Cytogenetic and Molecular Characterization of Plutonium-Induced Rat Osteosarcomas

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Osteosarcoma/Ionizing radiation/Alpha-particle emitter/Chromosome aberrations.

The association between ionizing radiation and the subsequent development of osteosarcoma has been well described, but little is known about the cytogenetic and molecular events, which could be involved in the formation of radiation-induced osteosarcomas. Here, we performed comparative genomic hybridization (CGH) to detect chromosomal copy number changes in a series of 16 rat osteosarcomas induced by injection of plutonium-238. Recurrent gains/amplifications were observed at chromosomal regions 3p12-q12, 3q41-qter, 6q12-q16, 7q22-q34, 8q11-q23, 9q11-q22, 10q32.1-qter, and 12q, whereas recurrent losses were observed at 1p, 1q, 3q23-q35, 5q21-q33, 8q24-q31, 10q22-q25, 15p, 15q, and 18q. The gained region at 7q22-q34 was homologous to human chromosome bands 12q13-q15/8q24/22q11-q13, including the loci of Mdm2, Cdk4, c-Myc and Pdgf-b genes. The lost regions at 5q21-q33, 10q22-q25 and 15q contained tumor suppressor genes such as p16INK4a/p19ARF, Tp53 and Rb1. To identify potential target gene(s) for the chromosomal aberrations, we compared the expression levels of several candidate genes, located within the regions of frequent chromosomal aberrations, between the tumors and normal osteoblasts by using quantitative RT-PCR analysis. The Cdk4, c-Myc, Pdgf-b and p57KIP2 genes were thought to be possible target genes for the frequent chromosomal gain at 7q22-34 and loss at 1q in the tumors, respectively. In addition, mutations of the Tp53 gene were found in 27% (4 of 15) osteosarcomas. Our data may contribute to further understanding of the molecular mechanisms underlying osteosarcomas induced by ionizing radiation in human.

INTRODUCTION

Sarcomas, including osteosarcomas, are relatively rare tumors in the general population. However, osteosarcoma is the most common primary tumor of bone in children and adolescents, and a leading cause of cancer-related death in this age group. This tumor is highly aggressive and is thought to arise primarily from bone-forming osteoblasts.

The association between ionizing radiation and the subsequent development of osteosarcoma has been well documented in prior studies. Osteosarcoma is known as one of the most frequent secondary malignant neoplasms occurring within the radiation field in patients, especially with retinoblastoma, treated with radiation therapy. The risk of osteosarcoma has been reported to be increased following the internal exposure to bone-seeking radioisotopes from occupational or medicinal use. It is also recognized that osteosarcoma belongs to a subset of cytogenetically complex sarcomas that typically develop as a secondary solid tumor following radiation exposure. Previous cytogenetic studies of human sarcomas, including osteosarcoma, developed after radiotherapy have reported that these tumors have chaotic karyotypes, with many structural and numerical changes of chromosomes. Although it seems that karyotypes of the radiation-induced sarcomas were relatively similar to those reported in the corresponding sporadic sarcomas, the higher frequency of polyclonal karyotypes and the frequent loss of 3p21-pter have been reported as unusual cytogenetic findings in radiation-induced sarcomas compared with sporadic cases. Furthermore, it has been observed that chromosome losses are more frequent than gains in radiation-induced sarcomas, whereas chromosome gains outnumber losses in sporadic cases. This feature has also been...
described in a recent comparative genomic hybridization (CGH) study conducted on 27 post-irradiation sarcomas.\textsuperscript{12} Interestingly, it has also reported that the chromosomal region 1p21-p31 is underlined as a major site of chromosomal loss in post-irradiation osteosarcomas comparatively to sporadic cases. However, to our knowledge, there are only limited numbers of reports describing the genetic changes in radiation-induced osteosarcomas.

