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Malignant Astrocytoma-Derived Region of Common Amplification in Chromosomal Band 17p12 Is Frequently Amplified in High-Grade Osteosarcomas

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Recently, we reported a new amplification event that involves marker D17S67 in 17p12 in three malignant astrocytomas of patients with a very short survival. The amplified region may contain an oncogene implicated in astrocytoma tumorigenesis. To determine the extent of the amplified regions, we constructed a yeast artificial chromosome contig spanning the D17S67 region and tested the amplification status of markers that map to the contig. We determined a commonly amplified region between markers D17S1311 and D17S1875 with a maximal length of 1,630 kb. By using marker 745R, from within the commonly amplified region, we screened 60 high-grade astrocytomas but could not detect additional tumors with the amplification event. This suggests that the incidence of the amplification event in high-grade astrocytoma is low (5%). It has recently been shown by comparative genomic hybridization that amplification of 17p11–p12 is a frequent event in high-grade osteosarcomas, occurring in 20–30% of cases. Since the commonly amplified region is within 17p12, we tested 745R in 20 osteosarcomas, including 6 lung metastases, and detected amplification in 9 cases (45%). Marker 745R was found to be amplified in 4 of the 6 lung metastases (66%). From this frequent involvement and the association with clinically aggressive astrocytomas we conclude that for both tumor types presence of the amplification event seems to correlate with aggressive clinical behaviour. *Genes Chromosom. Cancer 18:279–285, 1997.*

INTRODUCTION

The gliomas (astrocytomas, oligodendrogliomas, ependymomas) constitute a heterogeneous group of brain tumors. They are the most frequent primary neoplasms in the central nervous system. The various malignancy grades of astrocytic gliomas (astrocytoma, anaplastic astrocytoma, and glioblastoma multiforme) have been extensively studied for the presence of genetic alterations. The most frequent genetic changes are loss of heterozygosity (LOH) for 17p and *TP53* gene mutation, homozygous loss/inactivation of the *CDKN2* gene on chromosome arm 9p, LOH 10, and amplification of the *EGFR* gene on chromosome arm 7p. LOH 17p and *TP53* gene mutations are found in all malignancy grades. The other lesions are usually restricted to the high-grade astrocytomas (for review see Von Deimling et al., 1995). Next to *EGFR* gene amplification, which occurs in 30–40% of the glioblastomas multiforme, several other oncogenes have been found to be amplified in high-grade astrocytomas: *MDM2*, *CDK4*, and *SAS* as part of a large amplicon on 12q and *MET* on 7q in 10–15% of cases; *GLI*, within the amplicon on 12q, *PDGFRA* on 4q and others at lower frequencies (for review see Collins, 1995). Comparative genomic hybridization (CGH) has recently revealed the existence of amplified regions, notably on 1q, 9q, 12p, 17p, and 22q, which may contain additional oncogenes relevant to astrocytoma tumorigenesis (Fischer et al., 1994; Schröck et al., 1994; Mohapatra et al., 1995).

In a previous communication, we reported the identification of a new amplification event in 17p12 in 3 out of 30 malignant astrocytomas of patients with a very short survival (Bijlsma et al., 1994). The anonymous marker *D17S67* was found to be amplified in all three tumors. Since then, CGH analysis has shown amplification of 17p11–p12 in two out of 10 (20%) glial tumor cell lines (Mohapatra et al., 1995). Interestingly, amplification of 17p11–p12 has been reported to occur with considerable frequency in osteosarcomas (20–30%; Forus et al., 1995b; Tarkkanen et al., 1995), but seems to be an infre-

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quent event in other tumor types, such as soft tissue sarcomas (Forus et al., 1995a). D17S67 has been assigned to 17p12 (Wright et al., 1990) and may therefore be included in the amplified region on 17p in osteosarcomas.

To define more accurately the region that is amplified in the malignant astrocytomas, we determined the amplification status of markers adjacent to D17S67. Since D17S67 is not incorporated into current genetic and physical maps of chromosome arm 17p, we first identified yeast artificial chromosomes (YACs) carrying this marker and constructed a YAC contig encompassing D17S67. By establishing the amplification status of existing and newly developed markers, we were able to define a common region of amplification with a maximal length of 1630 kilobase pairs. To establish the importance of the amplification event, we determined its frequency in an extended series of high-grade astrocytomas and in high-grade osteosarcomas.

