American Journal of Pathology, Vol. 150, No. 2, February 1997

Gains, Losses, and Amplifications of DNA Sequences Evaluated by Comparative Genomic Hybridization in Chondrosarcomas

Marcelo L. Larramendy,*† Maija Tarkkanen,* Julio Valle,‡ Aarne H. Kivioja,§ Helena Ervasti,§ Erkki Karaharju,§ Tarja Salmivalli,‖ Inkeri Elomaa,¶ and Sakari Knuutila*

From the Department of Medical Genetics and Department of Pathology,‡ Haartman Institute, University of Helsinki, and the Departments of Orthopaedics and Traumatology,§ Radiology,‖ and Oncology,¶ Helsinki University Central Hospital, Helsinki, Finland; and the Laboratorio de Citogenética y Cátedra de Citología,† Facultad de Ciencias Naturales, Universidad Nacional de La Plata, La Plata, Argentina*

Comparative genomic hybridization was used to search for previously unknown gains and losses of DNA sequences along all chromosome arms in 29 chondrosarcoma specimens obtained from 23 patients. Extensive genetic aberrations, with a mean of 6 changes per tumor (range, 1 to 24), were detected in 21 of the 29 samples analyzed (72%). The majority of these changes were gains of whole chromosomes or whole chromosome arms. Gains of DNA sequence copy number were most frequent at 20q (38%), 17p (38%), 20p (31%), 1cen-q24 (28%), and 14q23-qter (28%). High-level amplifications of small chromosome regions were sporadic, detected in only 17% of the samples. The only recurrent high-level amplification, seen in two tumors (7%), affected the minimal common region 12cen-q15. Other amplifications, each encountered only once, involved 1p33-p35, 2p23-pter, 4p, 6p22-pter, 18q12-q22, 19p13.2, 19q13.2, and 20q13.1. Losses of DNA sequences were rare and were most commonly observed at 6cen-q22 (17%) and 9p (17%). (Am J Pathol 1997, 150:685–691)

Chondrosarcoma (CS) accounts for nearly 11% of all malignant bone tumors and is the second most common primary sarcoma of bone after osteosarcoma.[1] Most CSs arise in normal bone, but a small proportion originates from pre-existing benign cartilaginous lesions, such as enchondroma or osteochondroma. Approximately 11% of CSs arise in osteochondromas, typically in patients with the hereditary multiple exostoses syndrome characterized by the presence of multiple osteochondromas, and 3% of CSs originate from an enchondroma in patients with Ollier's disease (skeletal multiple enchondromatosis).[2] When a CS is radically resected, the prognosis is based mainly on the grade of the tumor, as the grade correlates with the ability of the tumor to metastasize. Metastases are rare in grade I CSs, whereas grade III CSs metastasize in approximately 70% of the patients.

Genetic changes underlying the initiation and progression of CS are poorly known. This is mainly due to the limitations of current research techniques for studying these neoplasms. Conventional cytogenetic analysis is often difficult due to the scarcity of mitotic cells and because of poor chromosome morphology and banding. A few cytogenetic studies on CS have shown that these tumors are characterized by complex karyotypes with inter- and intratumor heterogeneity.[3–6] Because the number of cytogenetically analyzed CSs is small, no specific or diagnostic cytogenetic markers have been established so far.[3–6]

In studies by Toguchida et al,[7] *p*53 mutations were present in almost one-half of the CSs analyzed. Immunohistochemical *p*53 positivity and/or *p*53 mutations, seen in 13 to 25% of CSs, were predominantly features of high-grade tumors, suggesting a role for *p*53 in the progression of CS.[8,9] A small number of

Supported by the Sigrid Jusélius Foundation, the Clinical Research Institute of the Helsinki University Central Hospital, the Finnish Cancer Society, the Finnish Medical Society Duodecim, and the Foundation of Orthopaedics and Traumatology, all from Finland, and by the National Council of Scientific and Technological Research (CONICET) and the National University of La Plata, from Argentina.

Accepted for publication September 22, 1996.