Abnormalities of genes involved in the retinoblastoma (Rb) and p53 tumor-suppressor pathways have been established as the most common features of the cytogenetically complex sarcomas.\textsuperscript{5,13} Deletions and structural alterations of the R\textit{B}1 gene have been frequently found in sporadic osteosarcomas.\textsuperscript{14–16} Loss of \textit{CDKN2A} gene (encoding \textit{p16\textsuperscript{INK4a}}) or amplification of \textit{CDK4} and \textit{CCND1} (Cyclin D1) genes has also been reported in some studies of this tumor,\textsuperscript{17–19} and abnormality of these genes also results in functional inactivation of the R\textit{B} gene. On the other hand, abrogation of the p53 pathway by loss of \textit{CDKN2A} gene (encoding \textit{p14\textsuperscript{ARF}}) or amplification of \textit{MDM2} gene has been suggested in osteosarcoma cell lines and sporadic osteosarcoma.\textsuperscript{20,21} Direct inactivation of the \textit{TP53} tumor suppressor gene by loss of heterozygosity (LOH) and/or mutation has also been observed in this tumor.\textsuperscript{22,23} The relationship between the abnormality of the Rb and p53 pathways and the development of osteosarcoma is further supported by the fact that patients with hereditary retinoblastoma and Li-Fraumeni syndrome, characterized by the presence of a germline mutation of the \textit{R\textit{B}1} and \textit{TP53} gene, respectively, have high risk for osteosarcoma.\textsuperscript{2,3,24} Like other tumors, abnormal activation of several growth factors, including TGF-\textit{β}, PDGF, and IGF-1, and their receptors has also been reported in sporadic osteosarcomas.\textsuperscript{25–27} These reports suggest that abnormal growth of osteosarcoma is sustained by the growth factor through the autocrine and paracrine mechanisms. Thus, abnormal control of the G1/S phase cell cycle checkpoint and the modulation of growth factor response are considered to play an important role in the pathogenesis of osteosarcoma.

It is known that osteosarcoma can be induced in laboratory animals by injection of bone-seeking alpha-emitting radionuclides.\textsuperscript{22,28,29} In the present study, we studied plutonium (Pu)-induced rat osteosarcomas as a model of radiation-induced bone tumors in human. Chromosomal imbalances in a series of 16 Pu-induced rat osteosarcomas were examined by CGH analysis. To identify potential target gene(s) involved in the recurrent chromosomal imbalances in Pu-induced rat osteosarcomas, the expression levels of several G1/S phase cell cycle-regulator and growth factor genes were analyzed by quantitative RT-PCR.

**MATERIALS AND METHODS**

**Plutonium Contamination and Tumor Specimens**

Sixteen osteoblastic osteosarcomas were obtained after intravenous injections of 238Pu, having a half-life of 87.7 years, as plutonium-citrate (about 30 kBq/animal) to 30 Sprague-Dawley (OFA) and 30 (Wistar-Furth x Fisher) F1 hybrid (IFFA-CREDO, France) male rats at the age of 12-weeks, following the experimental procedure previously described.\textsuperscript{40} The F1 hybrid rats were used for future LOH (loss of heterozygosity) studies in addition to the outbred Sprague-Dawley rats. Osteosarcomas developed from 12 to 18 months after the Pu-injection. The tumor characteristics and the estimated radiation-absorbed doses to bone have been described.\textsuperscript{30} The chemical toxicity of the Pu, a heavy metal, is inconsequential alongside the radiation effects and the incidence of spontaneous osteosarcomas is quite low. Therefore, the osteosarcomas developed after the Pu-injection in this study were considered as radiation-induced osteosarcomas. All animals were handled in accordance with the recommendations of the European Ethical Committee (86/609/EEC) and the French National Committee (87/848) for the care and use of laboratory animals.

