ELSEVIER

Microsatellite Markers as Tools for Characterization of DNA Amplifications Evaluated by Comparative Genomic Hybridization

Maija Wolf, Lauri A. Aaltonen, Jadwiga Szymanska, Maija Tarkkanen, Inkeri Elomaa, and Sakari Knuutila

ABSTRACT: *To test the applicability of microsatellite markers in the study of DNA amplifications evaluated by comparative genomic hybridization, we analyzed 55 highly polymorphic microsatellite marker loci from six liposarcoma tumors (seven specimens) and from one atypical lipoma with a gain or high-level amplification at 12q13-22. Twelve-trisomic neoplastic cells from a patient with B-cell chronic lymphocytic leukemia were used as a positive control, in which 74% of informative loci showed allelic imbalance. In every tumor specimen microsatellite marker loci analysis showed allelic imbalance. The amplicons were discontinuous, indicating the presence of separate amplicons in the 12q13-22 region. Not only gains but also losses as well as concomitant gains and losses of alleles were observed. The use of microsatellite markers has several advantages: gene loci as well as flanking DNA loci can be analyzed, it is fast and lends itself to automation, and allows a large number of marker loci to be analyzed simultaneously.* © Elsevier Science Inc., 1997

INTRODUCTION

Comparative genomic hybridization (CGH) has emerged as a powerful method to screen DNA copy number changes [1]. This method has rapidly revealed an increasing number of novel chromosomal areas with DNA copy number losses, gains, and amplifications [2–6]. These chromosomal areas, which usually range from a few megabases to tens of hundreds of megabases in length, have been successfully characterized further with single-copy probes by fluorescence in situ hybridization (FISH) [7, 8]. Although suitable probes, such as P1 clones for FISH, are readily available, the probes are expensive and it is laborious to score the FISH signals. Therefore the aim of our study was to test the usefulness of highly polymorphic microsatellite markers in the characterization of amplified DNA segments (amplicons). Microsatellite markers are tandem repeats of simple sequences that are abundant and well distributed throughout the human genome [9, 10] and have been successfully applied to the study of loss of heterozygosity (LOH) [11]. To evaluate the value of microsatellite markers in characterization of DNA amplifications we selected an amplicon in 12q, a frequent finding in lipomatous tumors [12, 13].

From the Department of Medical Genetics, Haartman Institute, University of Helsinki (M. W., L. A. A., J .S., M. T., S. K.), Department of Oncology, Helsinki University Central Hospital (I.E.), Helsinki, Finland.

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

Received August 13, 1996; accepted August 20, 1996.

MATERIALS AND METHODS

Patients

Seven patients (eight tumors; 1a and 1b from the same patient at a one-year interval) with an amplicon at 12q were selected. The material included three well-differentiated liposarcomas (cases 2, 5, and 6), liposarcoma grades II (case 1b) and III (case 1a), two dedifferentiated liposarcomas (cases 3 and 4) and one atypical lipoma (case 7). Table 1 shows the detailed copy number karyotypes and Figure 1 the average ratio profiles evaluated by CGH [12, 13]. A red-to-green ratio above 1.15 was the limit value for a gain in a chromosomal region. The cut-off value for high-level amplifications was 1.5.

As a positive control we used blood cells from a patient with B-cell chronic lymphocytic leukemia. Eighty percent of his blood cells were neoplastic B cells. CGH showed a gain of whole chromosome 12 (Fig. 1) and a morphology antibody chromosomes (MAC) study indicated that a great proportion of the neoplastic B cells contained the trisomy for chromosome 12 [14].

Microsatellite Markers

Fifty-five microsatellite markers from Généthon were selected for the PCR analysis. All marker loci were localized at

Cancer Genet Cytogenet 93:33–38 (1997)

655 Avenue of the Americas, New York, NY 10010

0165-4608/97/$17.00
PII S0165-4608(96)00291-9

Table 1 Gains and high-level amplifications detected by comparative genomic hybridization at 12q in six liposarcomas and one atypical lipoma

Tumor no[a]	Gain/high-level amplification
1a (7a)	12q/12q15
1b (7b)	12q12-23/12q14-21
2 (1)	12q13-23/12q14-21
3 (11)	12q12-23/12q13-22
4 (12)	12q13-21
5 (3)	12q14-21
6	12q14-22
7 (2)	12cen-q22/12q14-21

[a]In cases 1a–5 the numbers in brackets refer to Szymanska et al. 1996 [12] and in case 7 to Szymanska et al. 1996 [13]. Specimens 1a and 1b were obtained from the same patient at a one-year interval.

