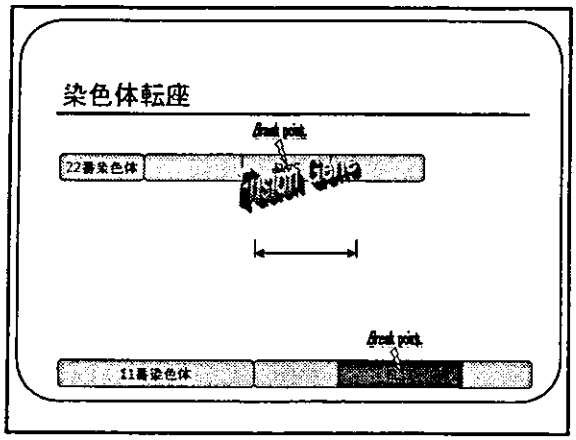
染色体転座
Break point
22番染色体
Fusion Gene
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研究発表

学会誌

Morimoto Y, Ouchida M, Ozaki T, Kawai A, Ito T, Yoshida A, Inoue H, Shimizu K.
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# Splicing isoform of SYT–SSX fusion protein accelerates transcriptional activity and cell proliferation

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**Abstract**

The human *SYT–SSX* gene has two splicing isoforms (type N and I), the latter of which contains an additional insertion of 93 bases. In the present study, we found increased transcriptional activity of the SYT–SSX type I protein in luciferase assay. When the *SYT–SSX* cDNAs were transfected to NIH3T3 cells, the type I transformant grew faster than the type N transformant. Furthermore, we evaluated the isoform ratio of the *SYT* or *SYT–SSX* transcripts in various tissues. Our results suggest that the SYT–SSX type I protein plays a critical role in the tumorigenesis of synovial sarcomas through increased transcriptional activity.


*Keywords:* SYT; SSX; Synovial sarcoma; Alternative splicing; Transcriptional activity; Cell growth

## 1. Introduction

Synovial sarcomas are soft tissue tumors that predominantly affect children and young adults. By the analysis of chromosomal translocation t (X;18) (p11.2;q11.2) cytogenetically characterized in synovial sarcomas, the *SYT* and *SSX* genes were identified [1]. Molecular analysis of the breakpoint has shown that the human *SYT* gene on chromosome 18q11.2 is translocated to one of the *SSX* family genes, *SSX1*, *SSX2* and *SSX4*, on chromosome Xp11.2, leading to the *SYT–SSX* fusion gene [1–3].

The *SYT* gene is ubiquitously expressed in most human tissues [4]. The SYT protein has no DNA binding domains and acts as a transcriptional coactivator [5]. The carboxyl-terminal region of SYT is rich in glutamines, prolines, glycines and tyrosines (the so-called QPGY domain) and is important for transcriptional activity [6]. Comparison database study has shown the presence of putative SH2 and SH3 binding domains that may be involved in protein–protein interaction [1]. It has been reported

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that SYT is associated with a chromatin remodeling factor hBRM/hSNF2 alpha protein [6,7]. SYT was associated with a histone acetyl transferase/transcriptional coactivator p300 protein in G1-arrested cells, and this interaction resulted in the activation of β1-integrin that regulated cell adhesion. The binding of SYT with p300 is retained in the SYT–SSX fusion protein, but the adhesion function is lost [8]. It was also shown that the SYT protein interacts with a putative transcriptional factor AF10 [9].

SSX transcripts are abundant in human adult testes, expressed at low levels in the thyroid, and not detectable in other normal tissues [10,11]. The SSX protein has no defined DNA-binding sequence and acts as a transcriptional corepressor [12,13]. The SYT–SSX fusion has been detected in nearly 97% cases of synovial sarcomas [14]. In most of the SYT–SSX fusion proteins, the C-terminal eight amino acids of SYT are replaced by C-terminal 78 amino acids of SSX [1,10,15]. As a consequence, a part of the QPGY domain of SYT is lost.

Recent studies have shown that the *SYT* gene has two splicing isoforms: the conventional *SYT* transcript (N-SYT) and a new variant *SYT* transcript (I-SYT), which contains an insertion of 93 bases between exon 8 and 9 of the conventional *SYT* transcript, and that the *SYT–SSX* fusion gene consequently has a conventional form (N-SYT–SSX) and a new variant (I-SYT–SSX) [16,17]. These reports indicate that both isoforms of the *SYT* gene are co-existent in human cells and that the expression ratio of isoform I/isoform N transcript (I/N ratio) of the *SYT–SSX* gene is higher in synovial sarcoma cells than the ratio of the *SYT* gene in normal tissues [16].

In this paper, to evaluate the function of the 93-base insertion, we examined the effect of I-SYT–SSX on transcriptional regulation and cell growth, and investigated the I/N ratio in synovial sarcomas, bone and soft tissue tumors, and normal tissues.

## 2. Materials and methods

### 2.1. Tissues

Tumor samples were obtained from the Department of Orthopaedic Surgery, Okayama University Hospital, between 1993 and 2002, after the acquisition of written informed consent from all patients concerned. All synovial sarcomas were diagnosed pathologically, and molecular-genetically, depending on the detection of the *SYT–SSX* fusion transcript by reverse transcription-polymerase chain reaction (RT-PCR) analysis described in our previous report [18]. All cases of synovial sarcomas were grade IIb. Histological diagnosis of other bone and soft tissue tumors were as follows: 13 malignant fibrous histiocytomas (MFH), 12 osteosarcomas, 10 liposarcomas, 10 Ewing's sarcomas, nine chondrosarcomas, six malignant peripheral nerve sheath tumors (MPNSTs), three leiomyosarcomas, and two rhabdomyosarcomas. Normal tissues were obtained from specimens which were resected on wide margin or amputation. The normal tissues comprised 12 normal muscle tissue samples (seven samples were obtained from patients with synovial sarcomas and five samples from patients with other sarcomas), four synovial membranes, three skin samples, three adipose tissue samples, and one nerve tissue sample.

### 2.2. RNA extraction and cDNA synthesis

Total RNA was extracted from frozen tumor tissues using ISOGEN reagent (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. One microgram RNA from each sample was reverse-transcribed using oligo(dT) primer with the reverse transcriptase ReverTra Ace (Toyobo, Osaka, Japan). In addition, a custom human total RNA master panel that included total RNAs from 20 normal tissues (BD Biosciences Clontech, Palo Alto, CA, USA) was reverse-transcribed as described above.

### 2.3. Construction of expression plasmids

To amplify the coding region of the N-SYT–SSX and I-SYT–SSX cDNA, PCR was performed with SYT-S1 (5′-TGG ATG GGC GGC AAC ATG TCT-3′) and SSX-AS1 primers (5′-GGA GTT ACT CGT CAT CTT CCT C-3′) using synovial sarcoma cDNA and LA-Taq DNA polymerase (Takara, Tokyo, Japan). Primers SYT-S1 and SYT-AS1 (5′-TTC ACT GCT GGT AAT TTC CAT AC-3′) were used to amplify the coding region of the N-SYT and I-SYT cDNA. Primers SSX-S1 (5′-GGT GCC ATG AAC

GGA GAC GA-3′) and SSX-AS1 were used to amplify the full length of the *SSX1* cDNA. The PCR process consisted of a denaturing step of 94 °C for 5 min, 35 cycles of a denaturing step at 94 °C for 30 s, an annealing step at 60 °C for 1 min and an extension step at 72 °C for 1 min, and a final extension step of 72 °C for 10 min. After the PCR products were cloned into pBluescript plasmid, the cDNA regions were confirmed by sequencing with a BigDye terminator cycle sequencing reaction kit and an ABI3100 sequencer (Applied Biosystems, Foster City, CA, USA). The N-SYT-SSX, I-SYT-SSX, N-SYT, I-SYT and *SSX1* cDNAs were ligated to the DNA binding domain of the *GAL4* gene on pCMV-BD plasmid in frame, to construct the GAL4 fusion proteins for luciferase assay. The N-SYT–SSX and I-SYT–SSX cDNAs were also subcloned into pCMV-Tag2b expression plasmid with FLAG tag, to construct pCMV-N-SYT–SSX and pCMV-I-SYT–SSX.