Address reprint requests to Dr. Sakari Knuutila, Department of Medical Genetics, Haartman Institute, University of Helsinki, PO Box 21 (Haartmaninkatu 3), FIN-00014 Helsinki, Finland.

CSs have been studied for *RB* alterations, but none have been identified.[10] In a small CS series, amplification of *CMYC* was reported in two of nine tumors.[11]

A recent study of sporadic CS demonstrated loss of heterozygosity at 8q24 and at the pericentromeric region of chromosome 11.[12] Both regions have been implicated in the hereditary multiple exostoses syndrome.[13,14] The loss of heterozygosity at 8q24 in CS and the deletions found in this region in osteochondromas[15] support the hypothesis that genes predisposing to the hereditary multiple exostoses syndrome have a tumor suppressor function and may participate in the tumorigenesis of cartilaginous neoplasms.

Comparative genomic hybridization (CGH) makes it possible to detect DNA sequence copy number changes in tumors without specific probes or previous knowledge of the main chromosomal rearrangements in the tumors.[16,17] The method is based on co-hybridization of differentially fluorochrome-labeled tumor DNA and normal DNA to normal metaphase chromosomes. Differences in tumor *versus* normal fluorescence intensities along the chromosomes reveal over- and under-representations of DNA sequences in the entire tumor genome. Digital image analysis provides a quantification of the copy number changes. In the present study, we used CGH to detect gains and losses of DNA sequences in CS.

Materials and Methods

Tumor Specimens

The material consisted of 29 CS samples obtained from 23 patients treated at the Department of Orthopaedics and Traumatology, Helsinki University Central Hospital. None of the patients had received chemo- or radiotherapy before the operation. For 4 patients (tumors 7, 12, 14, and 17), 2 samples were analyzed (coded a and b), and from 1 patient (tumor 8), 3 samples were included in the study (a, b, and c). The material consisted of 5 grade I CSs (tumors 1 to 5), 18 grade II CSs (tumors 6 to 17), 5 grade III CSs (tumors 18 to 22), and 1 grade IV CS (tumor 23). Histopathological and clinical characteristics of the samples are presented in Table 1. DNAs were extracted from 26 frozen tumor samples and from 3 paraffin blocks (samples 1, 7a, and 7b).

Comparative Genomic Hybridization

CGH was performed using direct fluorochrome-conjugated DNAs for all samples according to a recently published protocol with minor modifications.[16] Briefly, tumor DNA and normal DNA were labeled by nick translation with fluorescein-isothiocyanate-conjugated dUTP (DuPont, Boston, MA) and Texas-red-conjugated dUTP (DuPont), and DNA fragments of 600 to 2000 bp were obtained. The hybridization mixture consisted of 400 ng of tumor DNA, 400 ng of normal labeled DNA, and 10 μg of unlabeled human Cot-1 DNA (Gibco BRL, Life Technologies, Gaithersburg, MD) dissolved in 10 μl of hybridization buffer (50% formamide, 10% dextran sulfate, 2X standard saline citrate (SSC)). The mixture was denatured at 75°C for 5 minutes and then hybridized onto normal metaphases denatured in 70% formamide/2X SSC at 65°C for 2 minutes. Hybridizations were carried out at 37°C for 48 hours. The slides were washed three times in 50% formamide/2X SSC (pH 7.0), twice in 2X SSC, and once in 0.1X SSC (at 45°C for 10 minutes each), followed by washes in 2X SSC in a buffer containing 0.1 mol/L NaH_2PO_4 and 0.1 mol/L Na_2HPO_4 (pH 8.0) and in distilled water (at room temperature for 10 minutes each). The slides were counterstained with 4′,6-diamidino-2-phenylindole-dihydrochloride and mounted with Vectashield antifading buffer (Vector Laboratories, Burlingame, CA).