12q12-q24 according to the Entrez database (NCBI, NLM, NIH, Bethesda, Maryland, USA), spanning a 109.6 cM region [15]. The average distance between the markers was 1.99 cM.

Amplification of Microsatellite Loci

DNA samples from tumor and normal tissues were amplified by PCR in the following conditions: 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 μM each dATP, dGTP, and dTTP, 2.5 μM dCTP, 0.7 μCi of α-^{32}P-dCTP (3,000 Ci/mmol), 30 ng of each primer, 50 ng of DNA, 0.3 U Dynazyme Taq polymerase (Finnzymes, Espoo, Finland) in a volume of 10 μl. The thermal profile consisted of 27 cycles of 94°C for 30 seconds, 55°C for 75 seconds, 72°C for 15 seconds plus 72°C for 6 min after the last cycle.

The PCR products were subjected to electrophoresis in a 6% polyacrylamide gel. After electrophoresis the gels were dried and exposed to Kodak XAR film.

Blood was used as the normal reference in the liposarcoma patients and in the atypical lipoma patient. Skin fibroblast DNA was used as the normal reference in the control patient.

PCR results were evaluated visually by two researchers. Patients were designated uninformative when homozygous for the locus in question and informative when heterozygous. Allelic imbalance was detected from the heterozygous samples when one of the two alleles in the tumor track was either increased (GCN = gain in copy number) or reduced (LOH = loss of heterozygosity) in intensity relative to the remaining allele and those in the lymphocyte track (Fig. 2). The imbalance was interpreted as LOH or GCN only when one allele was almost totally missing or when a major increase in the intensity of one allele compared to the alleles in the normal tissue was observed. Changes of unidentified nature and changes that were not consistent were interpreted merely as an allelic imbalance. In some cases one of the alleles in the tumor sample showed an increase and the other a decrease in the intensity (Fig. 3). This situation is known as a concomitant gain and loss of alleles (GAL) [16].

To confirm the allelic imbalance detected by eye, we used laser scanning densitometry. An imbalance was observed when the ratios of the intensities between alleles in the tumor sample and the normal sample were different. The

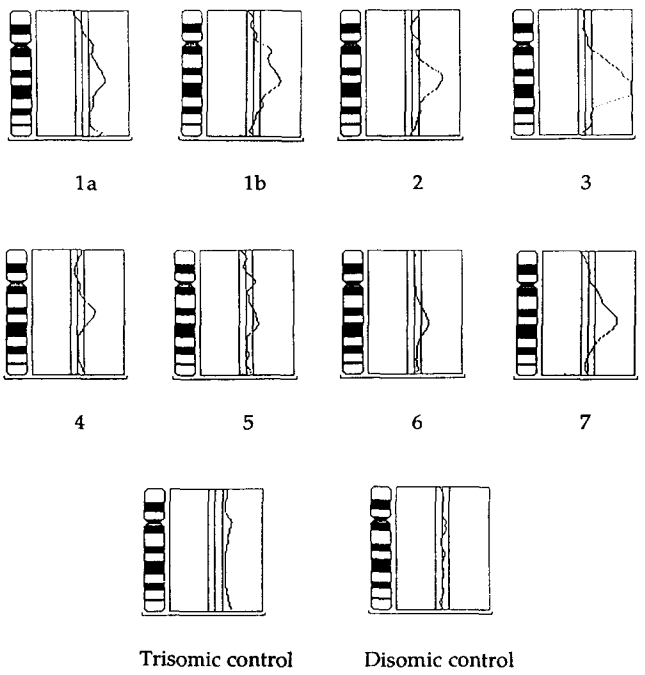

Figure 1 Mean red-to-green ratio profiles for chromosome 12. All cases show a gain or high-level amplification of the q-arm. Trisomic control shows the gain of whole chromosome 12. Disomic control represents a negative control from a sample with normal chromosome 12.