### 2.4. Luciferase assay

Each (0.4 μg) of the pCMV-BD-derived plasmids was co-transfected to human embryonic kidney 293 cells with 0.4 μg of luciferase reporter plasmid (pFR-luciferase) containing GAL4-binding DNA sequences and assayed by a dual-luciferase system on a Luminoskan (Thermo Labsystems, Vantaa, Finland) at 72 h after transfection. To complement the transfection efficiency, Renilla luciferase pRL-tk plasmid (0.2 μg) was also co-transfected as described in a previous report [19]. These assays were repeated at least twice in duplicate.

### 2.5. NIH3T3 cells expressing N-SYT–SSX and I-SYT–SSX

The expression plasmids, pCMV-N-SYT–SSX, pCMV-I-SYT–SSX and pCMV-Tag2b empty vector, were transfected into mouse normal fibroblast NIH3T3 cells by Effectene reagent (Qiagen, Hilden, Germany). After selection with 400 μg/ml of G418 (Sigma, St. Louis, MO, USA), the single colonies were purified and examined for the expression of N-SYT–SSX and I-SYT–SSX by RT-PCR analysis with SYT-S3 primer (5′-GAC CAA TAC AGT CAT GGT GGA C-3′) and SSX-AS1, and by Western blot analysis with anti-FLAG antibody (Sigma).

### 2.6. Western blot analysis

Western blot analysis was performed as described in a previous paper [20]. Briefly, following 12.5% polyacrylamide gel electrophoresis of cellular protein lysate (50 μg) from each culture, banded proteins were electrophoretically transferred to a polyvinylidene difluoride membrane (Clear blot membrane-p; ATTO Co., Tokyo, Japan) and then reacted with mouse monoclonal antibody against FLAG (M2; Sigma). The immunoreactive bands were incubated with alkaline phosphatase-labeled horse anti-mouse immunoglobulin (Ig) (Vectastain, Vector Laboratories Inc, Burlingame, CA, USA), followed by reactions with the substrate of CDP-Star (Roche Diagnostics, Mannheim, Germany) on a fluorescent image analyzer FLA-2000 (Fujifilm, Tokyo, Japan).

### 2.7. MTT assay

NIH3T3 transformant with pCMV-N-SYT–SSX, pCMV-I-SYT–SSX or pCMV empty vector, was plated as $1 \times 10^4$ cells per well on a 96-well plate and incubated for 48 h, and then the cell growth was measured with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide) assay kit (Chemicon International Inc, Temecula, CA, USA) according to the manufacturer's instructions. Each of the test samples was assayed in five wells ($n = 5$) at 570 nm on a Multiskan JX (Thermo Labsystems, Vantaa, Finland).

### 2.8. Detection of type I isoform transcripts in various tissues

RT-PCR was performed with SYT-S3 and SSX-AS1 primers to identify the I-SYT–SSX and N-SYT–SSX transcripts in synovial sarcomas. We also used the SYT-S3 and SYT-AS1 primers to detect I-SYT and N-SYT transcripts in the other tissues. *SYT–SSX1* and *SYT–SSX2* cDNA were identified as described in our previous report [18]. PCR products were electrophoresed on 2% agarose gel. The predicted sizes of the PCR products for the N-SYT, I-SYT, N-SYT–SSX and I-SYT–SSX cDNA were 345, 438, 558 and 651 base pairs (bp), respectively. By quantitative analysis of each band with Image Quant software (Molecular Dynamics, Sunnyvale, CA, USA),

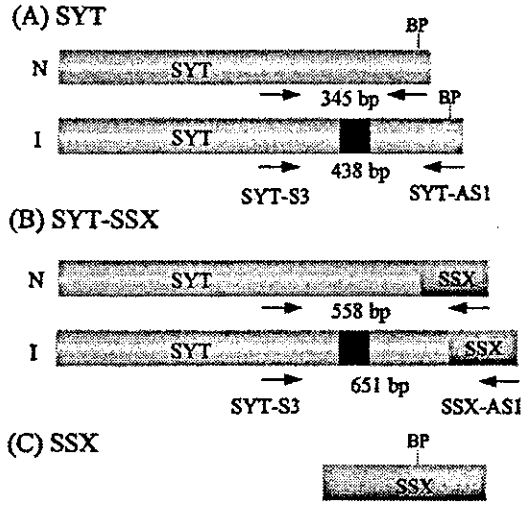


Fig. 1. Representation and analysis scheme of the SYT, SYT–SSX and SSX gene products. Black boxes show the 93-base insertion. To detect insertion on SYT (A) and SYT–SSX cDNA (B), a pair of SYT-S3 and SYT-AS1 primers and a pair of SYT-S3 and SSX-AS1 primers were designed. The predicted sizes of the PCR products are shown between the sense and antisense primers. N, isoform type N; I, isoform type I; BP, breakpoint.

the isoform I/N ratio was defined as I-SYT–SSX/N-SYT–SSX or I-SYT/N-SYT.

### 2.9. Statistical analysis

The isoform I/N ratios in different tissue groups were analyzed using the unpaired Student's t-test. Correlation between continuous variables was calculated by Pearson's correlation coefficient. A $p$-value below 0.05 was considered significant. All statistical analyses were performed using the .Statview 5 statistical package (SAS Institute Inc, Cary, NC, USA).

## 3. Results

### 3.1. Effect of the 93-base insertion on transcriptional regulation

Recent studies reported on novel isoform transcripts of the *SYT* and *SYT–SSX* genes (I-SYT and I-SYT–SSX) which contain an in-frame 93-base insertion in the conventional *SYT* and *SYT–SSX* transcripts (N-SYT and N-SYT–SSX, respectively) (Fig. 1) [16,17]. To evaluate the effect of the 93-base insertion on transcriptional regulation in luciferase assay, we constructed some expression vectors in which N-SYT–SSX, I-SYT–SSX, N-SYT, I-SYT and *SSX1* cDNA were ligated under the DNA binding domain of the *GAL4* gene in frame. When these plasmids were transfected into human embryonic kidney 293 cells with luciferase reporter plasmid bearing GAL4-binding sequences, we found that I-SYT and I-SYT–SSX proteins showed more increased transcriptional activity than N-SYT and N-SYT–SSX proteins did (Fig. 2).

### 3.2. Effect of the SYT–SSX isoform on cell growth

We constructed two cDNA expression plasmids containing N-SYT–SSX and I-SYT–SSX cDNA, to examine the effect of their insertion on cell growth. After the transfection of the two expression vectors and an empty vector into mouse fibroblast NIH3T3 cells, the cells were selected with G418. Single colony cells, which expressed the exogenous N-SYT–SSX or I-SYT–SSX with FLAG tag, were confirmed by detection of 558-bp or 651-bp PCR products on RT-PCR analysis (Figs. 1 and 3A). FLAG-specific bands with about 53 and 55 kDa of molecular weight were detected by Western blot analysis with anti-FLAG antibody (Fig. 3B). They seemed to correspond to the predicted sizes of 50 and 54 kDa for N- and I-SYT–SSX, respectively, and the faint bands in lane 3 may be non-specific signals. When the growth rates

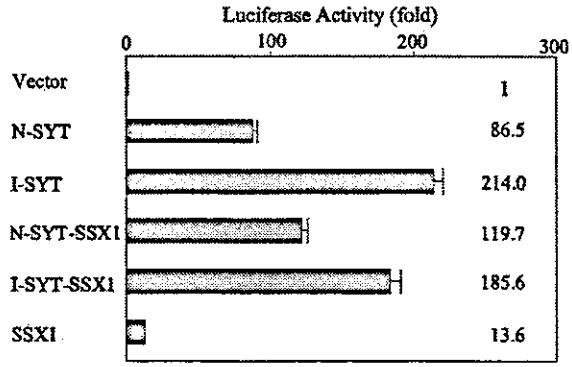


Fig. 2. Transcriptional activity of N and I type isoform proteins.N-SYT, I-SYT, N-SYT–SSX1, I-SYT–SSX1 and SSX1 were fused with the GAL4 DNA binding domain in frame, and the transcriptional activity was examined by luciferase assay using a reporter plasmid with GAL4 binding sequences.

