Comparative Genomic Hybridization of Postirradiation Sarcomas

Maija Tarkkanen, M.D., Ph.D.[1,2]
Tom A. Wiklund, M.D., Ph.D.[3]
Martti J. Virolainen, M.D., Ph.D.[4]
Marcelo L. Larramendy, Ph.D.[1,2,5]
Nils Mandahl, Ph.D.[6]
Fredrik Mertens, M.D., Ph.D.[6]
Carl P. Blomqvist, M.D., Ph.D.[3]
Erkki J. Tukiainen, M.D., Ph.D.[7]
Markku M. A. Miettinen, M.D., Ph.D.[4]
A. Inkeri Elomaa, M.D., Ph.D.[3]
Y. Sakari Knuutila, Ph.D.[1,2]

[1] Department of Medical Genetics, Haartman Institute, University of Helsinki, Helsinki, Finland.

[2] Laboratory of Medical Genetics, Helsinki University Central Hospital, Helsinki, Finland.

[3] Department of Oncology, Helsinki University Central Hospital, Helsinki, Finland.

[4] Department of Pathology, Haartman Institute, University of Helsinki, Finland.

[5] Laboratorio de Citogenética y Cátedra de Citologia, Facultad de Ciencias Naturales y Museo, National University of La Plata, La Plata, Argentina.

[6] Department of Clinical Genetics, Lund University Hospital, Lund, Sweden.

[7] Department of Plastic Surgery, Helsinki University Central Hospital, Helsinki, Finland.

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Address for reprints: Maija Tarkkanen, M.D., Ph.D., Department of Medical Genetics, Haartman Institute, University of Helsinki and Laboratory of Medical Genetics, Helsinki University Central Hospital, Haartmaninkatu 3, 4th Flr. (Box 21), FIN-00014 University of Helsinki, Finland; Fax: 358-(0)9-191 26788; E-mail: maija.tarkkanen@helsinki.fi

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BACKGROUND. Radiotherapy is a known risk factor for sarcoma development. Postirradiation sarcomas arise within the radiation field after a latency period of several years and usually are highly malignant. Very little is yet known about their genetic changes.

METHODS. Twenty-seven postirradiation sarcomas were analyzed by comparative genomic hybridization, which allows genome-wide screening of DNA sequence copy number changes.

RESULTS. Copy-number aberrations were detected in 20 (74%) tumors. The mean number of aberrations per tumor was 5.3 with gains outnumbering losses. The most frequent gains affected the minimal common regions of 7q11.2-q21 and 7q22 in 30% and 7p15-pter in 26%. Gain of 8q23-qter was detected in 22%. The most frequent losses affected 11q23-qter and 13q22-q32 in 22%. In osteosarcomas, the most frequent aberration was loss of 1p21-p31, in malignant fibrous histiocytomas (MFH) gain of 7cen-q22, and in fibrosarcomas gain of 7q22. The findings in postirradiation osteosarcomas and MFHs were compared with findings in sporadic osteosarcomas and MFHs, reported previously by the authors. In sporadic osteosarcomas, gains outnumbered losses, but, in postirradiation osteosarcomas, losses were more frequent than gains. Loss at 1p was rare in sporadic osteosarcoma (3%) but frequent (57%) in postirradiation osteosarcomas. Gains at 7q were frequent both in postirradiation and sporadic MFH.

CONCLUSIONS. According to previous studies on different types of sporadic sarcomas, gains at 7q or 8q are associated with poor prognosis or large tumor size. Thus, the frequent gains at 7q and 8q might have been responsible in part for the poor prognosis of postirradiation sarcomas. Also, however, some of their clinical features, i.e., high malignancy grade, late diagnosis, and central location, are associated with a poor prognosis. ***Cancer* 2001;92:1992–8.**

KEYWORDS: postirradiation sarcoma, comparative genomic hybridization, angiosarcoma, osteosarcoma, malignant fibrous histiocytoma (MFH), fibrosarcoma.

