ORIGINAL

Role of Epiligament in Ligamentum Flavum Hypertrophy in Patients with Lumbar Spinal Canal Stenosis : a Pilot Study

Nori Sato[1], Kosaku Higashino[1], Toshinori Sakai[1], Tomoya Terai[1], Vijay K. Goel[2], Ashok Biyani[2], Nabil Ebraheim[2], Yoichiro Takata[1], Fumio Hayashi[1], Kazuta Yamashita[1], Masatoshi Morimoto[1], Hiroaki Manabe[1], and Koichi Sairyo[1]

[1]*Department of Orthopedics, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima, Japan,* [2]*ECORE, Department of Bioengineering and Orthopedic Surgery, University of Toledo, Toledo, Ohio, United States*

Abstract : Ligamentum flavum (LF) hypertrophy is one of the main factors of lumbar spinal canal stenosis (LSCS). The primary object of this study is to clarify the existence of epiligament in the LF and its role in hypertrophy, and to develop an LF hypertrophy animal model. A cadaveric spine from a 30-year-old man was used to investigate the existence of epiligament in LF. Five LF samples from LSCS patients were obtained to evaluate hypertrophied LF. To create a rat model, we destabilized the lumbar spine. Each LF was sagittally cut for histological evaluation. The epiligament was clearly evident in normal LF specimens, which stained pink on Elastica van Gieson and green on Masson Trichrome. One layer was observed on the dural side and another on the dorsal side of the LF. LSCS patients had an enlarged dorsal epiligament, at around 30 times that of the regular thin epiligament on the dural side. The destabilized rat model showed an enlarged dorsal epiligament, with a mean thickness 8-fold that of the control. LF hypertrophy may be due to enlargement of the dorsal epiligament. Mechanical loading of the LF is an important factor for inducing hypertrophy in the rat model. J. Med. Invest. 65 : 85-89, February, 2018

Keywords **:** *Ligamentum flavum, Epiligament, Lumbar spinal canal stenosis, Collagenous fiber, Fibrosis*

INTRODUCTION

Lumbar spinal canal stenosis (LSCS) is a common lumbar disorder in the elderly population causing low back pain, radiculopathy, and cauda equina syndrome. Canal narrowing (stenosis) results partly from hypertrophy of the ligamentum flavum (LF), which mechanically compresses the nerve root or cauda equine (1-4). Although numerous investigations have been conducted to clarify the pathomechanism of LF hypertrophy, the exact mechanism has yet to be revealed (1-14). Therefore, surgeons are currently removing the hypertrophied LF since they cannot control its hypertrophy with drugs (15, 16). If the exact pathomechanism could be clarified, it may be possible to control the LF hypertrophy with drugs.

The epiligament is the surface layer of ligaments and consists of woven bundles of collagen fibers (17-19). Bray *et al.*(17) were the first to report the epiligament of the medial collateral ligament (MCL), which is considered to have several important functions including protecting the MCL against abrasion and being a source of extracellular matrix during ligament growth and ligament healing (18). They created an MCL hypertrophy animal model and showed that epiligament hypertrophy induced MCL hypertrophy in an unstable knee. Thus, the epiligament can be considered to play a major role in ligament hypertrophy. To date, however, no studies have reported on the role of epiligament in LF hypertrophy or even clarified its existence in the LF.

We hypothesized that epiligament surrounds the LF and is the main contributing tissue in LF hypertrophy such as MCL hypertrophy. The purpose of this study was to clarify the existence of epiligament in the LF, to elucidate its role in LF hypertrophy, and then to create an LF hypertrophy animal model.

Received for publication November 27, 2017 ; accepted January 2, 2018.

Address correspondence and reprint requests to Nori Sato, MD, PhD, Department of Orthopedics, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-88-633-0178.

MATERIALS AND METHODS

Institutional review board approval was obtained for this study, and subjects provided informed consent to participate.

