Bull Yamaguchi Med Sch 50(1-4):57-64, 2003

Difference in DNA copy number aberrations detected between Osteosarcoma and Malignant Fibrous Histiocytoma

Ritsuko Ohi

Department of Orthopedic Surgery, Yamaguchi University School of Medicine, Ube, Japan
Department of Pathology, Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan

Key words: comparative genomic hybridization, DNA copy number aberrations, osteosarcoma, malignant fibrous histiocytoma, differential diagnosis

Abstract Osteosarcoma (OS) and malignant fibrous histiocytoma (MFH) are the most frequent sarcomas of the bones and soft tissues, respectively. These tumors share a spectrum of histopathologic features. In order to elucidate the similarity and difference of tumor-specific genetic alterations between them, we analyzed DNA copy number aberrations of 7 OSs and 12 MFHs using comparative genomic hybridization (CGH). In OS, a DNA copy number gain of chromosome 1p21-pter was the most frequent aberration (85.7%; 6/7 tumors). Furthermore, gains of 6p12-p24, 8q13-q23, and 1q21.1-q25 and losses of 10p, 10q, 16p, 2q31-q36, 3p12-p21.3, 6q, 8p21.1-pter, 9p21-pter, 13q13-q21.1, and 19q13.2-qter were frequent. In MFH, gains of 5p12-p22.3, 7p12-p21, 7q, 9q13-q32, 12q13.1-q22.3, 14q11.2-q13, and 15q12-22.3 and losses of 1p31.2-pter, 9p21-pter, 10p, 10q, 19p, 19q and 13q were frequently detected. Gains of 1p21-pter, 6p12-p24 and 8q13-q23 were preferentially found in OS. A loss of 1p31.2-pter, gains of 5p13.1-15.3 and 15q11.2-23 were detected exclusively for MFH. These observations suggest that differences in these DNA copy number aberrations between OS and MFH could be an additional aid in the differential diagnosis of the two diseases.

Introduction

Bone and soft tissue sarcomas comprise approximately 1% of all human malignancies[3]. They are diverse in histologic type, and it is not always easy even for experienced pathologists to make an accurate diagnosis. Malignant fibrous histiocytoma (MFH) and osteosarcoma (OS) are the most common sarcomas of soft tissue and bone, respectively [4),5),6)]. Osteosarcoma is characterized by figures of osteoid production. However, osteoid formation is scarce in some OSs, in which an accurate diagnosis is difficult. Such cases may be erroneously diagnosed as MFH. The identification of specific non-random genetic alterations is useful to facilitate tumor diagnosis in some sarcomas, e.g., peripheral primitive neuroectodermal tumor/Ewing's sarcoma [7),8)], alveolar rhabdomyosarcoma[8),9)], and clear cell sarcoma[10),11)]. Although karyotyping with G-band staining was used for the cytogenetic analysis of OS[12)-15)] and MFH[15)-17)], it failed to find non-random genetic aberrations leading to a specific diagnosis. Comparative genomic hybridization (CGH) enables the testing of DNA copy number aberrations of tumors in a single hybridization procedure. It provides useful information concerning genetic alterations in various types of tumors[23),43)].

In this study, CGH analysis was performed to clarify the differences in DNA copy number aberrations between OS and MFH.

Materials and Methods

Tissue samples

In this study, we used frozen tissue specimens of 7 osteosarcomas (6 conventional, 1 small round cell), and 12 MFH. Patients with OS consisted of 5 males and 2 females with an average age of 19 years, ranging from 6 to 35 years old. Patients with MFH consisted of 11 males and 1 female with an average age of 68 years, ranging from 37 to 91 years old. Fourteen cases were primary tumors and 5 were recurrent tumors (one osteosarcoma and four MFH). Informed consent for this study was obtained from all patients.

Tissue preparation and microdissection

Tumors were removed surgically, frozen immediately in liquid nitrogen, and stored at −80℃ until use. Frozen tissue sections (20 μm thick) were prepared and an additional 4 μm section was made for detailed histological examination. All slides were stained with hematoxylin-eosin to identify tumor cell. Tumor fragments were microdissected from the surrounding stromal tissue with a sterile 26-gauge needle.

DNA Extraction

High molecular weight genomic DNA was extracted from each tumor specimen with a DNA extraction kit (Sepagene; Sankojunyaku Co. Ltd., Tokyo, Japan) according to the manufacturer's instructions. Control DNA was extracted from peripheral blood lymphocytes.

CGH and Digital Image Analysis

The CGH procedure was based on the protocol described by Kallioniemi et al., with a few modifications[1),2)]. Briefly, test DNA was directly labeled with Spectrum Green (Vysis, Inc., Downers Grove, IL) and reference DNA was labeled with Spectrum Red (Vysis, Inc.), both by nick translation. Nick-translated fragment sizes ranged from 400 to 2000 bp. Two hundred nanograms of each labeled DNA and 10μg of Cot-1 DNA (Life Technologies, Inc., Gaithersburg, MD) were mixed in 10μg of hybridization buffer and co-hybridized into normal denatured metaphase chromosomes for 72h at 37℃. Post-hybridization washes were performed with 2×SSC at 60℃. The slides were mounted in anti-fade solution containing 0.15 mg/ml 4',6-diamidino-2-phenylindole as counterstained. Digital images were captured with an Olympus BX60 fluorescence microscope equipped with a 100×UplanApo objective lens and a cooled charge-coupled device camera (SenSys 1400; Photometrics Ltd., Tucson, AZ), and approximately 20, but at least 10, representative images were analyzed. Losses of DNA sequences were defined as chromosomal regions where the average green-to-red ratio was below 0.8, with gains above 1.2. High-level copy number increase in the subregions (amplification) in contrast to whole arm gains were defined by a tumor: control ratio 1.4. The number of DNA sequence copy number aberrations is the total umber of chromosomal regions with alterations in the DNA sequence copy number.

Statistical analysis

Statistical analysis was performed with Statview software (SAS Institute Inc., Cary, NC). Fisher's exact test was performed to test reciprocal relations among MFHs and osteosarcomas, as appropriate. For all statistical tests, $p<0.05$ was considered significant.

Results

Genomic aberrations in osteosarcoma

In seven osteosarocmas, the mean number of genomic imbalances was 15.3 (8 gains and 7.3 losses). 1p21-pter gain or amplification was detected in all but one tumor, which was seen in a 34-years-old female. The DNA copy number increase was also frequent at 6p12-24 (5 cases), 8q13-23 (5 cases each), 1q21.1-25 (4 cases), 7p14-15.3 (3 cases), 9q13-32 (3 cases), 12q13.1-22 (3 cases), and 21q11.2-13.2 (3 cases). DNA copy number amplification was observed at 1p21-pter (3 cases), 1q21.1-25 (1 case), 6p 12-24 (1 case), 6q12-21 (1 case), 7p14-15.3 (1 case), 21q11.2-13.2 (1 case), and 4p13-15.3 (1 case) (Tables I & II). The frequent losses were 10p (4 cases), 10q (4 cases), and 16p (4 cases), 2q31-36 (3 cases), 3p12-21.3 (3 cases), 6q (3 cases), 8p21.1-pter (3 cases), 9p21-pter (3 cases), 13q13-21.1 (3 cases), and 19q13.2-qter (3 cases) (Tables I, II).

Table I. DNA copy number aberrations in OS and MFH
P, primary; R, recurrence; ampl, amplification

case	sex	age	histlogy	P/R	gain	loss	CNAs
1	M	17	OS	P	1p,1q21.1-25		1
2	M	16	OS	P	1p31.1-pter, ampl1q21.1-25, ampl4p14-pter ampl6p12-23, 8q, 9q22.3-23, 14q22-qter	1q32.2-qter, 2q24.3-qter, 3p, 6q, 7p, 8p, 9p, 10	17
3	F	25	OS	R	1p13.1-35, 1q21.1-24, 4p12-15.2, 6p12-22.2, 6q12-21, 8q12-22.3, 12q12-21.3, 14q11.2-13, 21q21-qter, 22q12.1-qter	8p, 9p, 16	14
4	F	35	OS	P		10,13q12.3-qter	3
5	M	6	OS	P	1p, 1q21.1-25, 6p, 7p13-21, 7q, 8, 9q21.2-32, 12q13.2-22, 14q23-qter, 17q21.3-24, 21q	2q14.1-36, 3p, 6q, 9p, 10q, 12q, 13q12.1-31, 16p, 19q, 22q	21
6	F	14	OS	P	1p13.1-35, 1q21.1-24, 2p12-16, 3q26.1-qter, 4q13.1-21.3, 5q, 6p21.3-22.3, 6q12-22.1, 7p14-15.3, 7q11.23-31.3, 8, 9q13-22.1, 12q13.1-21.3, ampl13q22-qter, 14q12-24.3	2q31-qter, 3q12-21.2, 10, 11p, 12p, 12q22-qter, 16p, 17p12-pter, 19	27
7	F	22	OS	P	1p22.1-31.1, 1q21.1-24, 2q14.1-qter, 5p, 6p, ampl7p, 8q12-22.3, 12p, 13q22-24.1 ampl21q	2p21-pter, 6q, 8p, 10p, 11q13.1-13.5, 12q, 13q12.3-21.3, 16p, 17, 19, 21q, 22q	24
1	M	64	MFH	R	ampl12q13.2-23	1q34.1-qter	2
2	F	69	MFH	R	9q13-21.3, 12q13.1-21.1		1
3	M	52	MFH	P		10	2
4	M	78	MFH	P	5p12-14, 7p, 7q11.21-32, 8q21.2-24.2, 9q21.1-34.1, 12q21.2-23, 14q11.2-13, 15q12-21.3, 17p11.2-12	9p21-pter, 10q21.2-qter, 13q, 18q	12
5	M	91	MFH	P	9q, 17p22-23, ampl22q11.2-13.1	1q31.2-pter, 3p, 8p, 8q12-21.2, 9p, 11q22.1-qter, 17q12-pter, 20p	11
6	M	72	MFH	P	1q23-qter, 2p13-16, 7p12-21, 7q11.21-22.1, 8	1q31.2-pter, 7q31.3-qter, 15q, 16p, 17, 19, 20q, 22q	16
7	M	73	MFH	R	ampl5p, 21q21-qter	6p21.1-21.3,7q,10	5
8	M	81	MFH	P	3p22-25, ampl5p, 5q33.1-qter, 7, ampl8q11.21-21.2, 12q14-qter, ampl14q11.2-13, 15q, 18q12.2-21.3, 20q11.2-13.2	1q31-qter, 2p22-pter, 2q13-31, 4p15.1-pter, 6p, 8q22.1-qter, 10, 11q, 16, 17q	22
9	M	79	MFH	P	1p21.1-21.3, 2p14-16, 7p12-21, 7q, 14q31-qter, 15q12-23, 17p	1p22.1-pter, 1q32.1-qter, 2q, 3p21.1-pter, 4q28-qter, 10, 11q22.1-qter, 13q, 16p, 18q	19
10	M	59	MFH	P	1p13.1-31.1, 3p, 4p12-15.1, ampl5p, 9q21.3-34.1, ampl14q12-21, 17p11.2-12, 17q22-23	1q41-qter, 2q32.3-qter, 9p21-pter, 10q21.1-qter, 13q, 16q, 19p, 21q	17
11	M	37	MFH	P	11p13-14, 11q21-23.2	1q33-pter, 17q22-qter, 19	23
12	M	59	MFH	R	ampl1q22-31, 2q32.1-33, 4p13-15.1, 5p14-15.2, 5q15-23.3, 6q12-31.3, ampl7p13-21, 9q13-33, 11p12-15.1, ampl12q13.1-21.3, ampl13q12.2-21.1, 14q21-qter, 18q12.2-21.3	1q31.2-pter, 3p14.1-22, 9p, 10, 16p, 17p, 19, 20q	5

Table II. Frequent gains and losses detected by CGH in OS and MFH

histololgy	gain/ loss	region	cases	%	histology	gain/ loss	region	cases	%
OS	gain	1p21-pter	6	86	MFH	loss	1p31.2-pter	6	50
	gain	6p12-24	5	71		loss	10q	6	50
	gain	8q13-q23	5	71		gain	7p12-21	5	42
	gain	1q21.1-25	4	57		gain	9q13-32	5	42
	loss	10p	4	57		gain	14q11.2-13	5	42
	loss	10q	4	57		loss	9p21-pter	5	42
	loss	16p	4	57		gain	5p13.1-15.3	4	33
	gain	7p14-15.3	3	43		gain	7q	4	33
	gain	9q13-32	3	43		gain	12q13.1-22	4	33
	gain	12q13.1-22	3	43		gain	15q11.2-23	4	33
	gain	21q11.2-13.2	3	43		loss	10p	4	33
	loss	2q31-36	3	43		loss	13q	4	33
	loss	3p12-14.3	3	43		loss	19p	4	33
	loss	6q	3	43					
	loss	8p21.1-qter	3	43					
	loss	9p	3	43					
	loss	13q13-21.1	3	43					
	loss	19q13.2-qter	3	43					

Genomic aberrations in MFH

In 12 MFHs, the mean number of genomic imbalances was 11.3 (5.2 gains, 6.1 losses). The DNA copy number increase was frequent at 7p12-21 (5 cases), 9q13-32 (5 cases), 14q11.2-13 (5 cases), 5p13.1-15.3 (4 cases), 7q (4 cases), 12q13.1-22.3 (4 cases), and 15q11.2-23 (4 cases). Amplification was detected at 5p13.1-15.3 (3 cases), 8q11.21-21.1(1 case), 12q13.1-22 (2 cases), and 14q11.2-13 (2 cases). The DNA copy number decrease was at 10q (6 cases), 1p31.2-pter (6 cases), 9p21-pter (5 cases), 10p (4 cases), 19p (4 cases), and 13q (4 cases). Three of 4 recurrent tumors showed a gain of 12q13.1-22.2 and two of these tumors demonstrated amplification of this locus (Tables I, II).

The differences in DNA copy number aberrations between OS and MFH

The frequency of gains at 1p21-pter, 6p12-p24 and 8q13-q23 were significantly more in OS than in MFH (P=0.0007, 0.0006 and 0.048 respectively)(Table III). Gains of 5p13.1-15.3 and 15q11.2-23 were detected exclusively for MFH. The loss of 1p31.2-pter, the most frequent loss in MFH, was not detected in OS. In contrast, gains of 9q13-32, 7p12-21, and 12q13.1-22.2 and losses of 10p, 10q, 9p21-pter, and 13q13-21.1 were found in both tumors (Fig. IA, IB).

Table III. Genomic aberrations of comparison between OS and MFH (Fisher's exact test)
CNAs : copy number aberrations

	OS CNAs(%)	MFH CNAs(%)	*p* value
gain of 1p21-pter	6 (87%)	1 (9%)	0.000743
gain of 6p12-24	5 (71)	0 (0)	0.000648
gain of 8q13-q23	5 (71)	3 (27)	0.048

	OS	MFH	*p* value
gain of 5p13.1-15.3	0(0)	4 (33)	0.085
gain of 15q11.2-23	0(0)	4 (33)	0.085
loss of 1p31.2-pter	0(0)	6 (42)	0.0237

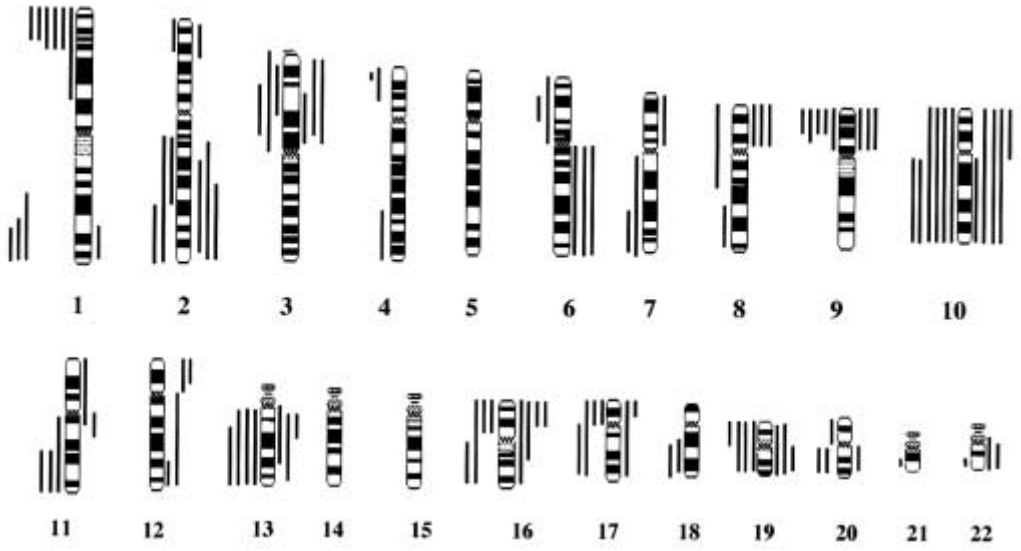

Fig.IA Ideograms of DNA copy number increases from 7 OSs and 12 MFHs.
Vertical lines on the right and left sides of chromosomes indicate OSs and MFHs.
Thicker lines indicate the amplification of genomic materials.

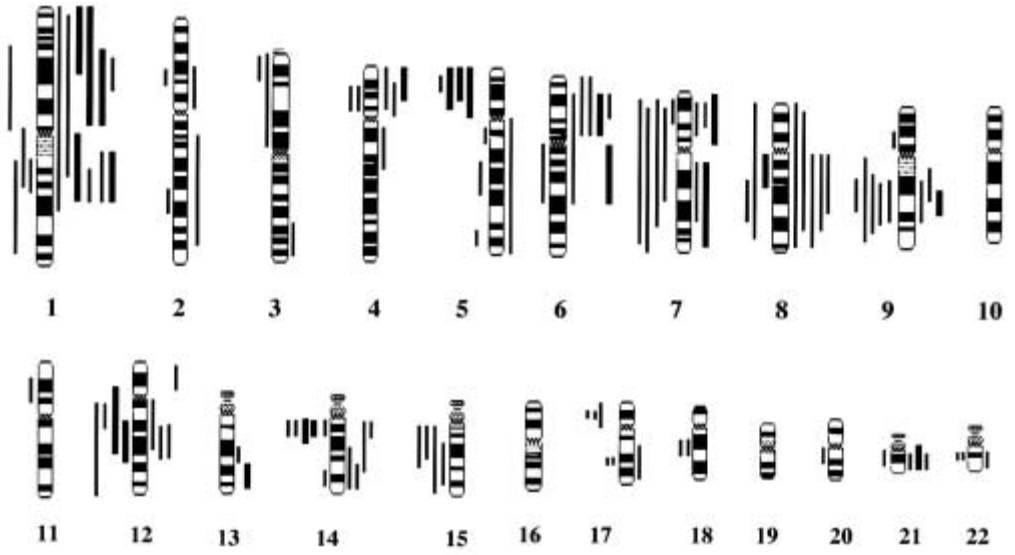

Fig.IB Ideograms of DNA copy number decreases from 7 osteosarcomas and 12 MFHs. Vertical lines on the right and left sides of chromosomes indicate OSs and MFHs.

Discussion

CGH analysis of OS revealed that gains of 1p, 5p, 8q, 12q12-15, and 17p and losses of 2 q, 8p, 9p, 10, 13q were frequent chromosomal aberrations[18)–22)]. Our results were similar to these observations. A gain of 1p21-pter was the most frequent aberration (6 cases) in OS, and amplification (high level gain) was found in 2 cases. In addition, we detected 8q13-25 gain in 5 of 7 OSs. These alterations were scarcely detected in MFHs. It is suggested that the chromosomal regions may contain oncogene(s) relevant to the development of OS. Tarkkanen[19)] found that the copy number increase at 8q13-q22 was statistically linked with poor survival and distant metastasis of OS. Genes involved in OS development are *myc*[23)], the osteoprotegerin gene, and the bone morphogenetic protein 1 (BMP1) gene [24),25)].

The morphologic heterogeneity[26),27),28)] of MFH sometimes precludes proper diagnosis and clinical analysis. A karyotype of MFH tends to be highly complex partly owing to its extensive intratumoral heterogeneity, and it is difficult to cytogenetically characterize this tumor. CGH analysis of MFH revealed a wide spectrum of genomic imbalances[29)–34)]. However, gains of 1q21-q22, 7p15-pter, 7q32, 12q13-15, 17q23-qter, and 20q, and losses of 2 p24-pter, 2q32-qter, 9p21-pter, 10q, 11q23-qter, and 13q10-q31 were common in their reports. These aberrations were also frequent in our present series including 12 MFHs. 12q13-15 gain was detected in MFH with high incidence and correlated with a poor outcome in studies by ourselves and others[32),33)]. This supports the hypothesis that gene(s) in this locus play an important role in the development of MFH [31)32)33)] and the chromosomal region of 12q 13-15 harbors MDM2, SAS, HMGIC, and CDK4 genes[39),42)].

The CGH profiles were very different between OS and MFH. Gains of 1p21-pter, 6p12-24, and 8q13-23, which have been reported as characteristic aberrations of OS[18)–22)], were scarce in MFH. In contrast, gains of 5p13.1-15.3 and 15q11.2-23 and the loss of 1p31.2-pter, which were frequent in MFH, were not detected in all OS. These chromosomal aberrations may be used as an aid in the differential diagnosis of difficult OS from MFH.

Losses of chromosome 10, 13q13-21.1 and 9 p21-pter were chromosomal aberrations common to OS and MFH in this study. The long arm of chromosome 10 harbors multiple tumor suppressor genes including PTEN (10 q23)[37),38)]. Although the loss of chromosome 10 is frequently detected in sarcomas [18),29),32),34),39)], the significance of PTEN loss has not been fully elucidated in sarcomas. Candidate genes for 9p21 and 13q10-q31 are p16NK4A and RB1, respectively[40),41)], and

these tumor suppressor genes are involved in various types of tumors. It is natural to speculate that losses of 10q, 9p21, and 13q13-21.1 are directly linked with the tumorigenic events in both tumors.

In conclusion, gains of 1p21-pter, 6p12-p24, 8q13-q23, and gains of 5p13.1-15.3 and 15q11.2-23, and the loss of 1p31.2-pter are cytogenetic markers for the differential diagnosis of these two diseases.

Acknowledgments

I gratefully thank Prof. K Sasaki, associate Prof. S Kawauchi of the Department of Pathology, and Prof. S Kawai, associated Prof. K Ihara of the Department of Orthopedic Surgery for advices and technical supports.

References

1) Kallioniemi A, Kallioniemi, O.P., Sudar, D., Rutovitz,D., Gray,J.W., Waldman,F., and Pinkel D. : Comparative genomic hybridyzation for molecular cytogenetic analysis of solid tumors. *Sience (Wash. DC),* **258** : 818-821, 1992.

2) Kallioniemi O.P., Kallioniemi, A., Sudar D., Rutovitz D., Gray J.W., Waldman F., and Pinkel D.: Comparative genomic hybridyzation: a rapid new method for detecting and mapping DNA amplification in tumors. *Semin Cancer Biol,* **4** : 41-46, 1993.

3) Parker SL, Tong T, Bolder W.: Cancer statiatics. CA Cancer. J Clin, **46** : 5, 1996.

4) Rydholm A, Berg NO, Gullberg B.: Epidemiology of soft tissue sarcoma in the locomotor system: a retrospective population-based study of the interrelationships between clinical and morphological variables. *Acta Pathol Microbiol Immunol Scand,* **92**A : 363, 1984.

5) Gustafson P.: Soft tissue sarcoma. Epidemiology and prognosis in 508 patients. *Acta Orthop Scand Suppl,* **259**: 1-31, 1994.

6) Dorfman HD, Czerniak B.: Bone Cancers. *Cancer,* **75** : 203-210, 1995.

7) Downing JR, Head DR, Parham DM, Douglass EC, Hulshof MG, Link MP, Motroni TA, Grier HE, Crucio-Brint AM, Shapiro DN. : Detection of the (11;22) (q24;q12) translocation of Ewing's sarcoma and peripheral neuroectodermal tumor by reverse transcription polymerase chain reaction. *Am J Pathol,* **143** : 1294-1300, 1993.

8) Athale UH, Shurtleff SA, Jenkins JJ, Poquette CA, Tan M, Downing JR, Pappo AS.: Use of reverse transcriptase polymerase chain reaction for diagnosis and staging of alveor rhabdomyosarcoma, Ewing sarcoma family of tumors, and desmoplastic small round cell tumor. *Am J Pediatr Hematol Oncol,* **23** : 99-104, 2001.

9) Koufos A, Hansen MF, Copeland NG, Jenkins NA, Lampkin BC, Cavenee WK.: Loss of heterozygosity in three embyonal tumors suggests a common pathologenetic mechanism. *Nature,* **316** : 330-334, 1985.

10) Antonescu CR, Tschernyavsky SJ, Woodruff JM, Jungbluth AA, Brennan MF, Lasanyi M.: Molecular diagnosis of clear cell sarcoma: detection of EWS-ATF1 and MITF-M transcripts and histopathological and ultrastructural analysis of 12 cases. *J Mol Diagn,* **4**: 44-52, 2002.

11) Limon J, Dibeiec-Rychter M, Nedoszytko B, Liberski PP, Babinska M, Szadowska A.: Aberrations of chromosome 22 and polysomy of chromosome 8 as non-random change in clear cell sarcoma. *Cancer Genet Cytogenet,* **72** : 141-145, 1994.

12) Fletcher JA, Gebhardt MC, Kozakewich HP. Cytogenetic aberrations in osteosarcomas. : Nonrandom deletions, rings, and double-minute chromosomes. *Cancer Genet Cytogenet,* **77** : 81-8, 1994.

