Int. J. Cancer: **109,** 363–369 (2004)

Publication of the International Union Against Cancer

AMPLIFICATION OF CHROMOSOME 1 SEQUENCES IN LIPOMATOUS TUMORS AND OTHER SARCOMAS

Malin NILSSON[1]*, Leonardo A. MEZA-ZEPEDA[2], Fredrik MERTENS[1], Anne FORUS[2], Ola MYKLEBOST[2] and Nils MANDAHL[1]

[1]*Department of Clinical Genetics, University Hospital, Lund, Sweden*
[2]*Department of Tumor Biology, The Norwegian Radium Hospital, Oslo, Norway*

Amplifications and gains involving 1q are common abnormalities in solid tumors. Recently, an amplicon originating from 1q21–23, containing the candidate oncogenes *COAS1*, *COAS2* and *COAS3* (Chromosome One Amplified Sequence) was identified. The presence, distribution and copy number level of extra *COAS* sequences were investigated in 48 bone and soft tissue tumor (BSTT) samples using metaphase FISH analysis. Amplification was seen in 27/48 (56%) samples. With few exceptions, all 3 genes were involved, but on average *COAS2* exhibited higher copy numbers. The presence of extra *COAS* signals, irrespective of copy numbers, was found at similar frequencies in different histologic tumor subtypes. However, medium or high level amplification was common in lipomatous tumors but rare in other, nonlipomatous tumors (9/21 *vs.* 2/27 samples). The most common localization of extra *COAS* signals in lipomatous tumors was in supernumerary ring and giant marker chromosomes. Among nonlipomatous tumors, the distribution of extra *COAS* genes was more disperse, being located in various unidentified chromosomal structures, including double minutes, and only rarely in ring chromosomes. Because *MDM2* is known to be amplified frequently in BSTTs, and in particular in atypical lipomatous tumors, cases with extra copies of *COAS* were studied also with an *MDM2* probe. Twelve out of 18 lipomatous tumors had extra copies of both *COAS* and *MDM2*, and the 2 genes were found to be coamplified and interspersed exclusively in ring and giant marker chromosomes. Also 12 out of 18 nonlipomatous tumors exhibited simultaneous gain of *COAS* and *MDM2*, but colocalization in the same chromosome was less frequent. The role of the frequent coamplification of *COAS*, or some other yet unknown gene in the 1q21–23 region, and *MDM2* remains to be elucidated.

Key words: *COAS1-3*; amplification; sarcoma; marker chromosome; cytogenetics

Bone and soft tissue tumors (BSTT) belong to a heterogeneous group of tumors that involve a large number of histological subtypes and affect persons of all ages.[1] The pattern of chromosome changes, including numerical and structural aberrations, is diverse.[2] Some tumor types exhibit highly specific translocations, occurring in 90–95% of a particular tumor type, such as t(12; 16)(q13;p11) in myxoid liposarcoma and t(X;18)(p11;q11) in synovial sarcoma. The molecular genetic consequence of these translocations is the formation of fusion genes encoding chimeric proteins. Other tumors exhibit characteristic, but not tumor-specific, aberrations, such as supernumerary ring chromosomes and giant marker chromosomes in atypical lipomatous tumors (ALT), low-grade malignant fibrous histiocytomas (MFH) and parosteal osteosarcomas. These BSTTs of borderline malignancy typically exhibit a low or moderate number of chromosomal aberrations. A third category of BSTTs, such as highly malignant osteosarcomas, MFHs and leiomyosarcomas, display an extensive cytogenetic heterogeneity with numerous structural and numerical aberrations.

Cytogenetic signs of gene amplification, *i.e.*, homogeneously staining regions (hsr) and double minutes (dmin), have been detected in less than 5% of BSTTs. However, also rings and giant marker chromosomes frequently contain amplified sequences. Little is known about the genes involved in the amplicons of BSTTs, apart from the extensively studied amplification of the *MDM2* gene in particular, but also of other genes flanking *MDM2* in 12q, such as *SAS*, *GLI, HMGA2* and *CDK4*.[3–8]

Studies of DNA copy number changes in BSTTs by comparative genomic hybridization (CGH)[9] have revealed several chromosome segments containing novel amplicons of interest. Amplifications and gains involving 1q are among the most common abnormalities in solid tumors.[10–12] Extra copies of sequences from chromosome region 1q21–23 have been described to occur frequently in BSTTs.[11,13–16] At further molecular characterization[17] and positional cloning studies of this potentially pathogenetically significant amplicon, 3 candidate oncogenes *COAS1*, *COAS2*, and *COAS3* (Chromosome One Amplified Sequence) were identified.[18] Especially *COAS2*, a member of the cycloporin-binding peptidylpropyl isomeras family, was highly amplified. Among lipomatous tumors, several genes on chromosome 12, including *MDM2*, are frequently coamplified.[9–21] In addition, some of these tumors exhibit amplification of sequences from 1q21–22,[11,14] the chromosome segment where *COAS1-3* are situated.

The localization of the extra *COAS* copies has not been studied, *i.e.*, it is unknown whether they are tandemly repeated at their native position, spread out over several chromosomes or clustered within 1 or a few aberrant chromosomes. By 3-color FISH analyses, using cosmid probes corresponding to the 3 *COAS* genes, we investigated the frequencies of different types of BSTTs exhibiting supernumerary COAS sequences, their chromosomal distribution, and the level of amplification. Tumors with *COAS* amplification were also investigated regarding the distribution and copy number status of *MDM2*.

MATERIAL AND METHODS

Patients

The study was carried out on 48 samples from 47 patients with BSTTs selected after G-band karyotyping (Table I). Except for 1 extraskeletal myxoid chondrosarcoma, none of the tumors had any specific tumor-associated translocation. The vast majority of samples, 30 primary lesions, 13 local recurrences and 5 distant metastases, were from soft tissue tumors, including 20 lipomatous tumors. The age of the patients, 23 men and 24 women, ranged from 24 to 87 years.

