

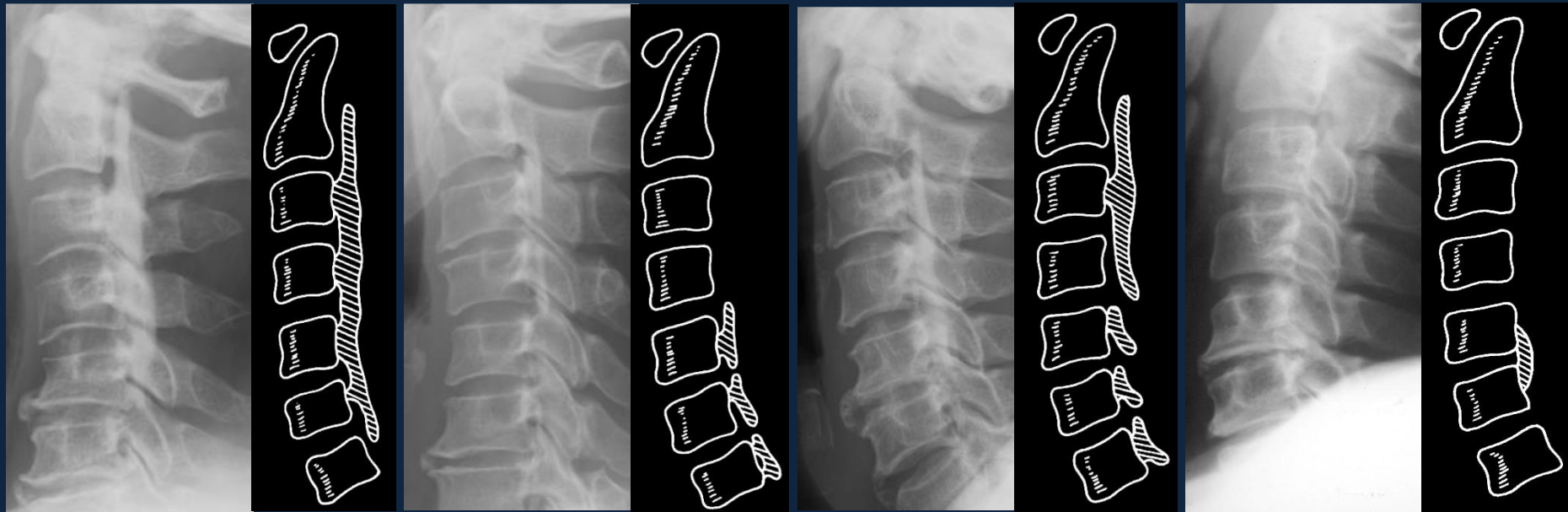
IndianHedgehog signaling induces
hypertrophy and differentiation of chondrocytes
in the ossification front of human cervical OPLL.

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Background: OPLL

Ossification of the posterior longitudinal ligament (OPLL) is characterized by **pathological bone formation** and causes serious neurological symptoms. In these days, it is thought as a subtype of DISH.