The majority of soft tissue and bone sarcomas arises spontaneously, but, in some patients, ionizing radiation can be identified as a predisposing factor. Criteria given by Cahan et al.[1] define postirradiation sarcomas as follows: evidence of initial nonmalignancy, development of the second malignant tumor in the irradiated tissue, a relatively long latency period, and histologic confirmation of sarcoma. Later, the first criterion was modified to include also malignant tumors of a different kind than the subsequent sarcoma.[2,3] The most common primary malignancies are breast carcinoma and cancers of the female reproductive organs according to the population-based study by Wiklund et al.[4] Median time interval between radiotherapy and the development of sarcoma in that study was 13.2 years, and the

most common histologic types were osteosarcoma, malignant fibrous histiocytoma (MFH), and fibrosarcoma.[4] The prognosis is poor, with 5-year survival rates varying from 11–29%.[4,5] The prognosis is, thus, clearly worse than in sporadic bone and soft tissue sarcomas with 5-year overall survival rates of 65% and 64%.[6,7]

Very little is known about the genetic changes involved in the tumorigenesis of postirradiation sarcomas. According to a recent study and review by Mertens et al.[8] regarding cytogenetic changes in postirradiation sarcoma, these tumors have complex karyotypes with loss of 3p21-pter being more frequent than in sporadic sarcomas. Also, polyclonal tumors with near-diploid chromosome numbers were observed.[8] Another recent cytogenetic study and review pointed out two distinct patterns: 1) polyclonal karyotypes, often with simple and balanced translocations, preferentially observed after long-term culturing and 2) monoclonal chromosomal alterations observed in highly aneuploid and complex karyotypes, usually detected in short-term cultures or in xenografts.[9] Alterations of the *RB1* and *TP53* tumor suppressor genes are frequent[10,11] and according to the study by Nakanishi et al.[10], the frequency of *TP53* mutations is higher in postirradiation sarcomas than in sporadic sarcomas.

Comparative genomic hybridization (CGH) allows for a genome-wide screening of losses, gains, and amplifications of DNA sequence copy numbers[12,13] (for reviews of amplifications and losses see Knuutila et al.,[14,15] which also are available at URL http://www.helsinki.fi/~lgl_www/CMG.html). In the current study, CGH was used to study 27 postirradiation sarcomas for the purpose of analyzing changes relevant to the tumorigenesis of these tumors. In addition, the findings in postirradiation osteosarcoma and MFH were compared with findings in sporadic osteosarcoma and MFH, reported previously by us.[16,17] To the best of our knowledge, this is the first report on postirradiation sarcoma studied by CGH (search covered Medline and pre-Medline, 1992–2000, online literature databases).

MATERIALS AND METHODS

Materials

Twenty-seven postirradiation sarcomas of 26 patients were analyzed by CGH. The tumors were selected to represent the most common histologic subtypes of postirradiation sarcoma, i.e., osteosarcoma, MFH, and fibrosarcoma,[4] on the basis of availability of fresh-frozen or paraffin-embedded tumor tissue. As angiosarcoma is very rare as a sporadic tumor but is a typical postirradiation sarcoma, this subtype was included also. Twenty-four tumors were identified from the Finnish Cancer Registry[4] and from patients treated by the Soft Tissue Sarcoma group at the Helsinki University Central Hospital.[6] Three tumors (Cases 4, 11, and 12) were from Sweden, and their CGH results have been published previously.[8] The material consisted of three angiosarcomas, seven osteosarcomas, eight MFHs, and nine fibrosarcomas (Table 1). The median time interval between the first, primary tumor and subsequent postirradiation sarcoma was 11 years (range, 4–33 yrs). Fifteen samples were from paraffin-embedded material, and 12 were fresh-frozen tumor samples.

In addition, 3 skin samples from irradiated but tumor-free areas from Patients 5, 20, and 26 were analyzed by CGH.

Comparative Genomic Hybridization

CGH was performed according to standard procedures[13] with the modification of using a mixture of fluorochromes conjugated to dCTP and dUTP nucleotides for nick translation.[18] Hybridizations, washings, and ISIS digital image analysis (Metasystems GmbH, Altlussheim, Germany) were performed as described elsewhere.[19] In each CGH experiment, a negative control (peripheral blood DNA from a healthy donor) and a positive control (DNA from a tumor with known DNA copy number aberrations) were included. Based on our earlier reports and the control results, 1.17 and 0.85 were used as cutoff levels for gains and losses, respectively. Gains exceeding the 1.5 threshold were termed high-level amplifications. All CGH results were confirmed by a 99% confidence interval.[20]

RESULTS

DNA sequence copy number aberrations were detected in 20 of 27 (74%) postirradiation sarcomas (Table 2). The mean number of changes per tumor was 5.3, with means of 3.0 gains, 2.3 losses, and 0.3 high-level amplifications per tumor. Gains were more frequent than losses by a ratio of 1.3:1. No copy number aberrations were detected in seven tumors: five were paraffin-embedded samples, and two were fresh-frozen tissue samples.

