Tensile stress regulation of NGF and NT3 in human dermal fibroblast

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Key Words : Tensile stress, Fibroblast, Neurotrophin, Transforming growth factor

Abstract

Fibroblast is constantly subjected to mechanical loads in connective tissues where mechanical signals are converted to intercellular biochemical events. The aim of this study is to understand the effects of tensile stress on the neurotrophin (NT) and transforming growth factor (TGF) expression of fibroblast in vitro. Nerve growth factor (NGF) stimulates fibroblast migration, and TGF is related to tissue repair. In this study, at the uniaxial stretch of 10% strain and frequency of 0.5 Hz, different resting times of 0, 20, and 60 min are placed in between 10 min stimulations periods. Results show increase in NGF mRNA levels and a substantial decrease in NT3 mRNA after 1 hr of stimulation, indicating that the tensile stress may regulate NGF and NT3, key factors for the neurocosmetic applications. The mRNA level for TGF-α and TGF-β2 had increased up to two-folds after 1 hr of stimulation, showing that the tensile stress may control TGF, an important part of wound healing.

1. Introduction

Mechanical forces play a important role in regulating tissue homeostasis (1). Fibroblast is a major type of mechanosensitive cells in connective tissues, which transfer mechanical signals to intercellular biochemical events (2, 3). Numerous previous studies have shown that the tensile stress alters ECM-related gene expressions in fibroblast (4, 5, 6, 7). In this study, we investigate the effects tensile stress on the neurotrophin (NT) and transforming growth factor (TGF) expression of dermal fibroblast *in vitro*. NGF and NT3, members of NT (neurotrophin) family, stimulate fibroblast migration and are related to wound healing(8, 9). Also, it is known that upregulation of NGF takes part in maintaining the biomechanical properties of the dermis (10). TGF, on the other hand, is related to tissue repair and Type I collagen synthesis, the main structural component of the extracellular matrix (ECM) (11,12). Here we observe NGF enhancement and NT3 reduction in response to the uniaxial stretch in human dermal fibroblast. Both neurotrophins and TGF exhibit tensile stress dependent behavior which could be an important part of neurocosmetic applications and wound healing.

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2. Uniaxial stretch regulates transcription level of NGF and NT3

In this study, seeded cells on the polydimethyl-siloxane (PDMS) substrate are stretched using a tensile stimulation device. With the uniaxial stretch of 10% strain at different frequencies, resting time 60 min were placed in between 10 min stimulation periods for the total stimulation of 1 hr.

To observe the change of NGF and NT3 mRNA level in response to uniaxial stretch, we performed real-time PCR analysis. Interestingly, NGF mRNA levels increased, and NT3 mRNA levels decreased after stimulation while showing with weak dependence on frequencies (Fig1).

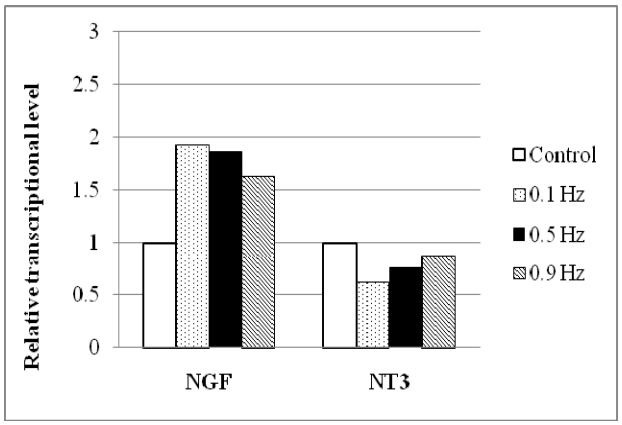

Fig 1. Uniaxial stretch enhances the mRNA level of NGF and reduces the mRNA level of NT3.