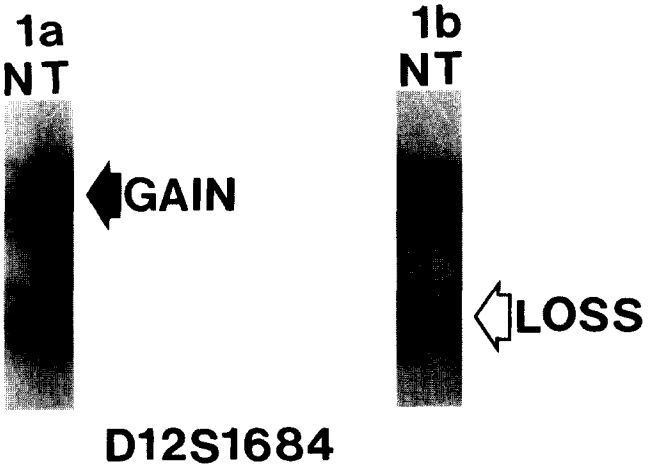

Figure 2 Allelic imbalance observed with marker D12S1684 from tumors 1a and 1b (obtained from the same patient at a one-year interval). Tumor 1a shows a gain of the upper allele when compared to the alleles in the normal tissue. Tumor 1b shows a loss of the lower allele. An allele consists of two fragments, separated by the interval of six nucleotides. N, normal tissue DNA; T, tumor tissue DNA.

Table 2 Intensity ratios determined by laser scanning densitometry between tumor and normal samples from loci with a gain in copy number

	Patients						
Markers	1a	1b	3	5	6	7	Control
D12S85							1.5
D12S359							1.4
D12S312							1.2
D12S83		1.7					2.1
D12S335	3.0	2.5	1.6	2.5	2.9		2.3
D12S313							1.5
D12S1703							1.3
D12S92			2.6				
D12S1711		3.3				7.0	1.9
D12S344						3.1	1.7
D12S350	2.7	2.5					
D12S1684	1.7						
D12S106						14.0	
D12S319					40.0		
D12S311							2.2
D12S95							1.4
D12S327					2.7		1.4
D12S348							1.2
D12S318	5.2	5.9					1.5
D12S78		1.4					1.3
D12S338							1.9
D12S353							1.1
D12S330							2.0
D12S105							1.6
D12S1612							2.0
D12S97							1.6

Figure 3 Concomitant gain and loss of alleles observed with marker D12S1676 from tumor 7. The lower allele shows a gain whereas the upper allele a loss. An allele consists of one clear band. A ladder of bands is seen because of "shadow bands" [28]. N, normal tissue DNA; T, tumor tissue DNA.

quotient of the signal intensity ratio for the alleles in the normal tissue sample and the signal intensity ratio for the alleles in the tumor sample ([n1/n2] /[t1/t2]) was determined so that it was not less than one. To study the consistency of the results, PCR reactions that showed allelic imbalance were performed two times and the results were evaluated separately.

RESULTS

Figure 4 shows the results from visual evaluations of PCR reactions and Table 2 the average values from densitometric measurements from the loci with a gain in copy number. The localization of some genes, which are known to be amplified in sarcomas, has been indicated in Figure 4 [8, 17–26].

All patients were informative in at least 32 loci. The average distance between the informative marker loci was 2.64 cM, which corresponds approximately to a 1.78 Mb long area, when 1 cM is expected to cover 675 kb of physical distance [24]. Seven of the eight samples (1a, 1b, 2, 3, 5, 6, and 7) contained allelic imbalances at several loci, resulting from either a gain of one allele (GCN) or a loss of the other (LOH), or both (GAL). Sample 4 showed an allelic imbalance at one locus only. Amplifications were detected in six tumor samples (cases 1a, 1b, 3, 5, 6, and 7), thus confirming our CGH results. In case 1a the average intensity ratio was 3.2 in amplified loci, 2.9 in case 1b, 2.1 in case 3, 2.5 in case 5, 15.2 in case 6, and 8.0 in case 7. All cases but one (case 1a) also contained allelic imbalances of unidentified nature. Interestingly, loss of heterozygosity was also found in several samples (1a, 1b, 2, 3, and 5). A concomitant gain and loss of alleles was detected in five tumor samples (cases 1a, 1b, 3, 6, and 7).

