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Malignant fibrous histiocytoma of bone: Analysis of genomic imbalances by comparative genomic hybridisation and C-MYC expression by immunohistochemistry

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ABSTRACT

Malignant fibrous histiocytoma (MFH) of bone is a rare, highly malignant tumour. As very little is known about its genetic alterations, 26 bone MFHs were analysed by comparative genomic hybridisation (CGH). Twenty-three tumours (89%) had DNA sequence copy number changes (mean, 7.2 changes per sample). Gains were more frequent than losses (gains: losses = 2.5:1). Minimal common regions for the most frequent gains were 8q21.3-qter (35%), 9q32-qter (35%), 7q22-q31 (35%), 1q21-q23 (31%), 7p12-pter (31%), 7cen-q11.2 (31%) and 15q21 (31%). Minimal common regions for the most frequent losses were 13q21-q22 (42%) and 18q12-q22 (27%). High-level amplifications were detected in 8 out of the 26 tumours (31%). The only recurrent amplifications, 1q21-q23 and 8q21.2-q22, were present in two samples (8%). As copy number increase at 8q24 (the locus of *C-MYC*) was frequent, the expression of *C-MYC* was studied by immunohistochemistry. Increased levels of c-myc protein were detected in 7 out of 21 tumours studied (33%). 81% of the samples studied both by CGH and immunohistochemistry showed concordant results. Furthermore, the findings of the present study were compared to previous publications on osteosarcoma, soft tissue MFH and fibrosarcoma of bone. Clear differences were detected in CGH aberration patterns, further supporting the concept of bone MFH as an individual bone tumour entity. Finally, the findings of the present study reflect well the high malignancy and aggressive nature of bone MFH.

1. Introduction

Malignant fibrous histiocytoma (MFH) of bone is a very rare, highly aggressive bone tumour. It accounts for less than 2% of all primary bone sarcomas.[1] It may occur at any age but is more common in adults,[2] in contrast to osteosarcoma. The most common sites of involvement are the metaphyseal regions of distal femur, proximal tibia and proximal femur.[3]

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Histologically MFH of bone is identical to MFH of soft tissue. It is usually composed of spindle cells sometimes arranged in a storiform pattern.[1,3] Giant tumour cells are also common. Histologically it may be difficult to distinguish MFH of bone from fibroblastic osteosarcoma. In these cases osteoid tumour formation favours the diagnosis of osteosarcoma. Without chemotherapy the prognosis of bone MFH is poor, with a 5-year survival rate of 15%.[4] With the addition of neoadjuvant chemotherapy, selected series have shown improved disease-free survival at rates of 67%, 69% and 59%.[5–7]

To the best of our knowledge and also according to the Mitelman Database of Chromosome Aberrations in Cancer,[8] only six cytogenetically analysed MFHs of bone have been published previously.[9–13] These karyotypes were characterised by great variation in chromosome number and extreme karyotypic complexity but without any similarities in their chromosomal aberrations. Specific genes in bone MFH have been analysed only sporadically. Based on the limited data published so far, it appears that *TP53* and *RB1* can be affected similarly to other sarcoma types[14,15] and that c-MET expression is very rare in bone MFH.[16]

More is known about genetic changes in soft tissue MFH. Karyotypes are usually complex with multiple numerical and structural rearrangements. Typical cytogenetic findings include telomeric associations, ring chromosomes, dicentric chromosomes, double minutes and homogeneously staining regions.[10,17,18] No diagnostically significant aberrations have been detected.[10,17,18] Amplification of 12q sequences is common and *SAS*, *CDK4* and *MDM2* are perhaps the most frequently amplified genes.[19]

In our previous studies of soft tissue MFH,[20] osteosarcoma[21] and fibrosarcoma of bone,[22] comparative genomic hybridisation (CGH) revealed several novel sites of DNA sequence copy number changes. The aim of the present study was to screen bone MFHs by CGH for DNA sequence copy number changes and to compare these to the findings in soft tissue MFH,[20] osteosarcoma[21] and fibrosarcoma of bone.[22] When CGH revealed that copy number increases at 8q are frequent in bone MFH, including the *C-MYC* locus, the expression of *C-MYC* was also studied by immunohistochemistry.