The most frequent DNA sequence copy number gains affected the minimal common regions of 7q11.2-q21 and 7q22 in 30%, 7p15-pter in 26%, and 8q23-qter and 15q24-qter in 22% of the 27 samples (Table 3). The most frequent losses affected 11q23-qter and 13q22-q32, seen in 22% of the samples. Recurrent high-level amplifications (green to red ratio > 1.5) were not detected. All aberrations in all tumor types are shown in Figure 1.

TABLE 1
Clinical and Histopathologic Characteristics of 27 Postirradiation Sarcomas

Case[a]	Gender	Age[b]	Primary tumor	Postirradiation sarcoma[c]	Type of sarcoma sample[d]	Location	Prior chemotherapy	Time interval to PIS (yrs)
1	F	75	Breast carcinoma	Angiosarcoma G4	P/F	Skin of breast	No	4
2	F	78	Bladder carcinoma	Angiosarcoma G4	LR/P	Abdominal wall	No	5
3	F	43	Breast carcinoma	Angiosarcoma G4	P/F	Skin of breast	No	6
4	F	17	Ewing sarcoma	Skeletal osteosarcoma OB G4	P/F	Humerus	Yes	6
5	F	73	Breast carcinoma	Extraskeletal osteosarcoma CB G4	P/F	Armpit	No	23
6	F	57	Giant cell tumor of bone	Extraskeletal osteosarcoma CB G4	P/F	Lower leg[e]	No	33
7 (7)	F	39	Ovarian carcinoma	Extraskeletal osteosarcoma G3	P/P	Abdominal wall	Yes	11
8 (27)	F	66	Uterus carcinoma	Skeletal osteosarcoma G3	P/P	Pelvis	No	7
9 (15)	F	52	Ovarian carcinoma	Extraskeletal osteosarcoma FS G3	P/P	Pelvis, pararectal space	Yes	11
10 (6)	F	27	Breast carcinoma	Skeletal osteosarcoma CB G4	P/P	Scapula	Yes	7
11	F	51	Breast carcinoma	MFH NOS G4	P/F	Shoulder, superficial	Yes	4
12	M	37	Astrocytoma G2–3	MFH NOS G4	P/F	Scalp	No	5
13	F	51	Breast carcinoma	MFH MYX G3	LR/P	Thoracic wall	No	11
14	F	60	Breast carcinoma	MFH ST-PL G4	P/P	Shoulder	No	10
15 (19)	F	63	Uterine cervix carcinoma	MFH GCT G3	P/P	Abdominal wall	No	12
16 (30)	F	82	Breast carcinoma	MFH G4	P/P	Subcutis of proximal humerus	No	16
17 (12)	F	59	Papillary thyroid carcinoma	MFH MYX G2	P/P	Neck	No	21
18	F	34	Chondrosarcoma	MFH ST-PL G4	P/F	Amputation stump	Yes	12
19 (28)	F	73	Larynx carcinoma	Fibrosarcoma G3	P/P	Neck	No	14
20a	F	50	Thyroid carcinoma	Fibrosarcoma G2	P/F	Neck	No	18
20b	F	50	Thyroid carcinoma	Fibrosarcoma G3	LR/F	Neck	No	18
21	F	36	Breast carcinoma	Fibrosarcoma G4	P/P	Thoracic wall	No	6
22	F		Breast carcinoma	Fibrosarcoma G4	P/F	Thoracic wall	No	11
23 (33)	M	84	Lymphoma	Fibrosarcoma G3	P/P	Back, scapular region	No	7
24 (18)	F	66	Breast carcinoma	Fibrosarcoma G4	P/P	Thoracic wall, axilla	No	15
25 (5)	F	20	Hodgkin disease	Fibrosarcoma G4	P/P	Thoracic wall	Yes	11
26	M	52	Lymphoma	Fibrosarcoma G4	P/F	Thoracic wall	Yes	8

G: grade.

[a] Corresponding case numbers in Wiklund et al., 1991[4] in parentheses.