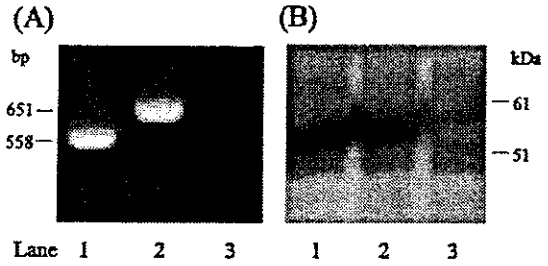


Fig. 3. Detection of exogenous SYT–SSX gene products in NIH3T3 transformants. NIH3T3 cells transformed with N-SYT–SSX cDNA (lane 1), I-SYT–SSX (lane 2) and empty vector (lane 3) were examined by RT-PCR with SYT-S3 and SSX-AS1 primers (A) and by Western blot analysis with anti-FLAG antibody (B). Molecular weight markers are shown on the right side of the Western blot and the predicted molecular weights are 50 and 54 kDa for types N and I, respectively.

of the transformants were examined by MTT assay, we found that the growth of NIH3T3 cells expressing I-SYT–SSX was significantly faster than that of the cells expressing N-SYT–SSX ($p = 0.016$) (Fig. 4), while both SYT-SSX transformants showed an increase in growth rate compared with the transformant carrying the empty vector.

### 3.3. Isoform I/N expression ratio in synovial sarcomas

It has been reported that the isoform I/N ratio of the *SYT–SSX* fusion gene in synovial sarcoma cells is higher than that of the *SYT* gene in normal cells [16]. Therefore, we analyzed the I/N ratio of both the *SYT–SSX* and the *SYT* transcripts in synovial sarcoma cells (Table 1). The I/N ratio of the *SYT–SSX* transcripts ranged from 0.20 to 6.73, with a mean value of 1.82, and that of the *SYT* transcripts ranged from 0.14 to 8.05, with an average value of 1.55 in synovial sarcomas. With regard to the *SYT–SSX* fusion type, no significant correlation was found in the I/N ratio between *SYT–SSX1* and *SYT–SSX2* ($p = 0.84$). When the synovial sarcomas were classified according to their tumor sizes, we observed a tendency that the higher I/N ratio of the *SYT–SSX* transcripts was shown in the group of large tumor size (5 cm or more) than the group of small size (less than 5 cm) ($p = 0.15$). There was no correlation between metastasis and the I/N ratio of the *SYT–SSX* transcripts in synovial sarcomas ($p = 0.67$). When the I/N ratio of the *SYT* and *SYT–SSX* transcripts was compared in the same samples, we found that the I/N ratio was almost the same for the normal and the fused genes (Fig. 5A).

### 3.4. Isoform I/N expression ratio in bone and soft tissue tumors

The I/N ratio of the *SYT* transcripts was detected in the tumor groups which were identified by histological diagnosis (Fig. 6). The highest ratio was detected in synovial sarcomas, with the average value of 1.82,

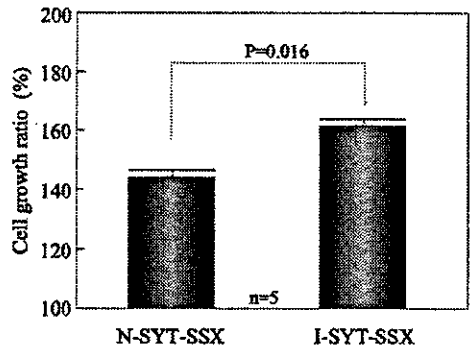


Fig. 4. Cell growth of NIH3T3 cells expressing SYT–SSX isoforms. NIH3T3 cells expressing N-SYT–SSX or I-SYT-SSX were measured by MTT assay at 48 h after plating. Growth of NIH3T3 transformed with the empty vector is shown as 100%.

Table 1
I/N ratio in synovial sarcomas

| | Cases | I/N ratio[a] | $p$ value |
|---|---|---|---|
| SYT | 18 | 1.55 ± 2.14 | 0.69 |
| SYT-SSX(1 + 2) | 18 | 1.82 ± 1.76 | |
| Fusion type | | | |
| SYT-SSX1 | 12 | 1.75 ± 1.82 | 0.84 |
| SYT-SSX2 | 6 | 1.94 ± 1.77 | |
| Tumor size | | | |
| >5 cm | 10 | 2.36 ± 2.18[b] | 0.15 |
| <5 cm | 8 | 1.14 ± 0.66[b] | |
| Metastasis | | | |
| + | 6 | 2.08 ± 1.74[c] | 0.67 |
| − | 12 | 1.69 ± 1.83[b] | |

[a] I/N ratio is expressed as mean ± SD.
[b] I/N ratio of the *SYT–SSX* transcripts in the synovial sarcomas is shown.
[c] I/N ratio of the *SYT–SSX* transcripts in the synovial sarcomas of the patients with metastasis is shown.

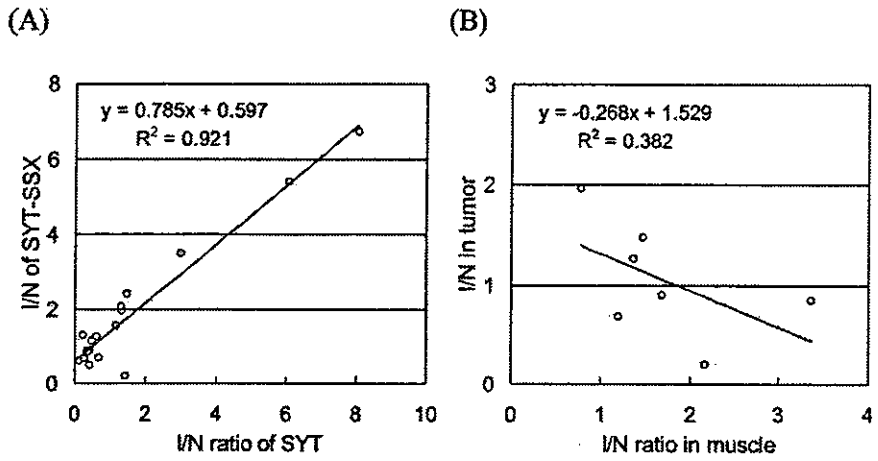


Fig. 5. Analysis of I/N ratio in synovial sarcomas. (A) Correlation of I/N ratio between the *SYT* and *SYT–SSX* transcripts in the same synovial sarcoma samples. (B) Correlation of I/N ratio of the *SYT* gene between synovial sarcoma and muscle tissue in the same patient. $R^2$ refers to the coefficient of determination.

and higher I/N ratios were more common in osteosarcomas (average value: 1.21) and malignant fibrous histiocytomas (MFHs) (average value: 1.01). The I/N ratio of the *SYT* transcripts in the other tumor samples (excluding synovial sarcomas, osteosarcomas and MFHs) ranged 0.11–1.70, with an average value of 0.59.