(i) Normal human LF specimens

LF was taken from a fresh cadaveric spine of a 30-year-old man at the Th11-12 level as a human LF control as it did not show severe degeneration or hypertrophy.

(ii) Hypertrophied human LF specimens

LF specimens were collected from 5 patients (1 man, 4 women ; mean age, 71.0 years ; age range, 66 to 79 years) during surgery for degenerative LSCS.

(iii) Rat model LF specimens

We created posterior destabilization to increase loading on the LF in 6 female Wistar rats (3 for the model and 3 for the control ; all 8 weeks old). Under general anesthesia with sodium pentobarbital (32.4 mg/kg body weight), the L5 spinous process, L4-6 supraspinous ligament, and L4-5 and L5-6 interspinous ligaments were removed. We paid special attention not to touch the lamina or LF during surgery. The rats were killed 8 weeks after the operation by pentobarbital overdose for histological study.

Histological processing

Ligaments in the human specimens were sagittally cut, fixed in 10% formalin for 48 h, and embedded into a paraffin block. Intact spinal column, including the vertebral body, disc, and lamina, was taken from the rat specimens at the L4-6 level and fixed in formalin for 48 h. Samples were decalcified with Plank-Rychlo's solution (Decalcifying Solution A ; Wako, Osaka, Japan) and then sagittally cut in the slightly para-sagittal plane from the midline to evaluate

the LF. Thin-sliced sections (3 μm) of the human and rat specimens were subjected to the following staining : Elastica van Gieson (EVG) to evaluate the condition of the elastic fibers and Masson Trichrome (MT) to evaluate the state of fibrosis.

RESULTS

(i) Normal human LF specimens

The LF consists of elastic fibers rather than collagen fibers. Thus, most parts of the LF stained black on EVG staining and pink on MT staining. In the human control samples, we found two superficial collagenous layers which stained red on EVG staining (Figures 1a-c) and green on MT staining (Figures 1d-f). One layer was present on the dural side and the other on the dorsal side of the LF, indicating that this collagenous layer surrounds the main elastic LF tissue ; these layers corresponded to the epiligament. Mean human epiligament thickness at the dorsal and dural aspects measured at five randomly selected sites was 140.9± 39.9 μm and 33.8± 7.9 μm, respectively. In the human samples, epiligament was thicker at the dorsal aspect than that at the dural aspect.

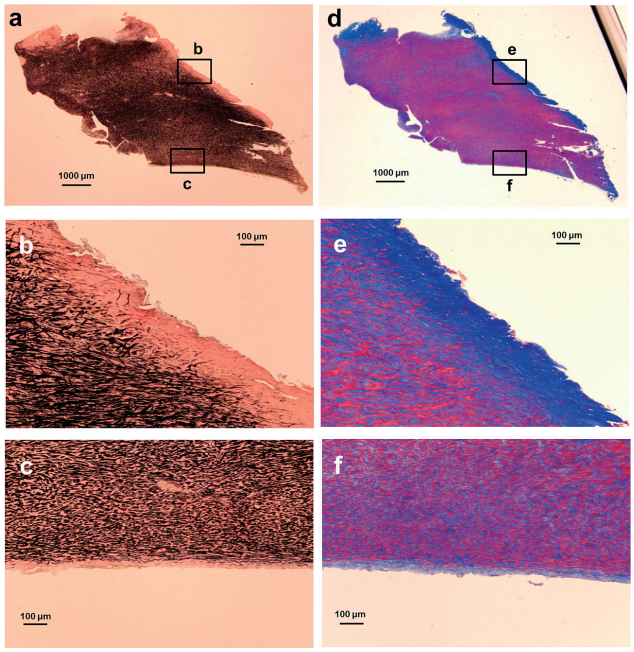

Figure 1. Photomicrographs of the ligamentum flavum (LF) specimen from the cadaveric spine of a 30-year-old man. Elastica van Gieson (EVG) (a-c) and Masson Trichrome (MT) (d-f) staining. The superficial dorsal layer (b and e) and the dural layer (c and f) stained red on EVG staining and green on MT staining. These layers correspond to the epiligament. Mean human epiligament thickness at the dorsal and dural aspects was 140.9± 39.9 μm and 33.8± 7.9 μm respectively.

