Ossification of the posterior longitudinal ligament of the cervical spine: histopathological findings around the calcification and ossification front

RYUICHIRO SATO, M.D.,¹ KENZO UCHIDA, M.D., Ph.D.,¹ SHIGERU KOBAYASHI, M.D., Ph.D.,¹ TAKAFUMI YAMAMA, M.D., Ph.D.,¹ YASUO KOKUBO, M.D., Ph.D.,¹ HIDEAKI NAKAJIMA, M.D., Ph.D.,¹ TAKAHARU TAKAMURA, M.D.,¹ ALEXANDER BANGIRANA, M.D., MMed.,² HIROSHI ITOH, M.D., Ph.D.,³ AND HISATOSHI BABA, M.D., Ph.D.¹

¹Division of Orthopaedics and Rehabilitation Medicine, Department of Surgery, and ²Department of Pathological Sciences, Faculty of Medical Sciences, The University of Fukui, Japan; and ³Department of Orthopaedic Surgery, Makerere University School of Medicine, Kampala, Republic of Uganda

Object. The authors studied the histological and immunohistochemical features of ossified posterior longitudinal ligament (PLL) of the cervical spine, especially in the calcification and ossification front.

Methods. Samples of en bloc ossified PLL plaque obtained in 31 patients were stained with H & E and immunohistochemically prepared for collagens (types I and II), vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β, and bone morphogenetic protein (BMP)-2, and by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling method for apoptosis.

Results. Enchondral ossification was evident between the ligamentous enthesis and deep layer of the ligament, with irregularly disorganized arrangement of elastic fibers in association with advancement of the degenerative process. In the ossification front, many hypertrophic metaplastic chondrocytes were noted in the ossifying plaque immediately contiguous to the ligament fibers, together with a considerable degree of neovascularization. Both TGFβ and BMP-2 were highly expressed in metaplastic hypertrophic chondrocytes in the ossification front, and BMP-2 was also expressed in fibroblastic cells near the ossified PLL plaque. Expression of type I collagen was significant in the matrix of the ossified PLL lesion, whereas that of type II was marked in metaplastic chondrocytes in the ossification front. Apoptotic hypertrophic chondrocytes were observed mainly in the fibrocartilaginous area near the calcification front.

Conclusions. The enchondral ossification process in the ossified PLL was closely associated with degenerative changes of elastic fibers and cartilaginous cartilage formation, together with the appearance of metaplastic hypertrophic cartilage cells and neovascularization. The authors also found that VEGF-positive metaplastic chondrocytes in the ossification front and different expression patterns of collagens probably play some role in the extension of the ossified PLL from the ossification front. (DOI: 10.3171/SPI-07/08/174)

KEY WORDS • calcification • cervical spine • histology • immunohistochemistry • ossification of the posterior longitudinal ligament

Ossification of the posterior longitudinal ligament of the spine may eventually cause serious spinal cord compromise. Ossification of the paravertebral tissue, including the spinal ligaments, may be evident in diffuse idiopathic skeletal hyperostosis, but in contrast to this, OPLL develops in isolation, frequently exhibiting significant ossification in the paravertebral areas. Several epidemiological, metabolic, mechanical and biological factors are suspected to contribute to the development and progression of OPLL. In addition, candidate genes have been analyzed to clarify the underlying genetic background based on the high prevalence of OPLL in certain countries and/or races. Thus, recent research on OPLL has involved undertaking association and/or genome-wide linkage analyses to determine the candidate genes, as well as proteomics analysis for detecting causative peptides in ossifying plaque. Histologically, abnormal enchondral ossification may play a role in OPLL, but because of the paucity of histological studies the underlying mechanisms of calcification and ossification processes remain obscure.
Cervical ossified posterior longitudinal ligament

Several investigators have described clustering of abnormal fibrocartilage cells, showing metachromasia and hypertrophy of the ligament fibers, as significant changes heralding ossification. In the presence of certain genetic background, mesenchymal fibroblasts or fibroblast-like cells within the deep layer of the PLL may proliferate in an abnormal manner. Tsukamoto et al. have recently confirmed experimentally that lumbosacral PLL cells in Wistar rats subjected to continuous mechanical distraction stress changed their cytological sensitivities in vivo to overexpress osteogenic cytokines (BMP-2, TGFβ), and they observed an increased appearance of mesenchymal cells in the ligament. These mesenchymal cells exhibiting marked metachromasia are speculated to transform to osteoblasts or osteoblast-like cells, contributing to development of OPLL. On the other hand, using a spinal hyperostotic mouse model (twy/twy), our group observed the appearance of mesenchymal metaplastic chondrocytes and osteoblast-like cells in the ossification front, followed by initiation of the ossification process and development of OPLL. Based on these studies of calcification of the spinal ligaments and herniated intervertebral discs, we examined human ossified PLL samples in vivo to gain greater insight into this pathological process.