### *3.5. Isoform I/N expression ratio in normal tissues*

We also investigated the isoform I/N ratio of the *SYT* transcripts in normal samples (Table 2). Muscle tissue samples showed a significantly higher ratio than adipose, skin and synovial tissues did. When we compared the I/N ratio between tumor tissues and normal muscle tissues obtained from seven patients with synovial sarcomas, there was no correlation in the I/N ratio of the *SYT* transcripts between tumor tissues and normal muscle tissues in same patients (Fig. 5B). We also performed an examination of the I/N ratio in the normal tissues of a commercially available human total RNA master panel (Fig. 7). The I/N ratio range varied (0.5–12.8), but tissues containing brain and spinal cord displayed a high I/N ratio of the *SYT* transcripts.

## 4. Discussion

The SYT protein is known to be a transcriptional coactivator, and previous studies have shown that the SYT protein interacts with a chromatin remodeling factor hBRM/hSNF2 alpha, an acetyl transferase/transcriptional coactivator p300, and a putative transcriptional factor AF10 [6–9]. Recently, Tamborini et al. and Brodin et al. have reported that the human *SYT* gene has a splicing variant, I-SYT, which has an insertion of 93 bases between exon 8 and 9 of the conventional *SYT* gene [16,17]. A database search revealed that the mouse *Syt* gene has four isoform transcripts (GenBank accession number: AY055726).

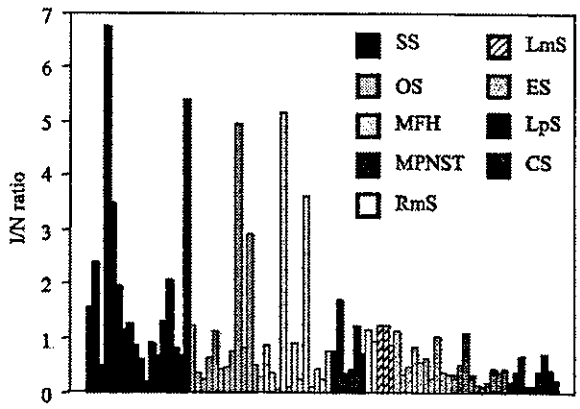


Fig. 6. Analysis of I/N ratio of SYT in a variety of bone and soft tissue sarcomas. SS, synovial sarcoma (18 cases); OS, osteosarcoma (12 cases); MFH, malignant fibrous histiocytoma (13 cases); MPNST, malignant peripheral nerve sheath tumor (6 cases); RmS, rhabdomyosarcoma (2 cases); LmS, leiomyosarcoma (3 cases); ES, Ewing's sarcoma (10 cases); LpS, liposarcoma (10 cases); CS, chondrosarcoma (9 cases).

Table 2
*I/N* ratio of SYT in normal tissues

| | Cases | I/N ratio[a] | *p* value[b] |
|---|---|---|---|
| Muscle | 12 | 2.22 ± 1.36 | - |
| Nerve | 1 | 0.83 | - |
| Adipose tissue | 3 | 0.47 ± 0.67 | 0.049 |
| Skin | 3 | 0.34 ± 0.13 | 0.037 |
| Synovium | 4 | 0.21 ± 0.10 | 0.012 |

[a] I/N ratio is expressed as mean ± SD.
[b] *p* value is against muscle.

Human I-SYT protein has 95.9% homology to mouse Syt alpha protein and human N-SYT protein has 95.6% homology to mouse Syt beta protein. The insertion is located in the QPGY domain of the SYT and the insertion itself is rich in glutamines, prolines, glycines and tyrosines, which are frequently observed in transcriptional activation domains [6]. This finding led us to hypothesize that the I-SYT protein possesses higher transcriptional activity than the N-SYT protein does. Therefore, we analyzed the transcriptional activity of isoforms by luciferase assay, and found that the transcriptional activity of the I-SYT and I-SYT–SSX proteins were higher than those of the N-SYT and N-SYT–SSX proteins. Furthermore, the NIH3T3 transformants expressing the I-SYT–SSX protein showed a higher growth rate than the transformants expressing the N-SYT–SSX. These results suggest that the I-SYT–SSX protein might play a critical role in the tumorigenesis of synovial sarcomas with the *SYT–SSX* gene translocation, at least through transcriptional activity. The insertion region is located between codon 299 and codon 329 on the I-SYT protein. Bearing in mind that p300 binds with the region between codon 1 and codon 250 of the SYT protein [8], and that AF10 binds with the region between codon 1 and codon 90 of SYT [9], the insertion region might interact with other proteins for transcriptional regulation and other regulation. The type I isoform proteins of SYT–SSX and SYT may effect the interaction with the BRM protein because the BRM protein binds with a major binding site between codon 60-158 and a secondary binding site between codon 158-387 of the SYT protein [6].

We were also interested in the formation rate of the isoform I/N of the *SYT–SSX* transcripts in synovial sarcomas. Although Tamborini et al. reported that a high I/N ratio was a unique occurrence in synovial sarcomas, they did not compare the I/N ratio between the *SYT* and *SYT–SSX* transcripts in same synovial sarcomas [16]. In the present study, we found that the I/N ratios of the *SYT* and *SYT–SSX* genes were almost identical in synovial sarcomas, and that the I/N ratio of the *SYT* gene was different in muscles and tumor tissues of the same patients, suggesting that the I/N ratio is dependent on tissue type, but not on individuals. In fact, similarity of the I/N ratio was observed in analogous tissues between leiomyosarcomas and rhabdomyosarcomas and between adipose tissues and liposarcomas. Our results showed that the I/N ratio in synovial sarcomas is different from that in normal synovial tissues, and it is not inconsistent with the previous reports that synovial sarcoma and normal synovial tissue are not derived from the same origin [21,22]. Tamborini et al. also reported that metastasis and recurrent cases of synovial sarcomas had a tendency to show increased expression levels of I-SYT–SSX. However, we could not examine the tendency in our cases, because we could not obtain RNA samples from both the primary sarcoma tissues and the paired metastasis or recurrent tissues in the same patients. Since our result show that NIH3T3 cells expressing the I-SYT–SSX protein grow faster than NIH3T3 cells expressing

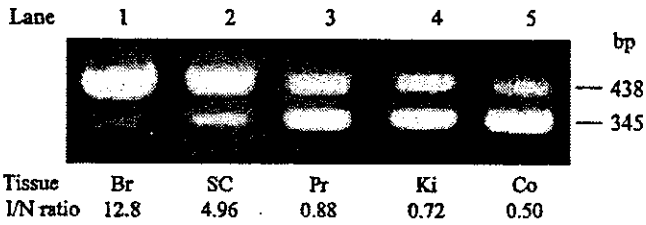


Fig. 7. RT-PCR analysis of SYT isoforms in normal tissues. Amplified products were analyzed by electrophoresis using 2% agarose gel. PCR products for the I and N types of SYT transcripts were 438 and 345 bp, respectively. Br, brain (whole); SC, spinal cord; Pr, prostate; Ki, kidney; Co, colon.

the N-SYT–SSX protein do, and that synovial sarcomas showed the highest I/N ratio among the tumor tissues we examined, synovial sarcomas or their precursor cells which dominantly express the I-SYT–SSX isoform may be selected and proceed to malignancies such as metastasis and recurrent sarcomas. Variation of the I/N ratio observed in osteosarcomas and MFHs may depend on their histological heterogeneity.

