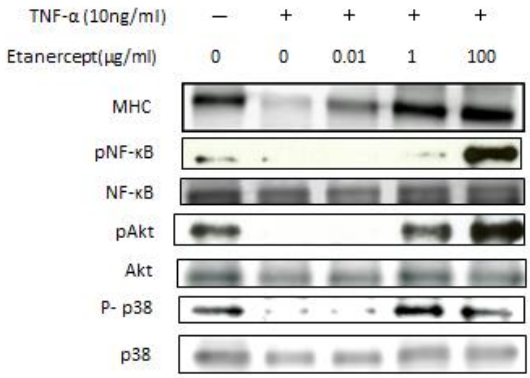


Fig.3



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Disclosures

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