continuous type

segmental type

mixed type

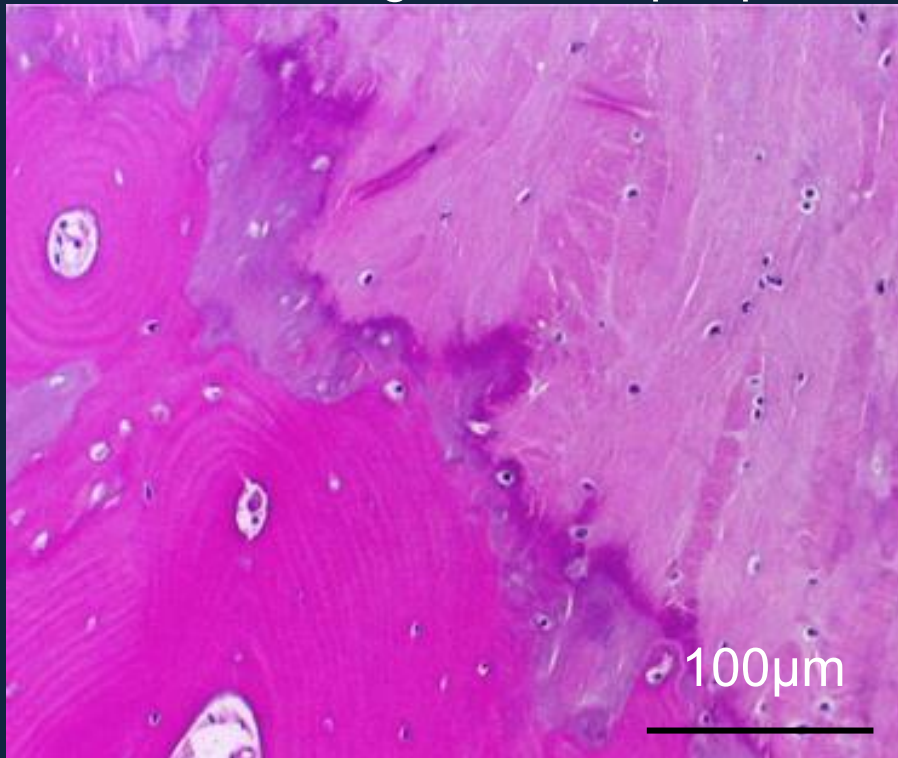
localized type

Character of OPLL

- Slowly progression of ossification
- Common in East Asia
- No critical treatment without operation

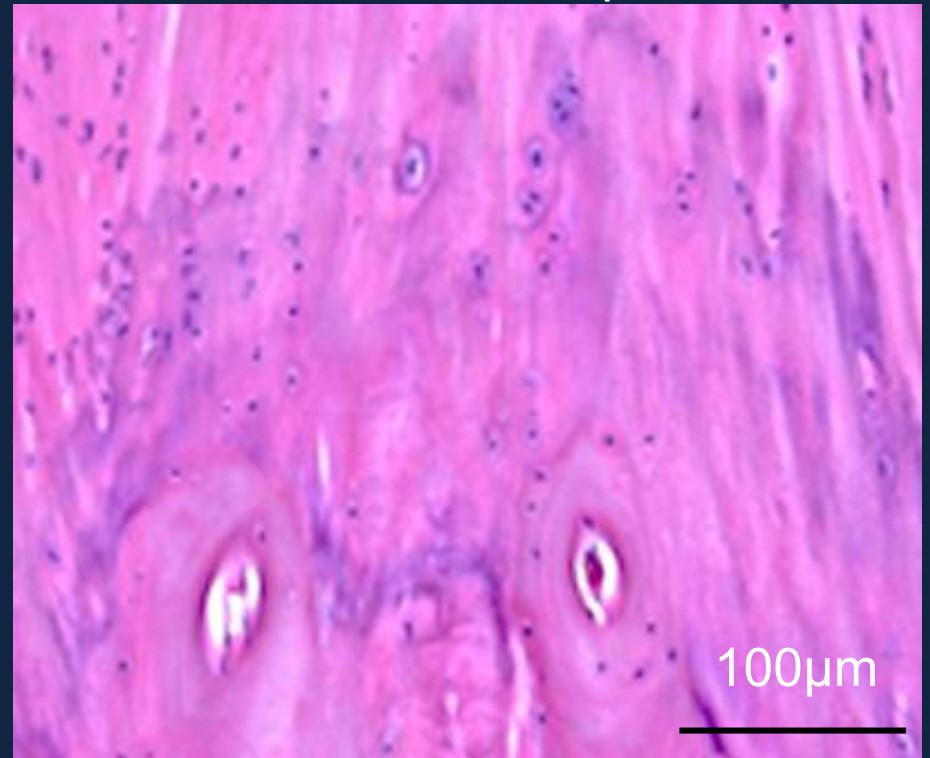
Background

HE staining for OPLL plaques derived from cervical OPLL patients



Segmental type

Ossification front is **thin**, and low cell concentration



Continuous type

Thick ossification front is consisted of matured bony layer and cartilage layer

Furusawa N et al. Eur J Histochem, 1996

Sato R et al. J Neurosurg Spine, 2007

Uchida K et al. Arthritis Res Ther, 2011

• The progression of OPLL plaque is similar to the **enchondral ossification**.