In case 1a allelic imbalances were present with six markers at a spacing of 44.7 cM. Four of the loci showed a gain of the other allele (D12S335, D12S350, D12S1684, and D12S318), the average intensity ratio being 3.2. In one locus one of the alleles was amplified whereas the other was lost revealing a concomitant gain and loss of alleles (D12S90). The average intensity ratio in this locus was 6.8. One LOH was also detected in this case (D12S353) with the average intensity ratio of 8.7. Case 1b contained an allelic imbalance with 17 markers at a 44.7 cM region. It contained more amplifications and losses than case 1a. Several loci showed also unidentified imbalances in case 1b. Case 2 contained an allelic imbalance with four markers at an 8.2 cM region. In case 3 allelic imbalances were present with 13 markers at a spacing of 25.4 cM. Case 4 contained an allelic imbalance with one marker (D12S313), case 5 with seven markers at a 37.8 cM region, case 6 with five markers at a 21.8 cm region, and case 7 with 10 markers at a spacing of 13.7 cm. The densitometric measurements showed that the average ratio between intensities of the alleles in amplified loci was 5.6. This means that the peak of intensity in the amplified allele was on average 5.6 times higher than in the normal allele. Because PCR is a semiquantitative

	Marker	Distance cM	Tumor 1a	Tumor 1b	Tumor 2	Tumor 3	Tumor 4	Tumor 5	Tumor 6	Tumor 7	Control
	D12S87	9.4	+	+	+	+	+	—	—	—	—
	D12S85	3.1	+	+	+	—	—	+	—	—	●
	D12S339	4.1	+	+	+	+	+	Nd	Nd	Nd	Nd
	D12S359	1.7	+	+	+	+	+	+	+	+	●
	D12S1586	0.6	+	+	+	+	+	+	+	+	○
	D12S1707	0	+	+	—	+	—	—	+	+	•
GADD153,	D12S312	0	+	+	—	—	+	Nd	+	—	●
GLI,	D12S1622	0	+	+	+	—	+	Nd	+	+	—
SAS,	D12S1724	1.7	+	+	+	+	+	Nd	+	+	—
CDK4	D12S90	0.6	◓	◓	+	—	—	+	—	+	—
	D12S1691	2.3	—	—	—	+	+	+	—	—	+
	D12S355	0.7	—	—	—	+	—	+	—	+	+
	D12S329	0	—	—	+	—	—	—	—	+	—
	D12S83	1.7	+	●	+	+	+	○	—	—	●
	D12S1702	0.6	—	—	+	◓	—	○	+	+	+
	D12S335	0.6	●	●	—	●	—	●	●	◓	●
	D12S1676	0.8	—	—	○	+	—	—	+	◓	—
MDM2	D12S313	1.6	+	○	•	+	•	+	+	•	●
	D12S1703	2.4	—	—	—	◓	Nd	—	◓	◓	●
	D12S92	0	—	—	•	●	+	+	+	•	+
	D12S1711	0	+	●	+	+	+	+	+	●	●
	D12S80	0.5	—	—	+	+	+	+	—	•	+
	D12S337	1.3	+	•	—	—	+	•	—	+	+
	D12S344	1.6	—	—	—	○	—	+	—	●	●
	D12S350	0	●	●	—	—	+	○	—	◓	—
	D12S1684	4.9	●	○	○	+	+	○	+	—	•
	D12S106	2.5	—	—	—	•	—	+	—	●	—
	D12S81	0.8	—	—	—	◓	+	+	—	—	—
	D12S319	0	+	+	—	—	—	—	●	+	—
	D12S88	1	—	—	+	◓	—	—	+	—	—
	D12S365	0.7	+	•	+	•	—	+	+	+	—
	D12S311	0	+	+	—	○	+	+	•	+	●
	D12S316	0	+	•	—	+	—	—	+	—	+
	D12S82	0.5	+	○	—	+	+	+	+	+	—
	D12S322	0.1	—	—	—	◓	+	+	—	+	—
	D12S351	0.6	+	•	—	◓	—	+	+	—	•
	D12S95	1.9	+	+	+	+	+	+	+	+	●
	D12S327	2.1	+	+	+	+	+	+	●	—	●
	D12S362	0.9	—	—	—	+	—	Nd	—	+	—
	D12S348	0.1	—	—	+	◓	—	Nd	+	—	●
	D12S309	2.7	+	+	+	+	+	—	—	+	—
	D12S1706	4	+	○	+	—	+	+	+	+	•
	D12S1607	2.5	+	•	+	+	+	+	+	+	—
	D12S318	0.8	●	●	+	+	+	+	+	—	●
	D12S360	0.6	—	—	—	—	+	+	+	—	Nd
	D12S78	0	+	●	+	+	+	+	+	—	●
	D12S338	3.3	—	—	+	+	+	•	+	+	●
	D12S353	0	○	○	—	+	—	—	+	—	●
	D12S330	2.3	+	+	+	+	+	+	+	—	●
	D12S105	7.2	—	—	—	—	—	+	+	+	●
	D12S79	11.4	+	+	+	+	—	—	—	+	—
	D12S76	3.5	+	+	+	+	+	—	+	+	+
	D12S1612	8.5	+	+	+	+	+	—	+	+	●
	D12S1658	11.4	—	—	+	—	—	+	+	—	+
	D12S97		—	—	+	—	—	—	+	+	●

