Original Article

Loss of *TP53* in sarcomas with 17p12~p11 gain. A fine-resolution oligonucleotide array comparative genomic hybridization study

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Abstract. The amplification or gain of the p-arm of chromosome 17 is common in sarcomas, suggesting its role in carcinogenesis. Here, we report the architectural structure and targets of 17p aberrations commonly shared by osteosarcoma (OS), leiomyosarcoma (LMS) and malignant fibrous histiocytoma (MFH) of soft tissue. Two low-grade and two high-grade soft tissue LMS, three OS, and two MFH samples were studied using fine-resolution oligonucleotide-based microarray comparative genomic hybridization. Eight of the nine samples showed a loss of 17pter→p13, the locus of tumor suppressor *TP53* preceding the amplified area 17p12→p11.2. The size and detailed architecture of the amplified region of 17p differed between the studied sarcoma entities. OS and high-grade LMS showed similar complex patterns of discontinuous amplifications with regions of gain in between. MFH and low-grade LMS showed continuous regions of gains and amplifications. Precise boundaries of the lost or gained regions were determined, and in addition to the previously suggested targets of the region, *ELAC* and *FLCN* were amplified in all the sarcoma entities.

The complex structural and numerical chromosomal aberrations accompanied by gene copy number alterations are characteristic features of several human sarcomas (Sreekantaiah et al., 1994). Recurrent gains or high-level amplifications at 17p with the minimal common region in 17p12→p11.2 are typical findings in osteosarcoma (OS), leiomyosarcoma (LMS), and malignant fibrous histiocytoma (MFH) of soft tissue, but rare in other malignancies (Knuutila et al., 1998, 2000). Amplifications of 17p12→p11.2 have been reported to occur in 13–32% of high-grade OS (Forus et al., 1995; Tarkkanen et al., 1995, 1999; Lau et al., 2004; Man et al., 2004). Recent studies by chromosomal comparative genomic hybridization (CGH) have shown that chromosome arm 17p is amplified in 24–40% of LMS (El-Rifai et al., 1998; Larramendy et al., 2006; Svarvar et al., 2006) and gained in 16–43% of MFH of soft tissue (Larramendy et al., 1997; Weng et al., 2003).

The suggested targets of the 17p12→p11.2 region in high-grade OS include *PMP22*, *TOP3A*, and *MAPK7* (van Dartel et al., 2002), but so far, to the best of our knowledge, no targets of 17p have been defined in LMS and MFH. To provide a comprehensive overview of the genetic characteristics of chromosome 17p in high-grade OS, high- and low-grade soft tissue LMS, and high-grade MFH of soft tissue, we applied high density fine resolution oligonucleotide array CGH (aCGH). This approach enabled us to determine the exact boundaries of gene amplifications, gains, and losses

Table 1. Clinical and chromosomal CGH (cCGH) data of nine sarcomas analyzed by array-CGH

Sample number[a]	Age/Sex	Tumor type[b]	Tumor grade	cCGH status of chromosome 17p	Reference[c]
1 [2]	36/M	LMS	Low grade	amp(17p)	Larramendy et al., 2006
2 [3]	64/F	LMS	Low grade	amp(17p)	Larramendy et al., 2006
3 [8]	80/F	LMS	High grade	enh(17p), amp(17p12)	Larramendy et al., 2006
4 [10]	46/M	LMS	High grade	amp(17p)	Larramendy et al., 2006
5 [3]	31/M	OS	G4	enh(17p), amp(17p11.2–p12)	Atiye et al., 2005
6 [12]	22/F	OS	G4	enh(17p)	Atiye et al., 2005
7 [8]	16/M	OS	G4	amp(17p)	Atiye et al., 2005
8	63/F	MFH	G4	enh(17), amp(17p11p12)	NPR
9	64/M	MFH	G4	enh(17), amp(17p12)	NPR

[a] Sample number in the reference publication in square brackets.
[b] LMS: soft tissue leiomyosarcoma; OS: osteosarcoma; MFH: malignant fibrous histiocytoma.
[c] NPR: not previously reported.

within the 17p region and to identify possible target genes within the amplified region to understand the genetic events underlying the pathogenicity of 17p amplicon-containing sarcomas. In addition, we searched for possible similarities and differences between the copy number changes in the studied sarcoma entities.