3. Dependence of total stimulation time for NGF and NT3 mRNA

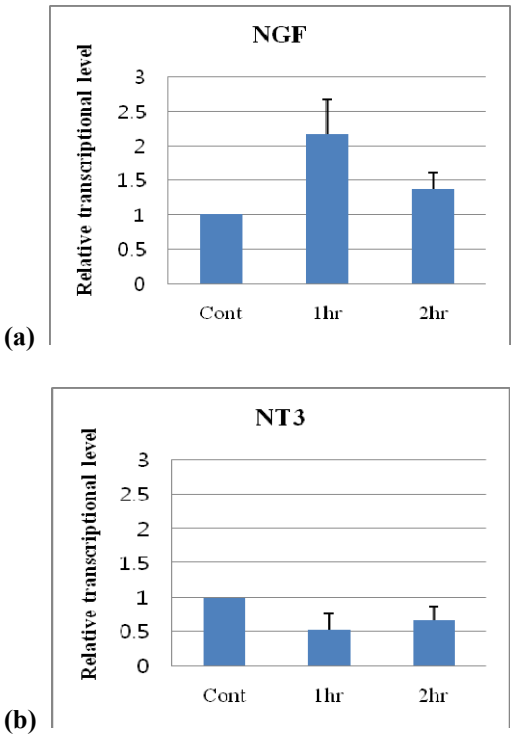

Fig 2. Effect of stimulation time on NGF and NT3 mRNA expression level

Figure 2 shows the effects of stimulation time on expression level of NGF and NT3. NGF mRNA was transcribed up to two-folds after 1hr stimulation. However, the level of mRNA transcription had decreased upon additional 1hr stimulation (Fig2.a). For NT3, mRNA level was decreased by half after 1hr stimulation, and not much difference was observed after additional stimulation (Fig2.b). Therefore, we concluded that stimulation longer than 1hr is not necessary to observe more dramatic result in this experiment.

4. Resting time during tensile stress and gene transcriptional regulation

Change in gene expression level requires resting time after stimulation (12, 13). Therefore, we investigated the effects of resting time on the gene expression. Resting times were given as 0min, 20min, and 60min between 10 min stimulations periods. The highest level of NGF mRNA and the lowest level of NT3 mRNA were observed after 60min of resting time (Fig3).

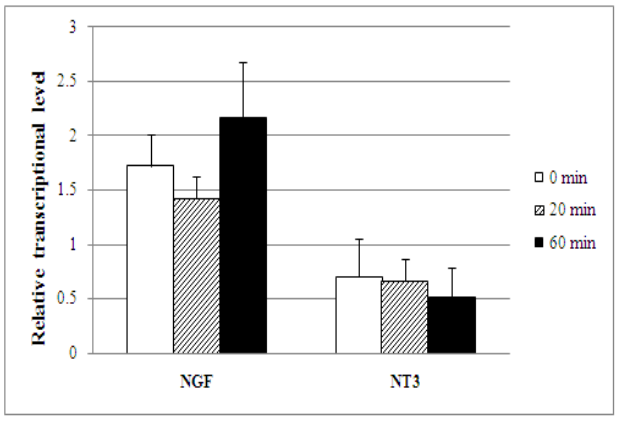

Fig 3. Resting time improves stimulatory effect.

5. Uniaxial stretch and other gene expression

Uniaxial stretch is known to induce collagen expression, and it is related to TGF-β1 pathway (6). To investigate the effect of uniaxial stretch on TGF-α and TGF-β family mRNA levels were observed. TGF-α and TGF-β2 mRNA levels had increased after stimulation (Fig4.a), while other TGF-β family genes are not changed in transcriptional level (Fig4.b). TGF-α and TGF-β are known to be related to wound healing process and tissue repair.

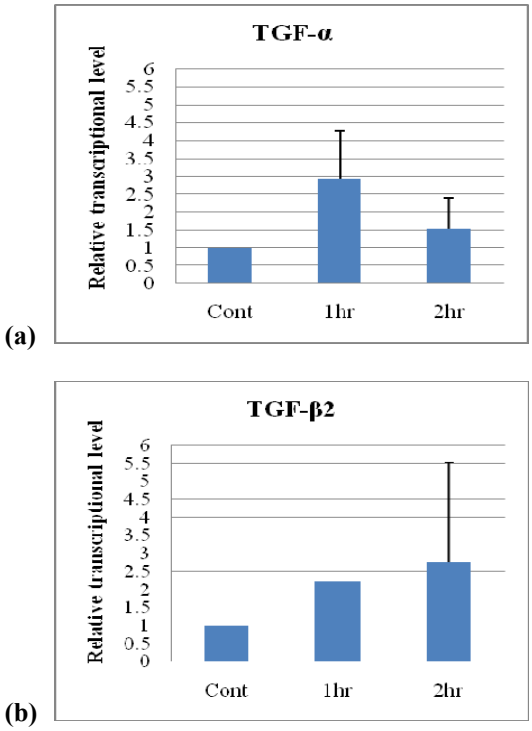

Fig 4. Uniaxial stretch effects on the TGF-α and TGF-β2 mRNA level.

6. Results

Tensile stress may regulate NGF and NT3 which could be a key factor for the neurocosmetic applications. Result for TGF indicates that the tensile stress may control TGF, an important part of wound healing.

Acknowledgement

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