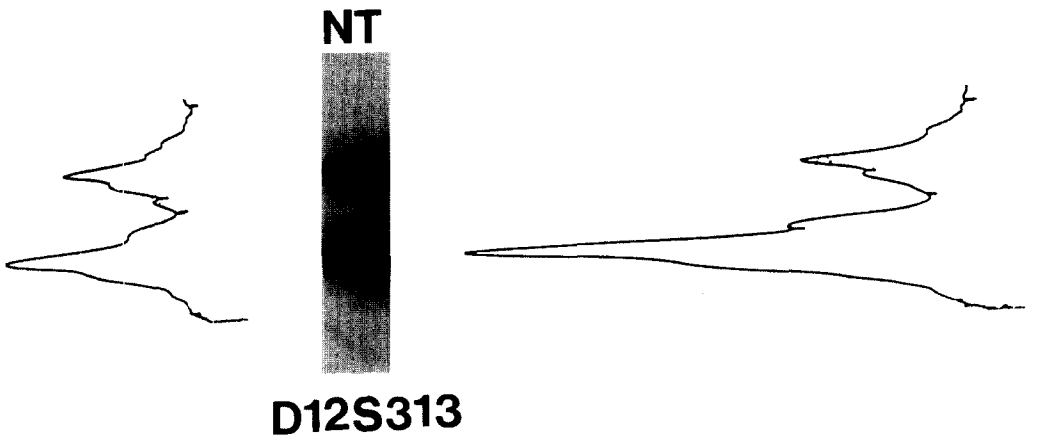

Figure 5 Allelic imbalance observed with marker D12S313 from the control sample. The lower allele shows a gain. An allele consists of one clear band. The ratio of imbalance detected by laser scanning densitometry is 1.5. On the left is the signal intensity profile for the normal sample and on the right is the signal intensity profile for the tumor sample. N, normal tissue DNA; T, tumor tissue DNA.

technique, the value is only relative; it does not imply how many copies the amplified allele in question contains. The highest differences in imbalance were in cases with a concomitant gain and loss of alleles, where the densitometric measurements gave average ratios ranging from 2.9 to infinite, when the other allele was totally missing. The great variation in GAL as well as LOH situations is probably caused by the normal cells in the samples.

Seventy-four percent of the loci analyzed showed an allelic imbalance in the case with trisomy 12 (control sample) and the average ratio of imbalance was 1.6 (Fig. 5).

DISCUSSION

Our results show that microsatellite markers can be used for the study of an amplicon. In all samples this method revealed an amplicon already detected by CGH, except in cases 2 and 7, which, however, contained an allelic imbalance. The method revealed also a large number of loci with LOH and some loci with a concomitant gain and loss of alleles, which had not been detected by CGH. Further, it revealed the discontinuity of the amplicon that cannot be detected by CGH, unless the studied sample contains high-level amplifications at 3-5 Mb interval.