2. Materials and methods

2.1. *Patients and tumour specimens*

Twenty-six bone MFH samples, 17 from the Rizzoli Institute (Nos. 1–16, Table 1) and nine from the Helsinki University Central Hospital (Nos. 17–24), were included in the study. Tumours were selected on the basis of availability of representative paraffin or fresh frozen tissue samples. Post-irradiation sarcomas were not included. Twenty-two samples represented primary tumours and four samples were from metastases (Table 1).

Table 1 – Clinical and histologic characteristics of 26 malignant fibrous histiocytomas of bone

Case number[a]	Sex, age at diagnosis	Tumour type[b]	Type of sample and operation	Location	Tumour cell percentage in paraffin blocks
1	M, 66	P	Biopsy#	Humerus	>90
2	F, 47	P	Resection	Femur	>90
3a	M, 14	P	Biopsy*	Humerus	>90
3b		M	Excision	Lung	80
4	F, 73	P	Biopsy#	Femur	>90
5	F, 43	P	Resection	Fibula	>90
6	F, 33	P	Biopsy*	Femur	>90
7±	F, 67	P	Resection	Sternum	>90
8±	M, 44	P	Biopsy	Humerus	90
9	F, 44	P	Resection	Femur	80
10	M, 50	P	Biopsy*	Tibia	>90
11	F, 75	P	Amputation	Fibula	>90
12	M, 8	P	Biopsy*	Humerus	80
13	M, 47	P	Biopsy*	Scapula	80
14	M, 18	P	Biopsy*	Femur	>90
15±	F, 40	P	Resection	Vertebra	>90
16	M, 28	P	Amputation	Femur	70
17	M, 48	P	Biopsy#	Femur	Fresh tumour tissue
18	F, 64	M[c]	Biopsy#	Lymph node of inguinal region	90%
19	M, 37	P	Biopsy#	Femur	70%
20	F, 53	P	Biopsy*	Femur	Fresh tumour tissue
21	M, 39	P	Biopsy#	Femur	Fresh tumour tissue
22±	M, 64	P	Biopsy*	Pelvis	>90%
23±	M, 55	M	Resection	Spine	80%
24a	M, 17	P	Biopsy*	Tibia	>90%
24b		M	Resection	Lung	>80%

Amputation or * resection followed the biopsy thus confirming the histologic diagnosis.
a Samples 3a–3b and 24a–24b are from the same patient; ± = metastatic disease at diagnosis.
b P = primary tumour; M = metastasis.
c Primary tumour in femur which developed after a fracture complicated by osteomyelitis.[23]

All samples were of high grade. The mean age of patients was 45 years (range, 8–73 years). The proportion of male patients was slightly higher than that of female patients (male:female ratio, 1.4:1). Five patients had metastatic disease at diagnosis (Nos. 7, 8, 15, 22 and 23, Table 1). One patient had a predisposing bone condition (No. 18, Table 1) as the tumour arose in a previous site of fracture and osteomyelitis.[23]

The diagnosis was based on radiology and histological analysis. Histological diagnosis was based on biopsy specimen and/or subsequent resected tumour specimen obtained at definitive surgery in all but one patient (No. 8, Table 1). The histology showed a highly cellular tumour, composed of spindle cells and in some cases large tumour cells with abundant cytoplasm. Numerous sections from the resection specimens were studied to exclude the possibility of a spindle cell osteosarcoma.

DNAs were extracted from paraffin blocks in 23 samples and from fresh tumour tissue in three samples, using standard methods.

2.2. *Comparative genomic hybridisation*

Comparative genomic hybridisation (CGH) was carried out as described previously.[24] Briefly, tumour DNA was labelled with FITC-dUTP (DuPont, Boston, MA, USA) and normal sex-matched DNA with Texas red-dUTP (DuPont) by nick translation. The hybridisation mixture contained 400 ng labelled tumour DNA, 400 ng labelled normal DNA and 20 μg cot-1-DNA (Gibco BRL, Life Technologies, Gaithersburg, MD, USA) dissolved in 10 μl of hybridisation buffer. Hybridisations on normal human metaphase spreads were carried out in 2–3 days. After washes the chromosomes were counterstained with DAPI in an antifade solution.