[b] Age at diagnosis of postirradiation sarcoma.

[c] Subtypes of postirradiation sarcoma (PIS), OB: osteoblastic, CB: chondroblastic, FS: fibrosarcomatous, MYX: myxoid, GCT: giant cell type, ST-PL: storiform-pleomorphic.

[d] Type of sarcoma sample, P: primary tumor, LR: local recurrence, P: paraffin sample, F: fresh-frozen sample.

[e] Soft tissue tumor attached to the tibia.

Postirradiation Angiosarcomas

DNA sequence copy number aberrations were detected in two out of three postirradiation angiosarcomas, both showing a single gain, one in 11q14-q23 and the other in 8q23-qter (Table 2).

Postirradiation Osteosarcomas

DNA sequence copy number aberrations were detected in six out of seven postirradiation osteosarcomas. The mean number of aberrations per sample was 8.4, with means of 3.7 gains, 4.7 losses, and 0.4 high-level amplifications per sample. Losses were more frequent than gains with the ratio of 1.3:1. The most frequent aberration was loss of 1p21-p31 in 4 samples (57%).

Postirradiation MFHs

DNA sequence copy number aberrations were detected in five out of eight postirradiation MFHs. The mean number of aberrations per sample was 2.9 (1.8 gains, 1.1 losses, and no high-level amplifications). Gains were more frequent than losses (1.6:1). The most frequent aberration was gain of 7cen-q22, which was seen in 3 samples (38%).

Postirradiation Fibrosarcomas

DNA sequence copy number aberrations were detected in seven out of nine postirradiation fibrosarcomas. The mean number of aberrations per sample was 6.6 (4.2 gains, 2.3 losses, and 0.6 high-level amplifications with the ratio of 1.8:1 of gains to losses). The

TABLE 2
DNA sequence Copy Number Changes in 27 Postirradiation Sarcomas by Comparative Genomic Hybridization (CGH)

Case	CGH findings
1	+11q14-q23
2	no changes
3	+8q23-qter
4	+1pter-q23, +2p22-q21, −2q33-qter, +3q24-q25, +5p, −5cen-q31.1, +9pter-q31, −11q13-qter, +15cen-q15, +19p, +20q
5	−1p13-p31, −2p23-pter, −3cen-p14, −6q12-qter, +7pter-q21, −10p11.2-q22, +12p, +12cen-q21/++12q13-q21, −13q22-qter, +14q/++14q31-qter, +15q22-qter, −18cen-q21
6	+1q21-q31, +8q/++8q23-qter, +14q24-qter, +15q22-qter, +17cen-p13, −Xq
7	−1p, +2p11.2-p12, −6q15-q22, +7p, −9p21-pter, −10, −11, −12q22-q23, −13q14-q32, +14q, −18q
8	−1p21-p32, −4q27-qter, +8q21.3-qter, −11p13-pter, −X
9	−1p21-p31, −3cen-p21, +4q, −6p21.3-pter, +7p15-pter, +8q, −9p21-pter, −10p, −11, −12p11.2-pter, −13q, −14q24-qter, +15q24-qter, −20p
10	no changes
11	−6p12-q16, +7cen-q22
12	+5p, +7pter-q32, +10q24-q26, −13q, +15q
13	−2pter-p23, −2q33-qter, +4q31.1-q33, +7cen-q31, −11q23-qter, −18q12-qter
14	+1pter-q41, +2p, +3p12-pter, +5p12-p14, −8p21-pter, +9q21-q32, +12p11.2-p12, +20q11.2-qter
15	−6p22-p23, −11q23-qter
16	no changes
17	no changes
18	no changes
19	+11q22
20a	−2q22-q31, +6p21.1-p21.3, +6q25-qter
20b	+6p11.2-pter, +6q24-qter, +7q22-qter, +14q22-qter, +16p, +17
21	−1q32-qter, −2p11.2-pter, −2q21-q24, +3p21-pter, −3q12-qter, −6p21.3-q23, −7p11.2-pter, +7q11.2-q31, −8p11.2-pter, −9p, −10pter-q21, −13q, −16, +17cen-p12, −18q12-qter, −21q, −Xp11.4-qter
22	no changes
23	+7, +8, +12
24	no changes
25	+1q21-qter, +2q21-qter, +3pter-q22, +5, +7pter-q22/++7p14-pter, +8, +14q, +15q, −Xq13-qter
26	+1q31-qter, +2/++2q33-qter, −3, +4pter-q13, +5p/++5p14-p15.2, −5q13-q15, +6q, +7, −9p, +10p11.2-pter, +10q24-qter, +11pter-q13, −11q22-qter, +12p11.2-pter, −13q21-qter, +15q21-qter, +20q, +21q/++21q22, +22q/++22q13, +Xq13-qter

CGH results of Cases 4, 11, and 12 have been published previously.[8]

most frequent aberration was gain of 7q22, which was seen in 5 samples (56%).

