Introduction
Many studies demonstrated the involvement of cyclooxygenase-2 (COX-2) in tumorigenicity of various types of malignant tumors such as colorectal cancer, breast cancer and lung cancer. Ependymoma is the most common intramedullary spinal cord tumor and accounts for approximately 60% of all spinal cord gliomas. Spinal cord ependymoma is essentially a benign tumor and standard treatment involves surgical resection. Complete tumor removal is often achieved, but not in all cases. The effectiveness of post-operative adjuvant therapy for cases of incomplete resection remains controversial, therefore new strategy for spinal cord ependymoma is required.

A few authors demonstrated COX-2 expression in intracranial ependymomas (1). However we were not able to find any previous reports of COX-2 expression in spinal cord ependymoma. Therefore we aimed to evaluate COX-2 expression in spinal cord ependymoma and investigate the role of COX-2 in tumor activity.

Material and Methods
Formalin-fixed and paraffinized spinal cord ependymoma tissues were obtained from consecutive patients who had undergone tumor resection in our institution from February 1995 to July 2004. Sixteen cases were available for this study. This series included 8 male and 8 female patients. The mean age was 45.6 years (range, 6-71 years). Thirteen cases were cellular-type ependyomas and the remained three cases were the myxopapillary-type. Since myxopapillary-type ependymomas are unique variant of ependymomas, we performed data analysis using two distinct categories: ‘group A’ consisted of all 16 cases including the myxopapillary-types and ‘group B’ consisted of the 13 cellular-type ependyomas excluding the myxopapillary-type.

The paraffinized tissues were cut into 4 μm thick serial sections and were subjected to immunohistochemical staining for COX-2, vascular endothelial growth factor (VEGF). Intratumoral micro-vessels were also stained immunohistochemically using anti-human von Willebrand factor antibody. The primary specific anti-rat COX-2 goat polyclonal antibody which has been shown to cross-react with human COX-2 (Santa Cruz Biotechnology, CA), anti-human VEGF rabbit polyclonal antibody (Santa Cruz Biotechnology, CA) and anti-human von Willebrand factor rabbit polyclonal antibody (DakoCytomation, CA) were applied at dilutions of 1:500, 1:500, 1:1000, respectively. Immunohistochemical staining was processed with a HISTFINE Kit® (Nichirei, Japan) and dianinobenzidine-containing substrate solution. COX-2- and VEGF-positive staining cells were counted under a light microscope in five different high-power fields (HPF) selected randomly at a magnification of 400×. The percentage of positively stained cells was calculated. In the same way intratumoral micro-vessels that were identified by positive staining for von Willebrand factor were counted in five different HPF selected randomly at a magnification of 100× and the averages of number of micro-vessels/HPF was calculated as the micro-vessel density (MVD).

Clinical features were also reviewed and were evaluated association with COX-2 expression. Mann-Whitney’s U test or chi-square test was used to compare the differences between two groups. Correlation was evaluated using Spearman’s correlation coefficient by rank. P-values of <0.05 were considered statistically significant.

Results
COX-2 expression was observed in 7 (43.8%) of the 16 ependymomas (group A). All of the three myxopapillary-type cases exhibited COX-2-positive staining. Excluding the myxopapillary-type cases (group B), COX-2 expression was identified in 4 (30.8%) of 13 cellular-type ependymomas. In all positive cases, all tumor cells exhibited positive staining and diffuse cytoplasmic staining. Therefore, the samples could be classified into COX-2-‘positive’ (Figure 1) and ‘negative’ (Figure 2) samples.

VEGF expression was observed in 9 cases (56.3%) in group A, compared with 6 cases (46.2%) in group B. All the COX-2-positive samples exhibited VEGF expression, whereas almost all the COX-2-negative samples exhibited negative staining for VEGF excluding 2 cases. The percentage of VEGF-positive cells was 17.9%±36.3% in COX-2-positive samples and 0.1%±0.1% in COX-2-negative samples in group A. In group B, the percentages of VEGF-positive cells were 3.1%±1.1% in the COX-2-positive samples and 0.1%±0.1% in COX-2-negative samples. In both group A and B, the percentages of VEGF-positive cells were increased significantly in COX-2-positive samples compared with COX-2-negative samples (p=0.0088 in group A, and p=0.0025 in group B). The MVD correlated significantly with the percentages of VEGF-positive staining cells in both group A and B (p=0.0021 and correlation coefficient = 0.706 in group A, p=0.0043 and correlation coefficient = 0.611 in group B). In regard to the association between COX-2 expression and MVD, MVD was increased significantly in the COX-2-positive samples (Figure 3) compared with COX-2-negative samples (Figure 4). MVD in the COX-2-positive samples were 18.1±6.4/HPF, whereas in the COX-2-negative samples were 5.5±6.2/HPF in group A (p=0.0095) (Figure 5). In group B, the MVD was 15.2±7.4/HPF in the COX-2-positive samples and 5.5±6.2/HPF in the COX-2-negative samples (p=0.0206). No significant differences in clinical features were detected between the COX-2-positive and –negative samples in groups A and B.

Discussion
COX-2 has been demonstrated to promote tumor growth through many pathways including up-regulation of growth factors such as VEGF. COX-2-derived prostaglandin E2 has been shown to dominantly regulate angiogenesis of tumors (2). Furthermore, a selective COX-2 inhibitor has been demonstrated to suppress tumor activity through not only induction of apoptosis but also down-regulation of VEGF (3). Present study showed COX-2 expression in 43.8% (group A) and 30.8% (group B) of spinal cord ependymomas. Furthermore, the association between COX-2 and VEGF expression that resulted in increase of intratumoral micro-vessels was demonstrated. These findings suggested a possibility that selective COX-2 inhibitors might be new therapeutic tools for spinal cord ependymomas through suppression of intratumoral angiogenesis. However, COX-2 might modulate angiogenesis as well as many other different aspects of tumor activities. Therefore, further study for the various interpretations of COX-2 expression in ependymomas of the spinal cord should be investigated.

1: Kim SK et al, Oncol Rep 12: 403-409, 2004