Hybridisations were analysed using an Olympus fluorescence microscope and the ISIS digital image analysis system (MetaSystems GmbH, Altlussheim, Germany) based on an integrated high-sensitivity CCD camera and automated CGH analysis software. Three-colour images (green for tumour DNA, red for normal DNA, and blue for DAPI) were captured from 12 metaphases in each sample. Chromosomal regions were interpreted as over-represented when the green-to-red ratio was higher than 1.17 (gains) and under-represented when the green-to-red ratio was lower than 0.85 (losses). These limits were based on control experiments with two differentially labelled normal DNAs. Gains exceeding the limit of 1.5 were classified as high-level amplifications.

A positive control (a tumour with known DNA sequence copy number aberrations) and a negative control (two differentially labelled normal DNAs) were included in each hybridisation.

2.3. *Immunohistochemistry for c-myc protein*

As copy number increase at 8q24, the locus of *C-MYC* (8q24.12-q24.13), was one of the most common aberrations, the expression of c-myc protein was studied by immunohistochemistry in 21 samples. Immunohistochemistry was performed using the avidin–biotin peroxidase complex method (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA) on formalin-fixed, paraffin-embedded tissue samples, as described previously.[25] Briefly, after de-waxing and blocking of endogenous peroxidase activity, the sections were treated with a citrate buffer solution (containing 0.01 mol/l citric acid and 0.01 mol/l sodium citrate, pH 6.0) in a microwave oven at 750 W for 3 cycles of 5 min each, in order to retrieve the antigen. Tissue sections were then incubated overnight at 4 °C with the 9E10 monoclonal antibody (Oncogene Research Products, Manhasset, NY, USA) at 1:50 dilution rate. The final reaction product was revealed with diaminobenzidine and nuclei were counterstained with Gill's hematoxylin. Only tumour cells showing a nuclear immunostaining were considered as positive, regardless of the staining intensity and the simultaneous presence or absence of cytoplasmic positivity. Tumour samples were scored as positive when a nuclear immunostaining was present in more than 5% of cells.

3. Results

3.1. *Comparative genomic hybridisation*

Out of the 26 samples studied by CGH, 23 (89%) had DNA sequence copy number changes (Fig. 1 and Table 2). Three primary tumour specimens did not show any changes by CGH: two were derived from paraffin blocks with a tumour cell percentage >90% (cases 6 and 7, Table 1) and one from fresh tumour tissue (case 21, Table 1) in which the tumour cell percentage was not known. The mean number of changes was 7.2 per sample. Gains were more frequent than losses (gains:losses = 2.5:1). High-level amplifications were detected in eight out of the 26 samples (31%), i.e., in 35% of the samples with aberrations.

Minimal common regions for the most frequent gains were 8q21.3-qter (35%), 9q32-qter (35%), 7q22-q31 (35%), 1q21-q23 (31%), 7p12-pter (31%), 7cen-q11.2 (31%), 15q21 (31%) and 1p21-p31 (27%) (Table 3). High-level amplifications of 1q21-q23 and 8q21.2-q22 were seen in two samples (8%). Minimal common regions for the most frequent losses were 13q21-q22 (42%) and 18q12-q22 (27%). The mean number of changes per tumour was 6.9 in primary tumour samples ($n = 22$) and 9.3 in metastases ($n = 4$). When the most common changes shown in Table 3 were analysed separately in primary tumour samples and in metastases, the most striking differences were seen in gains of 1q21-q23 (in 23% of primary tumours and in 75% of metastases) and of 9q32-qter (in 27% of primary tumours and 75% of metastases). Gain of distal 8q was detected at similar frequency both in primary tumours and metastases.

3.2. *Immunohistochemistry for c-myc protein*

Immunohistochemical detection of *C-MYC* was performed on paraffin-embedded tumour tissue sections from 21 out of the 26 samples analysed by CGH, including 18 primary tumours and three metastases (Table 2). Seven out of the 21 cases (33%) were positive for c-myc, showing a nuclear immunostaining that reflects the presence of increased levels of the protein (Fig. 2). No difference was found between the incidence of c-myc positivity in primary tumours (6/19 cases, 32%) and in metastases (1/3 cases, 33%). Among the cases positive for c-myc, the proportion of positive cells ranged from