Digital Image Analysis

The hybridizations were analyzed using an Olympus fluorescence microscope and the ISIS digital image analysis system (MetaSystems Hard & Software, Altlussheim, Germany) based on an integrated high-sensitivity monochrome CCD camera and automated CGH analysis software. Three-color images, green (fluorescein isothiocyanate) for the tumor DNA, red (Texas red) for the reference DNA, and blue (4′,6-diamidino-2-phenylindole-dihydrochloride) for the chromosome counterstain, were obtained of 12 metaphases in each sample. Chromosome regions were interpreted as over-represented when the green-to-red ratio was higher than 1.17 (gains) or 1.5 (high-level amplifications, >10-fold), and under-represented when the ratio was lower than 0.85 (losses). These cut-off values were derived from analyses of negative controls whereby two differentially labeled normal DNAs were hybridized against each other.[17] In each CGH experiment, a negative (peripheral blood DNA from normal controls) and a positive (tumor DNA with known copy number changes) control were included and run simultaneously with the tumor samples.

Telomeric and heterochromatic regions were excluded from the analysis when they appeared as the sole aberration present in the sample as these re-

Table 1. *Histopathological and Clinical Characteristics and DNA Sequence Copy Number Changes in 29 Chondrosarcoma Samples Analyzed by Comparative Genomic Hybridization*

Sample number	Sex	Age (years)	Location of tumor (size)	Grade	Copy number changes, CGH results
1*	F	25	Ulna (8 × 6 × 5) P	I	−X
2*	M	44	Pelvis (14 × 13 × 8) P	I	+Xq22-q26
3	F	46	Femur (NA) P	I	No changes
4*	F	44	Sacrum (NA) P	I	No changes
5	M	39	Talus (4) P	I	−18
6	M	32	Pelvis (NA) P	II	+2pter-q24, +8q
7a	M	39	Scapula (6 × 10 × 8) P	II	No changes
7b	M	40	Scapula (NA) R	II	+6p, +17p, +20
8a	M	63	Femur (NA) P	II	No changes
8b	M	64	Soft tissue (NA) R	II	+5q14-q32, +15q21-q25
8c	M	64	Soft tissue (15) R	II	+1cen-q24, +6p, −9p, +9q, +12q, +16q, +17, +20q
9	F	39	Pelvis (NA) P	II	No changes
10	F	36	Femur (3 × 6 × 6) P	II	−6, +16q, +17p, +18p, −18q, +20, +X
11	M	65	Tibia (NA) P	II	No changes
12a	M	37	Tibia (5 × 6 × 4) P	II	+17, +20q, +21
12b	M	38	Tibia (5 × 6 × 4) P	II	No changes
13	M	78	Rib VIII (20) P	II	+2, +5, +7, +8q, +14, +15, +16q, +17, +20, +21, +22
14a	F	34	Femur (15 × 3 × 5) P	II	+1, +2, +6, +7q, −9p, +10, +12pter-q15/**cen-q15**, + 16q, +17pter-q21, +20
14b	F	34	Femur (15 × 3 × 5) P	II	No changes
15	F	72	Pelvis (4 × 6 × 6) P	II	**+4p**, +7q, +8, +9p, +12, +14, +15, +17, +20, +Xq
16	M	73	Humerus (11) P	II	−X
17a*	F	52	Pelvis (8) P	II	+1/**p33-p35,** +2q33-qter, +6p, +7, +8p, +9p, +11, +12q22-qter, +14q23-qter, +15, +16q, +17, +18, +19/**p13.2, q13.2**, +20/**q13.1**, +21, +22
17b*	F	52	Pelvis (8) P	II	−1q31-qter, +3p, −9p, +12/cen-q24.2, +13q13-qter, +14q21-qter, +16q, +18/**q12-q22,** +Xq
18	M	58	Gluteal muscle (NA) M	III	−1p, +1q, +2q, −3p, +3q, +4, +5q, −6, +7, +8, −9p, +11, +12, −13, −22, +X
19	M	51	Femur (NA) P	III	−1p, +1q, +5, +7, +14, +18, +20, +21, +22, +X
20	M	24	Clavicle (4 × 5 × 3) P	III	+1, −2p22-pter, −3p, +3q, −5q, −6, +8q, −9p, +9q, −10, +11, −18, −22
21	M	27	Femur (5 × 3 × 5) P	III	+1pter-q23, +2/**p23-pter**, +3p22-pter, +4q, +5p, +6p/**p22-pter**, −6cen-q22, +6q23-qter, +7p, −7cen-q22, −8p, +8cen-q21.3, −10p, −11, −12p, +13, +14q22-qter, +15q21-qter, +17, +18, +20p, +21, +22, +Xp
22	F	43	Tibia (7 × 7 × 5) P	III	+2p, +6p, −6q, +14q23-qter, +15q23-qter, +16q, +17, +20q, +21, +22
23	M	53	Soft tissue (8 × 9 × 8) P	IV	+1, +2pter-q21, +3p, +4p, +5p, +8q, +9q13-31, +12q15-q22, +14, +15, +17, +20, +22