Materials and methods

Tumor specimens
Primary sarcomas with gains at 17p were selected from a previously published collection comprising 62 samples (Tarkkanen et al., 1995; Wolf et al., 1999; Atiye et al., 2005; Larramendy et al., 2006). The selected sample material consisted of two low-grade and two high-grade soft tissue LMS (Larramendy et al., 2006), three high-grade OS (Atiye et al., 2005), and two high-grade MFH of soft tissue. The samples were obtained from the Helsinki University Central Hospital, Helsinki, Finland and the diagnoses were confirmed by histopathological review and appropriate immunohistochemical analysis. DNA was extracted according to standard protocol from formalin-fixed paraffin-embedded tissues. Pooled lymphocytes from healthy male and female individuals were used as reference DNA in hybridizations and as male-to-male and male-to-female hybridization controls required by CGH Analytics software (Agilent Technologies, Palo Alto, CA, USA).

Oligonucleotide array-CGH and data analysis
Array-CGH was performed using oligonucleotide-based human genome CGH microarray 44B (Agilent Technologies). Labeling, hybridizations, and washes of the microarrays were done according to the manufacturer's protocols. Slides were scanned using Agilent's laser confocal scanner and data from the slide images was acquired with Feature Extraction software v8.1 (Agilent Technologies). The gene-level analysis was performed by using Agilent's CGH Analytics software v3.2.32, which shows the aberration copy number levels as fold changes (e.g., +2×, 2-fold gain). Negative fold levels were considered as losses, positive fold level changes as gains, and fold level changes exceeding the +2× level as amplifications. To identify recurrently amplified genes in the different sarcoma entities we compared the averaged copy number ratios of genes in the minimal common region of gain. Symbols, functions, and processes for each gene were retrieved from the NCBI database.

Results and discussion

In the present study we report the fine structure of the p-arm of chromosome 17 in OS, MFH, and LMS. Table 1 presents the clinicopathological data of the samples and the chromosomal CGH (cCGH) results of chromosome 17p. The cCGH data of OS and LMS samples are carried over from previous studies (Atiye et al., 2005; Larramendy et al., 2006), but the data of MFH samples are novel. Oligonucleotide-based array-CGH (aCGH) analysis enabled the detection of copy number changes at gene level, and the boundary regions for the breakpoints could be precisely determined. The DNA copy number profiles of 17p are shown in Fig. 1 and the exact breakpoints for the aberrations in Table 2.

A p-terminal loss was detected in all but one sample (case 8) before the gain/amplification, with *TP53–C17orf25* as the minimal common region and the proximal breakpoint within *TP53–GAS7* (7.5–10.1 Mb from the p-terminus). These copy number losses were not detected by cCGH (Table 1), probably due to its limited resolution. Losses in 17p have been detected in previous studies but no target genes were suggested. Wolf et al. (1999) used microsatellite markers to detect genomic imbalances in various sarcomas and found them in the p-arms of chromosome 17. However, no common region could be determined. Similar distal losses have been reported in a previous aCGH study, in which an 8-Mb region at 17p, containing *TP53*, was recurrently lost in OS (Squire et al., 2003). *TP53* is a well-known tumor suppressor gene involved in a variety of cancers, including OS (Andreassen et al., 1993). Point mutations, allelic losses, and gross gene rearrangements at *TP53* have been shown in OS, LMS, and MFH (Miller et al., 1990; Guo et al:, 1996; Simons et al., 2000; Sandberg and Bridge, 2003; Sandberg, 2005), indicating that it might be the target of loss in these cancer types. However, it is tempting to speculate whether the loss of the *TP53* region occurs before the amplification event or is it a pre-required alteration for the amplification process. In one sample (case 7), very low copy number ratios ap-