Analysis of the control sample showed that the method is sensitive enough to detect allelic imbalance in 74% of the informative loci when approximately 80% of the cells are trisomic.

If we compare this method with FISH, there are both advantages and disadvantages; concomitant gains and losses of alleles are not found by FISH from interphase nuclei, but FISH makes it possible to estimate the level of amplification and study the cellular morphology, which helps to avoid the risk of contaminating normal cells. Further, the individuals who are homozygous for the locus in question can be studied by FISH.

Our results from the 12q amplicon in liposarcoma have revealed the value of the microsatellite repeat analysis in the studies of DNA amplifications evaluated by CGH [27]. Analysis of microsatellite loci gives a more detailed description of the molecular structure of an amplicon. The method is fast and allows simultaneous investigation of a large number of marker loci. Compared to FISH, it is easier and quicker in the analysis of a large region. Known markers are available for genes as well as for flanking DNA segments. The amount of known $(CA)_n$ markers in the entire human genome is 5,264, with an average 1.6 cM spacing [15], and the primers are favorably priced. Further automation and better quantitation is possible by using a DNA sequencer. By using microdissection before PCR it is possible to select the target area more precisely and prevent contamination by normal cells.

This study was partly supported by the Research Institute of the Helsinki University Central Hospital and the Finnish Cancer Society.

Figure 4 Allelic imbalances found with microsatellite repeat analysis in six liposarcomas and one atypical lipoma. Tumors 1a and 1b were obtained from the same patient at a one-year interval. Some genes and the corresponding markers are indicated. The order of the markers is from centromere to telomere. Distances after markers refer to distance to following marker. Control DNA was extracted from leukemic 12-trisomic cells from a patient with chronic lymphocytic leukemia [9]. −, homozygous, not informative; +, normal heterozygous; •, allelic imbalance of unidentified nature; ●, gain in copy number (GCN); ◓, concomitant gain and loss of alleles (GAL); ○, loss of heterozygosity (LOH); Nd, not determined.

