Mechanical stretching induces skeletal muscle hypertrophy by increasing the expression of IRS-1 and myogenin

INTRODUCTION
Mammalian skeletal muscles exhibit tremendous plasticity in response to mechanical stress. For example, we gave mechanical stretching to skeletal muscle cells at the cyclic and the continuous pattern using a stretching system. Our results indicated that moderate mechanical cyclic stretching patterns induce responses, skeletal muscle hypertrophy. However, we did not explain why skeletal muscle hypertrophy was caused by moderate mechanical stretching patterns.

For understanding of the hypertrophy mechanism, we aimed to insulin-like growth factor-1 (IGF-1), MyoD and myogenin. IGF-1 is known as mitogenic and myogenic factor [1], and is secreted in response to exercise. Binding of IGF-1 to the IGF-1 receptor recruits the insulin receptor substrate (IRS-1). This stimulation leads to skeletal muscle hypertrophy via IGF-1 signaling pathways. MyoD and myogenin are known as the myogenic regulatory factors (MRFs) and play key regulatory roles in the development of skeletal muscle during myogenesis [2-3]. MyoD is expressed in proliferating, undifferentiated myoblasts, whereas myogenin is induced upon muscle differentiation.

We hypothesize that these factors are involved in the skeletal muscle hypertrophy after mechanical stretching.

So, we gave mechanical stretching to mouse cultured myoblast at different stretching patterns and measured expression of IRS-1, MyoD and myogenin by using real-time PCR and Western blotting.

MATERIALS AND METHODS

Cell culture
C2C12 cells, which were mouse skeletal muscle myoblasts, were used in this study.

Mechanical stretching of C2C12
The cells attached to the flexible collagen-coated silicone chamber bottom were incubated for 96 hours in Dulbecco’s modified Eagle’s medium supplemented with 2% horse serum until myotubes were performed before the mechanical stretching. Mechanical stretching was applied by using a uni-axial stretching system at 37deg C, 5% CO2, at cyclic stretching (10 cycle/min) and continuous stretching for 30 minutes.

Real-time PCR analyses
Total RNA was isolated using RNeasy Mini Kit (QIAGEN) at 1, 15 and 30 minutes after mechanical stretching. Complementary DNA was made from 1μg of total RNA using Omniscript RT Kit (QIAGEN). For quantitative analysis of the expression level of IGF-1, MyoD and myogenin mRNA, real-time PCR was performed with a SYBR Green gene expression assays. All gene expression patterns were normalized to the expression patterns of GAPDH. The products were analyzed with a thermal cycler (ABI 7500 Real Time PCR System).

Western blot analyses
Total proteins were isolated from each chamber with myotubes using RIPA buffer at 6, 12 and 24 hours after mechanical stretching. Extracted total protein contents of myotubes were measured using spectrophotometric analyzer. The proteins were separated by 8% SDS-polyacrylamide electrophoresis gel for IRS-1, 10% for MyoD, 10% for myogenin and 10% for β-Actin. Then the samples were transferred to nitrocellulose membranes that were used to perform immunostaining. Monoclonal anti-IRS-1 antibody, MyoD antibody and myogenin antibody were applied as primary antibodies. Monoclonal anti-β-Actin antibody was used for protein quantification. The horseradish peroxidase-conjugated secondary antibody was used for IRS-1, MyoD, myogenin and β-Actin.

RESULTS

Cyclic stretching culture has a higher percentage of long and wide myotubes than the others (Fig. 1 and 2). Table 1 shows extracted total nuclei, RNA, DNA and protein contents of myotubes in stretching and control cultures.

Messenger RNA expression of IRS-1 increased (3.5-fold) immediately after cyclic stretching (Fig. 3A). Messenger RNA expression of MyoD decreased immediately after stretching. Messenger RNA expression of myogenin after stretching slightly decreased.

Proteins of IRS-1 and myogenin increased at 12hours after stretching. In contrast, protein of MyoD decreased after cyclic stretching (Fig. 3B).

DISCUSSION
We gave mechanical stretching to skeletal muscle cells at the cyclic and the continuous conditions. Messenger RNA expression of IRS-1 increased immediately after cyclic stretching. Proteins of IRS-1 and myogenin increased after cyclic stretching.

These results suggest that cyclic stretching induces expression of IRS-1 and myogenin. These factors have potential for inducing hypertrophy of the skeletal muscle after mechanical stretching.

The findings from this study improve our understanding of the muscle hypertrophy process and may facilitate the development of novel ways to induce muscle hypertrophy by mechanical stretching.

REFERENCE