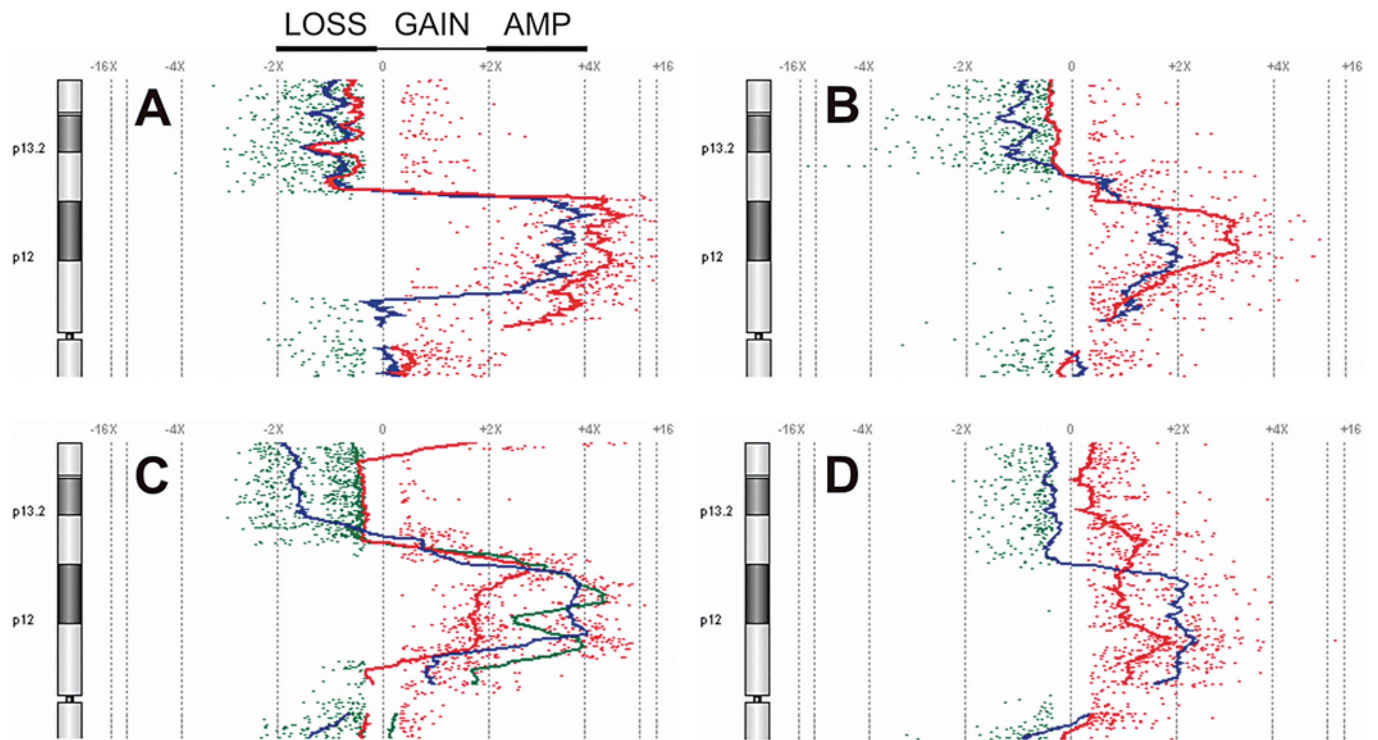

Fig. 1. Copy number levels at 17p in the three different sarcoma entities. The chromosomal positions of the features on the arrays (Y-axis) are plotted against the tumor-to-normal fluorescence intensity ratios (X-axis), and are shown as spots in the figure, as well as their moving averages of 2 Mb in red, blue, and green colour. The intensity ratios are shown as fold changes (for example +2× denotes a 2-fold increase as compared to the normal state). Threshold for loss, gain, and amplifications (AMP) are marked in the figure. Panel (**A**) shows low-grade leiomyosarcomas (sample 1 blue, sample 2 red), Panel (**B**) shows high-grade leiomyosarcomas (sample 3 blue, sample 4 red), Panel (**C**) shows osteosarcomas (sample 5 blue, sample 6 red, sample 7 green), and Panel (**D**) shows malignant fibrous histiocytomas (sample 8 red, sample 9 blue).

proaching homozygosity were observed in a 0.2-Mb segment (six genes) within the *TP53* region, further supporting the importance of *TP53* inactivation during tumorigenesis.