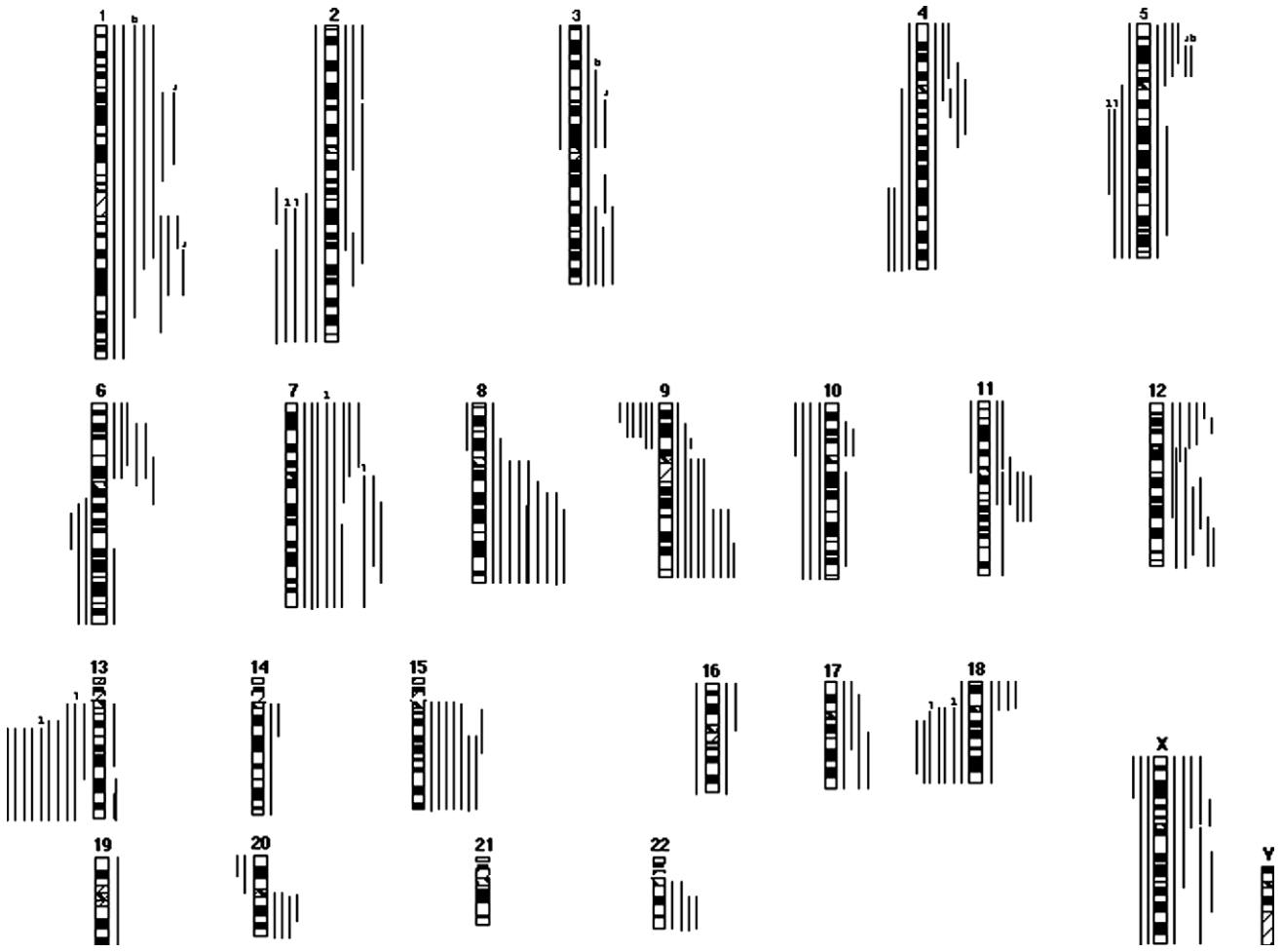

Fig. 1 – Summary of gains and losses of DNA sequence copy number in 26 malignant fibrous histiocytoma of bone specimens analysed by CGH. Each line represents an aberration seen in one sample. Gains are shown on the right side of the chromosomes and losses on the left. High-level amplifications are shown with thick lines. a = 24a; b = 24b; 1 = 3a; 2 = 3b.

5% to 60%, with strongest positivity in a metastatic lesion (case 24b, Fig. 2).