Chromosome preparations and G-banding

The cells were short-term cultured, harvested and G-banded as described by Mandahl.[22] In brief, the tumor tissue was disaggregated mechanically and then enzymatically in collagenase II for 3–5 hours. RPMI 1640 medium, supplemented with fetal bovine serum (17%), L-glutamine and antibiotics, was added to the cell suspension. The

Grant sponsor: The Swedish Cancer Society

*Correspondence to: Department of Clinical Genetics, University Hospital, SE-221 85 Lund, Sweden. Fax: +46-46-131061.
E-mail: malin.nilsson@klingen.lu.se

Received 3 June 2003; Revised 26 August 2003; Accepted 16 October 2003

DOI 10.1002/ijc.11716

TABLE I – HISTOPATHOLOGIC AND CLINICAL DATA ON 48 SOFT TISSUE AND BONE TUMORS

Case number	Diagnosis[1]	Source[2]	Age/sex	Size[3] (cm)	Location[3]
	Lipomatous tumors				
1	Lipoma	R	61/M	14	n.k.
2	Lipoma	P	53/F	11	Thigh
3	Atypical lipomatous tumor	P	85/F	9	Thigh
4	Atypical lipomatous tumor	P	75/F	10	Thigh
5	Atypical lipomatous tumor	R	55/F	9	Thigh
6	Atypical lipomatous tumor	R	73/M	n.k.	Retroperitoneal
7	Atypical lipomatous tumor	P	84/F	13	Neck
8	Atypical lipomatous tumor	P	68/M	23	Thigh
9	Atypical lipomatous tumor	P	64/M	25	Thigh
10	Atypical lipomatous tumor	P	67/M	18	Shoulder
11	Atypical lipomatous tumor	R	65/M	18	Thigh
12	Atypical lipomatous tumor	P	83/F	15	Thigh
13	Atypical lipomatous tumor	P	52/M	12	Knee
14	Atypical lipomatous tumor (DDLS, RCLS)	P	55/F	15	Thigh
15	Atypical lipomatous tumor (DDLS, SCLS)	R	85/F	20	Thigh
16	Liposarcoma, sclerosing	P	80/F	13	Upper arm
17a	Liposarcoma, sclerosing	R	64/M	12	Retroperitoneal
17b	Liposarcoma, sclerosing	R	66	7	Retroperitoneal
18	Liposarcoma, dedifferentiated	P	76/M	35	Retroperitoneal
19	Liposarcoma, dedifferentiated	P	77/M	16	Thigh
20	Liposarcoma, dedifferentiated	M	75/F	18	Thigh
	Fibroblastic tumors				
21	Myxofibrosarcoma	P	45/M	23	Lower leg
22	Myxofibrosarcoma	R	64/M	13	Groin
23	Myxofibrosarcoma	P	85/F	14	Hip
	Fibrohistiocytic tumors				
24	Malignant fibrous histiocytoma	P	55/F	11	Thigh
25	Malignant fibrous histiocytoma, inflammatory	P	67/M	3.5	Fingers
26	Malignant fibrous histiocytoma, myxoid	P	72/F	4.5	Knee
27	Malignant fibrous histiocytoma, myxoid	P	41/M	13	Thigh
28	Malignant fibrous histiocytoma, storiform	R	74/M	8	Abdominal wall
	Smooth muscle tumors				
29	Leiomyosarcoma	M	77/F	9	Groin
30	Leiomyosarcoma	R	74/F	8	Elbow
31	Leiomyosarcoma	P	83/M	5	Thigh
32	Leiomyosarcoma	P	87/M	4	Hip
33	Leiomyosarcoma	P	77/F	26	Thigh
34	Leiomyosarcoma	R	65/F	3	Axilla
35	Leiomyosarcoma	P	63/F	10	Thigh
36	Leiomyosarcoma	R	53/F	6	Axilla
37	Leiomyosarcoma, epithelioid	P	49/M	16	Thigh
	Other soft tissue tumors				
38	Mesenchymoma	M	81/F	9	Thigh
39	Myosarcoma, NOS	M	73/M	10	Knee
40	Malignant peripheral nerve sheath tumor[4]	P	30/F	8	Retroperitoneal
41	Malignant peripheral nerve sheath tumor	P	54/M	3	Back
42	Neurofibroma[4]	P	24/F	11	Back
43[5]	Pleomorphic sarcoma, NOS	P	59/F	6	Arm
44	Chondrosarcoma, extraskeletal	M	65/M	6	Groin
	Bone tumors				
45	Chondrosarcoma	P	86/F	2	Femur
46	Chondrosarcoma	P	52/M	0.7	Thorax
47	Chondroblastic osteosarcoma	R	75/M	4.5	Toe

[1]Abbreviations within parentheses indicate the presence of focal components of dedifferentiated liposarcoma (DDLS), round cell liposarcoma (RCLS), or sclerosing liposarcoma (SCLS). NOS = not otherwise specified.–[2]P, primary tumor; R, local recurrence and M, metastasis.–[3]n.k., not known.–[4]Patient with morbus Recklinghausen.–[5]Patient with earlier radiation treated lymphoma.

cells were cultured for 4 to 10 days, in plastic flasks and chamber slides, at 37°C in a humidified atmosphere containing 5% CO_2. Colcemid (0.02 μg/ml) was added to arrest dividing cells in metaphase. Prior to banding, chromosome preparations were treated in 2× SSC (saline sodium citrate) at 60°C to remove remaining cytoplasm. The chromosomes were stained according to a G-banding technique, using Wright's stain solution. The description of chromosome aberrations was according to ISCN (1995).[23]

Probes for FISH

Cosmid probes 37k4, 80N11 and 72i17, representing the genes *COAS1-3*, respectively, have been described earlier.[18] Although all 3 probes are localized to 1q21, signals can be seen at 1p36 as well, mainly for the *COAS2* probe, because of the existence of homologous sequences in this region.[24] *COAS2* is the most telomeric sequence, while *COAS3* is the most centromeric one. In 36 tumors, including all adipocytic cases, an *MDM2* yeast artificial chromosome (YAC) probe, 751a4 (CEPH, Paris), was used whenever possible to investigate coamplification with the *COAS* genes. Human DNA from the YAC clone was amplified by interAlu PCR, using the primers ILA3′ and ILA5′.[25] The probe DNA was directly labeled with Cy3-dUTP, Cy5-dUTP or FluorX-dCTP using Amersham's Megaprime kit (Amersham, Arlington Heights, IL). After labeling, the probe DNA was purified in a Sepharose CL-6B column (Pharmacia, Piscataway, NJ).