**Osteoblast Isolation and Cell Culture**

Osteoblasts were isolated from calvaria of two-day-old male and female Sprague-Dawley rats by sequential enzymatic digestion according to the method described previously.\textsuperscript{31} Cells pooled from six to seven rats in each group were grown in Petri dishes in phenol red-free DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 IU/ml penicillin, 50 μg/ml streptomycin, 2.5 μg/ml amphotericin B and 2.05 μg/ml sodium deoxycholate. The culture was maintained, up to 70% confluence, at 37°C in a humidified atmosphere of 5% CO₂.

**Comparative Genomic Hybridization (CGH) Analysis**

Genomic DNA from frozen tumor specimen and normal male rat tissue and slides with normal rat metaphases were prepared as previously described.\textsuperscript{32} CGH was performed after labeling of tumor and reference DNAs (1 μg) by nick-translation using Biotin-16-dUTP and digoxigenin-11-dUTP (Roche diagnostics), respectively. Tumor and reference DNAs were ethanol-precipitated with 20 μg of rat Cot-1 DNA (Rat Hybloc Competitor DNA - Applied Genetics Laboratories) and resuspended in hybridization buffer. Hybridization conditions and post-hybridization washes were the same as previously described.\textsuperscript{33} Biotin and digoxigenin probes were revealed by Avidin-FITC and anti-digoxigenin antibodies conjugated to rhodamine, respectively. After DAPI staining, three-color images from 15 well-hybridized metaphases were captured using IPLab Spectrum software from a cooled CCD camera (Princeton Instruments) mounted on a Microphot-FXA microscope (Nikon). Classification of G-banded chromosomes and the green-to-red fluorescence ratio along each individual chromosome were calculated by the QUIPS-CGH software (Vysis). Chromosome imbalances were analyzed on the basis of the
Genetic Alterations in Radiation-induced Osteosarcomas

Quantitative Real-Time Reverse Transcription-PCR (RT-PCR) Analysis

Total RNA was prepared from the 5 cell fractions of rat osteoblasts and 15 (of 16) osteosarcomas (cases A-M, O, P) using RNA-plus lysis reagent according to the manufacturer’s protocol (Q-Biogene) and quantified by Nanodrop spectrophotometer. For the first-strand cDNA synthesis, 1 μg of total RNA was reverse-transcribed using random hexamers (pd(N)6; Boehringer Mannheim) as described previously.33) PCR reactions were performed on an ABI PRISM 7300 sequence detection system (Applied Biosystems) using either the SYBR Green PCR Master Mix kit or the TaqMan Universal PCR Master Mix (Applied Biosystems). Quantification of each gene expression was calibrated using a reference standard curve obtained by serial dilutions of PCR product prepared from a mixture of cDNAs from several rat tissues. The geometric mean expression of three housekeeping genes (Hmbs, Hprt, and Ywhaz) was used to normalize the expression of genes of interest according to the published method.34) The sequences of PCR primers for the rat Cdk4, Mdm2, Wisp1, c-Myc, Pdgf-b, Vegf, E2F1, p19ARF, p57KIP2, RB1 and p16INK4a are available on request. The PCR primers and probes were purchased from Applied Biosystems for Hmbs, Hprt, Ywhaz, Cyclin D2, and p15INK4b genes (TaqMan Gene Expression Assays: assay ID Rn00565886_m1, Rn01527838_g1, Rn00755072_m1, Rn01492401_m1, and Rn00590746_m1, respectively).

Statistical Analysis

The Mann-Whitney U test was used for comparison between groups. A P value < 0.05 was considered as significant. Statistical analysis was performed using the StatView software (SAS Institute, Inc.).

Tp53 Mutation Analysis

Overlapping cDNA fragments, initially prepared by RT-PCR and covering the full length of the Tp53 coding sequence were directly sequenced using the dideoxynucleo-

Fig. 1. Summary of gains and losses of chromosomal materials in 16 Pu-induced rat osteosarcomas analysed by CGH. Ideograms of rat chromosomes correspond to G-banding. Vertical lines on the right and left sides of chromosomes indicate gains and losses, respectively. High level gains (amplifications) of genomic material are indicated by thick lines. The alphabets on the top of each line refer to case numbers.
otide method. Then, when a deletion was found, exons and parts of introns from genomic DNA were sequenced by the same technique in order to determine whether the cDNA deletion was the result of a splicing mutation or a genomic deletion. Primer sequences are available on request.