MATERIALS AND METHODS

Tumor Samples, DNA Isolation, and Southern Blot Analysis

The histopathological and clinical characteristics of the malignant astrocytomas with D17S67 amplification have been detailed previously (Bijlsma et al., 1994), those of the osteosarcomas are summarized in Table 3.

Genomic DNAs from tumor tissues and peripheral blood leukocytes were extracted according to Müllenbach et al. (1989). Southern blotting to nylon filters and hybridizations with ^{32}P-labeled probes were performed as described previously (Leenstra et al., 1992). Exons 5–8 of the *TP53* gene were sequenced as described in Baker et al. (1990).

Yeast Artificial Chromosome Clones

YAC clones were isolated from the CEPH mega YAC library. They were kindly provided by the YAC Screening Centre Leiden (YSCL). YAC DNAs were prepared in agarose blocks, separated by pulsed field gel electrophoresis, and transferred to nylon filters, essentially as described by Van der Drift et al. (1994). To identify D17S67-positive YACs, the filters were hybridized with ^{32}P-labeled probe LEW503 insert (Barker et al., 1987). YAC insert sizes were determined by hybridizing the filters with ^{32}P-labeled pBR322. Fluorescence in situ hybridization with biotinylated YAC probes on normal metaphase chromosomes was performed according to Hoovers et al. (1992) to check for possible chimerism. The investigated YACs hybridized predominantly to chromosome arm 17p. End clones of YAC inserts were rescued by the vectorette (Riley et al., 1990) or inverse PCR method (Silverman et al., 1991). Partial sequences of the end clones were determined by *Taq* polymerase cycle sequencing with fluorochrome-labeled dideoxy dye terminators and analysis of the sequencing products on a ABI 373 DNA Sequencing System (Perkin Elmer, Applied Biosystems Division). Primers were derived from the sequences for the generation of sequence-tagged sites (STSs).

PCR Conditions

Primer sequences, linkage, and contig information for *TP53*, D6S290, D13S124, D17S804, D17S947, D17S954, D17S969, D17S1311, D17S1692, and D17S1875 were taken from the CEPH-Généthon database, the Genome Data Base at Johns Hopkins University, the Cooperative Human Linkage Center database and the Center for Genome Research at the Whitehead Institute for Biomedical Research database by accession through the Internet.

For determination of LOH in tumor DNAs and STS content of YACs, PCR was performed in a final volume of 15 µl with 10 pmol of each primer, 30 ng genomic or 10 ng YAC DNA, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 200 µM each of dATP, dGTP, and dTTP, 2 µM dCTP, 0.1 ul [α-^{32}P]dCTP (3000 Ci/mMol), 0.001% gelatin, and 0.5 U *Taq*-polymerase. PCR conditions were 1 minute at 94°C, followed by 27 cycles of 1 minute at 94°C, 2 minutes at 55°C, 1 minute at 72°C, and one final incubation for 6 minutes at 72°C. After PCR, each reaction mixture was mixed with an equal volume of loading dye and 2 µl of the diluted mixture was loaded onto a 6% acrylamide gel. After electrophoresis, the PCR products were visualized via autoradiography for 1–3 hours at −80°C.

Determination of the Amplification Level of Markers

The protocol described in the previous section was also used for PCR-based determination of the amplification level of markers. For this purpose, master mixes were made with all components, except for the primers, for each test DNA (extracted from tumor, t) and for two control DNAs (extracted from blood leukocytes, l1 and l2). PCR was performed on each template in a final volume of 15 µl after mixing with a primer pair specific to a target marker (tar) or one of the two reference markers (D6S290 and D13S124, ref1 and ref2,

respectively). To ensure reproducibility, each PCR for a specific template-marker combination was performed twice. In control experiments, we determined that the respective target marker/ reference marker mean intensity ratios in the control DNAs differed by less than 25%. Only duplo signals of comparable intensities were used in the calculation of the amplification level of the target marker by the following formula:

$$\left\{ \frac{tar^t}{ref1^t} \times \frac{ref1^{l1}}{tar^{l1}} + \frac{tar^t}{ref1^t} \times \frac{ref1^{l2}}{tar^{l2}} + \frac{tar^t}{ref2^t} \times \frac{ref2^{l1}}{tar^{l1}} + \frac{tar^t}{ref2^t} \times \frac{ref2^{l2}}{tar^{l2}} \right\} \Big/ 4$$

in which tar^t represents the mean autoradiographic signal intensity generated by duplo PCR of the target marker in the tumor, etc. Signal intensities were quantified with a Phosphorimager (Molecular Dynamics) using Imagequant Software v.3.0. Amplification levels of 2.5 and higher were taken as evidence of true amplification (cf. Rollbrocker et al., 1996).

The amplification level for 745R was also determined on Southern blots according to a previously described procedure (Bijlsma et al., 1994). In short, the PCR-product was labeled with [α-^{32}P]dCTP and hybridized to Southern blots of *Eco*RI- or *Msp*I-digested tumor and reference (blood leukocytes) DNAs. The resulting autoradiographic signal (*Eco*RI fragment of 5.7 kb or *Msp*I fragment of 1.5 kb) was quantified with the Phosphorimager. Amplification levels were normalized for differences in sample loading by comparing the signal intensities obtained by hybridizing probe pVAW210M1, which identifies locus D17S115 on chromosome arm 17q.

RESULTS

YAC Contig of the Region Encompassing *D17S67* in 17p12

Marker D17S67 has been assigned to chromosomal band 17p12 (Wright et al., 1990). According to the CEPH YAC contig map of the human genome, this band is most probably represented in a contig between physical map positions 0.24 and 0.34 on chromosome 17 (Chumakov et al., 1995; Dib et al., 1996). To detect YACs that contain D17S67, we hybridized probe LEW503 (specific to D17S67) to blots of YACs selected from this contig. Initially, two positive YACs (745_f_5 and 838_b_10) were found. Additional D17S67-positive (821_f_3) and D17S67-negative YACs (822_b_11 and 899_g_8) were identified by testing of YACs that flank or overlap 745_f-5 and 838_b_10. These were selected on the basis of information provided by the Whitehead Institute database. To obtain additional nearby markers for the amplification studies, we isolated end clones of the inserts of all YACs, except for 821_f_3. We were unsuccessful in obtaining left-end clones. The right-end clones were converted into STSs by determination of their sequence and design of primers for PCR. The primer sequences are listed in Table 1.

TABLE 1. Characteristics of Isolated End Clones

End clone	Sequence	PCR product (bp)
822R	5′-CTTGGCTGAATTCTTTCTCCC-3′ 5′-AAAGGAAGGGAAGCAGAG-3′	168
899R	5′-ACGTTCCCATTAGAGCCCTT-3′ 5′-GCAGATCTCAGAAGCAGACTCA-3′	101
838R	5′-GCATCCACAGGTTCCAG-3′ 5′-GACCATGTCACAAAAAAGTG-3′	192
745R	5′-TTGGGTTTGGAACAAGAAGG-3′ 5′-GAGCTGGACATTTTCTCCCA-3′	164

Using the listed primers, we determined the STS content of each YAC by PCR analysis. Since the available data from the Whitehead Institute database and the Généthon human linkage map (Dib et al., 1996) suggest that markers D17S969, D17S1311, D17S1692, and D17S1875 may be located within the relevant region, these were also included in the PCR analysis. By combining all data, we could derive the most probable order of markers in the contig, which is shown in Figure 1.