Splicing variants, which are variously expressed in normal and tumor tissues, are known to exist in many genes, and several researchers are attempting to discover the meanings and functions of alternative splicing variants in tumors. In researching the *CD44* gene, analysis of the expression of the splicing variant was useful for detecting malignancy as a prognostic indicator [23–26]. In chondrosarcomas, the splicing pattern of the *tenascin-C* gene revealed to have clinical significance [27]. Research into the type I isoform of SYT–SSX proteins which are predominantly expressed in synovial sarcomas may be important to clarify the function of the 93-base insertion region in terms of protein–protein interaction and transcriptional regulation associated with pathogenesis of synovial sarcomas. Further study is necessary to shed light on this issue.

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# Genetic imbalances in benign bone tumors revealed by comparative genomic hybridization*

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There have been no reports on choromosomal aberrations of benign bone tumors revealed by comparative genomic hybridization (CGH). CGH analysis of benign tumors may be useful in understanding the mechanism of tumorigenesis with comparisons to malignant tumors. There were 4 tumors (2 enchondromas, one chondromyxoid fibroma, and one osteoid osteoma) and 8 tumor-like conditions (4 aneurysmal bone cysts (ABCs), one eosinophilic granuloma, one fibrous dysplasia, one solitary bone cyst, and one Rosai-Dorfman disease) available for analysis. One of 2 enchondromas and one of 4 ABCs exhibited rapid growth. Six lesions showed chromosomal aberrations, while 6 others did not. The most frequent aberrations were the loss of a whole chromosome-19 in 6 cases, the loss of chromosome-arm 22q in 4 cases, and the loss of chromosome-arm 17p in 3 cases. Gains were seen in 13q21 in 2 cartilaginous tumors and at 12q15-q21 in eosinophilic granulomas. Therefore, in benign bone tumors or tumor-like lesions, chromosomal aberrations are not frequent; however, some clear tendencies of clustering of aberrations can be observed.

*Key words: genetic imbalance, genomic hybridization, benign tumors, chromosomal aberrations*

Comparative genomic hybridization (CGH) is a relatively new technique for a genome-wide screening of DNA sequence copy number changes. In recent years, there have been several reports of CGH analysis of osteosarcoma [4, 11, 19, 21], Ewing tumor [10, 22], and chondrosarcoma [1, 9]. However, CGH analyses on benign bone tumors have, to the best of our knowledge, not yet been reported. Analyses of chromosomal instabilities in benign bone tumors may be important to understanding the mechanisms of tumorigenesis by comparing genetic aberrations with those of malignant bone tumors. In this study, we analyzed chromosomal instabilities in 12 benign bone tumors.

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## Material and methods

*Patients.* Twelve benign bone tumors or tumor-like lesions from 12 patients were available for this study (Tab. 1). All samples were obtained from frozen tissues, which were taken and preserved at –80 °C. Tumor tissues were taken from typical and viable tumor areas. In osteoid osteoma, the tissue was obtained from the nidus. In solitary bone cysts and aneurysmal bone cysts (ABCs), the tissues obtained by curettage from the cyst wall were used. Eleven tissues were taken from the primary lesions and one (Case 1) was from a local recurrence. Case 1 underwent curettage; however, local relapse developed 6 months after the initial surgery. The diagnosis was histologically confirmed as a benign bone lesion.

Of 12 lesions, 3 were cartilaginous tumors (including of 2 enchondromas and one chondromyxoid fibroma) and one was an osseous tumor (osteoid osteoma). Eight tumor-like lesions comprised 4 ABCs, one solitary bone cyst, one eo-

**Table 1. Patients with benign tumor and results of chromosomal aberrations**

| Case No. | Age (years) | Gender | Diagnosis | Site | Chr1 p | Chr1 q | Chr12 p | Chr12 q | Chr13 p | Chr13 q | Chr16 p | Chr16 q | Chr17 p | Chr17 q | Chr19 p | Chr19 q | Chr20 p | Chr20 q | Chr22 p | Chr22 q |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 36 | Male | Chondromyxoid fibroma | Metatarsal II | L* | | | L* | | G* | L | | L | | L | L | | L | | L |
| 2 | 27 | Male | Enchondroma | Metacarpal V | L* | | | | | G* | L | L | L | L | L | L | L | | | |
| 3 | 43 | Female | Enchondroma | Humerus, proximal | | | | | | | | | | | | L | L | | | L |
| 4 | 33 | Male | Osteoid osteoma | Femur, diaphysis | | | | | | | | | | | | | | | | |
| 5 | 13 | Female | Aneurysmal bone cyst | Femur, proximal | | | | | | | | | L | | L | L | | | | L |
| 6 | 14 | Female | Aneurysmal bone cyst | Humerus, distal | | | | | | | | | | | | | | | | |
| 7 | 11 | Male | Aneurysmal bone cyst | Calcaneus | | | | | | | | | | | | | | | | |
| 8 | 18 | Male | Aneurysmal bone cyst | Humerus, proximal | | | | | | | | | | | | | | | | |
| 9 | 9 | Male | Solitary bone cyst | Humerus, diaphysis | | | | | | | | | | | | | | | | |
| 10 | 14 | Male | Fibrous dysplasia | Femur, proximal | | | | | | | | | | | L | L | | | | |
| 11 | 11 | Male | Eosinophiles granuloma | Femur, diaphysis | | | | G* | | | | | | | L | L | | | | |
| 12 | 47 | Female | Rosai-Dorfmann disease | Radius, diaphysis | | | | | | | | | | | | | | | | |

G – chromosomal gain, L – chromosomal loss; *aberrations of small segmented of chromosome.

sinophilic granuloma, and one fibrous dysplasia, and one sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) [14] (Tab. 1). One of 2 enchondoromas abounds of cartilaginous cells and one of 4 ABCs was characterized by fast progression. No case showed local relapse after intralesional (curettage) procedures, excluding previous treatment in Case 1.

*Labeling procedures, comparative genomic hybridization and detection.* Reference DNA from healthy blood donors and tumor DNA were labeled by the nick translation method with digoxygenin-11-dUTP (Boehringer Mannheim, Germany) and biotin-14-dATP (Boehringer Mannheim, Germany), respectively. The hybridization was performed as described by KALLIONIEMI et al [7] with some modifications [2, 15].

Separate digitized gray level images of DAPI, FITC, and rhodamide fluorescence were taken with a CCD camera connected to a Leica DMRBE microscope. The image processing was carried out using Applied Imaging Software (Cytovision 3.1). Ratio profiles were averaged from 10 metaphases per sample (up to 20 chromosome homologues). Gains of DNA sequences were defined as chromosomal regions with a fluorescence ratio above 1.25 and losses as regions with a ratio below 0.75. A positive control with known aberrations and a negative control were included in each CGH experiment as quality controls. Over-representations were considered to be high-level gains when the fluorescence ratio exceeded 1.5. Heterochromatic regions near the centromeres and the entire X and Y chromosomes were excluded from the analysis. Judgment was based on a consensus of at least two of 3 authors in all cases without reference to the patient's clinical information.

## Results

Genomic imbalances were detected in 6 of 12 lesions (Tab. 1). Losses (19 incidences) were more frequent than gains (3 incidences). There were losses of whole chromosome-19's in 6 cases, a loss of chromosome-arm 22q in 4 cases, a loss of chromosome-arm 17p in 3 cases, a loss of chromosome-arm 16p in 2 cases, a loss of chromosome-arm 1p in 2 cases, and a loss of chromosome-arm 16q, 17q, or 20q in one case each. Loss of 1p was limited to the short region of 1p36 and loss of 12 was limited to the region of 12q24. Gains occurred at chromosome 13q21 in 2 cases and at 12q15-q21 in one case.

There were 16 aberrations in 3 cartilaginous tumors, none in one osseous tumor, and 6 in 8 tumor-like conditions. Of the cartilaginous tumors, all showed losses of chromosome 19 and 22q. Case 3 was characterized by a low cellularity and had a less aberrations than either Case 1 or 2; Case 1 was a relapsed tumor and Case 2 was an enchondroma with many cartilaginous cells. One of 4 ABCs had aberrations; this tumor had exhibited rapid growth.