FISH analyses

Three-colour metaphase FISH analyses were performed according to the protocol described by Gisselsson,[26] with minor modifications. Posthybridization washing was performed in 0.4× SSC at 72°C for 2 min before an unspecific counterstaining of chromosomes, using 0.5 mg/l 4,6-diamino-2-phenyl-indole (DAPI) (Boehringer Mannheim Biochemicals, Indianapolis, IN) in 2% 1,4-diazabicyclo-[2,2,2]-octan (DABCO) (Sigma Chemical Co., St. Louis, MO). The signals from the probes were detected in an epifluorescence microscope, coupled to a Cytovision ChromoFluor System (Applied Imaging, Newcastle, UK) and a CCD-camera. For each case, 3–25 metaphase cells were analyzed. Signal numbers barely exceeding the number expected in relation to the ploidy level were classified as low-level amplification, the presence of large segments or multiple sites with probe signals corresponding to at least 5 times more signals than expected was designated high-level amplification, whereas signal numbers/intensities between these 2 categories were classified as medium-level amplification. In 9 cases, FISH signals were detected in chromosomal structures that were not included among the clonal aberrations in the karyotype, based on 25 G-banded metaphase cells. This may be explained by cytogenetic heterogeneity.

RESULTS

Out of 48 samples subjected to metaphase FISH analysis, 27 samples (56%) exhibited amplification of 1 or more of the *COAS* genes (Table II). In almost all of these samples, extra copies of all 3 genes were present (Fig. 1). High-level amplification of *COAS*-sequences was seen in 4 samples, whereas amplification at a medium and low level was detected in 6 and 16 samples, respectively. In the remaining sample (case 33), there was a distinct difference between the hypertriploid clone that exhibited low level amplification and the near-hexaploid clone with high level amplification. In case 17, gain of *COAS* sequences was found only in the second of 2 local recurrences studied. In case 28, there was no gain of *COAS1* and in cases 26, 39, 40 and 41, extra copies of *COAS3* were barely detectable. In all other cases, all the *COAS* genes were amplified together. On average, the signal intensity for *COAS2* was higher than for *COAS1* and *COAS3*, and in no case the latter 2 sequences seemed to be amplified at a higher level than *COAS2*.

Although gain of *COAS* sequences was found at similar frequencies among the tumor types analyzed, except smooth muscle tumors, high and medium level amplification was primarily seen in lipomatous tumors (9/21 samples), the only exceptions being 1/5 fibrohistiocytic tumors and 1 clone in 1/9 smooth muscle tumors (Fig. 1*g–h*). In cases 23 and 44, low level amplification was obtained through partial or complete trisomy 1 in near-diploid karyotypes. Among the lipomatous tumors, where 13/21 samples exhibited gain of *COAS* sequences, signals were found in ring chromosomes in 11 samples, in giant marker chromosomes in 9 samples and in other chromosomes in 5 samples (Table II, Fig. 1*d*). Large clusters of signals were frequently seen in rings and giant markers (Fig. 1*a–c*), but ring chromosomes without any detectable *COAS* signals were present in 8 samples. Among the remaining, nonlipomatous tumors, the extra copies of *COAS* sequences were found in various unidentified chromosomes without any signs of signal clustering. Ring chromosomes were completely *COAS*-negative in 5 samples, but positive in cases 28 and 31. *COAS* signals were present in double minutes in 2, possibly 3, samples. Gain of *COAS* sequences was found at similar fractions of primary, locally recurrent and metastatic lesions, *i.e.*, 0.53, 0.54 and 0.60, respectively; none of the latter exhibited medium or high level amplification. The fraction of larger tumors (>5 cm) with gain of *COAS* was 0.58, whereas it was 0.44 in smaller tumors.

MDM2 exhibited amplification in 31 out of 36 cases analyzed (Table II). Simultaneous amplification of *COAS1-3* and *MDM2* was seen in 24 cases (Fig. 1*e–f*). Extra copies of *MDM2* were found in all 18 samples of lipomatous tumors analyzed and in 16/18 samples as high or medium level amplification. Out of 18 nonlipomatous tumors, 5 exhibited no gain of *MDM2* sequences, whereas 7 samples exhibited high or medium level amplification. In case 33, the amplification level of *MDM2* followed that of *COAS* in the 2 clones. Comparisons between copy numbers of *COAS* and *MDM2* revealed that they were similar in 20 samples. In another 15 samples there was a higher number of *MDM2* signals, and only in 1 sample (case 23) did the *COAS* signals outnumber the *MDM2* signals.

DISCUSSION

CGH and molecular genetic studies have revealed an amplicon at 1q12–q22 in different types of BSTTs, leukemias and epithelial tumors.[2,9,11,17,18] Among the sequences included in this amplicon, 3 novel genes *COAS1*, *COAS2* and *COAS3* were identified recently.[18] These genes were found to be amplified at different levels in lipomatous tumors, MFHs and osteosarcomas. However, many of these tumors had been selected because they were known to carry amplification of 1q21–q22. To further investigate the incidence of amplification and distribution of *COAS* among BSTTs, we studied a series of 47 tumors, representing several histotypes, which previously had been characterized by chromosome banding analysis after short-term culturing.

Extra copies of *COAS* genes were found in more than half of the tumors investigated and were present among all histotypes including tumors of both soft tissues and bone. This is consistent with previous findings of frequent partial or complete gain of 1q sequences in BSTTs, as shown by chromosome banding and CGH analyses.[2,9,33] The combined CGH data reveal that gain and/or amplification of chromosome region 1q12–22 is found in about 23% of MFHs, 16% of liposarcomas (including ALT and other subtypes) and 9% of osteosarcomas, which is to be compared to, for example, 4% of alveolar rhabdomyosarcomas and 2% of synovial sarcomas. In our study, no obvious correlations between the presence or absence of supernumerary *COAS* sequences and histological subtype, site, tumor size, tumor status or karyotypic complexity could be seen. Also when subdividing the tumors into 2 groups, lipomatous and nonlipomatous tumors, no difference was found (13/21 *vs.* 14/27 samples with gain of *COAS*). However, medium-high level amplification of *COAS* sequences differed by a factor of 6 (9/21 *vs.* 2/27) in favor of the lipomatous tumors ($p<0.004$, chi-square test).

