INTRODUCTION:
Decorin is a small leucine-rich proteoglycan. It has been reported that low levels of decorin expression are associated with worse prognosis in patients with breast, colon and ovarian cancer. However, the expression and role of decorin in human soft tissue tumors have not yet been characterized. To investigate whether decorin gene expression is a biomarker of aggressiveness in soft tissue sarcoma, 51 biopsy samples from 43 patients were studied.

PATIENTS AND METHODS:
Patients: This study was based on 51 tumor samples from 43 soft tissue tumor patients diagnosed from 1998 to 2001 at Mie University Hospital. The tumors included 8 benign neurogenic benign tumors, 6 malignant peripheral nerve sheath tumors (MPNST), 11 liposarcomas (LPS), 8 malignant fibrous histiocytomas (MFH), 8 synovial sarcomas (SS) and 2 clear cell sarcomas (CCS). Seven patients had recurrent lesions.
Tissue preparation: The tissue samples were obtained from patients undergoing open biopsy or surgical resection of tumors, after informed consent according to the institutional review board guidelines. The 51 tumor samples from 43 patients, consisted of 43 primary lesion, 5 local recurrences and 3 recurrent metastases.

RNA isolation and preparation of CDNA: Total RNA from frozen specimens was extracted using mRNA purification kit according to the manufacturer's protocol. The RNA was reverse transcribed using an Oligo(dT) primer according to the manufacturer's protocol.

Multiplex Real-Time PCR analysis: Quantitative analysis of decorin expression was measured by multiplex Real-Time PCR, using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems Japan, Tokyo). All reagents were obtained from Applied Biosystems. TaqMan primer; ID 1279800000 was used. Expression levels of each gene and sample were divided by the ß-actin expression level.

Immunohistochemistry: Immunohistochemistry was achieved with Ventana EX system using a DAB universal kit (Ventana Medical Systems, Inc, Tucson, Arizona, USA). Paraffin-embedded tumor tissue was immunohistochemically stained by using the streptavidin-biotinylated complex method. Antigen retrieval with 4.0 mM citric acid buffer was performed using a Panasonic microwave on the high setting 3 times for 25 seconds. The mouse monoclonal anti-human decorin antibody 6B6 (Seikagaku Corp.Japan) was diluted 1:2000 in antibody diluent buffer (Dako).

Statistics and analysis: Association with clinical-pathological variables was determined by Mann-Whitney or Kruskal-Wallis tests. Overall survival was defined the time from initial surgery to the date of death attributed to soft tissue sarcoma. For prognostic analysis, Kaplan-Meier survival analysis and log rank tests were performed. The analysis was performed using StatView statistical software (version 5.0; SAS Institute Inc. Cary, North Carolina).

RESULTS:
Expression of decorin in various soft tissue tumor: Reduced expression of decorin was significantly more frequent in the high-grade malignancies (MFH, SS, CCS) than in the benign neurogenic tumors or low-grade malignancies (LPS, MPNST) (p<0.05) (Fig. 1).
Expression of decorin in primary, recurrent, and metastatic lesions: In all 7 cases with current or metastatic lesions, the levels of decorin expression in secondary lesion were lower than in the primary lesion (Fig. 2). This result suggests that decorin expression decrease in association with disease progression in soft tissue sarcoma.

Relationship between decorin expression and overall survival in high-grade spindle cell sarcoma: Among 16 high-grade spindle cell sarcoma patients (MFH, SS), the patients with sarcoma expressing lower levels of decorin had a significantly lower overall survival (Logrank; p<0.01) (Fig. 3). These results suggest that low levels of decorin may be associated with a worse prognosis in high grade spindle cell sarcoma. To confirm the decorin protein expression, we examined anti-human decorin immunostaining. The peritumoral stroma were stained for decorin protein on the paraffin-embedding sections which showed high expression of decorin transcripts on real-time PCR (Fig 4).

DISCUSSION:
Recent data concerning decorin's inhibitory effects on tumor cell growth support that reduced decorin may facilitate tumorgenesis and growth in the following mechanism: 1) Decorin modulates growth factor activities and growth factor distribution attributable to its TGF-ß binding properties
2) Decorin interacts with the EGFR and triggers a signaling pathway that leads to growth suppression
3) Decorin suppress in vivo tumor growth by inhibiting the endogenous tumor cell production of VEGF.

However, the expression and role of decorin in human soft tissue tumors have not yet been characterized. Here we showed the relationship between decorin expression and outcome in soft tissue sarcoma. Cytoplasmic accumulation of decorin protein was detected in stromal cells. Decorin protein expression in stromal cells may play an important role in tumor progression of soft tissue sarcoma.

CONCLUSION:
Reduced expression of decorin, a small leucine-rich proteoglycan, in soft tissue tumor is associated with poor prognosis. Decorin gene expression is a biomarker of aggressiveness in soft tissue sarcoma.

REFERENCES: