Comparative Genomic Hybridization in Cartilaginous Tumors

TOSHIFUMI OZAKI[1], DANIEL WAI[2], KARL-LUDWIG SCHÄFER[2],
NORBERT LINDNER[1], WERNER BÖCKER[2], WINFRIED WINKELMANN[1],
BARBARA DOCKHORN-DWORNICZAK[3] and CHRISTOPHER POREMBA[2]

[1]Department of Orthopaedics and [2]Gerhard-Domagk-Institute of Pathology,
Westfaelische Wilhelms-University, Muenster; [3]Institute of Pathology, Kempten, Germany

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

Correspondence to: T. Ozaki, MD, Department of Orthopaedic Surgery, Okayama University Hospital, Okayama 700-8558, Japan. e-mail: tozaki@md.okayama-u.ac.jp

Key Words: Genomic hybridization, cartilaginous tumors, chondrosarcomas.

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).
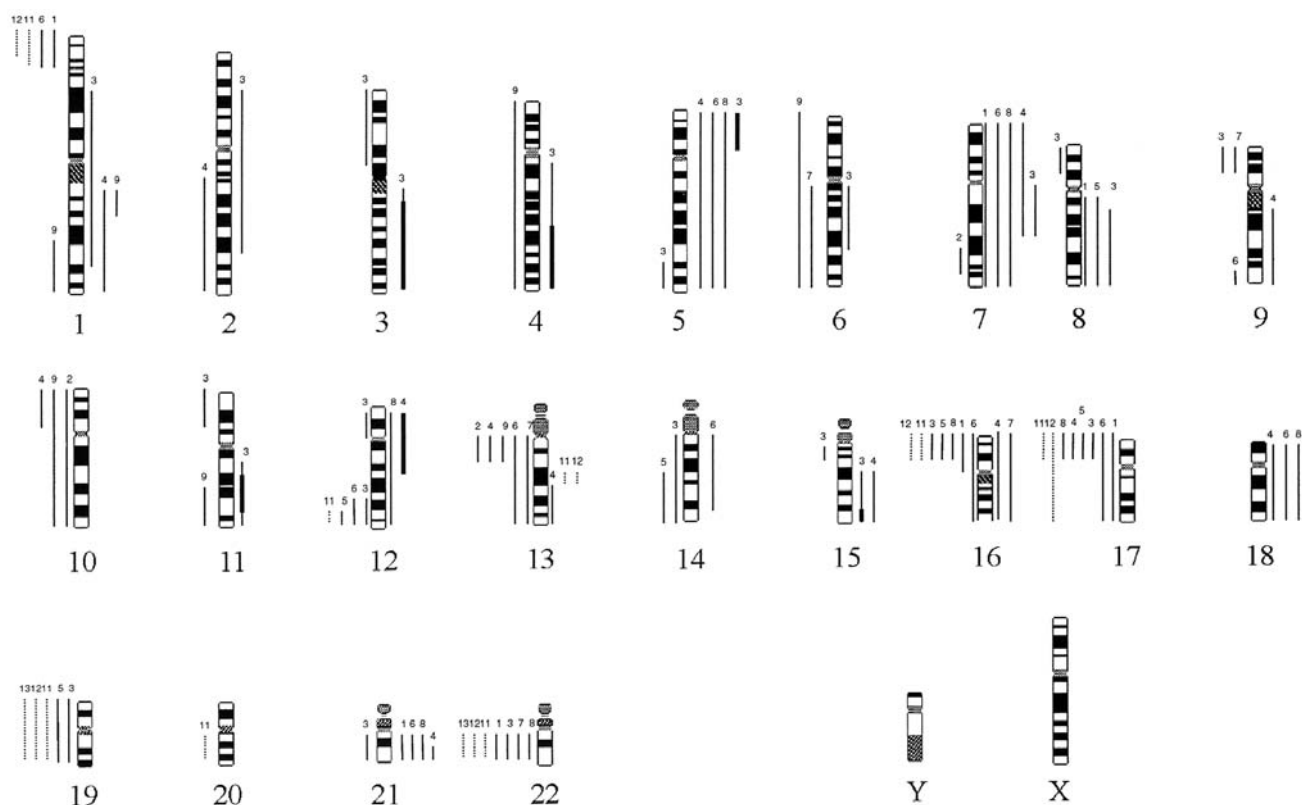
Discussion