(ii) Hypertrophied human LF specimens

In the elderly subjects with LSCS, the dorsal epiligament (thick fibrous area without elastic fibers) was enlarged in all 5 LF samples. Figure 2 shows a representative case of LSCS in a 70-year-old woman. The thick fibrotic area at the dorsal aspect, with loss of elastic fiber, was obvious (Figures 2a, b, d, and e). The mean thickness of the enlarged dorsal epiligament in this sample was 1450.8± 412.9 μm, and that of the enlarged dorsal epiligament in all 5 samples was 1162.6± 389.4 μm (Table 1). On the other hand, the mean thickness of the dural epiligament in this sample was 75.4± 8.4 μm, and that of the dural epiligament in all 5 samples was 42.6± 19.2 μm (Table 1). The dural aspect which had minimal fibrosis and multiple elastic fibers, was mostly of regular size (Figures 2c, f). Thus, the epiligament at the dorsal aspect was about 30-fold thicker than that at the dural aspect in the hypertrophied LF.

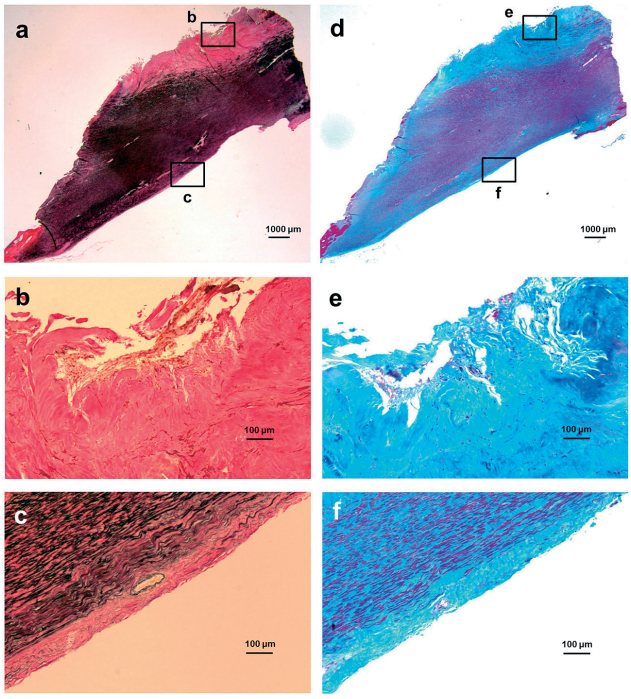

Figure 2. Photomicrographs of the LF specimen from a 70-year-old patient with lumbar spinal canal stenosis (LSCS). EVG staining (a-c) and MT staining (d-f). Enlarged dorsal epiligament with a thick fibrous area without elastic fibers (b and e). Regular thin dural epiligament (c and f). The mean thickness of the enlarged dorsal epiligament in this sample was 1450.8± 412.9 μm, and that of the dural epiligament was 75.4± 8.4 μm.

Table 1. Thickness of the epiligament of LF from patients with LSCS

Case#	Dorsal aspect (μm)	Dural aspect (μm)
#1	1450.8+/-412.9	75.4+/-8.4
#2	983.0+/-344.6	44.0+/-5.0
#3	851.1+/-60.5	29.7+/-3.7
#4	832.0+/-216.7	32.6+/-5.2
#5	1696.3+/-317.1	31.5+/-8.2
Mean	1162.6+/-389.4	42.6+/-19.2