## Discussion

To our knowledge, there have been no reports of CGH in these benign bone tumors or tumor-like conditions. An average of 5.3 and 0.75 aberrations were detected in cartilaginous tumors and tumor-like conditions, respectively, with genetic losses being more frequent than gains. Malignant bone tumors have a lot of aberrations with gain dominant. Osteosarcomas have been reported to have an average of 9.6 aberrations [21] or a median of 16.1 aberrations [11]. Ewing tumors [10] and chondrosarcomas [9] exhibit averages of 3.6 and 4.8 aberrations per tumor, respectively.

Solitary enchondroma is a benign cartilaginous tumor mainly arising in the small bones of the fingers with infrequent recurrence. Chondromyxoid fibroma is a benign tumor of bone characterized by a chondroid and myxoid differentiation in its basic tissue, and the chondroid compo-

nent (including pleomorphic tumor cells) can be mistaken for a chondrosarcoma [6]. These lesions have been reported to have a 25% local-recurrence rate [23]. GUNAWAN et al [5] reported a solitary enchondroma with t(8;17)(q23;p13) and loss of chromosomes 9, 19, and 22 as clonal changes. TARKKANEN et al [20] reported a chondromyxoid fibroma with clonal aberrations in chromosomes 2 and 5. In the moderately cellular cartilaginous component of dedifferentiated chondrosarcoma, CGH revealed deletions of chromosomes 4, 5, 13, 22, distal part of 16p, or 17p [1]. Osteoid osteoma is a benign osteoblastic lesion with a limited growth potential; the current case had no detectable chromosomal aberration. DAL CIN et al [3] reported that one of 6 cases of osteoid osteoma showed clonal structural change including chromosomes 1, 6, 14, and 17.

ABCs are benign, but often rapidly expanding, osteolytic lesions [16]. In this study, losses of 17p, 19, or 22q were detected by CGH. In a previous study, a normal karyotype was observed for 8 patients with ABCs [13]. PANOUTSAKOPOULOS et al [12] reported that 2 of 3 ABCs had a t(16;17)(q22;p13) rearrangement and one ABC had a del(16)(q22). SCIOT et al [16] reported that all ABCs showed a non-random involvement of chromosome segments 17p11-13 and/or 16q22.

A loss of whole chromosome-19 was the most frequent abnormality identified in this study. Our observations may indicate the existence of an important gene located on chromosome 19. The information on aberrations of chromosome 19q in human tumors is limited; however, a loss of 19q is commonly observed in gliomas [8, 17, 18].

In summary, a loss of chromosomes was more frequent than a gain of chromosomes in benign cartilaginous tumors. The significance of each individual chromosomal aberration, especially of chromosome-19 loss, will be understood in greater detail with further trials which include larger numbers of benign tumors.

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# Comparative Genomic Hybridization in Cartilaginous Tumors

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**Abstract.** *Genetic aberrations in cartilaginous tumors have not yet been well characterized. We analyzed the molecular-chromosomal aberrations in 10 chondrosarcomas (four Grade-3 tumors, four Grade-2 tumors and two Grade-1 tumors) and in three benign cartilaginous tumors (two enchondromas and one chondromyxoid fibroma). Genomic imbalances were detected in 9 out of 10 cases of chondrosarcomas. The median number of changes was 7.0 per tumor (range 0-23) and the gain-to-loss ratio was 1:1.4. The most frequent gains involved 7q, 5p, or 21q and the most frequent losses were 17p, 13q, 16p, or 22q. The three benign cartilaginous tumors each had two (0 gains and two losses), six (one gain and five losses) and eight (one gain and seven losses) chromosomal aberrations. Both of the gains occurred on 13q21 and losses were frequently observed on chromosomes 19 and 22q in all three cases. Loss of chromosomes 16p, 17p, 22q, or 19 loss were common in both chondrosarcomas and benign cartilaginous tumors. However, aberrations from chromosomes 2 to 11, 14, 15, 18, or 21 were detected only in chondrosarcomas. Therefore, although the number of aberrations between benign and malignant cartilaginous tumors appears to be similar, these two entities may be differentiated by determining which chromosomes are affected.*

Chondrosarcoma is the second most frequent malignant bone tumor after osteosarcoma (1). Chondrosarcomas show a variety of clinical features and biological behaviors. In addition to the common or conventional chondrosarcomas, several variants have been described (2). The basic neoplastic tissue of chondrosarcoma is cartilage without osteoid, formed directly by the sarcomatous stroma (3, 4). It is currently a problem to differentiate between enchondroma, a benign cartilaginous tumor and low-grade intramedullary chondrosarcoma (5). Moreover, chondrosarcomas sometimes metastasize (6) or exhibit tumor up-grading after local recurrence (7). Chondrosarcoma is one of the most difficult tumors to treat.

Comparative genomic hybridization (CGH) is a useful technique for a genome-wide screening of DNA sequence copy number changes. Up to now, there have only been two series of CGH analysis on chondrosarcoma (8, 9). Larramendy *et al.* reported CGH results on 50 chondrosarcomas in 45 patients with a gain of 8q24.1-qter as a possible prognostic factor (8). Bovee *et al.* (9) have reported a CGH analysis on the anaplastic and cartilaginous components of a de-differentaited chondrosarcoma The information obtained by CGH for chondrosarcomas is limited and CGH results of chondrosarcomas have not, to our knowledge, been compared with those of benign cartilaginous tumors. In this study, we analyzed the molecular-chromosomal aberrations in 10 chondrosarcomas and three benign cartilaginous tumors by CGH.

## Materials and Methods

*Patients.* Ten chondrosarcoma tissues, two enchondroma tissues and one chondromyxoid fibroma tissue from 13 patients who were treated between 1996 and 1998 were available for this study (Table I). All samples were obtained from frozen tissues, which were taken before preoperative treatment and preserved at -80°C. Tumor tissues were taken from typical and viable tumor areas. The diagnosis was histologically confirmed as cartilaginous tumors. One chondromyxoid fibroma (Case 11) was a locally-relapsed tumor, one chondrosarcoma (Case 2) was a secondary chondrosarcoma following Ollier's disease with multiple enchondroma and the others were primary tumors.

Among the 10 patients with chondrosarcoma, the male-to-female ratio was 6:4 and the patients' ages ranged between 20 and 63 years (median age = 49 years). Four tumors were proximally located (three pelvis and one scapula) and six were distally located (two femur, three tibia and one humerus). Tumor grade was classified according to the method by Evans *et al.* (10). In the benign tumor group, the ages of the three patients were 27, 36 and

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Table I. *Patient's information.*

| No. | Age (years) | Gender | Diagnosis | Subtype | Grade (Evans *et al.* (10)) | Site | Primary metastasis | Surgery | Surgical margin | Follow-up (months) | Prognosis |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 47 | M | Chondrosarcoma | Myxoid | 3 | Ilium | None | Resection | Wide | 51 | NED |
| 2 | 33 | M | Chondrosarcoma | Secondary | 3 | Tibia | None | Amputation | Radical | 24 | DOD |
| 3 | 56 | M | Chondrosarcoma | | 3 | Ischium | Multiple | Resection | Marginal | 8 | DOD |
| 4 | 20 | F | Chondrosarcoma | Mesenchymal | 3 | Tibia | Spleen | Amputation | Wide | 12 | DOD |
| 5 | 59 | F | Chondrosarcoma | | 2 | Femur | None | Resection | Wide | 28 | CDF |
| 6 | 30 | M | Chondrosarcoma | Clear cell | 2 | Humerus | None | Resection | Wide | 37 | CDF |
| 7 | 51 | M | Chondrosarcoma | | 2 | Scapula | None | Resection | Wide | 56 | CDF |
| 8 | 63 | F | Chondrosarcoma | | 2 | Tibia | None | Resection | Wide | 24 | CDF |
| 9 | 37 | F | Chondrosarcoma | | 1 | Ilium | None | Resection | Wide | 28 | CDF |
| 10 | 56 | M | Chondrosarcoma | | 1 | Femur | None | Resection | Wide | 36 | CDF |
| 11 | 36 | M | Chondromyxoid fibroma | | | Metatarsal II | None | Curettage | Intralesional | 51 | NED |
| 12 | 27 | M | Enchondroma | | | Metacarpal V | None | Curettage | Intralesional | 54 | CDF |
| 13 | 43 | F | Enchondroma | | | Humerus | None | Curettage | Intralesional | 47 | CDF |

CDF: Continuous disease-free, NED: No evidence of disease, DOD: Dead of disease

43 years. One tumor was located in the humerus, one was in the metatarsal II and one was in the metacarpal V.

