Introduction
Deposition of calcium crystal is currently known as “articular chondrocalcinosis” or the “pseudogout,” and acute or chronic inflammation caused by CPPD crystal deposition can occur in knee, hip, shoulder, spine, and other large joints (1). In the spinal region, there are observed in the supraspinous ligaments and intervertebral discs, and is especially abundant in the ligamentum flavum. Although a variety of studies of degeneration in the ligamentum flavum or CPPD crystal formation have been published, the histology of crystal deposition in the ligamentum flavum has not been elucidated. It has been thought that CPPD crystal deposition is accompanied by degeneration of ligaments, and that metabolic disorders or rheumatoid factor were involved in this process (2).

The purpose of the present study was to investigate, histologically and immunohistochemically, the role of chondrocytes in the crystal formation process in ligamentum flavum by immunohistochemistry.

Patients and methods
We examined the ligamentum flavum harvested from 119 surgical cases (43 men, 76 women; average age 69.7 years, range 43-85 years) with symptomatic lumbar spinal stenosis. Radiological examination showed that all patients had central or lateral type lumbar spine stenosis, which was not associated with inflammatory disease of the spine, pyogenic spondylitis, or a collagen disease. Ligamentum flavum tissue was harvested at L1-L2 in 3 patients, at L2-L3 in 11 patients, at L3-L4 in 44 patients, at L4-L5 in 106 patients, and at L5-S1 in 16 patients. Sections of the ligament were examined by scanning electron microscopy (SEM), energy dispersive X-ray microanalysis, and were immunostained for S-100 protein, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and CD34. The results were compared with those of ligament tissue harvested from 10 cases of lumbar disc herniation.

Result
The most superficial layer of the ligamentum flavum tissue obtained from lumbar disc herniation had a uniform appearance. The elastic fibres were in a regular arrangement, oriented parallel to the major axis of the ligament. In the lumbar spinal canal stenosis cases, elastic fibres were in an irregular arrangement and the fibres were of smaller diameter than in the lumbar disc herniation cases. In other areas, elastic fibres with abnormally small diameters were fragmented or separated by thick bundles of collagen fibres. Large fibrotic areas with a decreased elastic component and increased collagenous tissue were frequently observed. There were hypertrophic chondrocytes in these areas (Fig. 1).

In the ligamentum flavum around the calcium deposit, a number of hypertrophic chondrocytes and neutrophils were found, together with many small blood vessels. The elastic fibres appeared as ruptured and fragmented and abundant fibrocartilaginous tissue was present around the areas of crystal formation. In immunohistochemically, hypertrophic chondrocytes stained positively for S-100 protein, VEGF and bFGF (Fig. 2). The deposited calcium crystals were morphologically various, appearing as pin-like, rod-like, or rectangular crystals when observed under SEM. The diameter of these crystals was approximately 4 m or more. The ratio of calcium to phosphate in these crystals, as measured on X-ray microanalysis, was approximately 1 : 1, indicating that the crystals consisted of CPPD (Fig. 3).

Discussion
Crystal deposition in the ligamentum flavum occurs in ligaments where advanced fibrosis and chondrometaplasia exist, and the crystals are surrounded by chondrocytes, which are thought to be important for crystal formation and deposition (3, 4). The results of our present study indicate that the initial change that occurs in crystal deposition in the ligamentum flavum is rupture of elastic fibres. Collagen fibres then increase as if replenishing the sites where the elastic fibres ruptured, and chondrocytes are induced from fibroblasts. Chondrocytes induce growth of blood vessels by VEGF and bFGF, further fibrosis of the matrix by bFGF, and the expansion of chondrometaplastic areas. These matrix changes becomes the site of crystal deposition.

S-100 protein have specific binding sites for calcium crystals. Our results suggest that S-100 protein is one factor promoting crystal formation mediated by secretion of TGF- and subsequent calcium influx accompanied by angiogenesis and phosphate influx with increased PPi concentration, leading to mineralization of the matrix (5).

Conclusion: We speculate that hypertrophic chondrocytes play an important role in CPPD crystal formation. With respect to other factors involved in crystal deposition in ligaments, genetic factors, metabolic disorders, and systemic chondrocalcinosis are likely contributors.