(iii) Rat model LF specimens

The control rat had no fibrosis of the LF, which consisted mainly of elastic fibers. Regular thin epiligaments were seen at both the dorsal aspect and dural aspect (Figures 3a-f). The mean thickness of the dorsal epiligament was 20.9± 15.2 μm, and that of

the dural epiligament was 31.2±3.2 μm. On the other hand, a fibrotic area was found at the dorsal aspect in the rat unstable lumbar spine model on MT staining (Figures 4d, e). As this fibrotic area had few elastic fibers on EVG staining (Figures 4a, b), it indicated an enlarged LF epiligament. The mean thickness of the enlarged dorsal epiligament was 165.1±13.8 μm, which was notably thicker than in the control rat. In this model, the dural epiligament was not enlarged, with the mean thickness of 31.7±7.2 μm (Figures 4c, f). Taken together, posterior destabilization could cause enlargement of the dorsal side of the LF while keeping the dural side of the epiligament intact. These features are similar to the histological findings of the samples from LSCS patients.

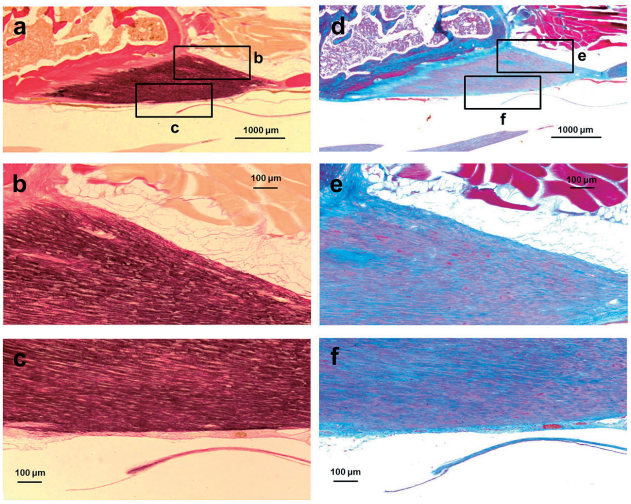

Figure 3. Photomicrographs of the LF specimen from a control rat. EVG staining (a-c) and MT staining (d-f). The control rat had no fibrosis of the LF, which consisted mainly of elastic fibers. Regular thin epiligaments were seen at both the dorsal aspect (20.9±15.2 μm ; b and e) and dural aspect (31.2±3.2 μm ; c and f).

In summary, the dorsal epiligament (thick fibrous area) was enlarged in both hypertrophied LF from LSCS patient (Figure 2) and the rat model (Figure 4).

DISCUSSION

This study revealed the following novel findings :

1) The human LF contains an epiligament consisting mainly of collagenous fibers, while the LF itself consists of elastic fibers. Thus, the epiligament has obvious histologically differences compared with the main LF.
2) All hypertrophied LF samples from the LSCS patients had an enlarged epiligament in the dorsal aspect consisting of collagenous tissue, not elastic fibers. Epiligament thickness at the dorsal spect was 30-fold that at the dural aspect.
3) Posterior destabilization in the rat spine probably caused the thickening of the dorsal epiligament, which was 8-fold thicker than that of the control rat. This histological finding was similar to the human samples from the LSCS patients. This is the first animal model of LF hypertrophy to be reported.