Gains of DNA sequences are marked with +, losses with −; high-level amplifications are in bold.
F, female; M, male; NA, data not available; P, primary tumor; R, recurrent tumor; M, metastasis.
*Multiple hereditary exostoses.

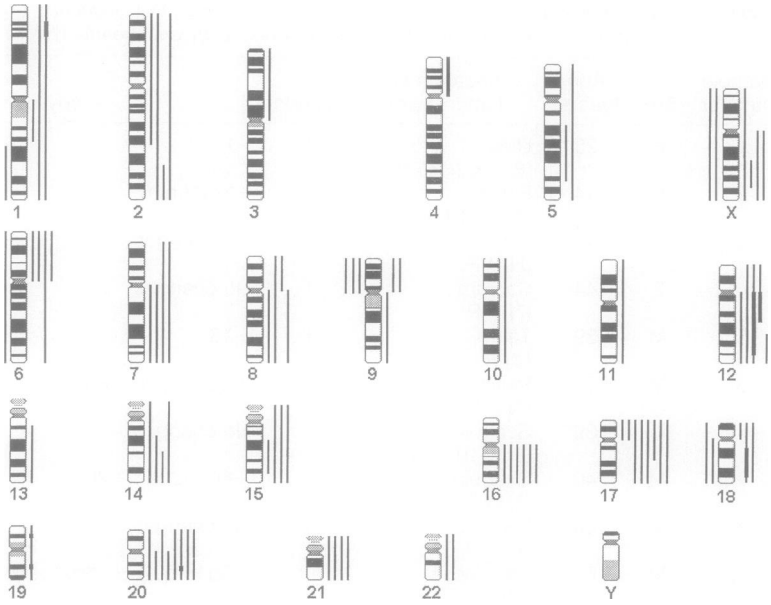

Figure 1. *Summary of gains and losses of DNA sequence copy number in 23 grade I or II CS samples analyzed by CGH. Losses are shown on the* left *and gains on the* right. *Each* line *represents a genetic aberration seen in one sample. High-level amplifications are shown as* thick lines.

gions cannot be evaluated reliably by CGH.[17] DNA copy number changes involving 16p and 19 were excluded from the analysis unless they represented a high-level amplification.[17,18] The exclusion of 16p and 19 was also based on a CGH experiment with reverse-labeled DNAs in randomly selected tumors.

Results

Overview of DNA Copy Number Changes

Of the 29 CS samples, 21 (72%) showed extensive changes (a mean of 6.0 ± 1.2 aberrations per sample; range, 1 to 24). The 8 samples without DNA copy number changes were all grade I CSs (tumors 3 and 4) or grade II CSs (tumors 7a, 8, 9, 11, 12b, and 14b; Table 1). Gains were more frequent than losses (gains:losses = 4.6:1). All chromosomal regions with an increased or decreased DNA sequence copy number are summarized in Figures 1 (CS grades I and II) and 2 (CS grades III-IV), and the most frequent changes are listed in Table 2.