**RESULTS**

**Genomic Imbalances in Pu-Induced Rat Osteosarcomas**

Genomic DNAs extracted from the 16 Pu-induced osteosarcomas, classified as osteoblastic osteosarcoma, were subjected to CGH analysis for identification of genomic imbalances. The CGH analysis revealed a complex pattern of chromosomal aberrations in all tumors (Fig. 1). On average, 90% of the RNO (Rattus Norvegicus) autosomal chromosomes presented a gain and/or a loss. The median number of changes was 27 per tumor (range 12–36) and each chromosome was found to be altered in at least 10 tumors. Gains (median 15, range 5–20) were more frequent than losses (median 12.5, range 7–18). High-level gains (amplifications) represented 7.5% of the genome of the tumors. The most frequently deleted RNO chromosomal regions were observed at 1p, 1q, 3q23-q35, 5q21-q33, 8q24-q31, 10q22-q25, 15p, 15q and 18q. Most recurrently gained RNO regions were mapped to chromosome 3p12-q12, 3q41-qter, 4q41-qter, 6q12-q16, 7q22-q34, 8q11-q23, 9q11-q22, 10q32.1-qter and 12q. High-level gains were frequently (40%) noticed in RNO chromosomes at 7q22-q34 and 12q.

**Identification of Potential Target Gene(s) Involved in the Frequent Chromosomal Imbalances in Pu-Induced Rat Osteosarcomas**

To identify potential target gene(s) involved in the recurrent chromosomal aberrations detected by the CGH analysis in Pu-induced rat osteosarcomas, we initially focused on the G1/S phase cell cycle regulator and growth factor genes. Using the rat genome database archived by NCBI, we found that some of the G1/S phase cell cycle regulator and growth factor genes were mapped to the regions of frequent chromosomal imbalances in Pu-induced rat osteosarcomas (Fig. 2A). These included 8 genes (c-Myc, Cdk4, Mdm2, E2F1, Ccn2/Cyclin D2, Vegf, Wisp1, and Pdgf-b) located at the regions of frequent chromosomal gain, and 5 genes (Cdkn2b/p15INK4b, Cdkn2a/p16INK4a, p19ARF, Rb1, and Cdkn1c/p57KIP2) located at the regions of frequent loss. By quantitative real-time RT-PCR analysis, the expression levels of these genes were measured in Pu-induced rat osteosarcomas and osteoblasts as normal counterparts (Fig. 2B–H). Among the genes located in the region of frequent chromosomal gains at 7q22-33, the expression levels of the c-Myc and Pdgf-b genes in the tumors were statistically significantly higher than that of normal osteoblasts (Fig. 2B, P < 0.05 and P < 0.005, respectively). We also observed that the expression of the Cdk4 gene was increased in many tumors (8 of 15 tumors). The expression level of the p57KIP2 gene, located in the region of chromosomal losses at 1q, was significantly reduced in the tumors compared with osteoblasts (Fig. 2F, P < 0.005). On the other hand, the mRNA for the p15INK4b, p16INK4a, and p19ARF tumor-suppressor genes were detected in many tumors. However, it should be noted that the expression of these genes was very low or undetectable in some tumor cases as well as in normal osteoblasts (Fig. 2G). In addition, we found hypermethylation of the p16INK4a promoter in tumor case M (data not shown), which showed undetectable level of the p16INK4a expression. Overall, there were no remarkable differences in the expression levels of the other genes between the tumors and osteoblasts, except for the Cyclin D2 gene. The expression levels of the Cyclin D2 gene were strongly decreased in many tumors, whereas this gene was located within the regions of the frequent chromosomal gain at 4q (Fig. 2D). With respect to this observation, recent studies have suggested that the expression of the Cyclin D2 is lost by the hypermethylation of its promoter region in several human cancers. Thus, the down-regulation of the Cyclin D2 in Pu-induced osteosarcomas may be occurring through the hypermethylation of its promoter. In conclusion, the Cdk4, c-Myc, Pdgf-b, and p57KIP2 genes were considered to be possible target genes for the frequent chromosomal gain at 7q22-34 and loss at 1q in the tumors, respectively.