Skin Samples from Irradiated Tumor-Free Areas

No copy number aberrations were seen in the skin samples taken from irradiated but tumor-free areas from Patients 5, 20, and 26.

TABLE 3
Most Frequent DNA-sequence Copy Number Aberrations in 27 Postirradiation Sarcomas by Comparative Genomic Hybridization (CGH)

Gains	*n* (%)	Losses	*n* (%)
7q11.2-q21	8 (30)	11q23-qter	6 (22)
7q22	8 (30)	13q22-q32	6 (22)
7p15-pter	7 (26)	1p21-p31	4 (15)
8q23-qter	6 (22)	6q15-q16	4 (15)
15q24-qter	6 (22)	9p21-pter	4 (15)
5p12-p14	5 (19)	10p11.2	4 (15)
14q24-qter	5 (19)	18q12-q21	4 (15)
1q21-q23	4 (15)	Xq13-qter	4 (15)
1q31	4 (15)		
2p11.2-p12	4 (15)		
12p11.2-p12	4 (15)		

DISCUSSION

The current study describes genetic changes in postirradiation sarcomas focusing on the most common histologic subtypes. Overall, the most frequent aberrations were copy number gains affecting chromosome 7, with the minimal common regions of 7q11.2-q21 and 7q22 in 30% and 7p15-pter in 26% of the tumors. Of these, 7q11.2-q21 and 7q22 were affected most frequently in MFH and fibrosarcoma, and 7p15-pter was affected more often in osteosarcoma and fibrosarcoma. As reported previously by us, gains affecting chromosome 7 are frequent also in sporadic MFH[17] and sporadic osteosarcoma.[16] To the best of our knowledge, there is not yet any data on copy number changes, studied by CGH, in sporadic fibrosarcoma. Expression of the hepatocyte growth factor HGF (gene located in 7q21) is common in sarcomas,[21] and, thus, this gene may be a target for copy number increases. No proof for such an association has been presented yet, and the possible other targets for gains on chromosome 7 are not yet known.

Gains were also common at the distal part of 8q (8q23-qter), most frequently in osteosarcoma. Gains at distal 8q have been also found to be frequent in sporadic osteosarcoma,[16] chondrosarcoma,[20] and leiomyosarcoma.[22] Furthermore, gain of the entire chromosome 8 is frequent in Ewing sarcoma and related tumors.[23] Taken together, overrepresentation of sequences at 8q seems to have a significant role in different sarcoma types. Chromosomal band 8q24 contains the *MYC* locus, but amplification of *MYC* is an uncommon event in sporadic osteosarcoma according to Ladanyi et al.[24] However, recently an increased *MYC* copy number was seen 7 of 16 osteosarcomas[25] and overexpression of MYC was frequent particularly in osteosarcomas which later relapsed.[26] *MYC* amplification has been reported only in a small