A good agreement was found between CGH and immunohistochemistry findings when 17 of the 21 tumours (81%) showed concordant results (Table 4). Five out of seven c-myc positive tumours had a copy number increase at the C-MYC locus (8q24.12-24.13) by CGH, whereas 12 out of 14 c-myc negative cases did not show any increase at this chromosomal region by CGH.

3.3. *Comparison between CGH findings in MFH of bone and soft tissue MFH*

All the most frequent aberrations in bone MFH (Table 3) have also been detected in soft tissue MFH,[20,26,27] with either identical, overlapping or slightly different minimal common regions. Losses of distal parts of 1q and 11q have been detected in 10% and 12%,[20] in 36% and 48%,[26] and in 45% and 55%[27] of soft tissue MFH, but were not seen in bone MFH in the present study. Gains of distal parts of 6q and 13q have been detected in 16%,[20] in 12% and 16%,[26] and in 24% and 21%[27] of soft tissue MFH, but were seen in MFH of bone only once (4%).

3.4. *Comparison between CGH findings in MFH of bone and osteosarcoma*

All the most frequent aberrations in bone MFH have also been detected in osteosarcoma previously by us[21] and by Lau and colleagues[28] with the same, overlapping or slightly different minimal common regions. None of the most frequent losses seen in the present bone MFH study were detected in the osteosarcomas studied by Stock and colleagues.[29] Losses at 1q and 3q are rare in osteosarcoma[21,28,29] and were not detected in bone MFH. Copy number increases at 4q have been detected in osteosarcomas[21,28,29] but not in bone MFH. Losses at 11p are more common in osteosarcoma than in bone MFH.[21,28,29] Losses at 15q in osteosarcoma[21,28] were not seen in bone MFH. High-level amplifications of 17p, characteristic to osteosarcoma[21,28] were not seen in bone MFH.

3.5. *Comparison between CGH findings in MFH of bone and fibrosarcoma of bone*

The most frequent aberration in fibrosarcoma of bone was gain of 22q, which was present in six out of nine tumours (67%).[22] One of the fibrosarcomas with the 22q gain had also

Table 2 – DNA sequence copy number changes in malignant fibrous histiocytoma of bone by comparative genomic hybridisation (CGH) and C-MYC expression by immunohistochemistry

Case number	CGH findings[a]	c-myc IHC[b] (% positive cells)
1	rev ish enh(20q11.2q13.1, Xq21q25)	Negative
2	rev ish enh(9q, 15q, 18p), dmin(2, 4, 10, 16, 18q, X)	Positive (20–30%)
3a	rev ish enh(7q), dmin(2q22qter, 5q13qter, 13q, 18q11.2qter)	Negative
3b	rev ish enh(1q21q31, 6p12p22, 7, 9q32qter, 11p11.2q13, Xp11.2pter), dmin(2q22qter, 3p, 5q13q23, 13q14qter, 18q), amp(1q21q24)	Negative
4	rev ish dmin(4q28qter, 10, 11p, 13q, X)	Negative
5	rev ish enh(11pterq13), dmin(5, 6q13qter, 13q13qter)	Negative
6	No changes	Negative
7	No changes	Negative
8	rev ish enh(1, 5p14pter, 7q22qter), dmin(13q14qter)	Negative
9	rev ish enh(1q21q23, 8q13qter, 9, 10p11.2p13, 12q22qter, 13cenq21, 15q, 17pterq21, 18), dmin(2q31qter, 6q14qter, 13q21qter)	Negative
10	rev ish enh(1pterq25, 2pterq12, 2q24q32, 3q23qter, 6p21.1q13, 7, 8q, 9p21qter, 11q12q14, 12q23qter, 15q, 18p, 20q), amp(1q21q23)	ND
11	rev ish enh(1pterq24, 2pterq24, 3q13.3q23, 4p13pter, 5, 6p21.1pter, 9q, 11q, 12p, 15q12q21, 16p, 17p12qter, 22q, Xpterq22), dmin(9p21-pter), amp(Xp11.4p21)	Negative
12	rev ish enh(2p16pter, 5p, 8q), dmin(2q21qter, 4q, 5q, 9p13pter, 13q14qter, 18q12qter)	Positive (5–10%)
13	rev ish enh(2p14q31, 3, 4p14q22, 7p, 8p12qter, 11cenq14, 12pterq12, 14q, 15q, 16, 17q21qter, 19, Xcenp11.4), dmin(4q28ter, 6q15q21, 9p21pter, 10p11.2pter, 13q13qter, 18q12q22, 20p), amp(3p13p21, 8cenq22, 15q11.2q13, 16p11.2pter, 22q)	Negative
14	rev ish enh(4, 6cenp22, 7, 8q, 9q22qter, 12q, 14cenq13, 15q, 17, 20q, X), dmin(8p12pter, 9p13pter, 10, 13q14qter, 18, 20p12pter,), amp(6p12-p21.3, 8q21.2qter)	Positive (5–10%)
15	rev ish enh(1p13p31, 4cenq13, 5p13pter, 7pterq11.2, 12pterq21), amp(12p11.2p12)	Positive (20–30%)
16	rev ish enh(9p13)	Negative
17	rev ish enh(3q25qter, 7p12pter, 8q21.1qter, Xp)	ND
18	rev ish enh(1q21q41, 9q, 10q21q25, 11cenq14, 15q21qter), dmin(9p21pter, Xp21pter)	Negative
19	rev ish enh(7q21q33, 8q21.3qter, 9q22qter, 12p11.2pter, 15q21qter, 18p), dmin(13cenq22, 18q)	Positive (40–50%)
20	rev ish enh(4pterq12, 7cenq31, 12q, Xq)	ND
21	No changes	Negative
22	rev ish enh(1, 5q14q33, 6p12pter, 7, 8, 11p11.2pter, 12p12, 12q15q24.2), dmin(9p22pter), amp(7p11.2p21)	Positive (5–10%)
23	rev ish enh(4p12q21, 12p13, 13q31qter, 22q12qter), amp(4cenq13, 13q32qter)	ND
24a	rev ish enh(1p21p31, 1q24q31, 3p12p14, 5p13p14)	ND
24b	rev ish enh(1pterq32, 3p12p21, 3q23qter, 5p13p14, 6p12pter, 6q22qter, 7, 8q21.1qter, 9q22qter, 10p11.2p12, 12q14q22, 20q11.2qter, 22q12qter), dmin(2q14.3q22, 13q14qter)	Positive (50–60%)