Hashizume Y. Acta Pathol Jpn, 1980

Liao CC et al. J Neurosurg, 1999

Background

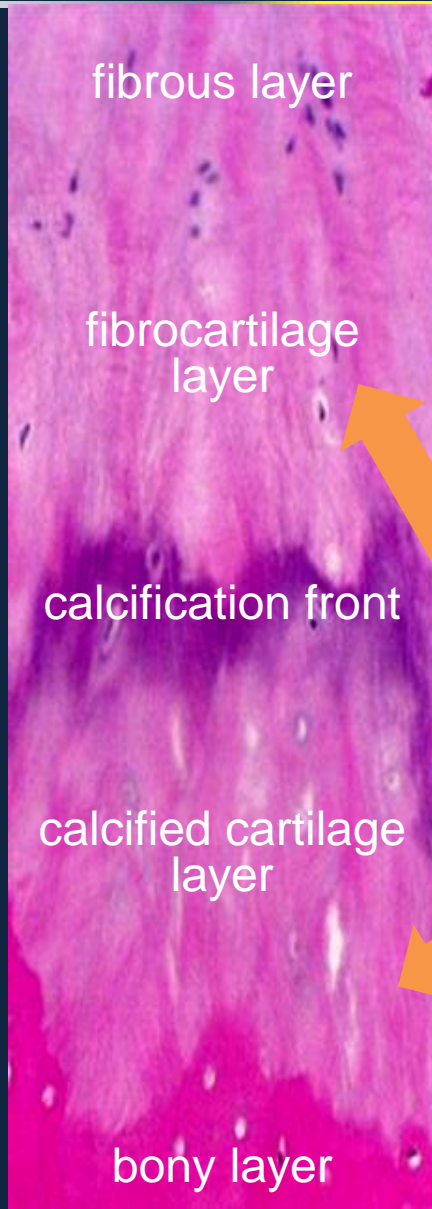
IndianHedgehog (Ihh) signaling is considered an important transcriptional factor for **enchondral ossification** in normal bone development

St-Jacques B et al. Genes Dev, 1999
Lanske B et al. Science, 1996

But, the exact role of Ihh signaling in the pathogenesis of OPLL remains unclear.

Purpose of this study

- To clarify the contribution of Ihh signaling for OPLL, assessing cultured cells derived from cervical OPLL patients



PTHrP

Sox9

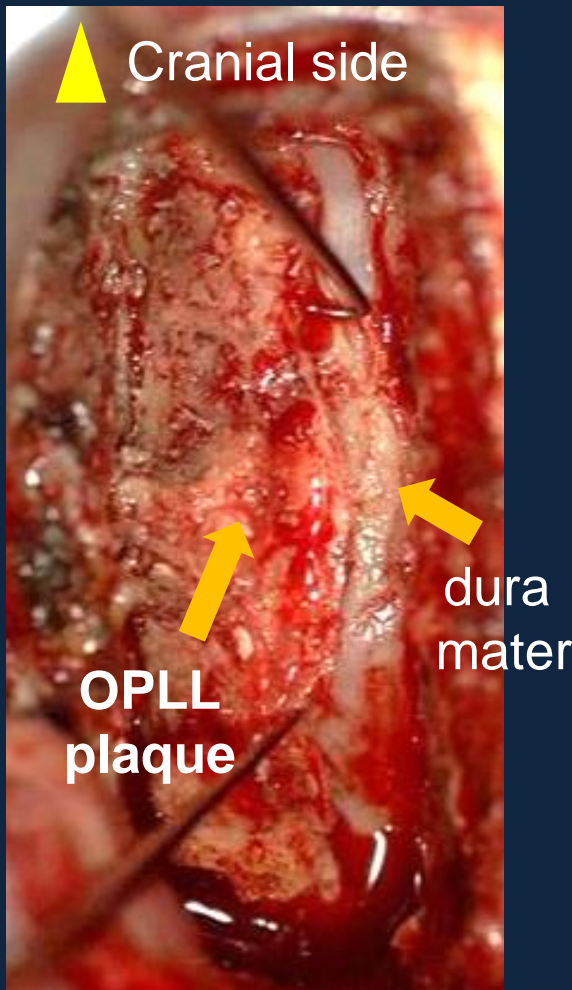
Ihh

Runx2

Ossification front of
Continuous type OPLL

Patients

We used ligamentous sections from 45 patients who were treated by anterior decompressive surgery for OPLL, and 10 PLL from patients without OPLL as a control



OPLL: 45 cases (35 Male, 10 Female)

Mean age: 63.1 (62-81)

preoperative JOA score: 11.2 (9-14)

control: 10 cases (5 Male, 5 Female)

Mean age: 62.8 (60-72)

preoperative JOA score: 12.8 (11-16)