Minimal Region of Common Amplification

To determine accurately the extent of the amplified region in the tumors, we established the amplification status of the markers that flank D17S67. Initially, we tried to use the PCR products of the respective markers as hybridization probes on Southern blots of the tumor DNAs. However, due to inefficient hybridization and the presence of repetitive sequences, causing background hybridization, marker-specific signals could not be generated by this procedure. The only exception was 745R, for which amplification levels of 22, 7, and 7 were found in tumor 1197, 1672, and 1683, respectively. The amplification status of the other markers, and also of *745R*, was determined by differential PCR. The results are summarized in Table 2. A representative autoradiograph, showing the preferential amplification of D17S969 and 745R but not of D17S1311 and D17S1875 in tumor 1197 relative to

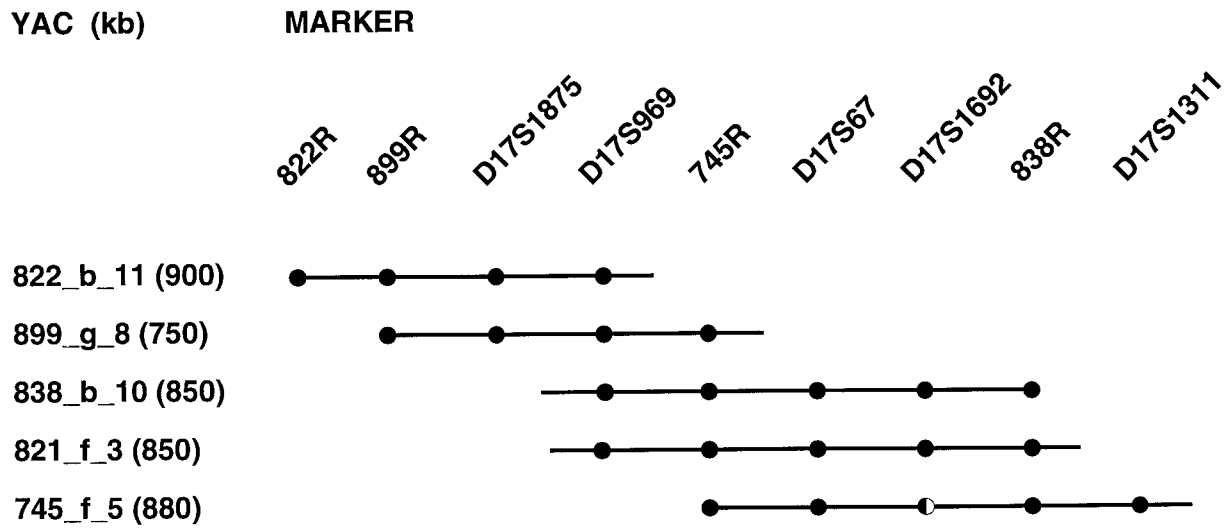

Figure 1. YAC contig of the region encompassing *D17S67* in 17p12. YAC names and estimated insert sizes (in parentheses) are indicated on the left. Each YAC is represented by a horizontal line. The marker content of each YAC was determined by PCR. Filled circle denotes presence of the marker on the YAC. Half-filled circle denotes presence of the marker on the basis of a PCR signal with reduced intensity. The positions of *D17S67* and *D17S1692* are interchangeable.

normal tissue (leuk1) is displayed in Figure 2. The PCR-based amplification levels of 745R in tumors 1197, 1672, and 1683 (9.6, 3.7, and 3.4, respectively) were approximately two times lower than those obtained by Southern blot analysis.

From the amplification profiles in Table 2 we conclude that the minimal region of common amplification is between D17S1875 and D17S1311. As shown in Figure 1, this region may include the total length of YACs 899_g_8 and 745_f_5, i.e., has a maximum length of 1630 kb.

Loss of Heterozygosity on 17p and *TP53* Gene Mutation

By using restriction fragment length polymorphism-based markers, we detected previously homozygous loss of the *TP53* gene in tumor 1197 and LOH for markers proximal and distal to the amplified segment in tumor 1672 (Bijlsma et al., 1994). Here we have applied highly informative microsatellite markers to define better the LOH profiles in the three tumors. We found additional LOH at locus D17S947 at the proximal side of the amplified segment in tumor 1197 (see Table 2). Moreover, we could confirm the LOH at loci flanking the amplified region in tumor 1672. In addition, this tumor now displayed LOH for the *TP53* gene. We sequenced exons 5–8 of the remaining *TP53* allele and detected a G → A transition in codon 237 in exon 7, which results in the replacement of methionine by isoleucine in the *TP53* protein (data not shown). Heterozygosity was retained at loci adjacent to the amplified segment of tumor 1683 and exons 5–8 of the *TP53* gene were not mutated in this tumor.