**Tp53 Mutations in Pu-Induced Rat Osteosarcomas**

Four of 15 (27%) tumors had a mutation in the Tp53 gene (Table 1). In the genomic DNA of tumor case B, a G:C > A:T transition was detected at base position 7567, within the exon 3. This point mutation is considered to create a novel donor splicing site, leading, in the mRNA, to a partial deletion of the exon 3. Furthermore, two missense mutations; CG:GC > AA:TT tandem mutation with both transition and transversion at codon 246, and G:C > A:T transition at

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**Fig. 2.** Identification of potential target gene(s) involved in the frequent chromosomal aberrations in Pu-induced rat osteosarcomas. (A) Candidate genes located within the region of frequent chromosomal aberrations were mapped to the chromosome ideograms using the rat Map Viewer of NCBI (http://www.ncbi.nlm.nih.gov/mapview/). Chromosomal syntenic regions from human (HSA) were displayed by vertical lines on the right side of each ideogram using the Rat Genome Database (RGD) Virtual Comparative Map tool (http://rgd.mcw.edu/VCMAP/). (B-H) Relative expression levels of c-Myc (7q33), Cdk4 (7q22), Mdm2 (7q22), E2F1 (3q41), Cyclin D2 (4q42), p15INK4b (5q32), p16INK4a (5q32), p19ARF (5q32), Rb1 (15q12), p57KIP2 (1q42), Vegf (9q12), Wisp1 (7q33), and Pdgf-b (7q34) genes between rat normal osteoblasts (OB, n = 5) and Pu-induced osteosarcomas (OS, n = 15).
Table 1. Mutations in the Tp53 Gene of Pu-Induced Rat Osteosarcomas

<table>
<thead>
<tr>
<th>Case</th>
<th>DNA</th>
<th>Mutation type</th>
<th>mRNA (Exon)</th>
<th>Consequence</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>B</td>
<td>E3  B7567</td>
<td>Point mutation</td>
<td>Co 103-123 (3) del 62 bp</td>
<td>Frameshift by splice-site alteration</td>
<td>Stop Co 109</td>
</tr>
<tr>
<td></td>
<td>CG &gt; T/A</td>
<td>Transition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>E5  B8759-8760</td>
<td>Tandem point</td>
<td>Co 246 (5) CG &gt; AA</td>
<td>Missense</td>
<td>Arg246Asn&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>CG/GC &gt; AA/TT</td>
<td>mutation Complex&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>E5  B8762</td>
<td>Point mutation</td>
<td>Co 247 (5) G &gt; A</td>
<td>Missense</td>
<td>Arg247Gln&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>G/C &gt; A/T</td>
<td>Transition</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>O</td>
<td>I7-E8 B10219-10282</td>
<td>Deletion</td>
<td>Co 330-364 (8) del 107 bp</td>
<td>Frameshift by splice-site alteration</td>
<td>Stop Co 343</td>
</tr>
<tr>
<td></td>
<td>del 64 bp</td>
<td></td>
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</table>

E: exon; I: intron; B: base position in the genomic sequence (GenBank accession number NC_005109.2, gene ID: 24842); del: deletion; bp: base pair; Co: codon. *Complex mutation consists of both transition and transversion. **Codons 246 and 247 correspond to codons 248 and 249 of the human Tp53 gene, respectively.

