INTRODUCTION:
Although chemotherapy and wide tumor excision have drastically improved the prognosis of the osteosarcoma patients, 30% to 50% of patients still die of lung metastasis. Therefore, control of lung metastasis might give a major impact on improving the prognosis of patients with osteosarcoma.

In order to isolate the putative genes for lung metastases of osteosarcoma, we screened genes that are differentially expressed between the Dunn osteosarcoma cell line (Dunn) and its subclone, the LM8 cell line (LM8) with high metastatic potential to the lung, using a differential display technique. And, we found reduced expression of decorin in LM8 (1). The present study investigates whether decorin inhibits pulmonary metastasis in vivo.

MATERIAL AND METHODS:
Cell lines
Murine LM8 osteosarcoma cell line (LM8), a variant with high metastatic potential to the lung, which is established from a murine Dunn osteosarcoma cell line (Dunn), was used.

Construction of decorin expression vector and generation of stably transfected clones
Human decorin gene was constructed into p3XFLAG-CMV-14 expression vector. Human decorin gene was transfected to LM8, followed by culture in selective medium containing G418.

Western blot analyses
Conditioned medium were used for SDS-PAGE, and transferred onto PVDF membranes. Immunodetection was performed using ANTI-FLAG M2 monoclonal antibody. Protein was detected with ECL Western blotting detection system.

Cell growth curve analysis
Three dishes for each cell line were simultaneously counted every other day.

Pulmonary metastasis assay in vivo.
Each cell line (10^7 cells/mouse) was inoculated subcutaneously into the back space of male C3H mice at 5weeks of age. Lungs were removed 4weeks later. After formalin-fixation, the lungs were paraffinembedded and cut at their maximum dimensions. The specimens were stained with hematoxylin-eosin to evaluate metastatic tumor nodules microscopically.

Cell motility and invasion assay in vitro
The motility experiments were performed using Biocoat migration chambers containing a 8.0-µm diameter pore size membrane for invasion assay, BD Biocoat Matrigel Invasion Chambers pretreated with matrigel. Protein was detected with ECL Western blotting detection system.

DISCUSSION and CONCLUSION:
Decorin has emerged as a powerful modulator of cell growth by affecting several key elements, including TGF-β, EGFR, growth factors, tyrosine kinase activity and angiogenesis (2,3,4) . It has been reported that low levels of decorin expression are associated with worse prognosis in patients with breast, colon and ovarian cancer (5) . And recently, we demonstrated that decorin protein produced by stromal myofibroblasts accumulated in the stromal extracellular space, and might play an important role in the stroma, protecting against tumor cell invasion in high-grade spindle cell sarcoma (6).

We examined the motility and invasion capacity through 8-µm diameter pore size membrane coated with the matrigel. Exogenous decorin inhibits the LM8 cell motility and invasion ability (P<0.05) (data not shown).

RESULTS:
Isolation of decorin expressing LM8
First, we isolated the subclones of LM8 cell lines which stably express decorin protein (DCN/LM8). Among six isolated subclones, clone #4 released the most amount of decorin protein into the culture medium (Fig.1). Therefore we decided to use the clone #4 as DCN/LM8. Next, we examined morphological differences between LM8, mock-transfected LM8 (mock/LM8) and DCN/LM8. No morphological differences were noted (data not shown).

Pulmonary metastasis assay in vivo
To investigate the effect of decorin protein on lung metastasis in vivo, we performed pulmonary metastasis assay. Less number of pulmonary metastases were significantly observed in mice with DCN/LM8 compared to mice inoculated with LM8 and mock/LM8 (P<0.05) (Fig.2a, 2b). No histological differences of the primary and metastatic lesions were observed.

Cell growth curve analysis
To clarify the reason of differences of the lung metastatic potential between three cell lines, we compared the growth rate between LM8, mock/LM8 and DCN/ LM8. Growth rates were not statistically different between three cell lines (Fig.3).

Cell motility and invasion assay in vitro
We examined the motility and invasion capacity through 8-µm diameter pore size membrane coated with the matrigel. Exogenous decorin inhibits the LM8 cell motility and invasion ability (P<0.05) (data not shown).

REFERENCES:
1) Morikawa J, Nishimura, Y, et al. ORS, 2001

52nd Annual Meeting of the Orthopaedic Research Society
Paper No: 1821

Decroin suppress lung metastases in murine osteosarcoma
+*Shintani, K; *Matsumine, A; *Matsuhara, T; *Satonaka, H; *Wakabayashi, T; *Morikawa, J; *Kusuzaki, K; *Uchida, A
+*Mie University School of Medicine, Mie, Japan
k-shinta@clin.medie.mie-u.ac.jp