Gains and High-Level Amplifications

Gains of DNA sequence copy number were most commonly observed in chromosomes 1 (28%), 8 (24%), 14 (28%), 15 (24%), 16 (24%), 17 (38%), 20 (38%), and 21 (24%). The most frequent minimal common regions were 20q, 17p, 20p, 1cen-q24, and 14q23-qter. Other minimal common regions of gains are presented in Table 2.

High-level amplifications of small chromosomal regions were found in 17% of the samples: in four grade II CSs (tumors 14a, 15, 17a, and 17b) and one grade III CS (tumor 21). Amplifications affected 12cen-q15 in sample 14a and 4p in sample 15. The two samples of tumor 17 contained different amplified regions involving 1p33-p35, 19p13.2, 19q13.2, and 20q13.1 (17a), and 12cen-q24.2 and 18q12-q22 (17b). In tumor 21 (grade III CS), high-level amplifications were detected at two sites, 2p23-pter and 6p22-pter (Table 2).

Losses

Losses most frequently affected chromosomes 1 (7%), 3 (7%), 6 (17%), 9 (17%), 10 (7%), 18 (10%), 22 (7%), and X (7%). Minimal common regions with the most frequent losses were 6cen-q22, 9p, and 18q. Less frequent losses affected 1p, 3p, 10p, 22, and X (Table 2).

Low-Grade CS (Grades I and II) versus *High-Grade CS (Grades III and IV)*

The mean numbers of DNA sequence copy number changes per sample in low-grade CSs and high-grade CSs were 3.8 ± 1.0 and 14.3 ± 1.9, respectively. In both categories, gains were more frequent than losses (low-grade CS, 78 gains and 9 losses = 8.7:1; high-grade CS, 64 gains and 22 losses = 2.9:1). Thus, the relative frequency of DNA sequence

losses in high-grade CS is three times higher than in low-grade CS.

Multiple Samples from the Same Patients

CGH did not reveal any changes in samples 7a, 12b, and 14b, whereas samples 7b, 12a, and 14a from these patients were found to contain changes. The most likely explanation for the normal findings is that the normal tissue contribution within the samples was greater than 50% of the total DNA content, making it impossible to detect the changes. Changes seen in samples 8b and 17a were different from those detected in samples 8c and 17b. A possible reason for this finding could be different clonal evolution among different tumor specimens (Table 1).

Discussion

Our study reports the first CGH analysis performed on chondrosarcomas. The analysis revealed that 72% of the 29 CS samples showed gains and/or losses of DNA sequences involving at least one, but typically several, different chromosomal regions. On average, the tumors had six copy number aberrations per sample, with almost all chromosome arms affected. Gains of whole chromosomes or chromosome arms were clearly more frequent than losses. Gains were most frequent at 1q, 8q, 12q, 14q, 16q, 17p, 20, and 21. High-level amplifications were rare. A similar complexity has also been seen in conventional cytogenetic studies, but often the complexity makes it impossible to interpret the karyotypes.[3–6]

The minimal common region of gain at 8q (8cen-q21.3) did not appear to include *CMYC* at 8q24.1. Recent findings show that, rather than *CMYC*, other oncogenes of 8q may be involved in the tumorigenesis of osteosarcomas with amplification of 8q.[19] Raskind et al[12] recently reported loss of heterozygosity at two of the three known hereditary multiple exostoses loci (*EXT*) in sporadic CS. Accordingly, the genes at *EXT1* (8q24) and *EXT2* (pericentromeric region of chromosome 11) are likely to have a tumor suppressor function significant in the development of CS. In the present study, we detected no losses of DNA sequences at 8q. Thus, the loss of heterozygosity reported in CS might be caused by mechanisms other than physical loss. It is also possible that the deletions are small and beyond the resolution capacity of CGH.