The present study was thus designed to analyze qualitatively the histological features of human ossified PLL harvested en bloc during anterior decompression. We also studied immunohistological properties around the ossified PLL plaque, ossification and calcification front, and in the ligamentous enthesis.

Materials and Methods

Patient Population and Samples of Ossified PLLs

Samples of ossified plaques were obtained in 31 patients who underwent anterior decompressive surgery for symptomatic cervical OPLL. The patients’ average age at surgery was 63.6 years (range 33–77 years). Three patients had continuous-type, eight segmental-type, 15 mixed-type, and five patients had localized-type OPLL based on radiographic classification (Fig. 1a–d). The continuous type was defined as an ossified mass lesion along the vertebral longitudinal axis with no disruption of the ossification; segmental type as a lesion primarily affecting some vertebral segments without ossification at the intervertebral disc level; mixed type as a longitudinally extended mass present continuously at some segments with other small similar lesions; and localized type as an extensive mass lesion primarily at one or two segments with no solitary lesions elsewhere. None of the patients had evidence of genetically bound bone, joint, or musculoskeletal tissue abnormalities, and none had taken specific medications such as corticosteroids, immunodepressants, bisphosphonate, and/or ethidronate sodium. None of the patients had renal osteodystrophy or hyperparathyroidism, and all were seronegative. Furthermore, survey radiography demonstrated the absence of ankylosing spondylitis and other forms of seronegative spondyloarthropathies, calcium pyrophosphate crystal deposition, diffuse idiopathic skeletal hyperostosis, and cervical osteoporosis.

The ossified cervical PLL, together with the surrounding nonossified area of the PLL, was excised en bloc anterior to the dura mater. Histopathological samples of the PLL and ligamentous enthesis obtained in 10 age-matched patients (average age 61.2 years, range 32–75 years) with spondylitis served as positive controls. The surgical technique of anterior decompression and resection of the ossified PLL has been described in our previous reports. Briefly, a left-sided anterolateral approach was used to expose the affected vertebral level(s). We performed a 2.0-cm–wide transverse vertebral resection at the level of vertebrectomy. Approximately one fifth of the posterior portion of the VBs, discs, and endplates at the adjacent levels were left intact for en bloc resection. Occasionally with the assistance of an operating microscope, we used a diamond bur, 2 to 3 mm in diameter, to cut and resect rectangularly the posterior cortices of the vertebrae and the ossified PLL together with the surrounding nonossified PLL. The OPLL plaque was then isolated circumferentially like an “island” upon the dura mater together with the PLL posteriorly, “floating” on the dura mater. Using a micro-Kerrison rongeur, the nonossified area of the ligament peripheral to the ossified lesion was carefully dissected and cut, and the ossified PLL plaque was removed en bloc together with the surrounding endplates, ligaments, and disc tissues (Fig. 2a–d). Written informed consent was obtained from each patient, and the study protocol strictly followed the human ethics review committee guidelines of our university.

Fig. 1. Radiographs demonstrating the continuous-type (a), mixed-type (b), segmental-type (c), and localized-type (d) cervical OPLL. Arrows in d show a localized “volcano”–like OPLL.
Histological Processing and Immunohistochemical Staining

Histopathologically, the resected specimens represented all four types of OPLL, although the mixed type was the most common (in 15 [48%] of 31 patients). Clinically, the localized lesion protrudes more posteriorly within the canal and exhibits regional progression. For these reasons, we examined the histopathological features of the mixed- and localized-type OPLL.

The resected ossified PLL plaque and the surrounding ligament and ligamentous enthesis were bisected midsagittally; the specimen was then fixed in 10% buffered formaldehyde for 48 hours at 4°C. The sample was then decalcified for 4 to 7 days at 4°C in 0.5 M ethylenediaminetetraacetic acid (0.5 M Tris-HCl buffer) at pH 7.6 and then embedded in paraffin using standard procedures. Serial 4-μm-thick cryostat sagittal sections of the ossified PLL–ligament-enthesis complex were prepared for H & E and toluidine blue (pH 7.4) staining.