Among the nonlipomatous tumors, only 1 MFH (case 24) and 1 leiomyosarcoma (case 33) exhibited medium and high level amplification, respectively. In the latter case, there was a dramatic difference in signal numbers between the hypertriploid cell population and the highly polyploid clone, with signals in multiple unidentified chromosomes and structures reminiscent of double minutes (dmin) that had passed undetected at the banding analysis. Whether this amplification was associated with the ploidy shift or a later event in the duplicated clone remains unknown. One other highly polyploid leiomyosarcoma (case 29) had only few extra copies of *COAS*. The only case of leiomyosarcoma with high level amplification was also the largest tumor (26 cm). In a previous CGH study, gain of 1q sequences was reported to be present only in large and very large leiomyosarcomas.[34]

The distribution profiles of supernumerary *COAS* signals exhibited some differences between lipomatous and nonlipomatous tumors. Among the former, they were nonrandomly distributed to ring and giant marker chromosomes, in particular in cases with medium-high level amplification. Only occasionally, and in cases with few extra *COAS* copies, were other chromosomes involved. Ring chromosomes were present in 9 cases of nonlipomatous tumors. Only 2 of these had extra *COAS* copies. In both cases, signals were located in both rings and unidentified chromosomes: in multiple cells in an MFH (case 28) and in sporadic cells in a leiomyosarcoma (case 31). This indicates that inclu-

TABLE II – SIGNAL DISTRIBUTION AND LEVEL OF AMPLIFICATION OF *COAS* AND *MDM2,* AND KARYOTYPIC DATA

Case number	*COAS* amplification			*MDM2* amplification		Karyotype
	Level	Location[1]	*COAS*[2] 1 2 3	Level[3]	Location	
1[4]	Medium	r, mar	1 = 2 > 3	Medium	r, mar, chr	47–49, XY, +1-3r, +1-2mar/93–95, XXYY, +1-3r, +mar/46, XY
2	High	r, mar	2 > 1 = 3	High	r, mar, chr 1, chr	44–48, XX, −4, +1-2r
3	High	r, mar	Equal	High	r, mar	45–49, XX, +1-3r, +mar/46, XX
4[4]	High	r, (chr)	2 > 1 > 3	High	r	47–49, XX, +9, +1-2r/93, XXXX, +r/46, XX
5	High	r, mar	Equal	High	r, mar	91–93, XXX, −X, −3, −4, −5, −6, −?10, −16, −22, +1-2r, +mar/46, XX
6[5]	Low	chr, (r)	Equal	Medium	r, mar	47, XY, +r/46, XY
7	No			High	mar, chr	44–47, XX, +r/83–90, XXXX, +1-3r/46, XX
8	No			High	r	47–48, XY, +1-2r/47, XY, +mar
9	Medium	r	1 = 2 > 3	n.a.		47–49, XY, +1-3r/46, XY
10	No			n.a.		47, XY, +r/46, XY
11	Medium	r, mar	Equal	Medium	r, mar	46–48, X, −Y, +1-3r/89–94, XX, −Y, −Y, +1-6r
12[4]	No			Low	r, mar	47–48, XX, +1-2r, +mar/46, XX
13[5]	No			Medium	r, mar	47–48, XY, +1-2r/46, XY
14	Low	mar, chr, (r)	Equal	High	r, mar, chr	47–49, XX, ?add(7)(p22), add(12)(q13), −14, −16, +1-3r, +mar/46, XX
15[4]	No			Medium	r	45–48, X, −X, +1-2r/74–137, XX, −X, −X, +1-4r, +2mar, dmin, inc
16	Low	mar, chr	Equal	Low	mar, chr	41–44, XX, der(2)t(2; 12)(q37; q13), del(4)(p14), del(11)(q13q21), −12, −12, −13, −14, −17, −18, add(19)(q13), +21, +?add(21)(q22), der(22)t(12; 22)(q13; q12), +2mar/82–88, idemx2/46, XX
17a[5]	No			n.a.		46–48, XY, +1-3r/43–49, X, −Y, der(13; 15)(q10; q10), −22, +1-6r, +1-3mar/50–51, X, −Y, −16, +4-5r, inc
17b	Low	chr, (r)	Equal	High	r, mar, chr	45–49, X, −Y, −13, +1-5r, +1-3mar
18	Medium	mar	2 = 3 > 1	High	mar	44–49, XY, +1-3r, +mar/91–94, XXYY, +4r, +mar/39–81, X?, +1-2r, +2-4mar, inc
19	Medium	r, mar	2 = 3 > 1	Medium	r, chr	42–47, XY, +1-3r/46, XY
20[6]	No			High	r	46–54, XX, der(11)t(11; 14)(p13; q11), −14, −17, −21, +1-4r, inc/47, XX, +X, add(1)(q44), der(5; 19)(q10; p10), ins(7;?)(p15;?), +8, +8, i(9)(q10), add(13)(q32), +14, der(14; 21) (q10; q10)/80–96, idemx2
21	Low	chr	Equal	Medium	chr	42, XY, dic(1;?)(p11;?), −2, der(3)t(3; ?7)(p12; q21), −9, −10, add(12)(p11), add(13)(p11), add(14)(q32), ?der(16)add(16)(p11)del(16)(q22), add(17)(p11), add(21)(p11), −22, −22
22[4]	No			High	r, mar	40–48, X, −Y, del(6)(q15), −9, −10, −?11, −15, +2r, inc
23	Low	chr 1	Equal	No		49–50, X, −X, +1, +der(1; 14)(p10; q10), der(1)add(1)(p22)add(1)(q32)x2, +5, add(5)(p15)x2, +12, ?del(16)(q22), ?dup(17)(q21q25), −18, +19, +22, +mar
24	Medium	chr	Equal	Medium	chr	73–76, X?, add(1)(q11), add(1)(q32), del(1)(q11), ?add(2)(q1?), add(3)(p13), add(4)(p16), del(5)(p13), del(6)(q15), add(12)(p13), del(12)(p12), inc
25	No			No		47–48, XY, +1-2r/94, idemx2
26	Low	mar, chr	1 = 2 > 3	Medium	mar, chr	32–35, X, −X, −1, del(2)(p11), −3, der(7; 15)(q10; q10), −8, add(9)(p11), −10, −11, del(11)(p11), −13, add(14)(p11), add(16)(q13), −17, −18, −19, −20, −21, −22, inc/62–68, idemx2, add(13)(p13)
27	No			No		85–87, X?, del(1)(q42), add(2)(q11), add(3)(q12)x2, ?add(6)(q15), del(7)(q13), del(11)(p13), ?del(11)(p23), ?del(12)(p11), add(15)(q22), add(17)(q25), add(19)(q13)x1-3, inc
28	Low	r, chr	2 = 3	Medium	r	47, XY, +mar/94, idemx2/48, XY, +2r/46, XY
29	Low	chr	Equal	Low	chr	100–200, X?, inc/46, XX
30	No					61–65, X, −X, −X, del(1)(p11), del(1)(q11), +del(1)(q12), −2, −3, −3, −4, −5, −5, −6, del(6)(q13), −7, add(7)(q32), del(7)(p11), −8, −8, −8, −9, −9, −9, −10, −11, del(11)(p14), ?add(12)(q22), del(12)(p12), −13, add(13)(q32), add(16)(q2?), −19, −19, −20, −21, +der(?)t(?;5)(?; q13), inc/46, XX
31	No	(r, chr)				59–63, XY, −X, +Y, −1, −3, del(3)(p12), −8, −9, −9, −10, −10, −13, −14, i(14)(q10), add(15)(p11), −16, −17, −18, −19, add(19)(q13), −20, −22, −22, +hsr(?), +1-3r, inc
32	No					70–134, XY?, add(1)(p11)x2-4, i(1)(p10), add (3)(q21), del(3)(q11), del(3)(p21), add(5)(q31), add(7)(q22), add(11)(p15), add(16)(p13), add(19)(p13), inc/46, XY
33	Low/high[7]	chr, (dmin?)	2 > 1 = 3	Low/high	chr	71–80, XXX, −8, −9, −11, −11, −17, −20, ?+22, +der(?)t(?; 11)(?; q12-13)/120–150, X?, inc/46, XX
34	No					68–71, XXX, del(1)(q12)x2, add(3)(q12), add(5)(p15) del(6)(q11), add(7)(p22), add(9)(q11), del(11)(q12)x2, add(13)(p11), add(17)(p11), ?add(18)(q23), inc
35	No					43–47, X?, +1-4r, inc/83–90, idemx2
36[8]	Low	chr	Equal	n.a.		89–94, XX, add(X)(q24), der(X; ?7)(q10; p10), der(2; ?18)(p10; q10), ?del(3)(q27), ?add(5)(p13), add(6)(q1?1), ?der(10; 14)(q10; q10), add(13)(q32), der(13; 14)(q10; q10)x2, add(16)(q24)x2, der(17)t(9; 17)(p13; p11)x2, add(19)(p13)x2-4, +der(?)t(?; 1)(?; q25), inc
37	No					79–92, XXYY, add(1)(q21)x2, t(11; 14)(q23; q32), −17, inc
38[6]	No			no		38–46, X, −X, der(1)dup(1)(p32p36)add(1)(p36), −13, −16, −17, −18, add(19)(q13), der(20)t(14; 20)(q11; p13)ins(20; ?)(p13; ?), −21, −22, inc/72–76, XX, −X, +1, der(1)dup(1) add(1)x2, −6, −17, add(19), der(20)t(14; 20)ins(20; ?)x2, −22, +r, inc/47, XX, +7/46, XX
39	Low	dmin, (chr), chr 1	1 = 2 > 3	Low	chr	62–63, X, add(X)(p22), −Y, +1, del(3)(p21p23), −4, −5, −5, add(5)(q31), −6, +7, −10, ?der(12)t(7; 12)(q11; p12), −13, −15, −16, −18, −19, add(19)(q13), +20, −21, 1–2dmin, inc
40	Low	dmin, chr, chr 1	1 = 2 > 3	Low	dmin, chr	78–80, XX, −X, +add(1)(p11), +2, +3, del(3)(q25)x2, +4, add(4)(q35)x2, +5, der(5)t(?1; 5)(p32; p15)x2, +6, +7, +8, −9, +11, +12, add(12)q(13)x2, +13, add(14)(q32)x2, −15, −15, +?add(16)(q?), −17, +18, −19, −19, +20, +21, +der(?)t(?; 17)(?; q11)x2, +?r, 2dmin
41	Low	mar, chr	1 = 2 > 3	Low	mar, chr	47, XY, der(2)t(X; 2)(q13; q37), +3, −9, add(15)(q22), dic(17; 19)(p11; p13), +18, +mar/45, X, −Y/46, XY
42	No					36–42, XX, del(13)(q12q13), −16, −17, +r, ?1-4dmin/70–83, idemx2, der(3; 17)(p10; q10), −4, −14, −15/46, XX