Frequency of the Amplification Event in High-Grade Astrocytomas and Osteosarcomas

To establish the frequency of the amplification event in 17p12, we determined the amplification status of 745R in 60 high-grade astrocytomas (anaplastic astrocytomas and glioblastomas multiforme). This series included the 30 tumors that were previously screened for D17S67 amplification (Bijlsma et al., 1994). The amplification status of 745R was determined by Southern blot analysis. Apart from the already identified tumors, no other astrocytomas with 745R amplification were found (data not shown). CGH experiments have shown that 17p11–p12 is amplified in 20–30% of high-grade osteosarcomas (Forus et al., 1995b; Tarkkanen et al., 1995). Since 745R is within 17p12, we screened a series of osteosarcomas for amplification of this marker. Only limited amounts of tissue were available for molecular analysis. For that reason, the amplification status of 745R was determined by differential PCR. As shown in Table 3, we detected significant amplification of 745R (3.2–12-fold) in 9 of 20 (45%) cases. Remarkably, of the six lung metastases in this series, four displayed amplification of 745R.

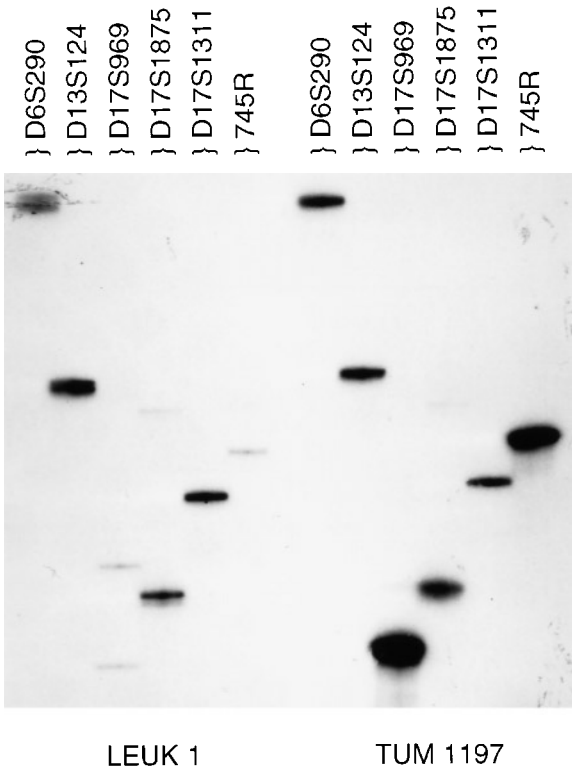

Figure 2. Autoradiograph showing determination of the amplification level of markers D17S969, D17S1875, D17S1311, and 745R in tumor 1197. PCR was performed on control DNA (leuk 1, **left)** or tumor 1197 DNA (**right)** with primer pairs specific to the reference markers D6S290 and D13S124 and the target markers D17S969, D17S1875, D17S1311, and 745R. Each PCR was performed twice. The ^{32}P-labeled PCR products of each primer-template combination were run pairwise in a sequencing gel and visualized by autoradiography. The signals were quantified with a Phosphorimager and used to calculate the amplification level for each target marker, as described in Materials and Methods. Note that control DNA (leuk 1) is not the corresponding leukocyte DNA of tumor 1197. The autoradiographic signals for D6S290 and D17S969 in control DNA are split because of heterozygosity at these loci.

DISCUSSION

The derivation of a physical map of the region encompassing D17S67 and the identification of markers flanking D17S67 (Fig. 1) allowed us to establish the extent of the amplified regions in the three malignant astrocytomas. By combining the amplification profiles (Table 2), we could derive a minimal region of common amplification that is bordered by D17S1311 at the proximal side and D17S1875 at the distal side and which has a maximal length of 1,630 kb. This is the total length of the two overlapping YACs, 745_f_5 and 899_g_8, on which D17S1311 and D17S1875, respectively, have been localized. Its minimal length is determined by the actual physical distance between the two markers. Because of the general instability of YACs, this distance cannot be derived accurately from the YAC contig. It needs to be determined from a corresponding bacterial clone contig, the construction of which is underway.