For immunohistochemical staining, serial 4-μm-thick sections were prepared from the paraffin-embedded specimens, deparaffinized with xylene, and treated with ethanol. After washing with water, the intrinsic peroxidase was blocked with 0.3% H₂O₂–methanol solution at 20°C for 10 minutes and washed with PBS (pH 7.4). In the next step, the sections were reacted with blocking medium (PBS containing carrier protein and 15 mM sodium azide; LSAB kit, Lot 00075, DAKO) at 20°C for 10 minutes. This was followed by reaction with the following primary antibodies, respectively, at 4°C overnight: monoclonal anti–type I collagen (mouse, Lot IH1229, R&D Systems); monoclonal anti–type II collagen (mouse, Lot GU1219, R&D Systems); monoclonal anti–VEGF (mouse, SC7269, Lot GU1219, R&D Systems); monoclonal anti–TGFβ (mouse, MAB1835, Lot CCI021021, R&D Systems; 0.2 μm filtered solution in PBS); and BMP-2 (mouse, AR004, Lot UED014091, R&D Systems; 0.2 μm filtered solution in PBS with 5% trehalose). Sections were further reacted with proteinase K solution (10 μg/ml in 10 mM Tris–HCl buffer, pH 7.4) and rinsed twice with PBS. The TUNEL reaction mixture (50 μl enzyme solution [terminal deoxynucleotidyl transferase from calf thymus in storage buffer] added to 450 μl label solution [nucleotide mixture in reaction buffer] and mixed well to equilibrate components) was prepared immediately before use, placed on slides, and incubated for 60 minutes at 37°C. For the negative control, label solution without terminal transferase was placed on slides instead of the TUNEL reaction mixture. After rinsing three times with PBS, anti-digoxigenin peroxidase was added, and the slides were incubated for 30 minutes at room temperature; they were rinsed three times with PBS, treated with DAB (Wako Chemicals) substrate (10 μg/ml in 10 mM Tris–HCl buffer, pH 7.4) and rinsed twice with PBS. The TUNEL reaction mixture (50 μl enzyme solution [terminal deoxynucleotidyl transferase from calf thymus in storage buffer] added to 450 μl label solution [nucleotide mixture in reaction buffer] and mixed well to equilibrate components) was prepared immediately before use, placed on slides, and incubated for 60 minutes at 37°C. For the negative control, label solution without terminal transferase was placed on slides instead of the TUNEL reaction mixture. After rinsing three times with PBS, anti-digoxigenin peroxidase was added, and the slides were incubated for 30 minutes at room temperature; they were rinsed three times with PBS, treated with DAB (Wako Chemicals) substrate (10 μg/ml in 10 mM Tris–HCl buffer, pH 7.4) and incubated for 10 minutes at room temperature. The slides were rinsed with distilled water and counterstained with methyl green (1%) for 5 minutes. After placing the samples on slides, they were analyzed under light microscopy.

Results

Histopathological Findings

Macroscopically, the dural side of the resected ossified PLL (mixed-type) sample was a semiround, often uneven or wov'en, protruding lesion with a yellowish-white surface (Fig. 3a). At low-power magnification, the ossified PLL (Fig. 3b) appeared as an ossified plaque, extending longitudinally and posteriorly, contiguous with the ligamentous enthesis to the VB and to the deep layer of the PLL. Ossification extended to a small area of the superficial layer of the PLL.
the PLL, but in most areas the ossification extended longitudinally rather than to the superficial layer. Histopathological examination also showed mature bone, lamellar structures, and haversian canal formation, transposed onto the calcified front along with the line of ossification (Fig. 4a). The ossification front exhibited an irregular arrangement of features. The calcified cartilaginous layer extended beyond the calcification front to the fibrocartilaginous area. In the calcified cartilaginous area, a number of hypertrophic chondrocyte-like cells were observed (Fig. 4b–f). In the fibrocartilaginous layer, scarce and degenerated elastic fibers were noted in association with a small blood vessel invasion, together with a small number of hypertrophic, seemingly mesenchymal, chondrocytes, relative to those in the calcified cartilaginous layer (Fig. 4b, d, and g). The elastic fibers in the fiber area tended to exhibit a normal configuration within the deep layer of the PLL (Fig. 4b, e, and f).