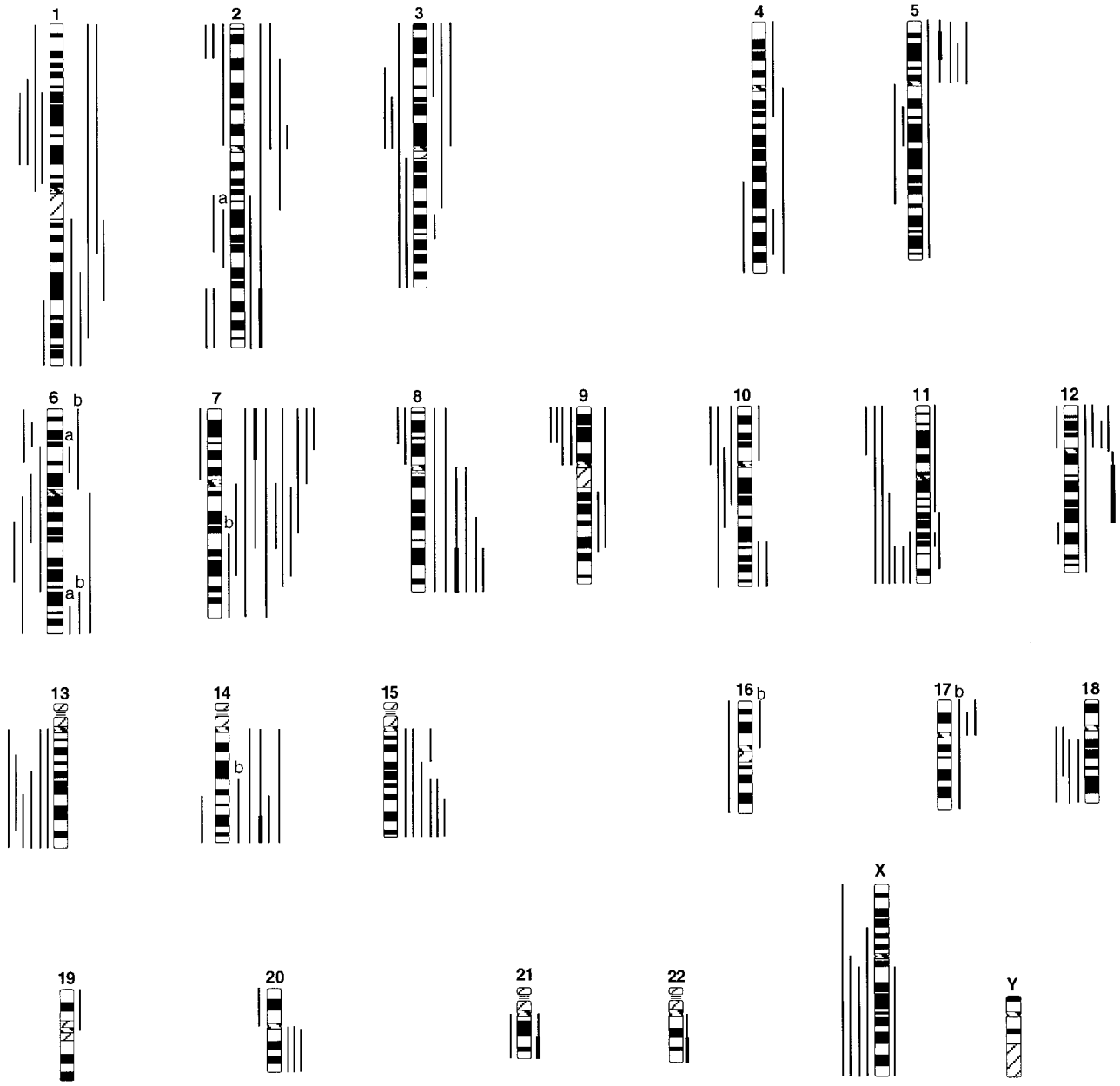

FIGURE 1. Summary of gains, high-level amplifications, and losses of DNA sequence copy number in 27 postirradiation sarcomas analyzed by CGH. Losses are shown on the left and gains on the right side of the chromosomes. Each line represents a genetic aberration seen in one sample. High-level amplifications are shown with thick lines. Notations a and b refer to Case 20.

fraction of chondrosarcomas[27] and soft tissue sarcomas.[28] The gains at 8q affect large chromosomal regions and the minimal common regions are slightly different in different tumor subtypes. Thus there probably are several target genes yet to be identified.

The most common losses affected 11q23-qter and 13q22-q32, both most frequently in osteosarcoma. Loss of distal 11q is common in several types of cancer[15] and also in sporadic MFH.[17] Loss of distal 11q was not detected by us in sporadic osteosarcoma, but these changes could have been masked by the frequent gains in 11q in this tumor type.[16] Candidate genes for losses in 11q include *ATM* (11q22-q23) and *PPP2R1B* (11q22-q24), but no data about their possible role in tumorigenesis of sarcomas exists yet. Interestingly, *ATM* is involved in cellular responses to ionizing radiation. Also, loss of distal 13q is frequent in several cancer types[15] and is seen also in both sporadic osteosarcoma[16] and MFH,[17] but no target genes in this region have been identified yet.