a According to ISCN 1995.
b IHC = immunohistochemistry.

a high-level amplification at 22q11.2-q12. In MFH of bone, only four cases (15%) had a copy number increase affecting 22q. All copy number increases at 22q were gains without high-level amplification. The second most common alteration in bone fibrosarcoma was gain of 8q24.1-qter, which was present in three tumours (33%). Our findings were similar, showing a gain with minimal common region of 8q21.3-qter in 35% of the bone MFH.

4. Discussion

The present study has shown the complex nature of genetic changes in MFH of bone. CGH revealed a high incidence of DNA sequence copy number changes. The mean number of aberrations per sample was 7.2. The changes affected each chromosome and each chromosome arm, except chromosome 21. The complexity of the changes seems to reflect the high malignancy grade of bone MFH. Clear clustering points with recurrent aberrations were also detected: loss of 13q21-q22 (42%) and gains of 8q21.3-qter (35%), 9q32-qter (35%) and 7q22-q31 (35%).

Gains of 8q21.3-qter harbour the locus of C-MYC (8q24.12-q24.13), an oncogene involved in the tumorigenesis and tumour progression of several bone and soft tissue sarcomas.[25,30,31] However, no data about C-MYC expression in MFH of bone have been reported previously. In the present study, a good agreement was found between the immunohistochemistry results and the CGH findings, which were concordant in

Table 3 – Most frequent DNA sequence copy number changes in 26 samples of bone MFH by comparative genomic hybridisation

Gains		High-level amplifications		Losses	
Minimal common region	*n* (%)	Minimal common region	*n* (%)	Minimal common region	*n* (%)
8q21.3-qter	9 (35%)	1q21-q23	2 (8%)	13q21-q22	11 (42%)
9q32-qter	9 (35%)	8q21.2-q22	2 (8%)	18q12-q22	7 (27%)
7q22-q31	9 (35%)			9p22-pter	6 (23%)
1q21-q23	8 (31%)				
7p12-pter	8 (31%)				
7cen-q11.2	8 (31%)				
15q21	8 (31%)				
1p21-p31	7 (27%)				
5p14	6 (23%)				
6p21.1	6 (23%)				
11q12-q13	6 (23%)				