Surgical margins of chondrosarcomas were classified according to the method described by Enneking *et al.* (11): radical in one patient, wide in eight patients and marginal in one patient. All patients with a benign tumor underwent curettage of the lesion (intralesional). In one patient with mesenchymal chondrosarcoma, chemotherapy was performed according to the protocol of Cooperative Ewing's Sarcoma Study 92 (12). The follow-up period ranged between 24 and 56 months (median 42 months).

*Labeling procedures, comparative genomic hybridization and detection.* Reference DNA from healthy blood donors and tumor DNA were labeled by the nick translation method with digoxygenin-11-dUTP (Boehringer Mannheim, Mannheim, Germany) and biotin-14-dATP (Boehringer Mannheim), respectively. The hybridization was performed as described by Kallioniemi *et al.* (13) with some modifications (14, 15). Separate digitized gray level images of DAPI, FITC and rhodamine fluorescence were taken with a CCD camera connected to a Leica DMRBE microscope. The image processing was carried out using Applied Imaging Software (Cytovision 3.1). Ratio profiles were averaged from 10 metaphases per sample (up to 20 chromosome homologues). Gains of DNA sequences were defined as chromosomal regions with a fluorescence ratio above 1.25 and losses as regions with a ratio below 0.75. A positive control with known aberrations and a negative control were included in each CGH experiment as quality controls. Over-representations were considered to be high-level gains when the fluoresence ratio exceeded 1.5. Heterochromatic regions near the centromeres and the entire X and Y chromosomes were excluded from the analysis. Judgement was based on a consensus of at least 2 out of 3 authors in all cases without reference to the patient's clinical information. The Mann-Whitney *U*-test evaluated differences of the mean rank between 2 groups.

## Results

Genomic imbalances were detected in 9 out of 10 chondrosarcomas (Table II). The median number of changes was 7.0 per tumor (range 0 - 23), while the average number of aberrations was 8.5 per tumor. There were 36 gains (median 2, range 0-10) including six high-level gains and 49 losses (median 4, range 0-14). The most frequent gains involved 7q (five cases), 5p (four cases) and 21q (four cases) (Figure 1). The most frequent losses were 17p (six cases), 13q (five cases), 16p (five cases) and 22q (four cases). Six high-level gains were observed in two Grade-3 chondrosarcomas.

Most of the aberrations in the benign tumors were losses. The three benign cartilaginous tumors each had 2, 6 and 8 aberrations (average = 5.3 per tumor) consisting of 0 gains and two losses, one gain and five losses and one gain and seven losses, respectively. Among these cases, a gain was observed only in 13q21 and losses were observed on chromosomes 19 (three cases), 22q (three cases), 17p (two cases), 16p (two cases), or 1p (two cases).

A loss of chromosomes 16p, 17p, 22q, or 19 was common in both malignant and benign groups. However, aberrations on chromosomes 2 to 11, 14, 15, or 21 were detected only in chondrosarcomas and not among benign cartilaginous tumors. We did not detect a difference in the median numbers of aberrations either between benign and malignant tumors or between Grade-3 and Grade-1 or -2 tumors (Mann-Whitney *U*-test).

## Discussion

Benign cartilaginous tumors exhibited an average of 5.3 aberrations per tumor with a 1:7 gain-to-loss ratio. Chondrosarcomas showed an average of 8.5 aberrations including 3.6 gains and 4.9 losses. The current study revealed that losses may be more frequent in chondrosarcomas than previously described; Larramendy *et al.* (8) reported an average of 4.8 aberrations in primary tumors and 3.4:1 gain-to-loss ratio. There does not appear to be a difference in the number of aberrations detected by CGH between benign and malignant cartilaginous tumors. An analysis of which chromosomes are affected may prove more fruitful in determining the tumor entity, the tumor stage, or the patient's prognosis.

Larramendy *et al.* (8) reported that frequent gains included 20q, 8q, 20p, or 14q and frequent losses included Xq, 6q, or 18q. Our study yielded somewhat different findings: we observed frequent gains of 7q, 5p, or 21q and frequent losses involving 17p, 13q, 16p, or 22q. These differences may be due to the small number of cases in the current study; nevertheless, a comparison of the CGH ideograms clearly reveals that gains of chromosomes 6p, 19, 20, or 22 are much less frequent and losses of 13p, 16p, 17p, or 19 are much more frequent in the current study than in the previous report. Further trials to clarify these disagreements are warranted.

There were frequent losses of chromosomes in benign cartilaginous tumors. Losses of whole chromosome 19 and of 22q were the most frequent. Similarly, a chromosome 22q loss was reported by Bovee *et al.* in the cartilaginous component of a de-differentiated chondrosarcoma (9) and, as mentioned above, we observed frequent losses of 22q in our chondrosarcomas. One candidate gene mapped to 22q is the breakpoint cluster region gene (BCR) which is located on 22q11.23 (16). Although we did not detect frequent chromosome 19 loss in our chondrosarcomas, loss of 19q has been commonly observed in gliomas (17-19).

A loss of 13q13-q14 was observed in five (50%) chondrosarcomas. These losses probably include the retinoblastoma 1 (*RB1*) locus on 13q14. Allele loss at polymorphic loci on 13q has been reported in 36% of chondrosarcomas (20); this incidence was higher in high-grade chondrosarcomas than in low-grade tumors. There may be more cases with 13q14 aberrations which can not be detected by CGH.

Six chondrosarcomas showed a loss of 17p and this may affect the tumor-suppressor gene *TP53* on 17p13.1. Bovee *et al.* (9) reported the loss of 17p in the cartilaginous component of a de-differentiated chondrosarcoma. Furthermore, loss of heterozygosity of 17p has been reported in 25% of chondrosarcomas (20).