Gains of DNA sequences in chromosomes 5, 6, and 12, although occurring at low frequencies, affected mainly 5q, 6p, and 12q, and a high-level amplification in 12q was observed in two low-grade tumor samples. Genes encoding several fundamental components of normal cartilage have been mapped to these chromosome arms. The gene for the human cartilage link protein necessary for proteoglycan-hyaluronate aggregation has been assigned to 5q13-q14.1,[20] and the gene for the α_2 chain of fibrillar collagen type XI (*COL11A2*), a minor structural component of cartilage fibrils, has been mapped to 6p21.3.[21] In chromosome 12, the gene for type II collagen (*COLQA1*), a major constituent of normal cartilage, has been mapped to 12q13.[22] Of the seven tumors with an increased copy number at

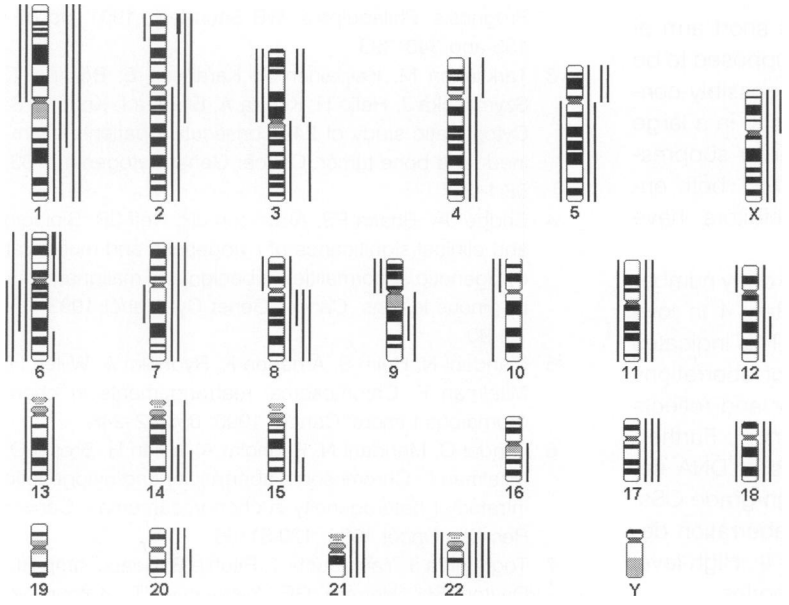

Figure 2. *Summary of gains and losses of DNA sequence copy number in six grade III or IV chondrosarcoma samples analyzed by CGH. Losses are shown on the* left *and* gains *on the right. Each* line *represents a genetic aberration seen in one sample. High-level amplifications are shown as* thick lines.

Table 2. *The Most Frequent Gains, High-Level Amplifications, and Losses of DNA Sequence Copy Number Detected by CGH in 29 Chondrosarcoma Samples*

Gains		High-level amplifications		Losses	
Location	Number of samples (%)	Location	Number of samples (%)	Location	Number of samples (%)
20q	11 (38)	12cen-q15	2 (7)	6cen-q22	5 (17)
17p	11 (38)	1p33-p35	1 (3)	9p	5 (17)
20p	9 (31)	2p23-pter	1 (3)	18q	3 (10)
1cen-q24	8 (28)	4p	1 (3)	1p	2 (7)
14q23-qter	8 (28)	6p22-pter	1 (3)	3p	2 (7)
8cen-q21.3	7 (24)	18q12-q22	1 (3)	10p	2 (7)
15q23-q25	7 (24)	19p13.2	1 (3)	22	2 (7)
16q	7 (24)	19q13.2	1 (3)	X	2 (7)
21	7 (24)	20q13.1	1 (3)		
2p	6 (21)				
2cen-q21	6 (21)				
6p	6 (21)				
7q	6 (21)				
12q15	6 (21)				
12q22	6 (21)				
22	6 (21)				
Xq22-q26	6 (21)				

12q, five showed a gain at 1q, which suggests that simultaneous gains at 1q and 12q might contribute to the development and progression of CS.

In 38% of the samples, a gain was seen at 17p. Similar observations have been reported in osteosarcoma.[19,23] The target gene or genes in these copy number increases are not known at present.

