The Differential Diagnosis between Well-Differentiated Liposarcoma and Lipoma to Detect Lipid Metabolic Genes

INTRODUCTION
Adipocytic tumors are the largest group of bone and soft tissue tumors. Lipoma is a benign tumor and the most popular soft tissue tumor in adults. Liposarcoma is a most common type of soft tissue sarcoma. Liposarcoma has some histological subtypes (well differentiated, myxoid, pleomorphic, and dedifferentiated), and its morphology, genetics, and prognosis vary among subtypes. Well-differentiated liposarcoma (WD) is a low-grade sarcoma, and rarely develops distant metastasis. WD has an overall survival rate over 90%, but WD sometimes dedifferentiate, especially in abdomen. WD and lipoma are similar in clinical and radiographic features, and then sometimes difficult to determine whether operation is need or not. Diagnose them using needle biopsy or frozen section is also difficult.

Previous gene expression profiling study reveals that the expression of some genes, including lipid metabolic genes, was significantly different between subtypes in adipocyte tumors. We assumed that these genes could distinguish from WD to lipoma. The aim of this study is to clarify whether the detection of lipid metabolism related genes are useful for differential diagnosis in WD and lipoma.

MATERIALS AND METHODS

Clinical specimens
The snap frozen tumor tissues stored at -80°C from 78 tumor patients (lipoma 44 and WD 34) were used for analysis. Written informed consent to store tumor sample was acquired from all patients. The ethics committee in our institute approved the usage of specimens for the analyses. All surgical specimens of the tumors were reviewed and diagnosed by two pathologists who were experienced in the diagnosis of bone and soft-tissue tumors.

RNA extraction and quantitative RT-PCR
The Tissues were disrupted using Beads Cell Disrupter MicroSmash (TOMY SEIKO, Japan), and total RNA was extracted using QuickGene SP kit (FUJIFILM, Japan) as per the manufacturer’s instructions. First-strand cDNA was synthesized from 0.5 μg total RNA using the ReverTra Ace-a (TOYOBO, Japan) according to the manufacturer’s instructions. Previous study showed that the differentially expressed genes between WD, dedifferentiated liposarcoma, and normal fat were 34. Twelve of them were reported to be associated with lipid metabolism (ADIPQ, ALDH2, C10orf116, CIDEA, CIDEC, GPD1, PRKAR2B, RBP4, SAA1, TOP2A, ZIC1, and PLIN), and we analyzed them in this study. To detect relative levels of each mRNA transcription, qRT-PCR was performed using Brilliant II SYBR Green QPCR Master Mix (Stratagene) under the following conditions: 94 °C for 10 min followed by 40 cycles of 94 °C for 0.5 min, 60 °C for 0.5 min and normalized to GAPDH. In every case, cycle threshold (Ct) taken for quantitation were in the linear portion of the amplification range. All PCRs were performed in triplicates using the Mx3000P Quantitative PCR system (Stratagene). RNA levels are reported as fold change compared with control using the comparative quantitation analysis software available with the Mx3000P. Change in expression (fold) is calculated as $2^{\Delta \Delta CT}$ where $\Delta CT = C_{T} − C_{\alpha}$ (housekeeping), and $\Delta (\Delta CT) = C_{T} − C_{\alpha}$. Reaction product purity was confirmed by examination of melting curves for a single peak.

Statistical methods
Statistical analysis (Welch’s test) was performed using JMP Statistical Discovery Software 8.0 (SAS Institute).

RESULTS
The mRNA expression level of CIDEA, PRKAR2B, SAA1, and TOP2A were not significantly different between lipoma and WD. ZIC1 indicated lower expression in WD than in lipoma. The expression of ADIPQ, ALDH2, C10orf116, CIDEC, GPD1, RBP4, and PLIN was higher in WD than in lipoma (Fig.1). In PLIN, 32 of 34 WD cases indicated $\Delta CT >= 0$. On the other hand, 7 of 44 lipoma cases indicated $\Delta CT <= 0$ (specificity 0.94 and sensitivity 0.84). In the cases of PLIN $\Delta CT < 0$, all of WD cases indicated RBP4 $\Delta CT > 2$, and 3 of 37 lipoma cases indicated RBP4 $\Delta CT > 2$. When we distinguished WD from lipoma with PLIN $\Delta CT >= 0$ and RBP4 $\Delta CT > 2$, the specificity and the sensitivity was 1.0 and 0.77, respectively.

REFERENCES
1. Singer S. et al., Cancer Res 67 (14), 6626-36, 2007

Fig.1, Expression levels of mRNAs in Lipoma and liposarcoma

DISCUSSION
Well-differentiated liposarcomas of the soft tissue closely mimic benign lipomas, although both tumors are different in biologic nature. The function of PLIN is known to restrain lipolysis by modulating the access of hormonesensitive lipase to the lipid surface. Increase of PLIN mRNA causes to inhibit the degradation of adipocyte. RBP4 is correlated with the growth of adipose tissue. The RNA expression of both genes was lower in WD than in lipoma, and the genes were useful to different diagnosis between them.

For clinical application, we must examine whether the expression of these genes vary in same tumor.