13) Bridge JA, Nelson M, McComb E, McGuire MH, Rosenthal H, Vergara G, Maale GE, Spanier S, Neff HR.: Cytogenetic findings in 73 osteosarcoma specimens and a review of the literature. *Cancer Genet Cytogenet,* **95** : 74-87, 1994.

14) Boehm AK, Squire JA, Bayani J, Nelson M, Neff J, Bridge JA. : Cyttogenetic findings in 35 osteosarcoma specimens and a review of the literature. *Pediatr Pathol Mol Med,* **19** : 359-76, 2000.

15) Sandberg AA, Bridge JA.: The cytogenetics of Bone and Soft Tissue Tumors. *R.G. Landes Company: Austine, Texas.* 1994.

16) Mandahl N, Heim S, Arheden K, Rydholm A, Willen H, Mitelman F.: Rings, Dicentrics, and telomeric associations in histipcytomas. *Cancer Genet Cytogenet,* **30** : 23-33, 1988.
17) Walter TA, Weh H-J, Schlag PM, Zornig C, Hossfeld DK.: Cytogenetic studies in malignant fibrous histiocytoma. *Cancer Genet Cytogenet,* **85** : 91-96, 1995.
18) Zielenska M, Bayani J, Pandita A, Toledo S, Marrano P, Andrade J, Pertilli A, Thorner P, Sorensen P, Squire J.A.: Comparative genomic hybridization analysis identifies gains of 1p35～p36 and chromosome 19 in osteosarcoma. *Cancer Genet Cytogenet,* **130** : 14-21, 2001.
19) Tarkkanen M, Elomaa I, Blomqvist C, Kivioja AH, Kellokumpulehtinen P, Bohling T Valle J, Knuutila S.: DNA sequnce copy number increase at 8q:a potential new prognostic marker in high-grade osteosarcoma. *Int J Cancer,* **84**: 114-21, 1999
20) Forus A, Weghuis DO, Smeets D, Fodstad O, Myklebost O, Geurts van Kessel A.: Comparative genomic hybridization analysis of human sarcoma: II. Identification of novel amplicons at 6p and 17p in osteosarcomas. *Genes Chromosom Cancer,* **14** : 15-21, 1995.
21) Tarkkanen M, Karhu R, Kallioniemi A, Elomaa I, Kivija AH, Nevalainen J, Bohling T, Karaharju E, Hyytinen E, Knuutila S.: Gain and losses of DNA sequences in osteosarcomas by comparative genomic hybridization. *Cancer Res,* **55** : 1334-8, 1995.
22) Stock C, Kager L, Fink FM, Gadner H, Ambros PF.: Chromosomal regions involved in the pathologenesis of osteosaromas. *Genes Chromosom Cancer,* **28** : 329-36, 2000.
23) Knuutila S, Bjorkqvist A-M, Autio K, Tarkkanen M, Wolf M, Monni O, Szymanska J, Larramendy ML, Tapper J, Pere H, El-Rifai W, Hemmer S, Wasenius V-M, Vidgren V, Zhu Y.: DNA copy number amplifications in human neoplasmas: review of comparative genomic hybridization studies. *Am J Pathol,* **152** : 1107-1123, 1998.
24) Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw Gegg L, Hughes TM, Hill D, Patison W, Campbell P, Sander S, Van G, Tarpley J. Derby P, Lee R, Amgen EST Program, Boyle WJ.: Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell, **89** : 309-319, 1997.
25) Tabas JA, Zasloff M, Wasmuth JJ, Emanuel BS, Alterr MR, McPherson JD, Wozney JM, Kaplan FS.: Bone morphogenetic protein: chromosomal localization of human genes for BMP1, BMP2A, and BMP3. *Genomics,* **9** : 283-289, 1991.
26) Orndal C, Rydholm A, Willen H, Mitelman F, Mandahl N.: Cytogenetic intratumor heterogeneity in soft tissue tumors. *Cancer Genet Cytogenet,* **78**: 127-137, 1994.
27) Dehner LP.: Malignant fibrous histiocytoma: Nonspecific morphologic pattern, specific pathologic entity, or both. *Arch Pathol Lab Med,* **112** : 236-237, 1998.
28) Hollowood K and Fletcher CD. Malignant fibrous histiocytoma: Morphologic pattern or pathologic entity. *Semin Diagn Pathol,* **12** : 210-220, 1995.
29) Simons A, Schepens M, Jeuken J, Sprenger S, van de Zande G, Bjerkehagen B, Forus A, Weibolt V, Molenaar I, van den Berg E, Myklebost O, Bridge J, Geurts van Kessel A, Suijkerbuijk R.: Frequnt loss of 9p21 (p16INK4A) and other genomic imbalance in human malignant fibrous histiocytoma. *Cancer Genet Cytogenet,* **118** : 89-98, 2000.
30) Forus A, Olde Weghuis D, Smeets D, Fodstad O, Myklebost O, Geurts van Kessel A.: Comparative genomic hybridization of human sarcomas: I. Occurrence of genomic imbalances and indetification of a novel major amplicon at 1q21-q22 in soft tissue sarcomas. *Genes Chromosom Cancer,* **14** : 8-14, 1995.
31) Larramendy ML, Tarkkanen M, Blomqvist C, Viriolainen M, Wiklund T, Asko-Seljavaara S, Elomaa I, Knuutila S.: Comparative genomic hybridization of