A gain at 20q was detected in 38% of the CS samples. Similar observations have been reported in breast cancer, with an increased copy number at 20q13.[24] As the only highly amplified site of 20q in CS affected the same chromosome band, this location may harbor novel oncogenes.

We observed recurrent losses, albeit rare, mainly in the long arm of chromosome 6 (with the minimal common region 6cen-q22) and in the short arm of chromosome 9. The deletion of 6q is supposed to be associated with regulatory gene loss, possibly contributing to primary cellular transformation in a large number of malignancies,[25] and two tumor suppressor genes, *p16* (*MTS1*) and *p15* (*MTS2*), both encoding cyclin-dependent kinase 4 inhibitors, have been mapped to 9p21.[26]

The mean number of DNA sequence copy number changes per sample was approximately 4 in low-grade CSs and 14 in high-grade CSs. This indicates a clear increase in the total number of aberrations with the increasing grade of the tumor and reflects the aggressiveness of high-grade tumors. Furthermore, the relative frequency of losses of DNA sequences was three times higher in high-grade CSs. All samples that did not contain any aberration detectable by CGH were of grade I or II. High-level amplifications were seen in both categories.

In conclusion, our CGH results reveal several novel chromosomal regions that may contain genes with an important role in the initiation and progression of chondrosarcomas. CGH demonstrated that gains of DNA sequences affected mainly whole chromosomes or chromosome arms, whereas high-level amplifications and losses were rare.

References

1. Mirra JM, Picci P, Gold RH: Bone Tumors: Clinical, Radiologic, and Pathologic Correlations. Philadelphia, Lea and Febiger, 1989, pp 439–690
2. Huvos AG: Bone Tumors: Diagnosis, Treatment, and Prognosis. Philadelphia, WB Saunders, 1991, pp 85–155 and 343–393
3. Tarkkanen M, Kaipainen A, Karaharju E, Böhling T, Szymanska J, Heliö H, Kivioja A, Elomaa I, Knuutila S: Cytogenetic study of 249 consecutive patients examined for a bone tumor. Cancer Genet Cytogenet 1993, 68:1–21
4. Bridge JA, Bhatia PS, Anderson JR, Neff JR: Biologic and clinical significance of cytogenetic and molecular cytogenetic abnormalities in benign and malignant cartilaginous lesions. Cancer Genet Cytogenet 1993, 69: 79–90
5. Mandahl N, Heim S, Arheden K, Rydholm A, Willén H, Mitelman F: Chromosomal rearrangements in chondromatous tumors. Cancer 1990, 65:242–248
6. Örndal C, Mandahl N, Rydholm A, Willén H, Brosjö O, Mitelman F: Chromosome aberrations and cytogenetic intratumor heterogeneity in chondrosarcomas. Cancer Res Clin Oncol 1993, 120:51–56
7. Toguchida J, Yamaguchi T, Ritchie B, Beauchamp RL, Dayton SH, Herrera GE, Yamamuro T, Kotoura Y,

Sasaki MS, Little JB, Weichselbaum RR, Ishizaki K, Yandell DW: Mutation spectrum of the p53 gene in bone and soft tissue sarcomas. Cancer Res 1992, 52: 6194–6199