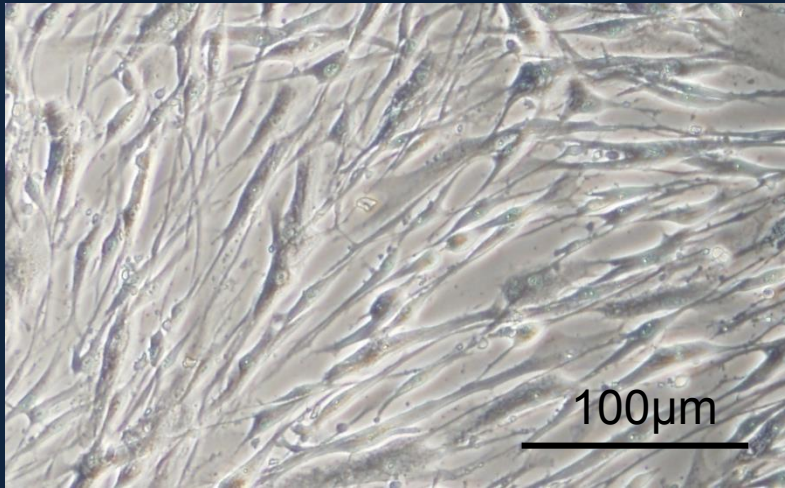
Experiment 1

To examine the **topographic expression** of transcriptional factors, serial 4 μ m thick sections were prepared from the paraffin-embedded specimens.

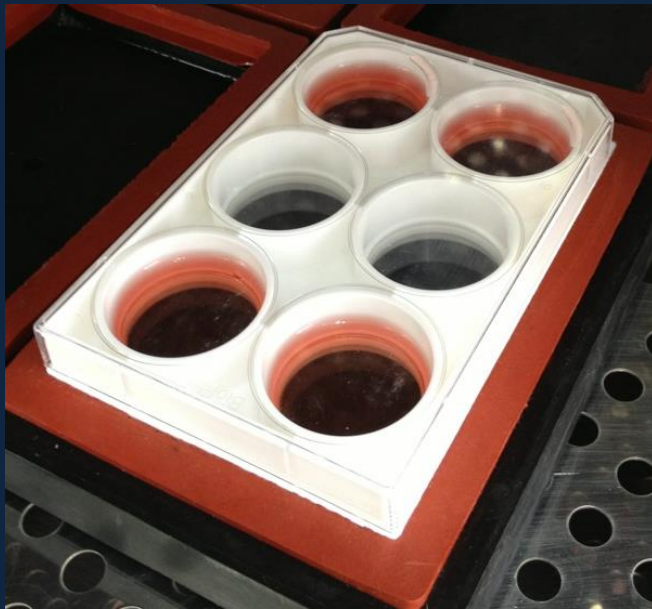
Primary antibodies

Ihh, Runx 2, Sox 9, PTHrP, Gli2, Gli3

Cyclic tensile strain application



Cultured cells from PLL
in patients with OPLL



Flexercell-3000

Experiment 2

The ligaments were harvested aseptically taken out the ossified tissue and explanted on dish.



We added **cyclic tensile strain**, and assessed the changes of expressions of transcriptional factors over time.

Cai HX et al. Spine, 2012

Condition of strain

Strength: 1Hz, 20% elongation

Duration: 0, 6, 12, 24 hours

Western Blotting

Ihh, Sox9, Gli2, Gli3

We quantified the band volume of Western blotting with the use of Image Quant LAS4000mini

Results: immunohistochemical staining

Ihh

Sox 9

Runx2

Ossified area

Ossification
front

n.c.

Ossified
area

Ossification
front

n.c.