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	1p	1q	2p	2q	3p	3q	4p	4q	5p	5q	6p	6q	7p	7q	8p	8q	9p	9q	10p	10q	11p	11q	12p	12q	13p	13q	14p	14q	15p	15q	16p	16q	17p	17q	18p	18q	19p	19q	20p	20q	21p	21q	22p	22q
1	Chondrosarcoma	3	7	3	4		L											G	G	G															L										G			L
2	Chondrosarcoma	3	3	0	3														L					L	L						L							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	H	G,H	G,H		L		L	L,H	G	L		L	G	L	L	G			L	G		L	L
4	Chondrosarcoma	3	14	10	4	G		L				G,H				G	G	G		L	G		G		L	G,H	G,H	L		G,L			G	G	G	G	L	G	G	L	L	L				L		L
5	Chondrosarcoma	3	6	1	5									G	G						G						L		L		L											L	L	L	L		G		
6	Chondrosarcoma	2	11	5	6		L							G	G	G	G	G	G	G			L								L		G	L	L	L	L	L		G	G					G			L
7	Chondrosarcoma	2	5	1	4											L							L											G	G														L
8	Chondrosarcoma	2	9	6	3									G	G	G	G	G	G	L						G	G		L		L				L	L				L		G	G				G		L
9	Chondrosarcoma	1	7	1	6	G,L				L	L			L	L	L	L							L	L	L	L	G	G		L	L						L		L		G	G						L
10	Chondrosarcoma	1	0	0	0																																												
11	Chondromyxoid fibroma		8	1	7	L																						L			G						L		L			L	L	L	L	L			L
12	Enchondroma		6	1	5	L																									G						L		L	L		L	L	L	L		G		L
13	Enchondroma		2	0	2																																						L	L					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.

Acknowledgements

References

1 Unni KK: Chondrosarcoma (primary, secondary, dedifferentiated, and clear cell). *In*: Dahlin's Bone Tumors. General Aspects and Data on 11,087 Cases. edited by Unni KK, Philadelphia, Lippincott-Raven, 1996, pp 71-108.

2 Schajowicz F: Tumor and Tumorlike Lesions of Bone. Pathology, Radiology, and Treatment. Berlin, Springer-Verlag, 1994.

3 Barnes R and Catto M: Chondrosarcoma of bone. J Bone Joint Surg Br *48*: 729-764, 1966.

4 Huvos AG: Chondrosarcoma including spindle-cell (dedifferentiated) and myxoid chondrosarcoma; mesenchymal chondrosarcoma. *In*: Bone Tumor. Diagnosis, Treatment, and Prognosis. (vol 2), edited by AG H, Philadelphia, WB Saunders, 1991, pp 343-393.

5 Meachim G: Histological grading of chondrosarcoma (editorial). J Bone Joint Surg Br *61*: 393-394, 1977.

6 Ozaki T, Hillmann A, Lindner N, Blasius S and Winkelmann W: Metastasis of chondrosarcoma. J Cancer Res Clin Oncol *122*: 625-628, 1996.

7 Bjornsson J, McLeod RA, Unni KK, Ilstrup DM and Pritchard DJ: Primary chondrosarcoma of long bones and limb girdles. Cancer *83*: 2105-2119, 1998

8 Larramendy ML, Mandahl N, Mertens F, Blomqvist C, Kivioja AH, Karaharju E, Valle J, Bohling T, Tarkkanen M, Rydholm A, Akerman M, Bauer HC, Anttila JP, Elomaa I and Knuutila S: Clinical significance of genetic imbalances revealed by comparative genomic hybridization in chondrosarcomas. Hum Pathol *30*: 1247-1253, 1999.

9 Bovee JV, Cleton-Jansen AM, Rosenberg C, Taminiau AH, Cornelisse CJ and Hogendoorn PC: Molecular genetic characterization of both components of a dedifferentiated chondrosarcoma, with implications for its histogenesis. J Pathol *189*: 454-462, 1999.

10 Evans HL, Ayala AG and Romsdahl MM: Prognostic factors in chondrosarcoma of bone: a clinicopathologic analysis with emphasis on histologic grading. Cancer *40*: 818-831, 1977.

11 Enneking WF, Spanier SS and Goodman MA: A system for the surgical staging of musculoskeletal sarcoma. Clin Orthop *153*: 106-120, 1980.

12 Paulussen M, Ahrens S, Braun-Munzinger G, Craft AW, Dockhorn-Dworniczak B, Dorffel W, Dunst J, Frohlich B, Gobel U, Haussler M, Klingebiel T, Koscielniak E, Mittler U, Rube C, Winkelmann W, Voute PA, Zoubek A and Jurgens H: EICESS 92 (European Intergroup Cooperative Ewing's Sarcoma Study)-- preliminary results. Klin Padiatr *211*: 276-283, 1999.