TABLE II – SISNAL DISTRIBUTION AND LEVEL OF AMPLIFICATION OF *COAS* AND *MDM2,* AND KARYOTYPIC DATA (CONTINUED)

Case number	*COAS* amplification			*MDM2* amplification		Karyotype
	Level	Location[1]	*COAS*[2] 1 2 3	Level[3]	Location	
43[8]	No			No		71–78, XX, −X, add(1)(q23), add(2)(q31)x1-2, der(2)del(2)(p14)add(2)(q35), −3, der(3) add(3)(p21)add(3)(q26)x2, −4, ?del(4)(q25), −5, −5, −5, −6, add(6)(q23), add(7)(q11), del(7)(p15), −8, −8, −9, −9, −10, add(10)(q24), −11, −11, der(11)add(11)(p15)add(11)(q21), +12, add(13)(p11), add(14)(q32), −15, add(16)(q13), −17, −17, −18, +19, +1-2r, inc
44[9]	Low	chr 1, (chr)	Equal	Low	chr	49, X, −Y, +7, +8, t(9; 22)(q22; q11-12), +12, add(16)(q?), +der(?)t(?;1)(?; q12)
45	Low	chr, (mar)	Equal	Low	chr	46, XX, t(5; 13)(q14; q32)/39–40, X, −X, +2, del(3)(q21), −4, −6, del(7)(q22), −8, add(11)(p11), add(13)(q34), ?i(14)(q10), −15, −15, der(18; 22)(q10; q10), +mar/46, XX
46	Low	mar	Equal	Medium	mar, chr	61–63, X, −X, −Y, −1, −2, −3, −4, +5, add(6)(p23), der(6)t(3; 6)(p23; p21), +7, −8, −9, −9, −10, −11, +12, −13, −16, −17, −18, +19, +20, +20, +21, −22, add(22)(q13)x2, +3mar
47	No					67–71, XY, −X, +Y, +2, −4, −9, −10, −11, +14, +14, −15, −16, −18, +19, +21, −22, +3mar

[1]Presence of *COAS* signals in mar = marker chromosome, r = ring chromosome, dmin = double minutes, chr = other, unspecified chromosome unless specified by a number; () indicates that signals were seen only in a subset of the metaphase cells analyzed.–[2]Semiquantitative estimation of the level of *COAS1, -2* and *-3* amplification by visual inspection.–[3]n.a., not available.–[4]Karyotype reported in Gisselsson et al.[27] tumor 22 is a later local recurrence of the reported case 26.–[5]Karyotype reported in Mandahl et al.[28] tumor 13 was also reported in Gisselsson et al.[29]–[6]Karyotype reported in Mertens et al.;[30] tumor 39 is a distant metastasis of the reported primary Lu 221.–[7]Different levels of amplification in the hypertriploid and more than pentaploid clones.–[8]Karyotype previously reported in Mertens et al.;[31] tumor 37 is a later local recurrence of the reported case 1620–96, and tumor 43 was reported as a spindle cell sarcoma.–[9]Karyotype reported in Panagopoulos et al.[32]

sion of *COAS* in ring chromosomes is associated rather to tumor histotype than to the formation of rings. *COAS* signals were found in dmin in 2, possibly 3, tumors (cases 33, 39 and 40), 2 of which were myogenic sarcomas. In all 3 tumors, signals were found on dmin and unidentified chromosomes and all had a low number of dmin, some of which might represent minute ring chromosomes. Roughly 1.5 % of BSTTs have dmin,[2] but little is known about their gene content, apart from the amplification of tumor specific fusion genes and of *MYCN* in alveolar rhabdomyosarcoma[35] and of *SAS* in MFH.[3] Our study shows that *COAS* genes may also be included in dmin. However, in none of the cases did gain of *COAS* sequences result in high level amplification, indicating that they do not represent the target genes in the amplicon.

