Introduction
Cartilage-derived retinoic acid-sensitive protein (CD-RAP) is a 11-kDa protein and isolated from bovine articular chondrocytes and human melanoma cell lines (melanoma inhibitory activity or MIA). In normal tissue, it is expressed specifically in cartilage cells, but in tumors it has been shown to be expressed in melanoma, chondrosarcoma, and breast cancer to date. CD-RAP/MIA is expressed at high levels in malignant melanomas and at lower levels in some benign melanocytic nevi, but is not detected in normal melanocytes. Serum levels of CD-RAP/MIA from malignant melanoma patients, increases with progression of the disease (1). Recently, in Swarm rat chondrosarcoma, serum levels of CD-RAP/MIA have been shown to correlate with the tumor onset and proliferation (2). Although serum levels of CD-RAP/MIA increase in animal models of chondrosarcoma, clinically, it has not been demonstrated to be at high levels in patients’ sample. The aim of this study is to determine serum levels of CD-RAP/MIA in patients with chondrosarcoma as well as other bone and soft tissue tumors, and to investigate whether serum CD-RAP/MIA is a useful marker of these tumors.

Materials and methods
The study was based on 86 patients pathologically diagnosed as bone or soft tissue tumors at Nagoya University Hospital. It included 21 cases with osteosarcoma, 14 with chondrosarcoma, 12 with giant cell tumor (GCT), 6 with liposarcoma, 4 with leiomyosarcoma, 4 with malignant fibrous histiocytoma (MFH), 3 with chordoma, 3 with desmoid, 3 with multiple exostosis, 3 with pigmented villonodular synovitis (PVNS), 2 with malignant peripheral nerve sheath tumor (MPNST), 2 with rhabdomyosarcoma, and 1 case each with adamantinoma, clear cell sarcoma, enchondroma, Ewing sarcoma, solitary exostosis, fibrosarcoma, fibrous dysplasia, hemangiomia, and synovial chondromatosis. Serum specimen were obtained pre-operatively from those patients, and stored at –80°C until use for ELISA, informed consent was obtained from each patient.

CD-RAP was measured using ELISA kit (Roche, Mannheim, Germany) according to manufacturer’s instruction. For immunohistochemistry of CD-RAP/MIA, formalin-fixed, paraffin-embedded, 8-µm-thick sections were boiled in citric acid buffer (PH6) to retrieve antigen activity followed by incubation with anti-human CD-RAP/MIA monoclonal antibody as primary antibody. The bound antibody was localized with biotinylated secondary antibody and an avidin-biotin-peroxidase detection kit (Nichirei, Tokyo, Japan), and the slides were developed with diaminobenzidine tetrahydrochloride (DAB), counterstained with hematoxylin.

Statistical analysis was performed by unpaired t test. Significance was determined at the p<0.05.

Result
Immunohistochemical study of CD-RAP/MIA demonstrated that tumor cells were positively stained, especially, in samples which were at higher serum levels of CD-RAP/MIA. The serum levels of CD-RAP/MIA were determined in 12 volunteers with no known diseases. The concentration of CD-RAP were 6.24± 1.83 ng/ml (mean ± SD) in normal samples, 10.65± 4.16 ng/ml in osteosarcoma, 8.58± 3.89 ng/ml in chondrosarcoma, 8.52± 3.98 ng/ml in GCT, 6.03± 2.30 ng/ml in liposarcoma, 5.17± 2.72 ng/ml in leiomyosarcoma, 8.86± 1.15 ng/ml in MFH, 15.06± 6.14 ng/ml in chordoma, 4.96± 2.41 ng/ml in desmoid, 12.92± 3.54 ng/ml in multiple exostosis, 5.41± 0.46 ng/ml in PVNS, 6.76± 2.81 ng/ml in MPNST, 7.60± 2.72 ng/ml in rhabdomyosarcoma, 6.95 ng/ml in adamantinoma, 9.18 ng/ml in clear cell sarcoma, 7.99 ng/ml in enchondroma, 8.15 ng/ml in Ewing sarcoma, 6.95 ng/ml in exostosis, 6.87 ng/ml in fibrosarcoma, 5.56 ng/ml in fibrous dysplasia, 5.60 ng/ml in hemangioma, and 5.07 ng/ml in synovial chondromatosis (Figure 1). In osteosarcoma, cases were divided into two groups; less than 20 years old, 20 and more than 20 years old, and the concentration were 12.58± 4.71 ng/ml, 8.53± 2.03 ng/ml, respectively (Figure 2).

The concentration of serum CD-RAP was significantly higher in osteosarcoma (p<0.003), in chordoma (p<0.045), MFH (p<0.027), and multiple exostosis (p<0.001) than in normal samples. In cases of osteosarcoma, concentration of CD-RAP/MIA in younger patients was significantly higher than older patients (p=0.021). However, the concentration in older osteosarcoma was still significantly higher than normal samples (p=0.012). As not expected, the serum levels of CD-RAP/MIA in chondrosarcoma patients.

Discussion
In epitherial malignant tumors, various kinds of specific marker has been used to evaluate the presence and activity of tumors. However, in bone and soft tissue tumor, useful markers have not been available clinically. In this study, it is demonstrated that serum levels of CD-RAP/MIA in chordoma, osteosarcoma, MFH, and multiple exostosis are significantly higher than those in normal sample. The reason why serum levels in chondrosarcoma were not high is unclear. One of the explanations is that tumor mass in Swarm rat chondrosarcoma is relatively bigger than in human chondrosarcoma. Although, serum levels of CD-RAP/MIA in younger patients, in which chondrogenesis is still activated, may be higher than older patients, older patients in osteosarcoma displayed higher serum levels than in normal samples. It suggests that CD-RAP/MIA would be a significant marker for these tumors. Although it is not well-known that the expression of CD-RAP/MIA in serum reflects the chondroblastic activity or other function, this molecule will be one of the useful markers of bone and soft tissue tumors.