codon 247, were identified within the exon 5 in cases J and M, respectively. These missense mutations at codon 246 and 247 are considered to result in the replacement of arginine by asparagine and glutamine, respectively. In addition, a genomic deletion of 64 base pairs (bp), leading to the removal of the acceptor splicing site in exon 8, was observed in case O. This genomic deletion encompassed the end of intron 7 and the beginning of exon 8 and was responsible for the complete deletion of exon 8 (107 bp). Collectively, the four genomic Tp53 mutations were characterized by one deletion of 64 bp (case O) and three point mutations: two transitions (cases B and M), and one complex mutation consisted of both transition and transversion (case J). Among these 3 point mutations, the C:G > A:T at codon 246 (base position 8759) in case J and the G:C > A:T at codon 247 (base position 8762) in case M, were located at CpG dinucleotides.

**DISCUSSION**

In the present study, Pu-induced rat osteosarcomas were characterized by highly complex chromosomal abnormalities by the CGH analysis, and this result was considered to be analogous to that of the cytogenetically complex sarcomas. The CGH study of Pu-induced rat osteosarcomas also identified frequent chromosomal gains/amplifications at 3p12-12, 3q41-ter, 4q41-ter, 6q12-1q6, 7q22-q34, 8q11-q23, 9q11-q22, 10q32.1-ter, 12q and losses at 1p, 1q, 3q23-q35, 5q21-q33, 8q24-q31, 10q22-q25, 15p, 15q, 18q (Fig. 1). Based on comparative map data, we found that the recurrently gained/amplified region RNO 7q22-34 consisted of three distinct homologous regions of human chromosome (HSA) 12q13-15, 8q24 and 22q11-13 (Fig. 2A). As we expected, it has been reported that amplification of the region HSA 12q13-15 is associated with a variety of human sarcomas, including osteosarcoma. Although this region contains numerous genes, it has been reported that some specific target genes, including the MDM2, CDK4, and SAS, are involved in the amplification of this region. Frequent chromosomal gains at the region HSA 8q23-24, where the c-MYC is located, has also been observed in several CGH studies of human sporadic sarcoma. Interestingly, frequent chromosomal gain of the region HSA 22q and overexpression of the PDGF-B gene (located at 22q13.1) have been reported in human fibrosarcomas of bone. In addition, we found that the chromosomal region RNO 9q12-21 corresponded to a part of the human chromosome arm 6p12-13, which is also frequently amplified in human sporadic osteosarcoma. In addition, we found that the other frequently gained regions in Pu-induced rat osteosarcomas included the Abl (3p12), Src (3q42), K-ras (4q44), N-myc (6q14), and Erbb2 (10q32.1) proto-oncogenes. On the other hand, we found that some tumor suppressor genes, including the Pten (1q41-43), p16INK4a (1p19), p16 (1p19), p19ARF (1q41-43), Rb1 (1q12), and Rb1 (1q12), were mapped to the chromosomal regions frequently lost in Pu-induced rat osteosarcomas. By quantitative RT-PCR analysis, the Cdk4, c-Myc and Pdgf-b genes were suggested to be probable target genes for the genomic gain at chromosomal region 7q22-34 in Pu-induced rat osteosarcomas (Fig. 2). These results were consistent with some previous reports in human sporadic sarcoma, including osteosarcoma. In this study, we did not always observe a clear correlation between the gene expression levels and the patterns of CGH alterations. This may be caused in part by the resolution of the conventional CGH analysis, for which the resolutions (10 Mb maximum) does not allow precise gene identification. Moreover, some gene expressions may be regulated by mechanisms other than genomic gain (or loss), such as negative (or positive) feedback regulations, mutated alleles, and promoter methylation. On the other hands, we observed that the expression level of the p57KIP2 gene, located in the region of chromosomal losses at 1q, was significantly reduced in the tumors compared to normal osteoblasts. Interestingly, human p57KIP2 gene is located at chromosome 11p15.5, and frequent loss of the human chro-
mosome 11p and down-regulation of the p57KIP2 gene by both chromosomal deletion and promoter hypermethylation has been reported in several human cancers such as Wilms’ tumor, bladder cancer, and gastric cancer.\textsuperscript{40–42} Therefore, the p57KIP2 gene may be a novel candidate gene for radiation-induced osteosarcomas. Taken together, abnormal control of the G1/S phase cell-cycle checkpoint and modulation of the growth factor response are considered to play an important role also in the pathogenesis of radiation-induced osteosarcoma.