In cases of localized-type OPLL, the histopathological findings were somewhat different (Fig. 5). The ossified layer, contiguous with the posterior cortex of the VB, was surrounded by a thin calcified cartilaginous layer, often directly facing the fibrocartilaginous layer and lacking the calcified cartilaginous layer (Fig. 5a). The ossified layer

often extended to the calcified cartilaginous layer showing a “shark teeth” pattern (Fig. 5b). In the calcified cartilaginous layer, a clearly defined calcification front was observed (Fig. 5b, c, and f), demarcating significantly calcified cartilaginous and fibrocartilaginous layers (Fig. 5b, d, and g). The histopathological features of the elastic fibers in the fiber area were essentially the same as those observed in the mixed-type ossified PLL (Fig. 5c and h).

**Immunohistological and Immunohistochemical Findings**

*Expression of Types I and II Collagen.* Expression of type I collagen was significant in the matrix of the ossified area of the mixed and localized types of the ossified PLL (Fig. 6a), whereas it was insignificant in the calcified cartilaginous and fibrocartilaginous layers. Expression of type II collagen, however, was significant in the calcified cartilaginous area (Fig. 6b) rather than in the ossification and the fibrocartilaginous areas. There were no differences in type II collagen expression features between mixed- and localized-type OPLL.

*Expression of TGFβ and BMP-2.* Expression of TGFβ was significant in the somatic bodies of hypertrophic chondrocytes in the calcified cartilaginous area (Fig. 7a). Significant expres-
sion of BMP-2 (Fig. 7d) was noted in the cell soma of mature chondrocytes in the calcified cartilaginous area near hypertrophic chondrocytes typically seen in the fibrocartilaginous area. In the fibrocartilaginous area of the ligament, expression of TGFβ and BMP-2 was also significant in the metaplastic fibroblast-like cells (Fig. 7b and e). Immunoreactivity for TGFβ and BMP-2 in calcified cartilage and the fibrocartilaginous area was significantly higher than in the fiber area (Fig. 7c and f).

Expression of VEGF. A significant expression level of VEGF was observed in hypertrophic chondrocytes in the calcification front and in the calcified cartilaginous area (Fig. 7g and h) in areas adjacent to the calcification front. The expression of VEGF, however, was insignificant in the fiber area of the PLL (Fig. 7i).

Staining With TUNEL. There were fewer TUNEL-positive hypertrophic chondrocytes in the calcified cartilage area (Fig. 8a) than in the fibrocartilaginous area. In the latter region, significantly higher numbers of TUNEL-positive cells were observed (Fig. 8b) in both continuous and localized types of OPLL. Furthermore, TUNEL-positive cells exhibited marked clustering in the fibrocartilaginous area near the calcification front.

Discussion

Although several investigators, have studied the origin of OPLL, the lesion’s histopathological features remain obscure. Sakou et al.23 and their colleagues17,19 were the first to identify histologically abnormal expression of collagen type Xla within the PLL as one of the candidate gene expressions in human OPLL. Our group2 found that the initiation and progression of OPLL in the spinal hyperostotic mouse (twy/twy) were associated with increased alkaline phosphatase activity in the mesenchymal osteoblast-like cells appearing within the enthesis of the PLL. Recently, the NPPS gene was identified in twy mouse spinal ligament ossification,12,21 showing abnormal mesenchymal osteoblast-like cell proliferation and enchondral ossification. Increased expression of type XI collagen was reported in the twy mouse, where our group postulated the
potential roles of types I and II collagens in the progression of ossified PLL plaque in the twy mouse. Although a number of authors have described origins, perhaps only the studies of Hirakawa et al., Yonemori et al., Ueno et al., and our own, including the present study, were dedicated to illustrating the histological properties of the OPLL process.