We identified gains and amplifications in 17p12→p11.2 in all samples (Table 2, Fig. 1). In only one OS sample (case 6) a 0.6-Mb segment of the p-terminus was amplified. The copy number profiles at region 17p12→p11.2 in low-grade LMS and MFH were similar with one continuously gained region, whereas the profiles of high-grade LMS and OS resembled each other by showing segments of gains and discontinuous amplifications (Fig. 1). The copy number profiles of the minimal common gained region shared by all samples (*FLJ45455-ULK2*) containing 63 genes are included in Supplementary Data (Appendix 1; www.helsinki.fi/cmg/microarray_data). For detailed information about the genes within the region 17p12→p11.2 in low- and high-grade LMS, OS, and MFH, along with varying log10 ratios between the three sarcoma types, see Appendix 1. Previous reports suggest *SCOI, C17orf48, MAP2K4, COX10, PMP22, ZNF286, NCORI, ADORA2B, ZNF287, CTGLF, COPS3, RASDI, PEMT, TOM1L2, SREBFI*, and *ALKBH5* as potential targets of the gained/amplified region in OS (Squire et al., 2003; van Dartel and Hulsebos, 2004a, b; van Dartel et al., 2004; Atiye et al., 2005). *COPS3*, *PMP22*, *TOP3A*, and *MAPK7* are known to be amplified and overexpressed in OS, and consequently are possible targets in all sarcomas with gain or amplification of 17p12→p11.2 (van Dartel et al., 2002, 2004; van Dartel and Hulsebos, 2004a, b). In addition, *COPS3* has been suggested to be involved in targeting *TP53* to proteasome-mediated degradation (Henriksen et al., 2003). Previous cDNA-based aCGH studies of OS and pooled LMS samples have revealed several common target genes, including *COPS3*, *PMP22*, *TOP3A* and *MAPK7* (Atiye et al., 2005; Larramendy et al., 2006), well in accordance with the present results. Table 3 shows the functions and cellular processes of ten genes that were commonly gained or amplified in all of the studied sarcoma types. Although *ELAC2* and *FLCN* have previously been implicated in cancer, their involvement in sarcoma has not been reported. *ELAC2* has been suggested as a candidate for prostate cancer susceptibility (Simard et al., 2002), whereas germ-line mutations at *FLCN* (alias *BDH*) are known to cause the Birt-Hogg-Dube (BHD) syndrome with predisposition for renal neoplasia (Vocke et al., 2005). Genes that were present in the 17p12→p11.2 region but not commonly amplified among the three studied sarcoma entities are listed in Supplementary Data (Appendix 1; www.helsinki.fi/cmg/microarray_data).

Table 2. DNA copy number gains, losses, and amplifications at 17p in individual samples according to CGH analytics software v3.2.32 (Agilent Technologies)