To date, there are only limited numbers of reports describing the genetic changes in radiation-induced sarcomas, including osteosarcomas. Interestingly, two studies have reported that chromosomal losses are more frequent than gains in post-irradiation osteosarcomas, whereas gains of genomic materials outnumber losses in sporadic osteosarcomas.\textsuperscript{9,12} This feature could allow to distinguish radiation-induced osteosarcomas from sporadic cases, however, there was no obvious difference between the proportion of the chromosomal gains and losses in Pu-induced rat osteosarcomas. In addition, the higher frequency of polyclonal tumors and the frequent loss of 3p21-ter have been reported as unusual cytogenetic findings in radiation-associated sarcomas compared to the observations in sporadic sarcomas.\textsuperscript{9,10} Using a total of 27 post-irradiation human sarcomas, representing the most common histological types, Tarkkanen et al., have also reported that chromosomal region 1p21-31 is underlined as a major site of chromosomal loss in the post-irradiation human sarcomas comparatively to sporadic cases by CGH analysis.\textsuperscript{12} In our CGH study, it remains unclear whether there are these radiation-specific cytogenetic features of frequent chromosomal losses in Pu-induced rat osteosarcomas since the human 1p21-31 region splits on rat chromosomes 2, 4, 5 and 14. Thus, further studies will be needed to determine whether there are specific changes in chromosomal aberrations in radiation-induced osteosarcoma.

Previous mutation spectrum studies have revealed the presence of characteristic patterns of DNA alteration induced by exogenous and endogenous mutagens in cancer-related genes. Inactivation of p53 by a mutational event is a key factor in cancer development and is known to be directly induced by exogenous carcinogens. For example, mutations in the TP53 gene are frequently observed in skin tissues highly exposed to UV radiation, lung in the smokers, or liver exposed to aflatoxins. In these cancers, specific patterns of TP53 mutations (mostly seen at codons 248, 249 and 273) have been reported to correspond to the precise exogenous carcinogen.\textsuperscript{9,34} Thus, it could be possible to identify a specific pattern of TP53 mutations associated with ionizing radiation by comparing the mutation spectrums in TP53 gene between radiation-induced and sporadic osteosarcomas. In the previous studies, specific patterns of TP53 mutations in radiation-induced sarcomas, including osteosarcomas, have been suggested as follows: the point mutations at non-CpG sites; the presence of recurrent mutation sites at codons 135 and 237.\textsuperscript{11,45} In contrast to these findings, two of the three point mutations found in this study were G:C > A:T transition at CpG sites and they were located at codons 246 and 247, corresponding to codons 248 and 249 in human, respectively. This discrepancy in the pattern of TP53 point mutations may be explained by the difference in energy deposition in the DNA molecule between high-LET alpha-particles studied here and low-LET radiation used for radiotherapy. On the other hand, the frequency of Tp53 mutations in Pu-induced osteosarcomas (27\%\,) was relatively similar to both sporadic (17\%\,) and radiation-induced sarcomas (37.5\%\,) in human.\textsuperscript{45}

To our knowledge, only limited information is available on the molecular features of radiation-induced osteosarcomas. Therefore, our data may contribute to further understanding of the molecular mechanisms underlying radiation-induced bone tumors in human.

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