Ossified area

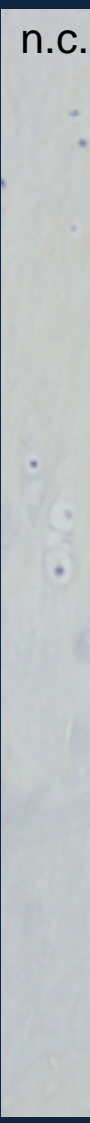
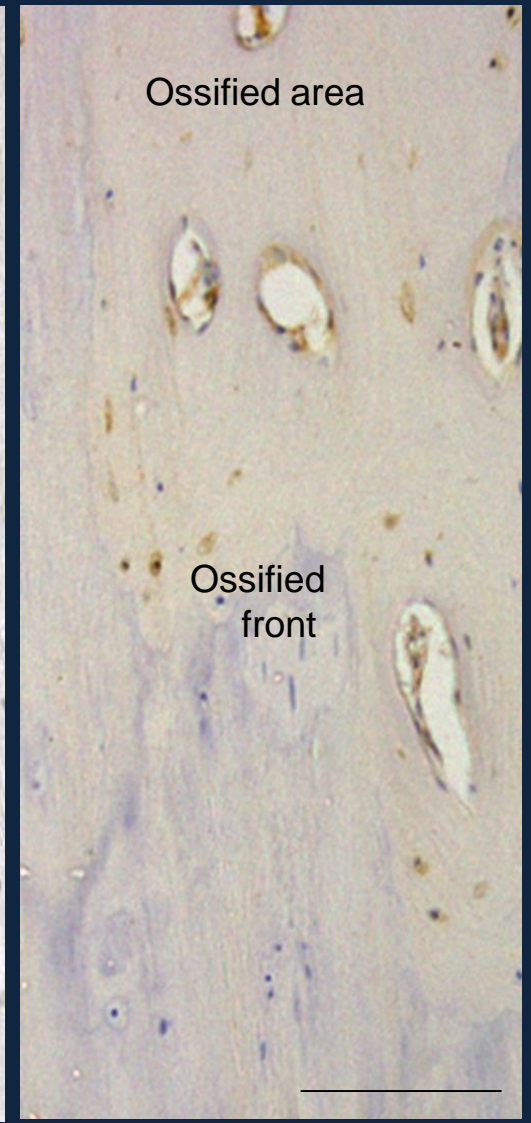
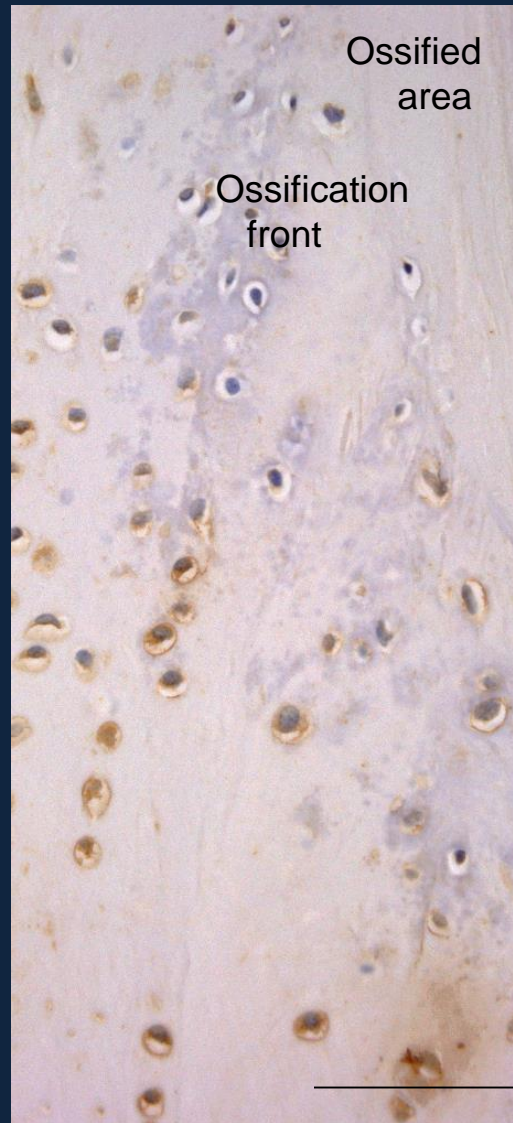
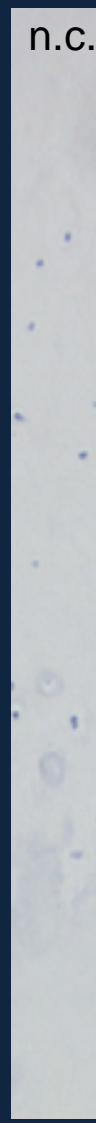
Ossified
front

n.c.