The elastic fibers of the PLL exhibit significant degeneration and derangement in their structural arrangement in the calcification front. The site between ossifying plaque and the calcified cartilaginous area contains clusters of mesenchymal fibroblast-like cells and hypertrophied cartilaginous cells. We also found marked ossification from the ligamentous enthesis to the middle portion of the deep layer of the PLL, but there was no evidence of ossification in the superficial layer of the ligament. In the ossification front we observed significant wave-shaped calcified cartilage. A significant immunoreactivity to BMP-2 was evident in the fibrocartilage close to the ossification front. The abnormal proliferation of chondrocytes (mostly fibrocartilage cells) is thought to contribute to the development of early stages of ossification. Others investigators have recently reported that cyclic mechanical distraction stress applied to the rat coccygeal vertebrae caused PLL hypertrophy in association with the appearance of abundant round chondrocyte-like cells within the ligamentous tissue. These chondrocyte-like cells were positive for S100 protein and Sox9 and were considered to produce matrix protein enriched with glycosaminoglycans. The Sox9 is known as a transcription factor and an activator of both chondrocyte differentiation and cartilage formation by regulating the type II collagen gene during the developmental stage of ossification. The expression of Sox9 is regulated through signaling by BMPs. In the present study, significant expression of BMP-2 was noted in the metaplastic fibroblastic round cells near the calcified cartilage and fibrocartilaginous areas. In agreement with Tsukamoto et al., these TGFβ- and BMP-2-positive cells may mature and differentiate into chondrocytes. On the other hand, marked TGFβ immunoreactivity was also noted in the soma of hypertrophied fibrocartilage cells in the transitional area between the ossified lesion and the nonossified degenerat-
ed ligament. Interestingly in the present study, we observed a longitudinally more extended and wider ossification front in cases of mixed-type OPLL (Fig. 4) than in localized-type OPLL (Fig. 5). This difference may imply a histological significance in terms of the feasibility of enhanced ossification by abnormal mechanical stress applied to a certain area of the ligament.27 Ossification is reported to progress longitudinally (4–7 mm in length) within an average period of 8 years in approximately 36 to 61% of OPLL cases, particularly mixed type in young patients less than 55 years of age. If the segment exhibiting a topographically more extended, wider ossification front is hypermobile and more degenerated by the excess magnitude of mechanical instability, one can formulate the hypothesis that mechanical and tensile stresses could facilitate ligament ossification with an extended ossification and calcification front in association with increased immunoreactivity to BMP-2 and TGFβ. However, we believe that this hypothesis should be tested in another study designed to investigate the relationship between radiographic motion analysis and histological examination.

Ishida and Kawai14 found a high turnover of chondrocytes and fibroblasts within the severely degenerated PLL associated with marked proliferation of small blood vessels, particularly in the region near the enthesis. They also reported that undifferentiated mesenchymal fibroblasts, as well as chondroblast-like cells within the hypertrophied ligaments, may undergo early calcification and subsequent ossification, together with proliferation of irregularly shaped fine collagen fibrils and increased activity of acid mucopolysaccharide. Although it is inappropriate to extrapolate observations from the twy mouse to human OPLL, our group28 confirmed the presence of a significant number of fibroblast- and osteoblast-like mesenchymal cells within the PLL. We reported overexpression of proliferative cell nuclear antigen and S100 protein in these mesenchymal chondroblast-like and osteoblast-like cells in twy mice.8 These findings suggest that cellular proliferation contributes to the early development of OPLL. A large number of matrix vesicles have been reported to appear near the collagen fibrils, seemingly degenerated and twisted in their arrangement, particularly in the enthesis.28 In the present study, we noted that such cells appeared prior to the development of an OPLL mass and persisted anterior to the ossified lesion within the PLL and the ligamentous enthesis. These observations and those noted in experimental works seem to portray an important picture of early ossification and subsequent development of mature ossified PLL lesions within the deep layer of the PLL.

Interestingly, we found a number of small blood vessels in the degenerated PLL, particularly in its deep layer, near the ligamentous enthesis and ossification front. In our previous studies, we made a similar finding of small blood vessel invasion in vivo within the torn PLL in patients with symptomatic herniated intervertebral discs18 and in human tissue in cases of degenerated ligamentum flavum.32 Degeneration of the elastic fiber within the fibrocartilage tissue could be associated, at least in part, with neovascularization. Ueno et al.30 recently described the essential role of VEGF and BMP-4 during the course of enchondral ossification in the grafted periosteum. Thus, our findings suggest that a virtual enchondral ossification occurs in the PLL, but essentially the trigger of this ossification pattern remains unknown. It is known that VEGF plays a role in prevention of apoptosis of intervertebral disc cells via its receptor Flt-1, and, perhaps, it may play some role in the differentiation of mesenchymal fibroblasts to metaplastic hypertrophic chondrocytes within the PLL.