TABLE 2. Amplification Status of Markers in and around 17p12 in Malignant Astrocytomas

		Tumor		
Marker	Position[a]	1197	1672	1683
TP53		D[b]	LOH	−[c]
D17S804	0.23	−	LOH	−
D17S954	0.24	−	LOH	−
D17S1875	0.26	− (2.4)	− (1.0)	− (0.5)
D17S969		+ (6.8)	+ (3.1)	+ (2.8)
745R		+ (9.6)	+ (3.7)	+ (3.4)
D17S1692		+ (10.6)	+ (3.2)	+ (3.1)
838R		+ (18)	+ (4.6)	ND[d]
D17S1311		− (1.0)	+ (3.1)	+ (2.6)
D17S947	0.34	LOH	+ (2.5)	− (0.6)
D17S58		−	LOH	−

[a]Position (in Morgans) on the Généthon genetic linkage map (Dib et al., 1996).
[b]D: homozygous deletion.
[c]+: Amplification; −: no amplification for D17S1875, D17S969, 745R, D17S1692, 838R, D17S1311, and D17S947 (amplification level in parantheses), no LOH for TP53, D17S804, D17S954, D17S1875, and D17S58.
[d]ND: not determined because of limited availability of tumor DNA.
The homozygous deletion of the *TP53* gene in tumor 1197 and the LOH data for D17S58 have been reported previously (Bijlsma et al., 1994).

TABLE 3. Amplification Status of Marker 745R in High Grade Osteosarcomas

Case no.	Location	Amplification[a]
1	right femur	+ (4.2)
2	right femur	−
3	lung metastasis	−
4	left femur	+ (4.0)
5	lung metastasis	+ (3.2)
6	right tibia	−
7	left tibia	+ (6.9)
8	right femur	+ (3.4)
9	right femur	−
10[b]	right os ilium	−
11	right femur	−
12	lung metastasis	+ (5.5)
13	right fibula/tibia	−
14	right femur	−
15	lung metastasis	+ (4.5)
16	lung metastasis	+ (12)
17	left femur	+ (7.4)
18	right humerus	−
19	lung metastasis	−
20	left femur	−

[a]+, Amplification (level in parentheses); −, no amplification (level of 2.5 or less).
[b]Intermediate grade osteosarcoma.

In tumors 1197 and 1672, we now demonstrate LOH at loci directly flanking the amplified segment on 17p (Table 2). As discussed earlier (Bijlsma et al., 1994), these observations support an amplification model in which excision of the DNA to be amplified results in the loss or rearrangement of that chromosome. The excised DNA is amplified extra-chromosomally before integration at a new position in the genome (cf. Passananti et al., 1987; Heard et al., 1991). LOH at loci adjacent to an amplified region has also been reported for amplification of the 12q13–q14 amplicon in malignant gliomas (Reifenberger et al., 1995), although this finding could not be confirmed by others (Rollbrocker et al., 1996). As reported previously (Bijlsma et al., 1994), the *TP53* gene is homozygously deleted in tumor 1197. In tumor 1672 we now detect loss of one copy of the *TP53* gene. The remaining copy carries a missense mutation in codon 237. The same mutation was found in a glioma cell line and it was demonstrated that this mutation results in functionally mutant p53 (Van Meir et al., 1994). Earlier we have shown that these tumors carry multiple genetic aberrations (Bijlsma et al., 1994). The new observations substantiate our original supposition, based on work of others (Livingstone et al., 1992), that the absence of functional p53 induces genetic instability, including the amplification events on 17p, in these tumors. We could not detect LOH on 17p in tumor 1683, nor could we find a mutation in exons 5–8 of the *TP53* gene in this tumor. Among other possibilities, this would suggest that other mechanisms of amplification are operative in this tumor.