Proliferating
chondrocytes

Proliferating
chondrocytes

Hypertrophic
chondrocytes

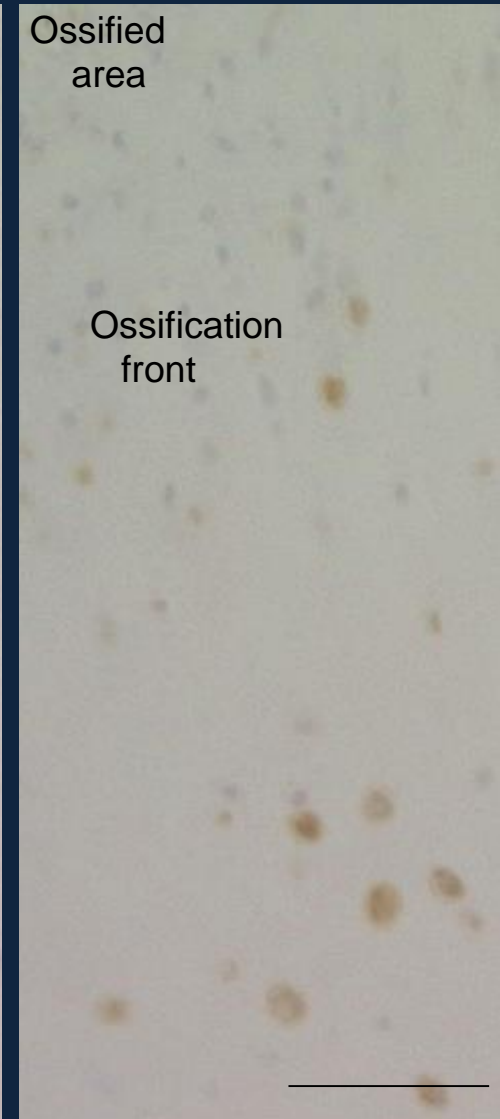
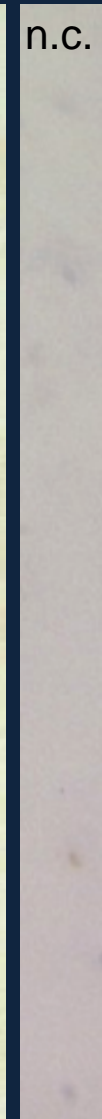
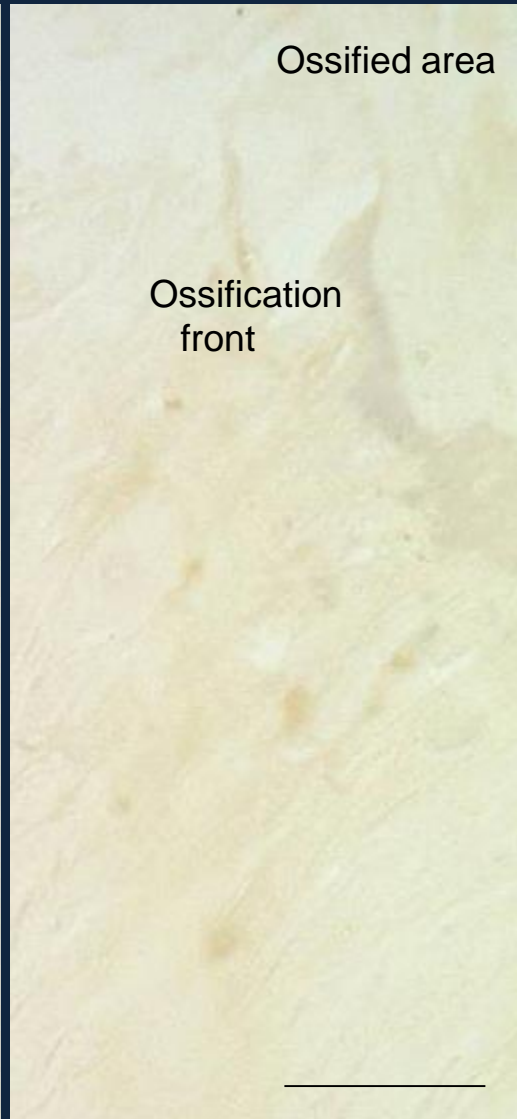