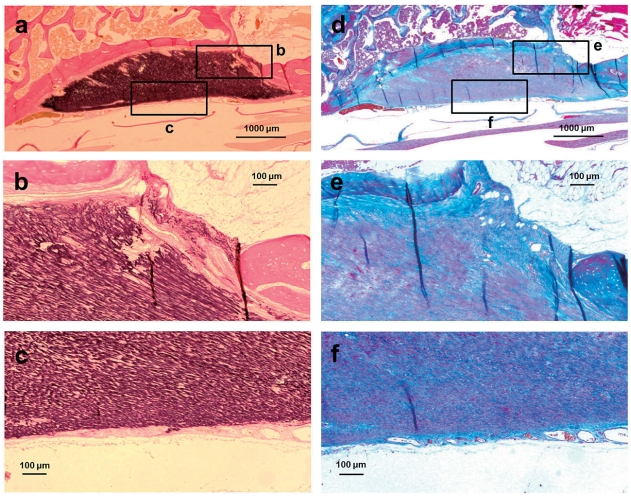

Figure 4. Photomicrographs of the LF specimen from a rat with an unstable lumbar spine. EVG staining (a-c) and MT staining (d-f). The fibrotic area of the dorsal aspect was stained with red on EVG staining (b) and green on MT staining (e). The mean thickness of the enlarged dorsal epiligament was 165.1±13.8 μm (b and e), which was notably thicker than in the control rat. The dural epiligament was not enlarged, with the mean thickness of 31.7±7.2 μm (c and f).

Epiligament of the LF

The epiligament constitutes the surface layer of ligaments and consists of woven bundles of collagen fibers (17-19). Knee MCL hypertrophy was reproduced in the animal knee instability model, and MCL hypertrophy of the epiligament induced MCL hypertrophy. Thus, the epiligament is considered to play a crucial role in ligament hypertrophy. To date, however, no studies have reported the role of the epiligament in LF hypertrophy. Moreover, the existence of the LF epiligament has been unknown. With this in mind, we first confirmed the existence of the LF epiligament. Normal LF was collected from the fresh cadaveric spine of a 30-year old man as a human control. This specimen was obtained from the thoracic spine, because even relatively young spines could have degenerative changes in the LF in the lumbar region. The sample clearly showed collagenous membranous tissue on both the dorsal and dural surfaces.

In the MCL investigation, it was difficult to differentiate the epiligament from the main MCL because both consisted mostly of type I collagen (17-19). On the other hand, the epiligament was obvious in the LF, making differentiation on MT and EVG staining easy. As the epiligament consists of collagenous fibers, while the LF consists of elastic fibers, staining is completely different.

Mechanism of LF Hypertrophy

LF hypertrophy is one of the major factors of canal narrowing in LSCS. Many studies have investigated the mechanism of LF hypertrophy using anatomical, histological, and biological methods (2, 9, 11-14, 20-22). Our group previously reported that hypertrophy occurs due to the accumulation of fibrosis (scarring) at the dorsal aspect of the LF (22-24). The present study revealed similar histological findings, with apparent enlargement of the thick fibrotic mass at the dorsal aspect of the LF epiligament ; the thickness was 30-fold that of the dural epiligament.

This study, as well as previous reports (22-24), indicates that the dorsal fibrous mass, which seems to correspond to enlargement of the dorsal epiligament, is the main pathology causing LF hypertrophy. Chowdhury *et al.* (18) reported that epiligament is a

source of extracellular matrix, cells, and vasculature during ligament growth and healing, and they further concluded that epiligament is the main source of cells that form ligament scars during ligament healing. Matthews *et al.* (25) created an MCL hypertrophy model using a canine knee joint and induced hypertrophy by transecting the anterior cruciate ligament to destabilize the knee joint. In the hypertrophied MCL, a dense, scar-like tissue mass was found at the medial aspect, and histological findings indicated that the scar-like fibrous mass was connected to the medial epiligament. Their histological findings were similar to those of Chowdhury *et al.* (18). Thus, for the LF hypertrophy mechanism, it is not difficult to assume that (i) micro injury occurs at the dorsal aspect of the main LF and (ii) healing of the LF causes the dorsal epiligament to create a thick fibrotic mass in the dorsal aspect of the LF.

The present finding are in agreement with previous findings (22, 23) that the dorsal aspect of the LF showed thick scarring and the dural aspect was mostly intact. We previously reported that mechanical stress at the dorsal aspect was about 5-fold higher than that at the dural aspect of the LF (22). Thus, during daily activities, higher mechanical stress is likely to be applied to the dorsal aspect, which may induce micro injury on the dorsal side, rather than on the dural side. During the process of micro injury healing, a thick fibrotic mass may be produced, which could cause LF hypertrophy.