When the findings in postirradiation osteosarcoma and MFH were further compared with findings in sporadic osteosarcoma and sporadic MFH, as reported previously by us,[16,17] some differences were found: Although the mean number of aberrations per tumor was similar in postirradiation and sporadic osteosarcomas, in sporadic osteosarcomas gains were more frequent than losses (1.9:1), but, in postirradiation osteosarcomas, losses outnumbered gains (1.3:1). Loss at 1p was seen in only one sporadic osteosarcoma (3%), but loss at 1p was frequent (57%) in postirradiation osteosarcomas. Loss at 1p is a frequent event in different types of cancer[15] and is seen also in malignant mesothelioma,[29] a cancer strongly associated with exposure to asbestos. Conversely, gains at Xp and 6p were frequent in sporadic osteosarcomas but were not seen in postirradiation osteosarcomas. At present, we believe that there is no data on CGH findings in extraskeletal osteosarcomas and, therefore, the CGH findings in postirradiation osteosarcomas (three skeletal and four extraskeletal tumors) were compared with skeletal osteosarcomas.[16]

In postirradiation MFH, the mean number of aberrations per tumor was somewhat lower than in sporadic MFH, which could be because of the slightly larger proportion of tumors without any aberrations in the current study. In postirradiation MFH, the most frequent aberration was gain of 7cen-q22, seen in 38%. In sporadic MFH, gains affecting chromosome 7 also were frequent: 7cen-q22 in 17% and 7q32 in 24%. Because of a lack of CGH data on sporadic tumors, similar comparisons can not be carried out at present in angiosarcoma or fibrosarcoma.

A great variability characterizes the findings of the present study, from cases with complex aberrations to cases with a few aberrations to cases with a single or no aberration. Several factors can explain why no copy number changes were detected in some cases: CGH does not detect balanced aberrations, and, thus, tumors containing only such changes will appear normal by CGH. Furthermore, CGH detects only clonal aberrations occurring in a relatively large subset of the tumor cell population, and changes present in a subclone can remain undetected by CGH.[13] Thus, intratumoral heterogeneity[30] and polyclonality[8] can prevent detection of existing changes. The analysis of the primary tumor and its local recurrence in Case 20 is illustrative of such heterogeneity. These samples share two imbalances but show also unique copy number changes, the local recurrence showing a more complex aberration pattern than the primary tumor. The local recurrence was detected more than 2 years after the primary tumor was operated. Also, the recurrence was a high-grade tumor, whereas the primary tumor was of Grade II. Thus clonal evolution during tumor progression is a likely explanation for the different aberration patterns in this tumor pair.

According to the recent study and review by Mertens et al.,[8] loss at 3p (3p21-pter) is frequent in postirradiation sarcomas by cytogenetic analysis (12 out of 18 cases). In the current study, loss at 3p was detected in only three tumors with the minimal common region of 3cen-p14. This discrepancy might have been due to complex marker chromosomes that contain material from 3p, not detectable by conventional cytogenetic analysis, or due to intratumoral heterogeneity with subclonal variation.[30]

In conclusion, according to our findings, postirradiation osteosarcomas differ genetically from sporadic osteosarcomas,[16] most notably in more frequent losses of DNA sequences in postirradiation osteosarcoma, whereas gains are more frequent in sporadic osteosarcoma. Furthermore, loss at 1p was rare in sporadic osteosarcoma but frequent in postirradiation osteosarcoma. Copy number gains at 7q were frequent in postirradiation sarcomas, especially in MFH and fibrosarcoma. In a previous study of sporadic MFH, we demonstrated that gain of 7q32 is associated with shorter metastasis-free and overall survival.[17] Also, gain of distal 8q was frequent in postirradiation sarcomas, especially in osteosarcoma. This aberration is associated with poor prognosis in sporadic osteosarcoma,[16] chondrosarcoma,[20] and Ewing tumors.[23] Gain of distal 8q also has been associated with large tumor size in leiomyosarcoma[22] and synovial sarcoma.[31] Thus, the frequent gains at 7q and 8q may explain, in part, the poor prognosis of patients with postirradiation sarcomas. However, the poor outcome of these patients also may be due to some of the typical clinical features of postirradiation sarcomas: high malignancy grade, late diagnosis, and central location.

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