REFERENCES

1. Kallioniemi O-P, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW, Pinkel D (1994): Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosom Cancer 10:231–243.
2. Kallioniemi A, Kallioniemi O-P, Piper J, Tanner M, Stokke T, Chen L, Smith HS, Pinkel D, Gray JW, Waldman FM (1994): Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. Proc Natl Acad Sci USA 91:2156–2160.
3. Tarkkanen M, Karhu R, Kallioniemi A, Elomaa I, Kivioja AH, Nevalainen J, Böhling T, Karaharju E, Hyytinen E, Knuutila S, Kallioniemi O-P (1995): Gains and losses of DNA sequences in osteosarcomas by comparative genomic hybridization. Cancer Res 55:1334–1338.
4. Visakorpi T, Kallioniemi AH, Syvänen A-C, Hyytinen ER, Karhu R, Tammela T, Isola JJ, Kallioniemi O-P (1995): Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization. Cancer Res 55:342–347.
5. Monni O, Joensuu H, Franssila K, Knuutila S (1996): DNA copy number changes in diffuse large B-cell lymphoma—Comparative genomic hybridization study. Blood 87:5269–5278.
6. El-Rifai W, Sarlomo-Rikala M, Miettinen M, Knuutila S, Andersson LC (1996): DNA copy number losses in chromosome 14: An early change in gastrointestinal stromal tumors. Cancer Res 36:3230–3233.
7. Thompson CT, Gray JW (1993): Cytogenetic profiling using fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH). J Cell Biochem 17G:139–143.
8. Mitchell ELD, White GRM, Santibanez-Koref MF, Varley JM, Heighway J (1995): Mapping of gene loci in the Q13-Q15 region of chromosome 12. Chrom Res 3:261–262.
9. Hamada H, Kakunaga T (1982): Potential Z-DNA forming sequences are highly dispersed in the human genome. Nature 298:396–398.
10. Tautz D, Renz M (1984): Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res 12:4127–4138.
11. Jones MH, Nakamura Y (1992): Detection of loss of heterozygosity at the human *TP53* locus using a dinucleotide repeat polymorphism. Genes Chrom Cancer 5:89–90.
12. Szymanska J, Tarkkanen M, Wiklund T, Virolainen M, Blomqvist C, Asko-Seljavaara S, Tukiainen E, Elomaa I, Knuutila S (1996): Gains and losses of DNA sequences in liposarcomas evaluated by comparative genomic hybridization. Genes Chrom Cancer 15:89–94.
13. Szymanska J, Virolainen M, Tarkkanen M, Wiklund T, Asko-Seljavaara S, Tukiainen E, Elomaa I, Blomqvist C, Knuutila S (1997): Overrepresentation of 1q21-23 and 12q13-21 in lipoma-like liposarcomas but not in benign lipomas. A comparative genomic hybridization study. Cancer Genet Cytogenet (in press).
14. Knuutila S, Elonen E, Teerenhovi L, Rossi L, Leskinen R, Bloomfield CD, de la Chapelle A (1986): Trisomy 12 in B cells of patients with B-cell chronic lymphocytic leukemia. New Engl J Med 314:865–869.
15. Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J (1996): A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154.
16. Loupart M-L, Armour J, Walker R, Adams S, Brammar W, Varley J (1995): Allelic imbalance on chromosome 1 in human breast cancer. I. Minisatellite and RFLP analysis. Genes Chrom Cancer 12:16–23.
17. Roberts WM, Douglass EC, Peiper SC, Houghton PJ, Look AT (1989): Amplification of the *gli* gene in childhood sarcomas. Cancer Res 49:5407–5413.
18. Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B (1992): Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature 358:80–83.
19. Smith SH, Weiss SW, Jankowski SA, Coccia MA, Meltzer PS (1992): *SAS* amplification in soft tissue sarcomas. Cancer Res 52:3746–3749.
20. Forus A, Florenes VA, Maelandsmo GM, Meltzer PS, Fodstad Ø, Myklebost O (1993): Mapping of amplification units in the q13-14 region of chromosome 12 in human sarcomas: Some amplica do not include *MDM2*. Cell Growth Differ 4:1065–1070.
21. Khatib ZA, Matsushime H, Valentine M, Shapiro DN, Sherr CJ, Look AT (1993): Coamplification of the *CDK4* gene with *MDM2* and *GLI* in human sarcomas. Cancer Res 53:5535–5541.
22. Florenes VA, Maelandsmo GM, Forus A, Andreassen Å, Myklebost O, Fodstad Ø (1994): *MDM2* gene amplification and transcript levels in human sarcomas: Relationship to *TP53* gene status. J Natl Cancer Inst 86:1297–1302.
23. Forus A, Florenes VA, Maelandsmo GM, Fodstad Ø, Myklebost O (1994): The protooncogene *CHOP/GADD153*, involved in growth arrest and DNA damage response, is amplified in a subset of human sarcomas. Cancer Genet Cytogenet 78:165–171.
24. Krauter K, Montgomery K, Yoon S-J, LeBlanc-Straceski J, Renault B, Marondel I, Herdman V, Cupelli L, Banks A, Lieman J, Menninger J, Bray-Ward P, Nadkarni P, Weissenbach J, Le Paslier D, Rigault P, Chumakov I, Cohen D, Miller P, Ward D, Kucherlapati R (1995): A second-generation YAC contig map of human chromosome 12. Nature 377:321–333.
25. Nilbert M, Rydholm A, Mitelman F, Meltzer PS, Mandahl N (1995): Characterization of the 12q13-15 amplicon in soft tissue tumors. Cancer Genet Cytogenet 83:32–36.
26. Elkahloun AG, Meltzer PS, Guan X-Y, McNinch JS, Trent JM, de Jong PJ (1996): Isolation of a cosmic sublibrary for a region of chromosome 12 frequently amplified in human cancers using a complex chromosome microdissection probe. Genomics 31:343–347.
27. Wolf M, Aaltonen LA, Szymanska J, Tarkkanen M, Blomqvist C, Berner J-M, Myklebost O, Knuutila S (1996): Complexity of 12q13-22 amplicon in liposarcoma: Microsatellite repeat analysis. Genes Chrom Cancer (in press).
28. Hauge XY, Litt M (1993): A study of the origin of "shadow bands" seen when typing dinucleotide repeat polymorphisms by the PCR. Human Molec Genet 2:411–415.