8. Dobashi Y, Sugimura H, Sato A, Hirabayashi T, Kanda H, Kitagawa T, Kawaguchi N, Imamura T, Machinami R: Possible association of p53 overexpression and mutation with high-grade chondrosarcoma. Diagn Mol Pathol 1993, 2:257–263
9. Wadayama B, Toguchida J, Yamaguchi T, Sasaki MS, Kotoura Y, Yamamuro T: p53 expression and its relationship to DNA alterations in bone and soft tissue sarcomas. Br J Cancer 1993, 68:1134–1139
10. 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 1991, 83:194–200
11. Castresana JS, Barrios C, Gómez L, Kreicbergs A: Amplification of the c-*myc* proto-oncogene in human chondrosarcoma. Diagn Mol Pathol 1992, 1:235–238
12. Raskind WH, Conrad EU, Chansky H, Matsushita M: Loss of heterozygosity in chondrosarcomas for markers linked to hereditary multiple exostoses loci on chromosomes 8 and 11. Am J Hum Genet 1995, 56:1132–1139
13. Cook A, Raskind W, Halloran-Blanton S, Pauli RM, Gregg RG, Francomano CA, Puffenberger E, Conrad EU, Schmale G, Schellenberg G, Wijsman E, Hecht JT, Wells D, Wagner MJ: Genetic heterogeneity in families with hereditary multiple exostoses. Am J Hum Genet 1993, 53:71–79
14. Wu Y-Q, Heutink P, de Vries BBA, Sandkuijl LA, van den Ouweland AMW, Niermeijer MF, Galjaard H, Reyniers E, Willems PJ, Halley DJJ: Assignment of a second locus for multiple exostoses to the pericentromeric region of chromosome 11. Hum Mol Genet 1994, 3:167–171
15. Mertens F, Rydholm A, Kreicbergs A, Willén H, Jonsson K, Heim S, Mitelman F, Mandahl N: Loss of chromosome band 8q24 in sporadic osteocartilaginous exostoses. Genes Chromosomes & Cancer 1994, 9:8–12
16. Kallioniemi A, Kallioniemi O-P, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D: Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992, 258:818–821
17. Kallioniemi O-P, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW, Pinkel D: Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes & Cancer 1994, 10:231–243
18. Kallioniemi A, Kallioniemi O-P, Citro G, Sauter G, DeVries S, Kerschmann R, Caroll P, Waldman F: Identification of gains and losses of DNA sequences in primary bladder cancer by comparative genomic hybridization. Genes Chromosomes & Cancer 1995, 12: 213–219
19. Forus A, Weghuis DO, Smeets D, Fodstad Ø, Myklebost O, van Kessel AG: Comparative genomic hybridization analysis of human sarcomas. II. Identification of novel amplicons at 6p and 17p in osteosarcomas. Genes Chromosomes & Cancer 1995, 14:15–21
20. Osborne-Lawrence SL, Sinclair AK, Hicks RC, Lacey SW, Eddy RL, Byers MG, Shows TB, Duby AL: Complete amino acid sequence of human cartilage link protein (CRTL1) deduced from cDNA clones and chromosomal assignment of the gene. Genomics 1990, 8:562–567
21. Law ML, Chan SDH, Berger R, Jones C, Kao FT, Solomon E, Cheah KSE: The gene for the $\alpha 2$ chain of human fibrillar collagen type XI (COL11A2) assigned to the short arm of chromosome 6. Ann Hum Genet 1990, 54:23–29
22. Takahashi E, Hori T, O'Connell P, Leppert M, White R: R-banding and nonisotopic *in-situ* hybridization: precise localization of the human type II collagen gene (COLqA1). Hum Genet 1990, 86:14–16
23. Tarkkanen M, Karhu R, Kallioniemi A, Elomaa I, Kivioja AH, Nevalainen J, Böhling T, Karaharju E, Hyytinen E, Knuutila S, Kallioniemi O-P: Gains and losses of DNA sequences in osteosarcomas by comparative genomic hybridization. Cancer Res 1995, 55:1334–1338
24. Tanner MM, Tirkkonen M, Kallioniemi A, Collins C, Stokke T, Karhu R, Kowbel D, Shadravan F, Hintz M, Kuo W-L, Waldman FM, Isola JJ, Gray JW: Increased copy number at 20q13 in breast cancer: defining the critical region and exclusion of candidate genes. Cancer Res 1994, 54:4257–4260
25. Meloni AM, Stephenson CF, Li FP, Sandberg AA: del(6q) as possible primary change in malignant mesothelioma. Cancer Genet Cytogenet 1992, 59:57–61
26. Kamb A, Gruis NA, Weaver-Feldhaus J, Li Q, Harshmn K, Tavtigian SV, Stockert E, Johnson BE, Skolnick MH: A cell cycle regulator potentially involved in genesis of many tumor types. Science 1994, 264:436–440