Rat LF Hypertrophy Model

In the MCL hypertrophy model of Matthews *et al.* (25), the dense, scar-like tissue mass found at the medial aspect had similar histological features to the hypertrophied LF from the LSCS patients. Based on the model of Matthews *et al.* (25), we surmised that hyper-mechanical stress on the LF could cause hypertrophy in an animal model. Stress in the LF is mainly longitudinal (tensile) stress. Thus, flexion is the most important motion for inducing mechanical stress in the LF (22). Based on this concept, we induced posterior destabilization in the rat model to increase flexion. To avoid damaging the LF during surgery, we removed the L5 spinous process, supraspinous ligament, and interspinous ligaments.

The LF of control rat was surrounded by epiligament, as in the normal human LF. Posterior destabilization in the rat spine caused thickening of the dorsal epiligament to 8-fold that of the control rat. This histological finding was similar to that of the LSCS patients. The control rat had a regular thin epiligament at the dural aspect, with the LF consisting of elastic fibers. Thus, these histological features indicate that the LF in the destabilized model is the same as that in human hypertrophied LF. To our knowledge, this is the first report of an animal model of LF hypertrophy.

In conclusion, the LF has two distinctive collagenous layers ; one on the dural side and the other on the dorsal side. An enlarged dorsal epiligament is present in hypertrophied LF.

CONCLUSIONS

This is the first report on the existence of epiligament in the LF and its role hypertrophy. LSCS patients had an enlarged dorsal epiligament and a regular thin dural side epiligament. The epiligament at the dorsal aspect was about 30-fold thicker than that at the dural aspect in the hypertrophied LF. To clarify the existence of epiligament in the LF and its role hypertrophy, we created a rat model with an unstable lumbar spine. The rat model showed an enlarged dorsal epiligament surrounded by thick fibrosis, with a mean thickness 8-fold that of the control. Posterior destabilization could cause enlargement of the dorsal side of the LF while keeping the dural side of the epiligament intact.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

