INTRODUCTION
Osteosarcoma is the most common primary malignant tumor of bone in humans and accounts for 20% of primary osseous neoplasms. Clinically evident metastatic disease in lungs or bones is present at the time of diagnosis in 10 to 20% of patients, and a large proportion of patients eventually succumb to pulmonary metastatic disease despite recent progress in survival after adjuvant chemotherapy [1].

Tumor necrosis factor alpha (TNF-α) is a key player in the malignant tumor microenvironment and is involved in the pathogenesis of cancer [2]. TNF-α also stimulates the production of IL-6, and it is a potent growth factor and directly stimulates angiogenesis [3]. It is an important factor for malignant tumor progression [4].

Infliximab is an anti-TNF-α monoclonal antibody. In this study, we investigated that infliximab inhibit lung metastasis of osteosarcoma cell line (143B) in vitro and in vivo.

MATERIALS AND METHODS
Osteosarcoma cells. The human osteosarcoma cell line 143B was cultured in MEM supplemented with 10% FBS.

Analysis of TNF-α Expression.
We assessed the expression of TNF-α and TNF receptor in 143B by RT-PCR.

Cell viability test in vitro
We analyzed the effect of infliximab to tumor cells viability with WST-1 assay. Each wells contained MEM with infliximab (0, 10, or 100 μg/ml). After seeding at 24, 48 and 72hour, WST-1 assay was performed.

Migration assays in vitro.
Boyden chamber were used for migration assays. Each chamber contained MEM with infliximab (0, or 10 μg/ml). 143B cells were added to the upper component of the chamber. After incubation, cells on the upper surface of the membrane were thoroughly removed. Quantitation of the migration was performed by staining membranes and counting the average number of cells in a membrane.

Effect of infliximab on tumor growth in vivo, and on lung metastasis in vivo.
For investigation into the effect of infliximab to tumor growth and lung metastases, 143B cells were injected in the right tibia of 5-week-old SCID mice. Infliximab was administered intraperitoneally at dose 10 mg/kg on every once a week after tumor cell inoculation. Untreated mice received saline by intraperitoneal injection. Mice were sacrificed 4 weeks after inoculation, tumor and lung were resected to macroscopic and pathological examination. The tumor volume was calculated, from the measured values of the maximum and minimum diameters as minimum diameter^2 x maximum diameter /2.

Statistical analysis
Statistical analysis was performed using the Stat View statistical software. Significant differences were evaluated using ANOVA.

RESULTS
TNF-α Expression in 143B cells
TNF-α mRNA and TNF-RI mRNA was expressed in 143B cells (data not shown).

Cell viability test in vitro
There was no significant difference in each group (data not shown).

Migration assays in vitro
Cell number was counted the moved cells through a membrane.

Effect of infliximab on tumor growth in vivo.
There was no significant difference in each group (Fig 2).

Effect of infliximab on lung metastasis in vivo.
Four weeks after tumor inoculation, the number of metastasis lesion was investigated per mouse or per lung in a mouse. The frequency of lung metastasis in treated mice was lower than that in untreated mice (Fig 3).

DISCUSSION and CONCLUSIONS
Tumor cell-derived TNF-α plays a profound role in malignant tumors [2, 5-7]. In this report, we demonstrated that anti-TNF-α therapy using infliximab suppressed tumor migration in vitro, and lung metastasis in vivo. These results suggested that infliximab suppressed lung metastases, following with inhibition of tumor migration. Our study showed that inhibition of TNF-α represents a promising therapeutic option.