malignant fibrous histiocytoma reveals a novel prognosis marker. *Am J Pathol,* **151** : 1153-1161, 1997.
32) Hinze R, Schagdarsurengin U, Taubert H, Meye A, Wurl P, Holzhausen HJ, Rath FW, Schmidt H.: Assessment of genomic imbalances in malignant fibrous histiocytomas by comparative hybridization. *Int J Mol Med,* **3** : 75-79, 1999.
33) Sakabe T, Shinomiya T, Mori T, Ariyama Y, Fukuda Y, Fijiwara T, Nakamura Y, Inazawa J.: Identification of a novel gene, MASL1, within an amplicon at 8p23.1 detected in malignant fibrous histiocytomas by comparative genomic hybridization. *Cancer Res,* **59** : 511-515, 1999.
34) Marial A, Terrier P, Chibon F, Sastre X, Lecesne A, Aurias A.: Loss of chromosome 13 is the most frequent genomic imbalance in malignant fibrous histiocytomas. A comparative genomic hybridization analysis of a series of 30 cases. *Cancer Gent Cytogenet,* **111** : 134-138, 1999.
35) Levy B, Mukherjee T, Hirschhorn K.: Molecular cytogenetic analysis of uterine leiomyoma and leiomyosarcoma by comparative genomic hybridization. *Cancer Genet Cytogenet,* **121** : 1-8, 2000.
36) Lushnikova T, Knnutila S, Miettinen M.: DNA copy number changes in epithelioid sarcoma and its variants: a comparative genomic hybridization study. *Mod Pathol,* **13** : 1092-6, 2000.
37) Maier D, Comparone D, Taylor E, Zhang Z, Gratzl O, van Meir EG, Scott RJ, Merlo A.: New deletion in low-grade oligodendroglioma at the glioblastoma suppressor locus on chromosome 10q25-26. *Oncogene,* **15** : 997-1000, 1997.
38) Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanelli BC, Ottmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R.: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science,* **275**: 1043-2503, 1997.
39) Schmidt H, Taubert H, Wurl P, Kappler M, Lange H, Bartel H, Bache M, Holzhausen H.J, Hinze R.: Gain of 12q are the most frequent genomic imbalances in adult fibrosarcoma and are correlated with a poor outcome. *Genes Chromosom Cancer,* **34** : 69-77, 2002.
40) Wunder JS, Czitrom AA, Kandel R, Andrulis IL.: Analysis of alterations in the retinoblastoma gene and tumor grade in bone and soft-tissue sarcomas. *J Natl Cancer Inst,* **83** : 194-200, 1991.
41) Kamb A, Gruis NA, Weaver-Felfhaus J, Liu Q, Harshman K, Tavtigian SV, Stockert E, Day RS Ⅲ, Johnson BE, Skolnick MH.: A cell cycle regulator potentially involved in genesis of many tumor types. *Science,* **264** : 436-440, 1994.
42) Bartel F, Meye A, Wurl P, Kappler M, Bache M, Lautenschlager C, Grunbaum U, Schmidt H, Tauber H.: Amplification of the MDM2 gene, but not expression of splice variants of MDM2 mRNA, is associated with prognosis in soft tissue sarcoma. *Int J Cancer,* **95** : 168-175, 2001.
43) Stephanie S, Martine DF, Pascale CL.: Compilation of published comparative genomic hybridization studies. *Cancer Genet Cytogenet,* **135** : 63-90, 2002