Supernumerary rings and giant marker chromosomes, the cytogenetic hallmark of ALTs, invariably contain amplified sequences from 12q, with few exceptions including *MDM2*.[19–21] Material from other chromosomes is frequently present in these chromosomes, in particular sequences from chromosome 1.[20] Our study shows that chromosome 1 sequences are amplified and that the *COAS* genes are part of this amplicon, which is consistent with CGH data showing preferential involvement of 1q21–22.[36,37] All 18 samples of lipomatous tumors that could be analyzed for *MDM2* copy numbers had extra *MDM2* signals. Among the 12 samples with gain of both *COAS* and *MDM2*, the distribution of ectopic signals showed great similarities in the majority of cases. Coamplified signals were primarily seen in supernumerary ring and/or giant marker chromosomes. Complete colocalization was seen in rings (9 cases), and markers (8 cases) but not in other chromosomes. The estimated levels of amplification of *COAS* and *MDM2*, respectively, were similar in the majority of cases (8/12 samples), and in cases showing differences, *MDM2* was amplified at a higher level.

The frequent coamplification and colocalization of sequences from chromosomes 1 and 12 in rings and giant markers in ALTs raises the question whether this is related to the mechanism of formation of these supernumerary chromosomes or to the pathogenetic process. In the present material, absence or presence of extra copies of *COAS* genes could not be correlated with any clinical or histopathologic parameter. The similar number of signals of *COAS1*, *COAS2* and *COAS3* in almost all cases seems to reflect their close native colocalization and does not give any indication of selective amplification or loss in later stages of tumor development. The 2 local recurrences of case 17, obtained within an interval of more than 2 years, demonstrate that *COAS* sequences may be lost or gained during tumor development, which is consistent with the constant remodeling, through breakage-fusion-bridge cycles, of these mitotically unstable structures.[38] In addition, in some ALTs, albeit a minority, with rings and/or giant markers no extra *COAS* copies could be detected, and in samples 6, 14 and 17b, representing 3 of the 4 samples with low level gain of *COAS*, signals were seen only sporadically in rings that invariably exhibited medium or high level amplification of *MDM2*. Finally, it has been shown that elevated *COAS2* expression was seen in pleomorphic and undifferentiated liposarcomas but was almost undetectable in well-differentiated liposarcomas.[18] Currently it is unclear to what extent amplification of the different *COAS* genes contribute to the tumor phenotype and whether there may be other nearby genes that are more consistently amplified. The structure of the 1q amplicon is very heterogeneous, and we are investigating it further using genomic microarrays containing BACs representing the complete tiling path of the relevant segments.

Out of 18 cases of nonlipomatous tumors analyzed with probes for both *COAS* and *MDM2*, 13 had gain of *COAS* sequences. Twelve of these also had extra *MDM2* copies, the only exception being case 23 with gain of *COAS* due to trisomy 1. In 8 of these 12 cases, both *COAS* and *MDM2* signals were present in at least 1 chromosome. The *COAS* amplification level never exceeded that of *MDM2*, whereas the reverse was seen. Thus, the present findings indicate that sequences from chromosomes 1 and 12 frequently end up together in structurally rearranged chromosomes in BSTTs. The significance of simultaneous gain or coamplification of the *COAS* genes and *MDM2* remains unknown and needs further studies to be explained.

FIGURE 1 – Metaphase FISH showing amplification of *COAS1-3* and *MDM2*. (*a–c*) *COAS1* (green), *COAS2* (red) and *COAS3* (yellow) in ALT, case 5, where signals are clustered in a ring (arrowhead) and a giant marker chromosome (arrow). (*d*) *COAS2* (red) and *COAS3* (yellow) in chondrosarcoma, case 44, located on 2 normal chromosomes 1 (long arrows), 1 extra copy of 1q (arrowhead), and 1 unidentified chromosome (short arrow). (*e*) *COAS1* (green) and *COAS3* (yellow) co-amplified with *MDM2* (red) in ALT, case 3, where all signals are seen together in rings (arrowheads), a giant marker chromosome (long arrow) and a marker chromosome (short arrow), while *MDM2* signals are seen on multiple unidentified chromosomes. (*f*) *COAS3* (yellow) co-amplified with *MDM2* (red) in lipoma, case 2, seen in rings (arrowheads) and a giant marker chromosome (arrow). (*g*) *COAS1* (yellow) and *COAS2* (red) amplified at a high level in leiomyosarcoma, case 33. (*h*) The same cell without any probe signals.