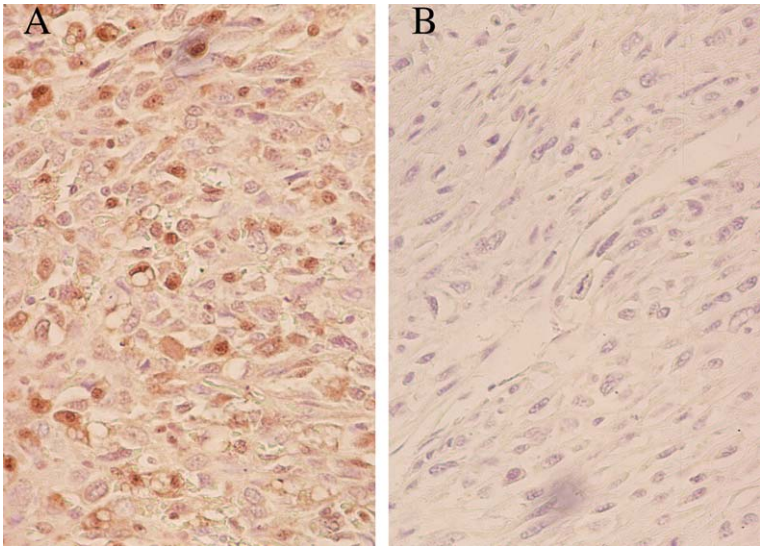

Fig. 2 – Immunohistochemistry for c-myc protein in paraffin-embedded malignant fibrous histiocytoma of bone tissue samples: (A) the immunohistochemical positivity for c-myc is localised in the tumour cells (case number 24b, Table 1); (B) c-myc negative sample (case number 13, Table 1). Original magnification 500×.

Table 4 – Correlation of copy number increase at 8q24.12-24.13 by CGH and C-MYC expression levels as detected by immunohistochemistry in 21 malignant fibrous histiocytomas of bone

CGH findings	c-myc	
	Positive	Negative
Copy number increase at 8q24.12-24.13	5[a]	2
No copy number increase at 8q24.12-24.13	2	12[a]
Total	7	14

a Concordance between CGH and immunohistochemistry: 17/21 cases (81%).

81% of cases. Positivity to c-myc protein was revealed by immunohistochemistry in seven out of 21 tumours (33%), in five of which CGH detected a copy number increase at 8q24. The chromosomal region of copy number increase at 8q in the two discrepant cases may have been too small to be detected by CGH. The level of amplification affects the sensitivity of CGH: the higher the copy number of specific sequences is, the smaller is the region that can still be detected by CGH. With a high-level amplification, even a 1 Mb region can be detected by CGH.[32] Mechanisms other than genomic amplification can also be responsible for the increased C-MYC expression. A good agreement with CGH was found also for C-MYC negative cases, as 12 out of 14 did not show any aberration involving 8q24. It should be noted that one of the discordant samples had a high-level amplification at 8cen-q22 and a large region at 8q was gained in both. Thus, in these two cases the copy number increases most probably affected other target genes rather than C-MYC.

As very little is known of cytogenetic and molecular genetic changes in MFH of bone, only speculations about the background of these changes can be presented. Gains at 1q21-q23 (in 31% in the present study) are frequently also seen in other sarcomas, e.g., osteosarcoma,[33,34] Ewing's sarcoma,[35] chondrosarcoma,[24] liposarcoma[36,37] and soft tissue

MFH.[36] Amplifications of *FLG*, *SPRR3* and *NTRK1*, located in 1q21, have been reported in some human sarcoma samples,[38] and amplification and overexpression of *PRUNE* (1q21.3) has been detected in sarcomas and breast cancer.[39] Copy number increases at chromosome 7 were also frequent findings in the present study, affecting the whole chromosome or shorter regions and thus contributing to the formation of three minimal common regions, affected in 31–35% of the cases. Trisomy 7 is a common secondary aberration in many cancers and copy number increases of chromosome 7 have been detected by CGH in many tumour types, including several sarcomas.[40] Amplification and overexpression of *c-MET* (7q31) has been shown in some sarcoma types,[41,42] but it is a very rare event in bone MFH.[16] Gains of 9q are very rarely seen in other tumour types but have been detected in soft tissue MFH[20,26,27] and in the present bone MFH study, suggesting that gains affecting 9q might be linked to the tumorigenesis of MFH. Gains of 12q were less frequent than the changes discussed above, as 19% of the samples had gain of 12q15-q21. One possible target is *MDM2*, typically amplified in different sarcoma types.[43,44]