It is possible that a different pattern of collagens is present among the protein subtypes (types I, II, and III collagens) in human OPLL. It is known that the expression levels of collagen types I and II in human PLL vary with age, but in OPLL the expression levels of these collagens were increased in certain areas. We also noted marked expression of type I collagen in the ossified lesion site, whereas that of type III was marked in chondroblast-like cells and hypertrophied chondrocytes in the ossific front and in the adjacent ligament. What does this mean in relation to the development of OPLL? We have previously demonstrated that enchondral ossification correlates with changes

**Fig. 6.** Photomicrographs with immunohistological staining for type I and type II collagens (mixed-type OPLL). a: Expression of type I collagen in the matrix of ossified area. b: Expression of type II collagen was significant in the calcified cartilaginous area. Original magnification × 20.
in matrix proteins, including the expression of yet unknown different collagens, constituting the fibrocartilaginous ligament, such as chondroitin 4-sulfate proteoglycan or other proteoglycans. The authors of molecular genetic studies have identified several candidate genes for susceptibility to OPLL, and COL11A2, which encodes collagen type XIa2, was identified in a sib-pair linkage study of the human leukocyte antigen region on chromosome 6p21.12,17 A genome-wide sib-pair linkage study followed by linkage equilibrium mapping identified COL6A1, which encodes collagen VIα1 on chromosome 21q22.3, as a novel candidate, and this was further confirmed recently in a large-scale genetic association study by Horikoshi et al.12 To date, a total of 35 candidate genes has been identified during the initiation and development of OPLL. A total of 109 single nucleotide polymorphisms in 35 candidate genes was genotyped in 711 sporadic cases of OPLL and 896 healthy volunteers. Among those, COL11A2 and COL6A1 may play the most active roles in ossification, but the precise pathomechanisms of gene expression and histopathology were not elucidated in the present study.

We investigated the extent of apoptosis in the calcification and ossification areas. The TUNEL-positive hypertrophic chondrocytes were primarily found in the fibrocartilaginous area near the calcification front. This area also contained numerous mesenchymal hypertrophic chondrocytes, whereas no such finding was observed in the non-calcified area far from the calcification front. In experimental OPLL, proliferative cell nuclear antigen–positive osteoblast-like cells were found in the fibrocartilaginous area near the calcification front.8 We cannot formulate a pattern based on the current findings, but it is possible that enhanced extension of the calcified area within the degenerated noncalcified area and initiation of the ossification process from the calcification may be associated with the appearance of TUNEL-positive hypertrophic chondrocytes. The appearance of TUNEL-positive cells in the fibrocartilaginous area adjacent to the calcification front perhaps suggests an enhanced turnover of cellular life,9 including mesenchymal cells, within the PLL. In addition, VEGF overexpression around the TUNEL-positive hypertrophic chondrocytes may serve to prevent apoptosis of these cells, although further investigation is warranted.

We examined here only some areas and the ossifying process in a limited number of samples. Thus, there is a need for further clarification, both immunohistochemically and quantitatively, of the main mechanism of early ossification and development of OPLL in the ligamentous enthesis and deep layer of the PLL. Furthermore, we need to identify those cytokines responsible for the ossification process. Genetic background and the candidate gene(s) responsible for the enchondral ossification process of OPLL.

Fig. 7. Photomicrographs with immunohistological staining in a representative mixed-type OPLL for TGFβ (a–c), BMP-2 (d–f), and VEGF (g–i). Regions include the calcified cartilage area (a, d, and g), fibrocartilaginous area (b, e, and h), and fiber area (c, f, and i). Original magnification x 40.
Elucidation of the mechanism by which OPLL develops may allow design therapies targeting regulation of expression of specific cytokines and proteins that cause ligamentous ossification.

Conclusions

We studied the histological and immunohistochemical features of human cervical OPLL, particularly at the calcification and ossification front. The enchondral ossification process of OPLL was closely associated with degenerative changes of elastic fibers and cartilaginous cartilage formation, together with the appearance of metaplastic hypertrophic cartilage cells and neovascularization. We found that VEGF-positive metaplastic chondrocytes in the ossification front and different expression patterns of collagens play at least some role in the extension of the ossified PLL from the ossification front.

References

13. Inoue I, Ikeda R, Tsukahara S: Current topics in pharmacological
Cervical ossified posterior longitudinal ligament


This work was supported in part by grants (2004–2006) from the Investigation Committee on Ossification of the Spinal Ligaments, the Public Health Bureau of the Japanese Ministry of Health and Welfare, and by Grants-in-Aid (Nos. 16390435 and 18390411) for General Scientific Research from the Japanese Ministry of Education, Science and Culture.

Address reprint requests to: Ryuichiro Sato, M.D., Division of Orthopaedics and Rehabilitation Medicine, Department of Surgery, Faculty of Medical Sciences, The University of Fukui, Matsuoka Shimoaizuki 23, Eiheiji, Fukui 910-1193, Japan. email: drs@fmrrsa.fukui-med.ac.jp.