Gene Expression Profile of Poorly Differentiated Synovial Sarcoma
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Introduction
In our previous study, we analyzed gene expression in 105 samples from 10 types of soft tissue tumors and reported strong correlation between gene expression and their histology and availability of histological diagnosis of soft tissue sarcomas based on gene expression using microarray technology (1). Since soft tissue sarcomas are rare malignancies and show highly heterogeneous histological types, we have focused on analyzing gene expression of each tumor type which has both homogeneous background in molecular pathology and distinct subtypes to be genetically characterized. Synovial sarcoma (SS) is one of the common soft tissue sarcomas in adolescents and young adults accounting for 5-10% of all primary soft tissue sarcomas. The great majority of SS bear a distinctive chromosomal translocation, t(X,18)(p11;q11), resulting in SS specific ST7-SSX fusion transcript and now these somatic changes are considered to play an important role in the initial step of oncogenesis in SS. This unique genetic background causes a distinct expression profile of SS samples when compared with other sarcoma types (1).

In general, SS is considered to be classified into three major histological subtypes: monophasic type synovial sarcoma (MSS) uniformly composed of spindle cells; biphasic type synovial sarcoma (BSS) containing both epithelial cells arranged in glandular structures and spindle cells; and poorly differentiated type synovial sarcoma (PDSS) characterized by predominantly small round cell or short spindled cell features. However, this classification is based on morphology alone and there are great differences among pathologists in the criteria distinguishing the SS subtypes. Among these subtypes, PDSS has been posing a special problem in diagnosis since it is basically indistinguishable from Ewing sarcoma and other small round cell sarcomas by routine light microscopy and immunohistochemistry and, moreover, it bears few specific markers for diagnosis to distinguish it from other subtypes of SS. Some thought that PDSS is not a single entity but a form of histological progression that can occur any of the SS subtypes and categorized the tumors as MSS or BSS in their studies. Thus, even the incidence of PDSS among SS is difficult to estimate. Recognition of PDSS is of practical importance not only because it poses a special problem in diagnosis but also because it is strongly associated with poor prognosis. A number of clinicopathological studies have been carried out to find that tumor size, histological grade (grade 3), histological subtype (PDSS) are adverse prognostic factors of SS. It is clear that tumors with poorly differentiated areas generally behave more aggressively and metastasize in a higher percentage of cases than those without such areas. Although a number of reports from clinicopathological studies showed strong association between PDSS and a poor prognosis, little has been studied on the molecular characterization of PDSS. In this study, we analyze gene expression from 34 SS samples to show different expression patterns among its subtypes and identify distinct expression profile of PDSS.

Material and Methods
34 patients received histological diagnosis of SS in each institute, (NCCH, Keio Univ. Hosp., Niigata Univ. Hosp.). Tumor samples were collected from the part with macroscopically high tumor content immediately after surgical excision and cryopreserved until use in each hospital. Expression of the SS specific fusion transcript, ST7-SSX was confirmed by RT-PCR in all samples. This study was approved by the ethical committee of each hospital and conducted according to tenets of the Declaration of Helsinki.

Total RNA was isolated using IsoGen (Nippon gene, Toyama, Japan) according to the manufacturer’s instruction. Samples were analyzed with a GeneChip Human Genome U133 plus 2.0 (Affymetrix, Santa Clara, CA, USA) which enables us to analyze the expression of more than 47,000 transcripts. Gene expression data were subsequently imported into GeneSpring GX7.2 (Agilent Technologies, Santa Clara, CA, USA) to be statistically analyzed.

Results
Gene expression data of 34 ST7-SSX positive SS samples were obtained using an oligonucleotide microarray containing 54,675 probe sets. To overview the gene expression of all samples, we performed principal component analysis. In this analysis, 34 SS samples were roughly classified into three subgroups based on the X and Y axis, which were highly correlated with the three histological subtypes of SS. To identify genes whose expression differed in a statistically significant manner among three histological subtypes, we performed an analysis of variance (ANOVA) among three histological subtypes and selected 403 probe sets with P-values of less than 0.001.

We performed two-dimensional hierarchical clustering analysis using 254 probe sets whose expression differed in a statistically significant manner among 3 genetic subgroups (ANOVA, P<0.0001). To identify differentially expressed genes in each subgroup, we performed Student’s t-test between one and other two subgroups and classified them “biological process”, “cellular component”, and “molecular function” based on Gene Ontology Annotation. In BSS, which is histologically characterized by the epithelial glandular differentiation, the genes associated with epithelial tissue, such as keratins, were up-regulated, whereas COL2, NCAM1, BCL2 and so on were down-regulated. Interestingly, the genes associated with neuronal and skeletal development and cell-cell adhesion were down-regulated in PDSS, and remarkably outnumbered those up-regulated in PDSS. We did not find any specific function of the up-regulated genes in PDSS based on Gene Ontology Annotation; however, we found that 4 genes, especially selected with high statistical values, shared the same genomic locus.

To analyze the expression pattern of four genes on the locus up-regulated in PDSS, we displayed the expression values of these genes in each sample and showed that the four genes on the locus were consistently up-regulated among PDSS samples, while other genes statistically up-regulated in PDSS, such as MYC or ADAMTS8, were not. Next, we analyzed genomic locus of the four genes in more detail, and showed that these four genes existed in a continuous manner, suggesting that some specific changes are occurring at this locus.

Discussion
In an unsupervised analysis with unsel ected probe sets, 34 SS samples clearly fell into three subgroups based on gene expression, which were highly correlated with 3 histological subtypes (MSS, BSS, and PDSS). Based on our results, PDSS was shown to be a single entity which shares a distinct gene expression profile. Although immunohistological diagnosis has not been established in this study due to the lack of appropriate antibodies, we believe that, from now on, PDSS should be included as one of the clearly-established subtypes of SS in the clinicopathological studies. On the other hand, these results strongly suggested the availability of gene expression analysis using microarray technology not only in histological diagnosis but also in subtype diagnosis in each tumor. PDSS showed a distinct gene expression profile. Of note, the down-regulated genes included those associated with neuronal and skeletal development and cell-cell adhesion and strongly outnumbered the up-regulated genes. In this gene expression profile that the characteristic morphology and biology of PDSS must be based on. In addition, down-regulation of those associated with cell-cell adhesion probably affected high incidence of metastatic disease and poor prognosis. Moreover, we found that the up-regulated genes in PDSS were located in a single genomic locus. We now hypothesize that the locus specific up-regulation is associated with PDSS oncogenesis and possibly plays an important role in the suppression of the genes associated with neuronal and skeletal development and cell-cell adhesion.

Reference