Results: immunohistochemical staining

PTHrP

Gli2

Gli3



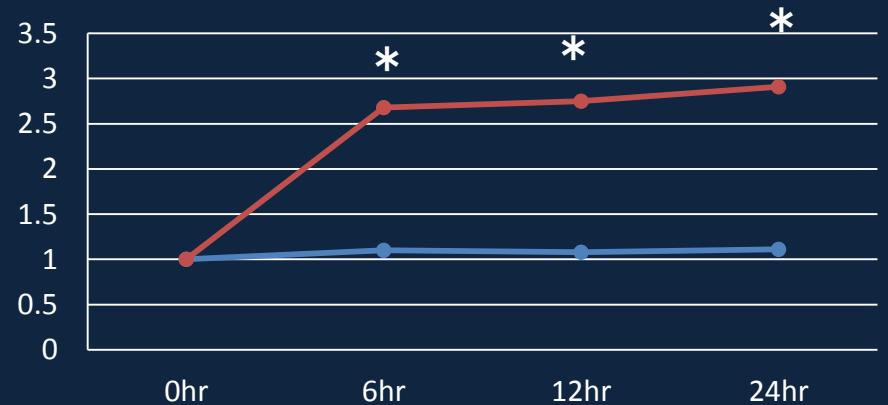
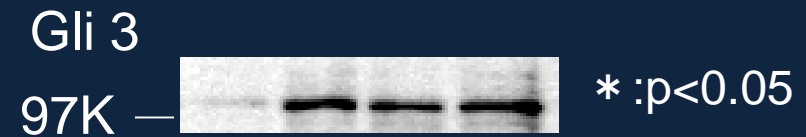
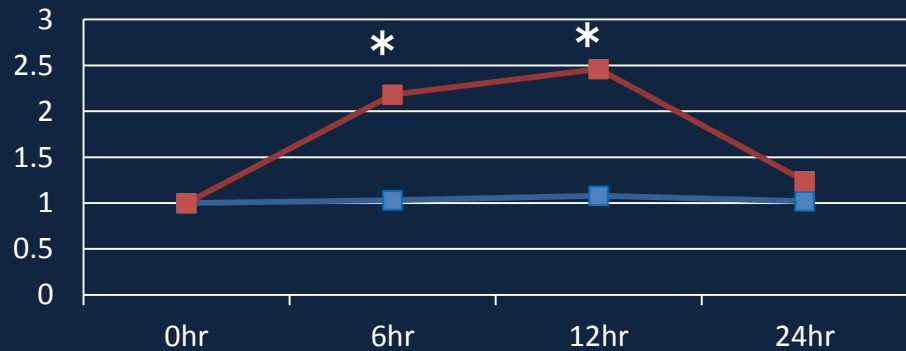
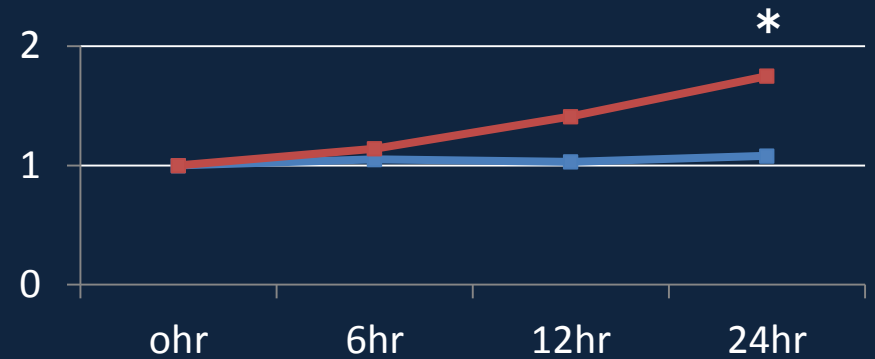
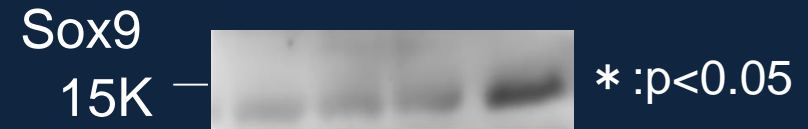
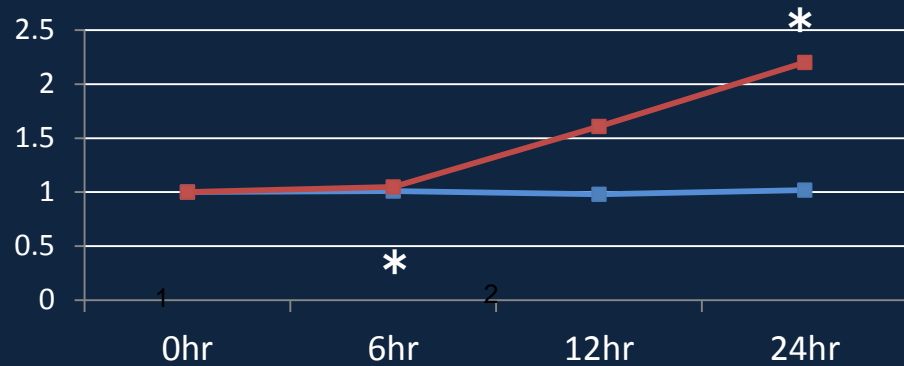
Proliferating and hypertrophic chondrocytes