REFERENCES

1. Beamer YB, Garner JT, Shelden CH : Hypertrophied ligamentum flavum. Clinical and surgical significance. Archives of surgery (Chicago, Ill : 1960) 106 : 289-292, 1973
2. Park JB, Chang H, Lee JK : Quantitative analysis of transforming growth factor-beta 1 in ligamentum flavum of lumbar spinal stenosis and disc herniation. Spine 26 : E492-495, 2001
3. Towne EB, Reichert FL : Compression of the Lumbosacral Roots of the Spinal Cord by Thickened Ligamenta Flava. Annals of surgery 94 : 327-336, 1931
4. Ullrich CG, Binet EF, Sanecki MG, Kieffer SA : Quantitative assessment of the lumbar spinal canal by computed tomography. Radiology 134 : 137-143, 1980
5. Evans JH, Nachemson AL : Biomechanical study of human lumbar ligamentum flavum. Journal of anatomy 105 : 188-189, 1969
6. Fukuyama S, Nakamura T, Ikeda T, Takagi K : The effect of mechanical stress on hypertrophy of the lumbar ligamentum flavum. Journal of spinal disorders 8 : 126-130, 1995
7. Nachemson AL, Evans JH : Some mechanical properties of the third human lumbar interlaminar ligament (ligamentum flavum). Journal of biomechanics 1 : 211-220, 1968
8. Nakatani T, Marui T, Hitora T, Doita M, Nishida K, Kurosaka M : Mechanical stretching force promotes collagen synthesis by cultured cells from human ligamentum flavum via transforming growth factor-beta1. Journal of orthopaedic research : official publication of the Orthopaedic Research Society 20 : 1380-1386, 2002
9. Okuda T, Baba I, Fujimoto Y, Tanaka N, Sumida T, Manabe H, Hayashi Y, Ochi M : The pathology of ligamentum flavum in degenerative lumbar disease. Spine 29 : 1689-1697, 2004
10. Olszewski AD, Yaszemski MJ, White AA, 3rd : The anatomy of the human lumbar ligamentum flavum. New observations and their surgical importance. Spine 21 : 2307-2312, 1996
11. Postacchini F, Gumina S, Cinotti G, Perugia D, DeMartino C : Ligamenta flava in lumbar disc herniation and spinal stenosis. Light and electron microscopic morphology. Spine 19 : 917-922, 1994
12. Ramsey RH : The anatomy of the ligamenta flava. Clinical orthopaedics and related research 44 : 129-140, 1966
13. Schrader PK, Grob D, Rahn BA, Cordey J, Dvorak J : Histology of the ligamentum flavum in patients with degenerative lumbar spinal stenosis. European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society 8 : 323-328, 1999
14. Yoshida M, Shima K, Taniguchi Y, Tamaki T, Tanaka T : Hypertrophied ligamentum flavum in lumbar spinal canal stenosis. Pathogenesis and morphologic and immunohistochemical observation. Spine 17 : 1353-1360, 1992
15. Ikuta K, Arima J, Tanaka T, Oga M, Nakano S, Sasaki K, Goshi K, Yo M, Fukagawa S : Short-term results of microendoscopic posterior decompression for lumbar spinal stenosis. Technical note. Journal of neurosurgery Spine 2 : 624-633, 2005
16. Wada K, Sairyo K, Sakai T, Yasui N : Minimally invasive endoscopic bilateral decompression with a unilateral approach (endo-BiDUA) for elderly patients with lumbar spinal canal stenosis. Minimally invasive neurosurgery : MIN 53 : 65-68, 2010

17. Bray RC, Fisher AW, Frank CB : Fine vascular anatomy of adult rabbit knee ligaments. Journal of anatomy 172 : 69-79, 1990
18. Chowdhury P, Matyas JR, Frank CB : The "epiligament" of the rabbit medial collateral ligament : a quantitative morphological study. Connective tissue research 27 : 33-50, 1991
19. Frank CB : Ligament structure, physiology and function. Journal of musculoskeletal & neuronal interactions 4 : 199-201, 2004
20. Mensor MC, Fender FA : The ligamentum flavum : Its relationship to low back pain. . Surg Gynecol Obstet 73 : 822-827, 1941
21. Naffziger HC, Inman V, Saunders JB : Lesions of the intervertebral disc and ligamentum flava. Surg Gynecol Obstet 66 : 288-299, 1938
22. Sairyo K, Biyani A, Goel V, Leaman D, Booth R, Jr., Thomas J, Gehling D, Vishnubhotla L, Long R, Ebraheim N : Pathomechanism of ligamentum flavum hypertrophy : a multidisciplinary investigation based on clinical, biomechanical, histologic, and biologic assessments. Spine 30 : 2649-2656, 2005
23. Kosaka H, Sairyo K, Biyani A, Leaman D, Yeasting R, Higashino K, Sakai T, Katoh S, Sano T, Goel VK, Yasui N : Pathomechanism of loss of elasticity and hypertrophy of lumbar ligamentum flavum in elderly patients with lumbar spinal canal stenosis. Spine 32 : 2805-2811, 2007
24. Sairyo K, Biyani A, Goel VK, Leaman DW, Booth R, Jr., Thomas J, Ebraheim NA, Cowgill IA, Mohan SE : Lumbar ligamentum flavum hypertrophy is due to accumulation of inflammation-related scar tissue. Spine 32 : E340-347, 2007
25. Matthews JL, Chung M, Matyas JR : Indirect injury stimulates scar formation-adaptation or pathology? Connective tissue research 45 : 94-100, 2004