The commonly amplified region in 17p12 may harbour an oncogene involved in the development of high-grade astrocytomas. However, the amplification event was found only in 3 of 60 high-grade tumors (5%), indicating that amplification of this presumptive oncogene region is not a major genetic event in these tumors. CGH analysis has revealed frequent amplification of the chromosomal region 17p11–p12 in osteosarcomas (Forus et al., 1995b; Tarkkanen et al., 1995). Since the commonly amplified region lies within 17p12, we investigated whether it is implicated in the amplification process in osteosarcomas. By using marker 745R, we examined 20 tumors, including 6 lung metastases, and found significant amplification levels in 9 cases (45%, Table 3). From this frequent involvement, we conclude that amplification of the presumptive oncogene region in 17p12 is an important genetic event in the development of osteosarcomas. We detected amplification in 4 of the 6 cases of lung metastases (66%). In case of the astrocytomas, the amplification event was only found in very aggressive tumors, as judged from the very short survival of the patients (i.e., less than one month, Bijlsma et al., 1994). These data indicate that for both tumor types presence of the amplification event correlates with aggressive clinical behaviour. CGH analysis of the osteosarcomas is underway and has already resulted in the conformation of the amplification event in the lung metastases of cases 12 and 15 (J. Bras et al., unpublished data). It remains to be seen whether the amplification events on 17p in osteosarcoma can be of help in further narrowing the minimal region of common amplification. On the one hand, the CGH-detected amplified regions seem to be quite large, involving two chromosomal bands on 17p. On the other hand, we detected amplification events in a higher percentage of the osteosarcomas than by CGH (45% and 20–30%, respectively). This suggests that in some cases the amplified region is small and possibly undetectable by CGH. It will be of interest to determine amplification profiles in these tumors to establish the extent of the amplified segment in 17p12. Finally, to gain more insight into the mechanism of the amplification process, we will determine in these osteosarcomas the *TP53* gene status and the LOH status of loci flanking the amplified segment.

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REFERENCES

Baker SJ, Preisinger AC, Jessup JM, Paraskeva C, Markowitz S, Willson JK (1990) p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. Cancer Res 50:7717–7722.

Barker D, Wright E, Fain P, Goldgar D, Skolnick M, Latt S, Willard H (1987) Thirty new chromosome 17 DNA markers. Cytogenet Cell Genet 46:576.

Bijlsma EK, Leenstra S, Westerveld A, Bosch DA, Hulsebos TJM (1994) Amplification of the anonymous marker D17S67 in malignant astrocytomas. Genes Chromosom Cancer 9:148–152.

Chumakov IM, Rigault P, Le Gall I, Bellanné-Chantelot C, Billault A, Guillou S, Soularue P, Guasconi G, Poullier E, Gros I, Belova M, Sambucy J-L, Susini L, Gervy P, Glibert F, Beaufils S, Bui H, Massart C, de Tand M-F, Dukasz F, Lecoulant S, Ougen P, Perrot V, Saumier M, Soravito C, Bahouayila R, Cohen-Akenine A, Barillot E, Bertrand S, Codani J-J, Caterina D, Georges I, Lacroix B, Lucotte G, Sahbatou M, Schmit C, Sangouard M, Tubacher E, Dib C, Fauré S, Fizames C, Gyapay G, Millasseau P, NGuyen S, Muselet D, Vignal A, Morissette J, Menninger J, Lieman J, Desai T, Banks A, Bray-Ward P, Ward D, Hudson T, Gerety S, Foote S, Stein L, Page DC, Lander ES, Weissenbach J, Le Paslier D, Cohen D (1995) A YAC contig map of the human genome. Nature 377S:175–279.

Collins VP (1995) Gene amplification in human gliomas. Glia 15:289–296.

Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morisette J, Weissenbach J (1996) A comprehensive genetic map

of the human genome based on 5,264 microsatellites. Nature 380:152–154.

Fischer U, Wullich B, Sattler H-P, Göttert E, Zang KD, Meese E (1994) Coamplification on chromosomes 7p12–13 and 9q12–13 identified by reverse chromosome painting in a glioblastoma multiforme. Hum Genet 93:331–334.

Forus A, Olde Weghuis D, Smeets D, Fodstad O, Myklebost O, Geurts van Kessel A (1995a) 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 Chromosom Cancer 14:8–14.

