INTRODUCTION
Ossification of the posterior longitudinal ligament of the spine (OPLL) is characterized by ectopic ossification in the spinal ligaments, mainly through endochondral ossification. Recently, Nakanishi cloned a mRNA predominantly expressed in chondrocytes by differential display PCR and found that its gene, named hcs24, was identical with that of connective tissue growth factor (CTGF). It has been shown that CTGF/Hcs24 plays a major role in endochondral ossification. The present study was carried out to clarify the involvement of CTGF/Hcs24 in OPLL. First, the expression of CTGF/Hcs24 in ossified ligament tissues from OPLL patients. Furthermore, effects of addition of CTGF/Hcs24 on the cultured spinal ligament cells were analyzed to reveal involvement of CTGF/Hcs24 in the initiation of OPLL.

METHODS
The Ethics Committee of Hirosaki Univ. School of Med. has approved this study. Ossified ligament tissues were taken from OPLL patients during surgery. Paraffin sections of specimens were stained by H-E and Safranin O. Subsequently, immunohistochemical staining by antibodies against CTGF/Hcs24 and type II collagen was performed. Spinal ligament cells were isolated from OPLL as well as non-OPLL patients. After addition of recombinant CTGF/Hcs24, the expression of osteoblastic and chondrocytic marker in cells was analyzed by RT-PCR. In the same way, effects of TGF-β were examined.

RESULTS
Immunohistochemical staining revealed that chondrocytes in the transitional region from nonossified to ossified ligament were stained with antibodies against CTGF/Hcs24. CTGF/Hcs24 enhanced the expression of ALP mRNA in cells from OPLL groups, whereas the expression remained unchanged in cells from non-OPLL groups. TGF-β increased the expression of CTGF/Hcs24 in cells from both groups. However, TGF-β differentially enhanced the expression of ALP mRNA in the two groups. Furthermore, the effects of TGF-β on the expression of ALP were similar to that of CTGF. In the presence of ascorbic acid, CTGF/Hcs24 induced the expression of type II collagen on cells from both groups.

DISCUSSION
The present study demonstrated immunoreactivity to CTGF/Hcs24 in chondrocytes around the ossified tissue in OPLL, suggesting that CTGF/Hcs24 plays an important factor in the development of OPLL.

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