Sample	Aberration type	Cytogenetic position	Genomic position (Mb from pter)	Size (Mb)	First and last gene
1	Loss	pter–17p13.1	0.0–10.1	10.1	*RPH3AL–GAS7*
	Amplification	17p13.1–p11.2	10.1–18.8	8.7	*MYH13–SLC5A10*
2	Loss	pter–17p13	0.0–10.1	10.1	*RPH3AL–GAS7*
	Gain	17p13.1–cen	10.1–21.4	11.3	*MYH13–cen*
3	Loss	pter–17p13.1	0.0–8.0	8.0	*RPH3AL–ALOEXE3*
	Gain	17p13.1	8.0–8.6	0.6	*HES7–FLJ32734*
	Gain	17p12–p11.2	10.2–21.0	10.8	*MYH13–DKFZp566O084*
4	Loss	pter–17p13.1	0.0–8.3	8.3	*RPH3AL–RPL26*
	Gain	17p13.1–p12	8.3–11.4	3.1	*NDEL1–FLJ45455*
	Amplification	17p12–p11.2	11.4–16.1	4.7	*LOC284033–PIGL1*
	Gain	cen–17p11.2	16.1–21.8	5.7	*PIGL1–cen*
5	Loss	17p13.3–p13.1	0.0–7.5	7.5	*RPH3AL–TP53*
	Gain	17p13.1	7.5–10.3	2.8	*FLJ10385–MYH1*
	Amplification	17p13.1–p11.2	10.3–17.7	7.4	*MYH2–TOMIL*
	Gain	cen–17p11.2	18.6–22.0	3.4	*FLJ36492–cen*
6	Amplification	17pter	0.0–0.6	0.6	*RPH3AL–RNMTL*
	Loss	17p13.3–p13.1	0.6–9.7	9.1	*C17orf25–GLP2R*
	Amplification	17p13.1–p12	9.7–12.3	2.6	*GLP2R–MAP2K4*
	Gain	17p12–p11.2	12.3–18.8	6.5	*FLJ34690–GRAP*
7	Loss	pter–17p13.1	0.0–8.9	8.9	*RPH3AL–NTN1*
	Gain	17p13.1	8.9–9.7	0.8	*NTN1–GLP2R*
	Amplification	17p12	9.8–14.9	5.1	*GAS–FLJ45831*
	Gain	17p12–p11.2	15.0–16.3	1.3	*PMP22–PRR6*
	Amplification	17p11.2	16.3–18.8	2.5	*THC2089016–SLC5A10*
	Gain	17p11	19.2–21.8	2.6	*EPN1–cen*
8	Gain	pter–17p11.2	6.7–22.0	15.3	*MGC49942–cen*
9	Loss	pter–17p12	0.0–11.1	11.1	*RPH3AL–FLJ45455*
	Gain	17p12–p11.2	11.1–22.0	10.9	*FLJ45455–cen*

Table 3. Recurrently gained/amplified genes in amplicon 17p12→p11.2 in low and high grade LMS, OS and MFH

Gene name[a]	GenBank code[a]	Gene function [b,c]	Process [b,c]
DNAH9	AJ404468	ATP binding; ATPase activity; microtubule motor activity; nucleotide binding	Cell motility; microtubule-based movement
ELAC2	AK125030	Endonuclease activity; hydrolase activity; metal ion binding	tRNA processing
COX10	BC000060	Protoheme IX farnesyltransferase activity; transferase activity	Heme biosynthesis
FLJ36674	BC029542	Binding; transporter activity	Transport
FLJ35696	AK093015	ND	ND
FLCN	AF517523	ND	Cell cycle; negative regulation of progression through cell cycle
COPS3	BC035838	Protein binding	ND
ATPAF2	BC004114	Unfolded protein binding	Protein folding
FLJ20308	BC062339	ND	ND
SMCR7	AK128310	ND	ND

[a] According to CGH Analytics v 3.2.32 (Agilent Technologies).
[b] According to the NCBI database (http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi).
[c] ND: not determined.

In summary, we report the structure of 17p in three sarcoma entities (OS, LMS, and MFH). Discontinuous amplicons within gained 17p12→p11.2 regions in OS and high-grade LMS distinguished them from MFH and low-grade LMS with continuous amplifications and gains. OS and high-grade LMS, and low-grade LMS and MFH may possess different mechanisms of amplicon formation. The p-terminal portion of the chromosomal arm (containing *TP53*) was lost in all but one of the cases, suggesting a potential role for *TP53* in sarcomagenesis. Further gene expression studies are required to distinguish the real target amplified genes from the co-amplifiers. The original microarray data will be available at www.cangem.org.

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