Forus A, Olde Weghuis D, Smeets D, Fodstad O, Myklebost O, Geurts van Kessel A (1995b) Comparative genomic hybridization analysis of human sarcomas: II. Identification of novel amplicons at 6p and 17p in osteosarcomas. Genes Chromosom Cancer 14:15–21.

Heard E, Williams SV, Sheer D, Fried M (1991) Gene amplification accompanied by the loss of a chromosome containing the native allele and the appearance of the amplified DNA at a new chromosomal location. Proc Natl Acad Sci U S A 88:8242–8246.

Hoovers JMN, Mannens M, John R, Bliek J, van Heyningen V, Porteous DJ, Leschot NJ, Westerveld A, Little PF (1992) High resolution localization of 69 potential human zinc finger protein genes: A number are clustered. Genomics 12:254–263.

Leenstra S, Troost D, Westerveld A, Bosch DA, Hulsebos TJM (1992) Molecular characterization of areas with low grade tumor or satellitosis in human malignant astrocytomas. Cancer Res 52:1568–1572.

Livingstone LR, White A, Sprouse J, Livanos E, Jacks T, Tlsty TD (1992) Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 70:923–935.

Mohapatra G, Kim DH, Feuerstein BG (1995) Detection of multiple gains and losses of genetic material in ten glioma cell lines by comparative genomic hybridization. Genes Chromosom Cancer 13:86–93.

Müllenbach R, Lagoda PJL, Welter C (1989) An efficient salt-chloroform extraction of DNA from blood and tissues. Trends Genet 5:391.

Passananti C, Davies B, Ford M, Fried M (1987) Structure of an inverted duplication formed as a first step in a gene amplification event: Implications for a model of gene amplification. EMBO J 6:1697–1703.

Reifenberger G, Reifenberger J, Ichimura K, Collins VP (1995) Amplification at 12q13–14 in human malignant gliomas is frequently accompanied by loss of heterozygosity at loci proximal and distal to the amplification site. Cancer Res 55:731–734.

Riley J, Butler R, Ogilvie D, Finniear R, Jenner D, Powell S, Anand R, Smith JC, Markham AF (1990) A novel, rapid method for the isolation of terminal sequences from yeast artificial chromosome (YAC) clones. Nucleic Acids Res 18:2887–2890.

Rollbrocker B, Waha A, Louis DN, Wiestler OD, von Deimling A (1996) Amplification of the cyclin-dependent kinase 4 (CDK4) gene is associated with high cdk4 protein levels in glioblastoma multiforme. Acta Neuropathol 92:70–74.

Schröck E, Thiel G, Lozanova T, du Manoir S, Meffert MC, Jauch A, Speicher MR, Nürnberg P, Vogel S, Jänisch W, Donis-Keller H, Ried T, Witkowski R, Cremer T (1994) Comparative genomic hybridization of human malignant gliomas reveals multiple amplification sites and nonrandom chromosomal gains and losses. Am J Pathol 144:1203–1218.

Silverman GA, Jockel JI, Domer PH, Mohr RM, Taillon-Miller P, Korsmeyer SJ (1991) Yeast artificial chromosome cloning of a two-megabase-size contig within chromosomal band 18q21 establishes physical linkage between BCL2 and plasminogen activator inhibitor type-2. Genomics 9:219–228.

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.

Van der Drift P, Chan A, van Roy N, Laureys G, Westerveld A, Speleman F, Versteeg R (1994) A multimegabase cluster of snRNA and tRNA genes on chromosome 1p36 harbours an adenovirus/SV40 hybrid virus integration site. Hum Mol Genet 3:2131–2136.

Van Meir EG, Kikuchi T, Tada M, Li H, Diserens A-C, Wojcik BE, Huang H-J S, Friedmann T, de Tribolet N, Cavenee WK (1994) Analysis of the p53 gene and its expression in human glioblastoma cells. Cancer Res 54:649–652.

Von Deimling A, Louis DN, Wiestler OD (1995) Molecular pathways in the formation of gliomas. Glia 15:328–338.

Wright EC, Goldgar DE, Fain PR, Barker DF, Skolnick MH (1990) A genetic map of human chromosome 17p. Genomics 7:103–109.