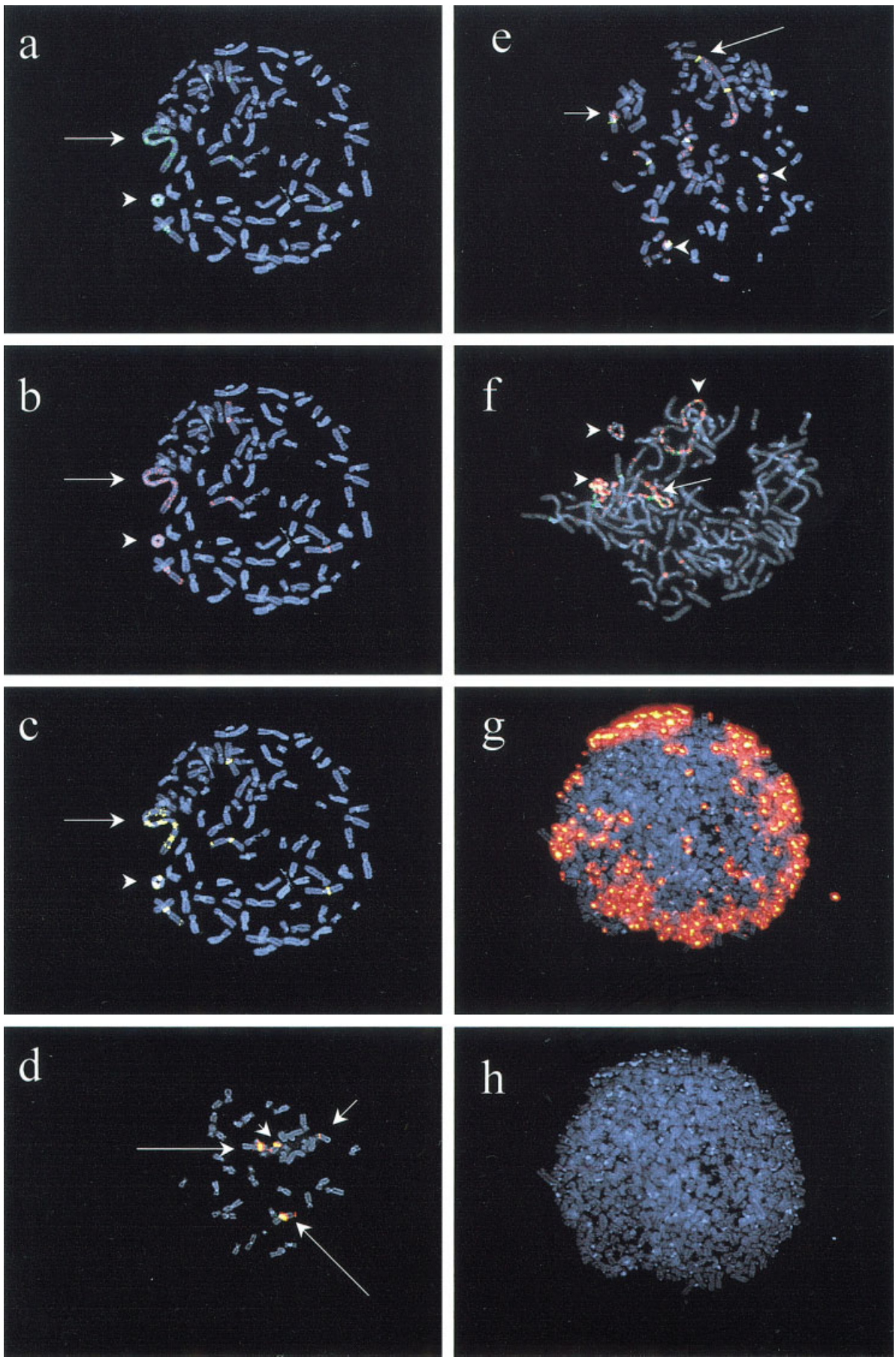

Figure 1.