Losses affecting chromosome 13 had the minimal common region of 13q21-q22 in 42% of the samples but in ten tumours (38%) also 13q14 (locus of *RB1*) was affected by a loss. 9p22-pter loss was seen in 23%. Martignetti and colleagues found frequent loss of heterozygosity (LOH) at 9p21-22, the region that is linked to diaphyseal medullary stenosis with malignant fibrous histiocytoma syndrome and is likely to contain a putative tumour suppressor gene relevant to the tumorigenesis of bone MFH.[45] Also, loss of 9p21 was frequent in the present study, suggesting the involvement of $p16^{INK4A}$ tumour suppressor gene.

The mean number of changes per tumour was higher in metastases than in primary tumour samples. When the most common changes were analysed separately in primary tumours and in metastases, the most striking differences were gains of proximal 1q and of distal 9q. Both gains were clearly more frequent in metastases. No difference was detected in the frequency of gain at 8q or expression of *C-MYC*, when primary tumours and metastases were analysed separately. From two patients, a primary tumour and its metastasis were paired and analysed by CGH (patients 3 and 24, Tables 1 and 2). Both pairs showed increase in changes during tumour progression and new aberrations could be detected in the metastases. In addition to quantitative changes, identical aberrations were seen in both pairs. These findings are in agreement with our previous study of primary tumours and their metastases.[46]

The present CGH findings of bone MFH are very similar to those reported in soft tissue MFH.[20,26,27] All the most frequent aberrations in bone MFH have also been detected in soft tissue MFH, with either identical, overlapping or slightly different minimal common regions. Differences in the aberration frequencies and minimal common regions are most probably related to the relatively small sizes of the materials studied and to the heterogeneity of these tumours. Yet some differences should be pointed out. Loss of distal part of 1q and 11q, both relatively frequent in soft tissue MFH,[20,26,27] were not detected in the present bone MFH study. In addition, gains of distal parts of 6q and 13q are more frequent in soft tissue MFH[20,26,27] than in bone MFH. Based on the limited data on *C-MYC* amplification in soft tissue MFH,[31] the increase in *C-MYC* copy number and *C-MYC* expression appear to occur in about one-third of the cases. Thus, the incidence coincides with the copy number increase we detected at the *C-MYC* locus and in *C-MYC* expression in bone MFH.

The CGH findings in bone MFH and osteosarcoma are also similar considering that all the most frequent aberrations in bone MFH have also been detected in osteosarcoma with the same, overlapping or slightly different minimal common regions.[21,28] However, distinct differences can be found between osteosarcoma and bone MFH: losses at 1q, 3q, 11p and 15q, recurrent in osteosarcoma, were not detected or they were clearly less frequent in bone MFH.[21,28,29] Most notably, high-level amplifications in 6p and 17p, characteristic to osteosarcoma,[21,28,29] were not detected in bone MFH. In osteosarcoma, c-myc is overexpressed in a high proportion of relapsed tumours and metastases as well as in primary lesions which developed metastases, indicating that this oncogene is related to osteosarcoma tumorigenesis and tumour progression.[30]

The comparison between CGH findings in bone MFH and bone fibrosarcoma supports the assumption that these two neoplasms are closely related but distinct tumour entities.[22] However, due to the small sample size in the bone fibrosarcoma study this comparison is more arbitrary than that between soft tissue MFH and osteosarcoma. Copy number increase at 22q seems to distinguish bone fibrosarcoma from bone MFH: it is a distinctive feature in bone fibrosarcoma but not in bone MFH, in which it has been a rarely reported aberration.[22] It is also worthwhile to note that the second most common alteration in bone fibrosarcoma, present in one-third of the tumours, was gain of 8q24.1-qter, similarly to what was observed in the present bone MFH study.

In conclusion, the present findings reflect well the high malignancy grade of bone MFH. The CGH results are somewhat different when compared to those reported in osteosarcoma, bone fibrosarcoma and soft tissue MFH. These findings support the notion that bone MFH is an individual tumour entity with a characteristic aberration pattern.

Conflict of interest statement

None declared.

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