13 Kallioniemi OP, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW and Pinkel D: Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer *10*: 231-243, 1994.

14 Brinkschmidt C, Poremba C, Christiansen H, Simon R, Schafer KL, Terpe HJ, Lampert F, Boecker W and Dockhorn-Dworniczak B: Comparative genomic hybridization and telomerase activity analysis identify two biologically different groups of 4s neuroblastomas. Br J Cancer *77*: 2223-2229, 1998.

15 Scheel C, Schaefer KL, Jauch A, Keller M, Wai D, Brinkschmidt C, van Valen F, Boecker W, Dockhorn-Dworniczak B and Poremba C: Alternative lengthening of telomeres is associated with chromosomal instability in osteosarcomas. Oncogene *20*: 3835-3844, 2001.

16 O'Donovan M, Russell JM, O'Leary JJ, Gillan JA, Lawler MP and Gaffney EF: Abl expression, tumour grade, and apoptosis in chondrosarcoma. Mol Pathol *52*: 341-344, 1999.

17 Kohda T, Asai A, Kuroiwa Y, Kobayashi S, Aisaka K, Nagashima G, Yoshida MC, Kondo Y, Kagiyama N, Kirino T, Kaneko-Ishino T and Ishino F: Tumour suppressor activity of human imprinted gene PEG3 in a glioma cell line. Genes Cells *6*: 237-247, 2001.

18 Smith JS, Tachibana I, Lee HK, Qian J, Pohl U, Mohrenweiser HW, Borell TJ, Hosek SM, Soderberg CL, von Deimling A, Perry A, Scheithauer BW, Louis DN and Jenkins RB: Mapping of the chromosome 19 q-arm glioma tumor suppressor gene using fluorescence *in situ* hybridization and novel microsatellite markers. Genes Chromosomes Cancer *29*: 16-25, 2000.

19 Smith JS, Tachibana I, Pohl U, Lee HK, Thanarajasingam U, Portier BP, Ueki K, Ramaswamy S, Billings SJ, Mohrenweiser HW, Louis DN and Jenkins RB: A transcript map of the chromosome 19q-arm glioma tumor suppressor region. Genomics *64*: 44-50, 2000.

20 Yamaguchi T, Toguchida J, Wadayama B, Kanoe H, Nakayama T, Ishizaki K, Ikenaga M, Kotoura Y and Sasaki MS: Loss of heterozygosity and tumor suppressor gene mutations in chondrosarcomas. Anticancer Res *16*: 2009-2015, 1996.

21 Balsara BR, Sonoda G, du Manoir S, Siegfried JM, Gabrielson E and Testa JR: Comparative genomic hybridization analysis detects frequent, often high- level, overrepresentation of DNA sequences at 3q, 5p, 7p, and 8q in human non-small cell lung carcinomas. Cancer Res *57*: 2116-2120, 1997.

22 Tarkkanen M, Elomaa I, Blomqvist C, Kivioja AH, Kellokumpu-Lehtinen P, Bohling T, Valle J and Knuutila S: DNA sequence copy number increase at 8q: a potential new prognostic marker in high-grade osteosarcoma. Int J Cancer *84*: 114-121, 1999.

23 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, van Kessel AG and Suijkerbuijk R: Frequent loss of 9p21 (p16 (INK4A)) and other genomic imbalances in human malignant fibrous histiocytoma. Cancer Genet Cytogenet *118*: 89-98, 2000.

24 Zhang H, Kobayashi R, Galaktionov K and Beach D: p19Skp1 and p45Skp2 are essential elements of the cyclin A-CDK2 S phase kinase. Cell *82*: 915-925, 1995.

25 Ouyang P: An *in vitro* model to study mesenchymal-epithelial transformation. Biochem Biophys Res Commun *246*: 771-776, 1998.

26 Naka T, Iwamoto Y, Shinohara N, Ushijima M, Chuman H and Tsuneyoshi M: Expression of c-met proto-oncogene product (c-MET) in benign and malignant bone tumors. Mod Pathol *10*: 832-838, 1997.

27 Sakai Y, Saijo M, Coelho K, Kishino T, Niikawa N and Taya Y: cDNA sequence and chromosomal localization of a novel human protein, RBQ-1 (RBBP6), that binds to the retinoblastoma gene product. Genomics *30*: 98-101, 1995.