Proliferating chondrocytes

Proliferating chondrocytes

Results; Western Blotting

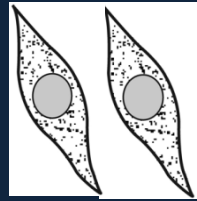
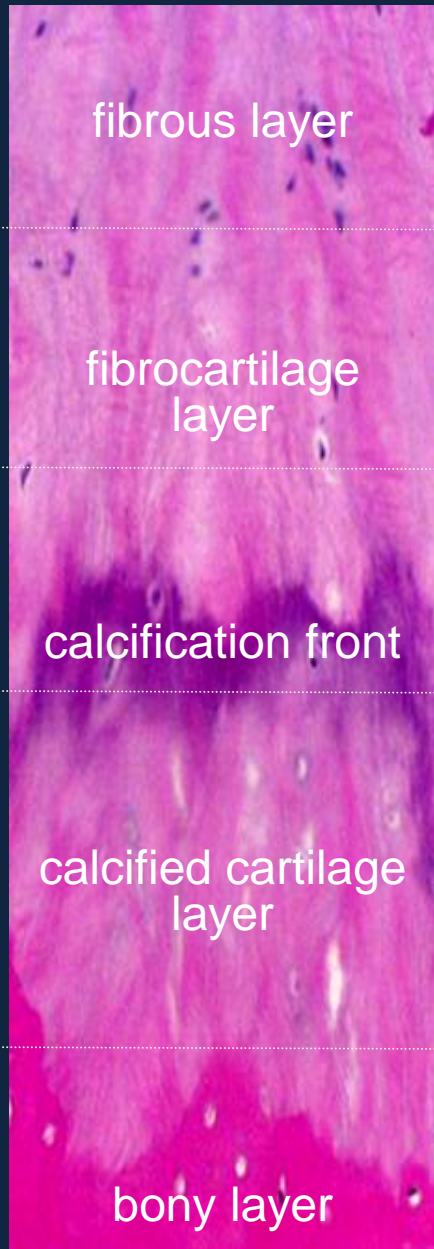
(Relative Expression)



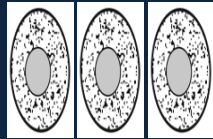
■ : OPLL cell

■ : NON-OPLL cell

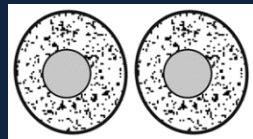
Discussion



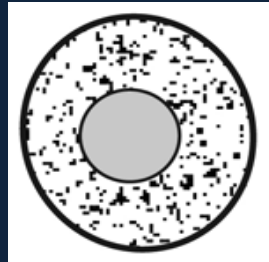
mesenchymal cells



proliferating chondrocytes



prehypertrophic chondrocytes



hypertrophic chondrocytes



apoptosis

Ihh signaling is concerned with the differentiation and hypertrophic change of chondrocytes with or without

Sox9

Sox9 and PTHrP

Shimoyama A et al. Mol Biol Cell, 2007

PTHrP

Gli2, Gli3

Wnt/B catenin signaling

Ihh

Runx2

BMP2

Ihh signaling induces the Maturation of chondrocytes with Runx2.

Amano K et al. Mol Biol Cell, 2009

Discussion

Cyclic tensile strain

**Upregulation of
Ihh signaling**

**Degenerative
change of
ligament**

Angiogenesis

**Mesenchymal cell
infiltration**

**Osteoblast
differentiation**

**Chondrocytes
maturation**

Apoptosis

Cyclic tensile strain is thought as A important local factors. It may induces over expressions of some transcriptional factors, such as VEGF, TNF- α , and Wnt/ β -catenin.

Matsunaga S, et al., Clin Orthop, 1994
Furukawa KI. J Pharmacol Sci, 2006
Uchida K et al. Arthritis Res Ther, 2011
Cai HX et al. Spine, 2012

•Cyclic tensile strain induces overexpression of transcriptional factors and degenerative change of ligament of cervical spine.

Summary

- We investigated about immunohistochemical topography and biological characteristics about transcriptional factors of Ihh signaling concerned with hypertrophy of chondrocytes or osteoblast differentiation
- Ihh signaling (Ihh, Gli2, Gli3) , Runx2, and Sox9 were positive at ossification front, and they had the topography of expression in the ossification front.
- The expression levels of these transcriptional factors were irregularly higher under cyclic tensile strain