REFERENCES

1. Fletcher CDM, Unni KK, Mertens F, eds. WHO classification of tumours. Pathology and genetics of tumors of soft tissue and bone. Lyon: IARC Press, 2002. 427p
2. Mitelman Database of Chromosome Aberrations in Cancer. Mitelman F, Johansson B, Mertens F, eds. http://cgap.nci.nih.gov/Chromosomes/Mitelman.
3. Smith S, Weiss S, Jankowski S, Coccia M, Meltzer P. *SAS* amplification in soft tissue sarcomas. Cancer Res 1992;52:3746–9.
4. Wei G, Lonardo F, Ueda T, Kim T, Huvos AG, Healey JH, Ladanyi M. *CDK4* gene amplification in osteosarcoma: reciprocal relationship with *INK4A* gene alterations and mapping of 12q13 amplicons. Int J Cancer 1999;80:199–204.
5. Zhao J, Roth J, Bode-Lesniewska B, Pfaltz M, Heitz PU, Komminoth P. Combined comparative genomic hybridization and genomic microarray for detection of gene amplification in pulmonary artery intimal sarcomas and adrenocortical tumors. Genes Chromosomes Cancer 2002;34:48–57.
6. Berner J-M, Forus A, El Kahloun A, Meltzer PS, Fodstad Ø, Myklebost O. Separate amplified regions encompassing *CDK4* and *MDM2* in human sarcomas. Genes Chromosomes Cancer 1996;17:254–9.
7. Berner JM, Meza-Zepeda LA, Kools PFJ, Forus A, Schoenmakers E, Van de Ven WJM, Fodstad O, Myklebost O. HMGIC, the gene for an architectural transcription factor, is amplified and rearranged in a subset of human sarcomas. Oncogene 1997;14:2935–41.
8. Meza-Zepeda LA, Berner JM, Henriksen J, South AP, Pedeutour F, Dahlberg AB, Godager LH, Nizetic D, Forus A, Myklebost O. Ectopic sequences from truncated *HMGIC* in liposarcomas are derived from various amplified chromosomal regions. Genes Chromosomes Cancer 2001;31:264–73.
9. CGH Database. http://www.helsinki.fi/cmg/CGH-data/Boneandsorf tissue.htm
10. 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;55:1334–8.
11. Forus A, Weghuis DO, Smeets D, Fodstad O, Myklebost O, Guerts van Kessel A. Comparative genomic hybridization analysis of human sarcomas: I. Occurrence of genomic imbalances and identification of a novel major amplicon at 1q21–q22 in soft tissue sarcomas. Genes Chromosomes Cancer 1995;14:8–14.
12. Mertens F, Johansson B, Höglund M, Mitelman F. Chromosomal imbalance maps of malignant solid tumors: a cytogenetic survey of 3185 neoplasms. Cancer Res 1997;57:2765–80.
13. Forus A, Weghuis DO, Smeets D, Fodstad O, Myklebost O, Guerts van Kessel A. 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.
14. Szymanska J, Tarkkanen M, Wiklund T, Virolainen M, Blomqvist C, Asko-Seljavaara S, Tukiainen E, Elomaa I, Knuutila S. Gains and losses of DNA sequences in liposarcomas evaluated by comparative genomic hybridization. Genes Chromosomes Cancer 1996;15:89–94.
15. Szymanska J, Virolainen M, Tarkkanen M, Wiklund T, Asko-Seljavaara S, Tukiainen E, Elomaa I, Blomqvist C, Knuutila S. Overrepresentation of 1q21–23 and 12q13–21 in lipoma-like liposarcomas but not in benign lipomas: a comparative genomic hybridization study. Cancer Genet Cytogenet 1997;99:14–18.
16. Armengol G, Tarkkanen M, Virolainen M, Forus A, Valle J, Böhling T, Asko-Seljavaara S, Blomqvist C, Elomaa I, Karaharju E, Kivioja AH, Siimes MA, Tukiainen E, Caballin MR, Myklebost O, Knuutila S. Recurrent gains of 1q, 8 and 12 in the Ewing family of tumors by comparative genomic hybridization. Br J Cancer 1997;75:1403–9.
17. Forus A, Berner JM, Meza-Zepeda LA, Saeter G, Mischke D, Fodstad O, Myklebost O. Molecular characterization of a novel amplicon at 1q21–q22 frequently observed in human sarcomas. Br J Cancer 1998;78:495–503.
18. Meza-Zepeda L, Forus A, Lygren B, Dahlberg A, Godager L, South A, Marenholz I, Lioumi M, Flörenes V, Maelandsmo G, Serra M, Mischke D, et al. Positional cloning identifies a novel cyclophilin as a candidate amplified oncogene in 1q21. Oncogene 2002;21:2261–9.
19. Forus A, Flörenes V, Maelandsmo G, Fodstad Ö, Myklebost O. The protooncogene *CHOP/GADD153*, involved in growth arrest and DNA damage response, is amplified in a subset of human sarcomas. Cancer Genet Cytogenet 1994;78:165–71.
20. Pedeutour F, Suijkerbuijk R, Forus A, Van Gaal J, Van de Klundert W, Coindre J, Nicolo G, Collin F, Van Haelst U, Huffermann K, Turc-Carel C. Complex composition and co-amplification of *SAS* and *MDM2* in ring and giant rod marker chromosomes in well-differentiated liposarcoma. Genes Chromosomes Cancer 1994;10:85–94.
21. Nilbert M, Rydholm A, Mitelman F, Meltzer P, Mandahl N. Characterization of the 12q13–15 amplicon in soft tissue tumors. Cancer Genet Cytogenet 1995;83:32–6.
22. Mandahl N. Methods in solid tumor cytogenetics. In: Rooney DE. Human Cytogenetics: malignancy and acquired abnormalities, 3rd ed. New York: Oxford University Press, 2001. 165–203.
23. Mitelman F, ed. ISCN (1995) An international system for human cytogenetic nomenclature. Basel: S Karger, 1995. 114p
24. Hardas BD, Zhang J, Trent JM, Elder JT. Direct evidence for homologous sequences on the paracentric regions of human chromosome 1. Genomics 1994;21:359–63.
25. Höglund M, Sidén T, Åman P, Mandahl N, Mitelman F. Isolation and characterisation of radiation hybrids for human chromosome 12. Cytogenet Cell Genet 1995;69:240–5.
26. Gisselsson D. Refined characterization of chromosome aberrations in tumors by multicolor banding and electronic mapping resources. Methods Cell Sci 2001;23:23–8.
27. Gisselsson D, Höglund M, Mertens F, Johansson B, Dal Cin P, Van den Berghe H, Earnshaw WC, Mitelman F, Mandahl N. The structure and dynamics of ring chromosomes in human neoplastic and non-neoplastic cells. Hum Genet 1999;104:315–25.
28. Mandahl N, Mertens F, Willén H, Rydholm A, Kreicbergs A, Mitelman F. Nonrandom pattern of telomeric associations in atypical lipomatous tumors with ring and giant marker chromosomes. Cancer Genet Cytogenet 1998;103:25–34.
29. Gisselsson D, Höglund M, Mertens F, Mitelman F, Mandahl N. Chromosomal organization of amplified chromosome 12 sequences in mesenchymal tumors detected by fluorescence in situ hybridization. Genes Chromosomes Cancer 1998;23:203–12.
30. Mertens F, Fletcher CDM, Dal Cin P, De Wever I, Mandahl N, Mitelamn F, Rosai J, Rydhom A, Sciot R, Tallini G, Van den Berghe H, Vanni R, Willén H. Cytogenetic analysis of 46 pleomorphic soft tissue sarcomas and correlation with morphologic and clinical features: a report of the CHAMP study group. Genes Chromosomes Cancer 1998;22:16–25.
31. Mertens F, Larramendy M, Gustavsson A, Gisselsson D, Rydholm A, Brosjö O, Mitelman F, Knuutila S, Mandahl N. Radiation-associated sarcomas are characterized by complex karyotypes with frequent rearrangements of chromosome arm 3p. Cancer Genet Cytogenet 2000;116:89–96.
32. Panagopoulos I, Mertens F, Isaksson M, Domanski HA, Brosjö O, Heim S, Bjerkehagen B, Sciot R, Dal Cin P, Fletcher JA, Fletcher CDM, Mandahl N. Molecular genetic characterization of the *EWS/CHN* and *RBP56/CHN* fusion genes in extraskeletal myxoid chondrosarcoma. Genes Chromosomes Cancer 2002;35:340–52.
33. Struski S, Doco-Fenzy M, Cornillet-Lefebvre P. Compilation of published comparative genomic hybridization studies. Cancer Genet Cytogenet 2002;135:63–90.
34. El-Rifai W, Sarlomo-Rikala M, Knuutila S, Miettinen M. DNA copy number changes in development and progression in leiomyosarcomas of soft tissues. Am J Pathol 1998;153:985–90.
35. Weber-Hall S, McManus A, Anderson J, Nojima T, Abe S, Pritchard-Jones K, Shipley J. Novel formation and amplification of the PAX7-FKHR fusion gene in a case of alveolar rhabdomyosarcoma. Genes Chromosomes Cancer 1996;17:7–13.
36. Micci F, Teixeira MR, Bjerkehagen B, Heim S. Characterization of supernumerary rings and giant marker chromosomes in well-differentiated lipomatous tumors by a combination of G-banding, CGH, M-FISH, and chromosome- and locus-specific FISH. Cytogenet Genome Res 2002;97:13–9.
37. Pedeutour F, Forus A, Coindre J-M, Berner J-M, Nicolo G, Michiels J-F, Terrier P, Ranchere-Vince D, Collin F, Myklebost O, Turc-Carel C. Structure of the supernumerary ring and giant rod chromosomes in adipose tissue tumors. Genes Chromosomes Cancer 1999;24:30–41.
38. Gisselsson D, Pettersson L, Höglund M, Heidenblad M, Gorunova L, Wiegant J, Mertens F, Dal Cin P, Mitelman F, Mandahl N. Chromosomal breakage-fusion-bridge events cause genetic intratumor heterogeneity. Proc Natl Acad Sci USA 2000;97:5357–62.