A chromosome 5p gain was observed in 4 chondrosarcomas and is also common in lung cancers (21),

Table II. *CGH results.*

| No. | Diagnosis | Grade | Total Number of Aberrations | Gain | Loss | 1 p | 1 q | 2 p | 2 q | 3 p | 3 q | 4 p | 4 q | 5 p | 5 q | 6 p | 6 q | 7 p | 7 q | 8 p | 8 q | 9 p | 9 q | 10 p | 10 q | 11 p | 11 q | 12 p | 12 q | 13 p | 13 q | 14 p | 14 q | 15 p | 15 q | 16 p | 16 q | 17 p | 17 q | 18 p | 18 q | 19 p | 19 q | 20 p | 20 q | 21 p | 21 q | 22 p | 22 q |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Chondrosarcoma | 3 | 7 | 3 | 4 | L | | | | | | | | | | | | G | G | G | | | | | | | | | | | | | | | | | | | | | | | | | | G | L | | |
| 2 | Chondrosarcoma | 3 | 3 | 0 | 3 | | | | | | | | | | | | | | | L | | | | L | L | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 | Chondrosarcoma | 3 | 23 | 9 | 14 | G | G | G | G | L | G,H | G,H | H | L | G | | G | L | G | L | G | L | | | | L | H | L | L | | L | | L | | L,H | L | L | | L | | L | L | | | | | | |
| 4 | Chondrosarcoma | 3 | 14 | 10 | 4 | | G | | L | | | | | G | G | | G | G | | | | G | L | | | G,H | G,H | | G,L | | G | | G | | G | G | G | L | | G | G | | | | | | | |
| 5 | Chondrosarcoma | 2 | 6 | 1 | 5 | | | | | | | | | | | | | | | G | | | | | | | | L | | L | | L | | L | | L | | L | | L | L | | | | | | | |
| 6 | Chondrosarcoma | 2 | 11 | 5 | 6 | L | | | | | | | | G | G | | G | G | | | | L | | | | | | L | | L | G | | L | | L | L | L | G | G | | | | | | | | | |
| 7 | Chondrosarcoma | 2 | 5 | 1 | 4 | | | | | | | | | | | L | | | | | | L | | | | | | | | L | | | | G | G | | | | | | | | | | | | | |
| 8 | Chondrosarcoma | 2 | 9 | 6 | 3 | | | | | | | | | G | G | | G | G | | | | | | | | | | G | G | | | | | | L | | L | | G | L | | | | | | | | |
| 9 | Chondrosarcoma | 1 | 7 | 1 | 6 | G,L | | | | | | L | L | | | L | L | | | | | | | L | L | L | | | | L | | | | | | | | | | | | G | L | | | | | |
| 10 | Chondrosarcoma | 1 | 0 | 0 | 0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 11 | Chondromyxoid fibroma | | 8 | 1 | 7 | L | | | | | | | | | | | | | | | | | | | | | | | | L | G | | | | | L | | L | | | L | L | L | | | | L | |
| 12 | Enchondroma | | 6 | 1 | 5 | L | | | | | | | | | | | | | | | | | | | | | | | | | | | | G | | | L | L | L | | | | L | L | | | L | |
| 13 | Enchondroma | | 2 | 0 | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | L | |

G: gain, L: loss, H: high-level gain

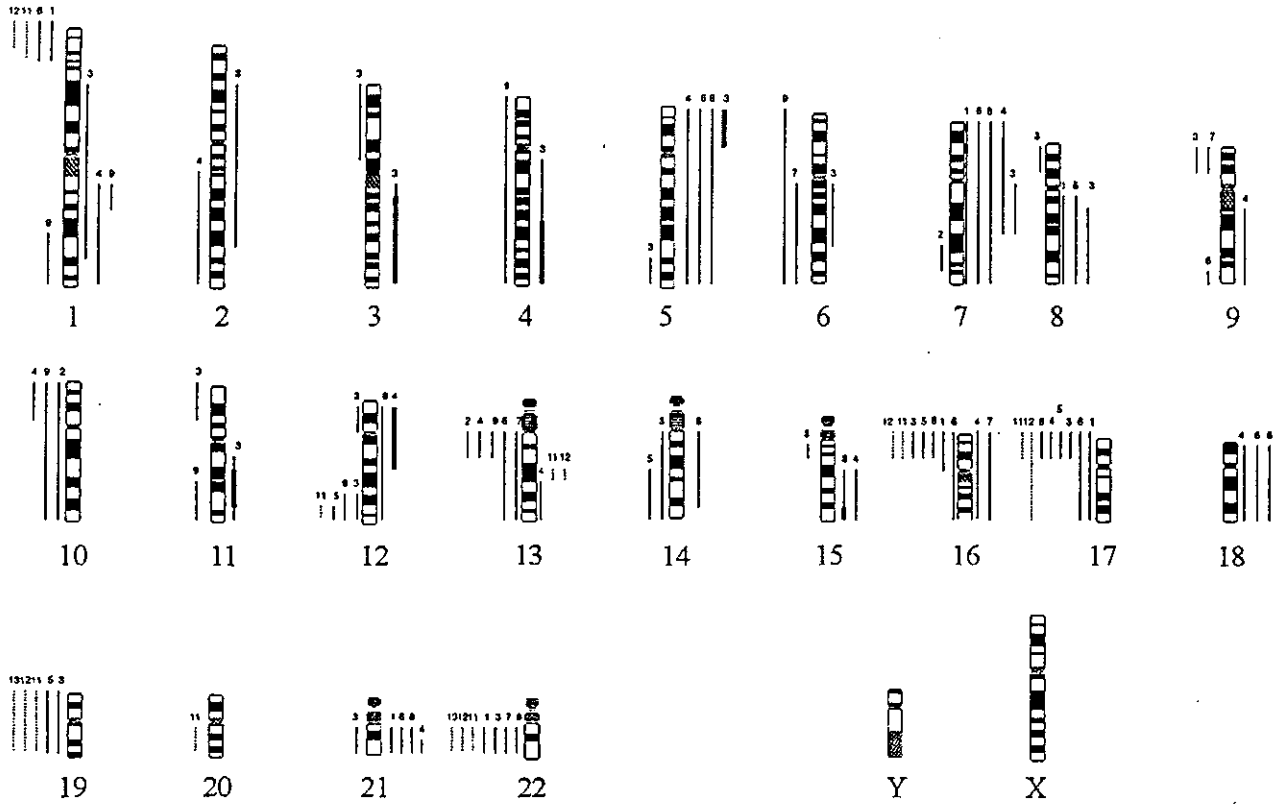


Figure 1. *Chromosomal aberrations of cartilaginous tumors by comparative genomic hybridization. The lines on the left side of chromosome mean loss and the lines on the right side of chromosome mean gain. The broad lines mean high-level gain. Dotted lines mean benign tumors. The number above each lines mean the case number (Table I).*

osteosarcoma (22) and malignant-fibrous histiocytoma (23). One candidate gene mapped to 5p13 is S-phase kinase-associated protein 2 (*SKP2*) which encodes an essential element of the cyclin A/cyclin-dependent kinase 2 S-phase kinase (24). Gains of 7q were also detected in 5 chondrosarcomas. This region includes the genes encoding hepatocyte growth factor (HGF) and its receptor, MET, which are located on 7q21.1 and 7q31, respectively. A chondrosarcoma cell line (SW 1353) with a fibroblast-like phenotype is known to secrete hepatic growth factor and to express a large amount of p140c-met, the receptor tyrosine kinase for HGF/SF (25). c-MET (7q31) expression was observed in 54% of chondrosarcomas (26). The occurrence of chondrosarcoma may be associated with these factors.

Loss of 16p was also observed by Bovee *et al.* (9) in the cartilagenous component of de-differentiated chondrosarcoma. However, Larramendy *et al.* (8) did not report about 16p loss in 50 chondrosarcomas. Retinoblastoma binding protein 6 (RBBP6) is located on 16p11.2-p12 (9). Loss of function of this protein may be concerned with the ocurrence of chondrosarcomas.

Genomic imbalances were detected in 9 out of 10 chondrosarcomas. Loss was more frequent than gain. The most frequent gains involved 7q, 5p and 21q and the most frequent losses were 17p, 13q, 16p and 22q. The average number of aberrations in chondrosarcoma (8.5) were not significantly different from those of benign cartilaginous tumors (5.3). On the other hand, most of the aberrations in benign tumors included losses: frequently in 19 and 22p. Although losses of 16p, 17p, 22q and 19 were common in both groups, chromosomes 2 to 11, 14, 15, 18 and 21 aberrations were detected only in chondrosarcomas. The differential diagnosis between benign and malignant cartilaginous tumor seems to be difficult by the number of aberrations in CGH; however, it may be possible by the type of aberrations.

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