INTRODUCTION

Ossification of the posterior longitudinal ligament of the spine (OPLL) is characterized by ectopic bone formation in the spinal ligaments. Mechanical stress, which acts on the posterior ligaments, is thought to be an important factor in the progression of OPLL. Recently, the spinal ligament cells derived from OPLL patients (OPLL cells) have shown several phenotypes for osteoblastic behavior, suggesting the metaplasia of spinal ligament cells to osteoprogenitor cells in OPLL. From this evidence, we hypothesized that in OPLL, mechanical signals may be transmitted to induce differentiation of spinal ligament cells to osteoblasts. On the other hand, it was reported that core binding factor alpha 1 (Cbfa1), a runt-related osteoblast specific transcription factor, plays an important role in osteoblast differentiation and function. To elucidate the effect of mechanical stress on OPLL development and participation of Cbfa1 in ossification by using OPLL cells, we investigated transcriptional responses of OPLL cells to uni-axial cyclic stretch.

METHODS

1. RT-PCR

The ligaments harvested aseptically from 10 each OPLL and non-OPLL patients during surgery were extirpated carefully from a non-ossified site to avoid any possible contamination with osteogenic cells. The cells (5th passages) derived from OPLL patients (OPLL cell) and non-OPLL patients (non-OPLL cell) were subjected to uni-axial cyclic stretch by using a four point bending apparatus (Scholertec Corp., Japan), in 120% peak to peak, at 0.5Hz for 0, 3, 6, 9 hours. The mRNA expressions of type I collagen, alkaline phosphatase (ALP), integrin β1 and Cbfa1 were quantified by RT-PCR.

2. Immunohistochemistry

The cervical interspinous ligaments without ossified area were harvested en bloc from OPLL and non-OPLL patients. The ligaments were attached to the silicon chamber by ligature and subjected to cyclic stretch in 120% peak to peak, at 0.5Hz for 4 hours. After stimulation ligaments were analyzed by immunohistochemistry using an antibody against Cbfa1.

3. Effect of MAPK/ERK kinase (MEK) inhibitor

Cells were treated with 40 µM of U0126, a specific inhibitor of MEK for 9 hours or subjected to cyclic stretch for 9 hours. Effect of U0126 on stretch-induced expressions of Cbfa1 and ALP mRNA in OPLL and non-OPLL cells were analyzed by RT-PCR.

This study was approved by the Ethical Committees of Hirosaki University and all participants gave written informed consent.

ESSENTIAL RESULTS

1. In OPLL cells, 9-hour cyclic stretch significantly increased the mRNA expression of Cbfa1, type I collagen, ALP and integrin β1 about 170%, 180%, 250% and 200% respectively in OPLL cells, whereas no change was observed in non-OPLL cells.

2. In the 4-hour stretched ligaments from OPLL patients, Cbfa1 expressed weakly in chondrocytes at the site of enthesis and the expression increased in a lot of the fibroblast at the center of 4-hour stretched ligament significantly more than in the non-stretched ligaments. In ligaments from the non-OPLL patients there were no changes of expression of Cbfa1 by cyclic stretch.

3. U0126 suppressed stretch-induced expressions of Cbfa1 and ALP in OPLL cells, whereas no changes were observed in non-OPLL.

DISCUSSION

In this study, we hypothesize that mechanical stress is converted by integrin β1 to intracellular signaling and Cbfa1 is activated through the MAP kinase pathway. Type I collagen, ALP and Cbfa1 itself are transcribed by activated Cbfa1. Type I collagen and ALP as marker proteins of osteoblast differentiation lead OPLL cells to differentiate into osteogenic cells (Fig.1). These results suggest that Cbfa1 expression by mechanical stress plays a key role in the progression of OPLL through integrin β1 and the MAP kinase pathway.