ANTICANCER RESEARCH *25*: 2951-2956 (2005)

Postradiation Malignant Fibrous Histiocytoma and Osteosarcoma of a Patient with High Telomerase Activities

TOSHIHIRO MATSUO[1], TAKASHI SUGITA[2], SHOJI SHIMOSE[1], TADAHIKO KUBO[1], YUJI YASUNAGA[1], EISO HIYAMA[3] and MITSUO OCHI[1]

[1]Department of Orthopaedic Surgery and [3]Natural Science Center for Basic Research and Development, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima; [2]Department of Orthopaedic Surgery, Hiroshima Prefectural Hospital, Hiroshima, Japan

Abstract. *Background: An extremely rare case of postradiation malignant fibrous histiocytoma (MFH) and osteosarcoma (OS) secondary to radiation therapy for leukemia-related osteolytic lesions is presented. In addition, the telomere biology of these tumors was investigated. Case Report: A 14-year-old boy was diagnosed with acute lymphocytic leukemia. The right tibia was irradiated at a total dose of 60 Gy, and the left tibia was irradiated at a total dose of 40 Gy. The left tibia developed MFH and the right tibia developed OS. Results: Telomere reduction (MFH 70.2, OS 70.0%) and high telomerase activities (MFH 12.1, OS 17.7 TPG) were observed. These results reflect an aggressive feature of postradiation sarcomas. Conclusion: Prognosis for patients diagnosed with postradiation sarcoma is poor due to its aggressiveness. However, even if sarcoma occurs after irradiation in more than two fields in a single patient, improvements in prognosis are anticipated with appropriate chemotherapies and wide resection.*

The incidence of postradiation of bone sarcoma is very low, being estimated to occur in less than 1% of irradiated patients, but postradiation sarcoma is a serious, long-term complication of radiation therapy (1, 2). Here, the first case of postradiation malignant fibrous histiocytoma (MFH) and osteosarcoma (OS), which developed in the bilateral proximal tibia in the same patient, following radiation therapy for leukemia-related osteolytic lesions, is reported. In addition, the telomere biology of these tumors was investigated for parameters of aggressiveness.

Correspondence to: Toshihiro Matsuo, MD, Ph.D., Department of Orthopaedic Surgery, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Tel: +81-82-257-5233, Fax: +81-82-257-5234, e-mail: tmatsuo@hiroshima-u.ac.jp

Key Words: Postradiation sarcoma, malignant fibrous histiocytoma, osteosarcoma, telomerase activity, telomere length.

Case Report

A 14-year-old boy experienced high fever in October 1989 and was diagnosed with acute lymphocytic leukemia. After diagnosis, he underwent chemotherapy with methotrexate (MTX), cyclophosphamide (EDX), adriamycin, vincristine (VCR), cytarabine (Ar-C), prednisolone (PSL) and 6-mercaptopurine (6-MP). In May 1990, he experienced pain in his right knee. Plain radiograph and MRI revealed an osseous lesion of the right tibia due to leukemia. The right tibia was irradiated at a dose of 20 Gy. In October 1990, at 15 years of age, he experienced pain in both knees. Irradiation of the bilateral proximal tibia at a dose of 20 Gy was performed because bone invasion in both proximal tibias was noted on MRI. The radiation field was subsequently expanded to both distal femurs, and both knees were irradiated at a dose of 20 Gy. The right tibia received a total dose of 60 Gy, and the left tibia received a total dose of 40 Gy. In November 1991, at 16 years of age, outpatient treatment with intravenous administration of MTX (12 mg/m^2), VCR (1.5 mg/m^2), Ar-C (100 mg/m^2), EDX (600 mg/m^2) and PSL (60 mg/m^2) was initiated, and no enlargement of the lesions in either knee was noted on MRI. Oral administration of 6-MP, MTX and PSL began in December 1993. In May 1999, at 23 years of age, the patient developed a fever and swelling of the left knee. MRI of the left proximal tibia revealed abnormal findings (Figure 1). An open biopsy was performed and the patient was diagnosed with MFH (giant cell type) (Figure 2). For neoadjuvant chemotherapy, administration of carboplatin (CBDCA) (100 mg/m^2), etoposide (VP-16) (100 mg/m^2) and MTX (8～12 g/m^2) were started, and a total of four courses were performed. After the chemotherapy, left above-knee amputation and open biopsy of the right proximal tibia were performed in August 1999. CBDCA (300 mg/m^2), VP-16 (200 mg/m^2) and MTX (12 g/m^2) were administered for a total of six courses after surgery. In January 2003, at 27 years of age, the patient noticed swelling and local heat in the right knee. MRI of the

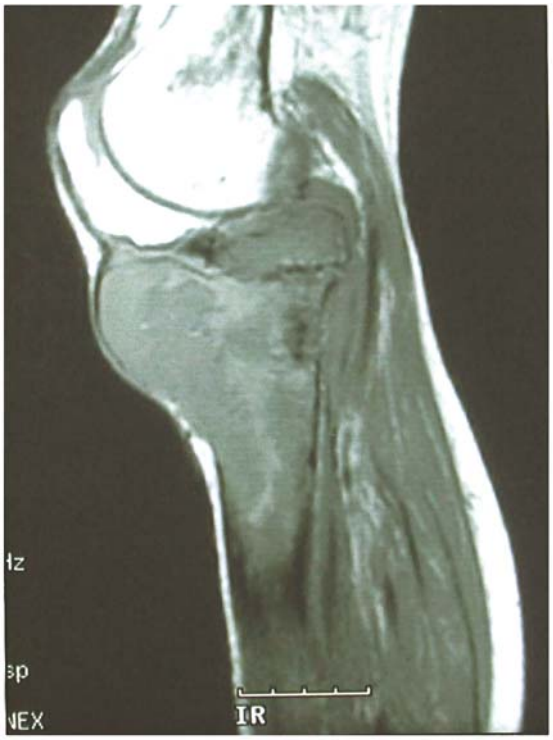

Figure 1. *T1-weighted sagittal MRI (TR 516/ TE 12) demonstrates a non-homogeneous large mass of low signal intensity protruding from the left tibia.*

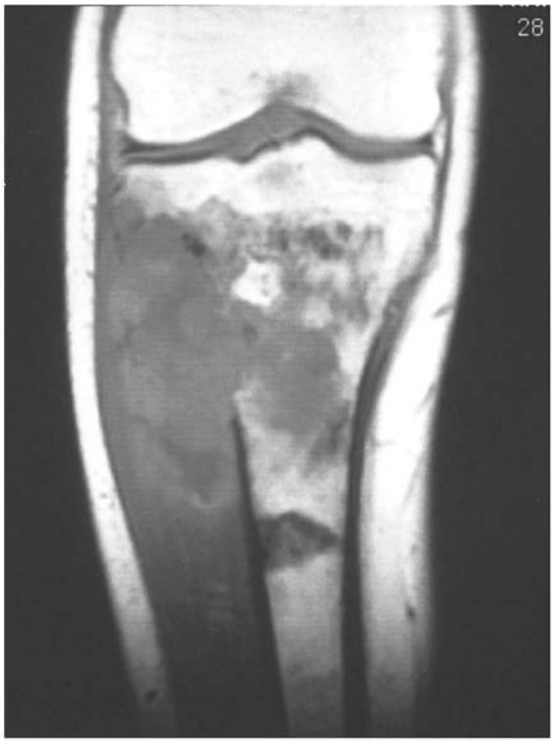

Figure 3. *T1-weighted coronal MRI (TR 466/ TE 9) demonstrates a non-homogeneous mass of low signal intensity protruding from the right tibia.*

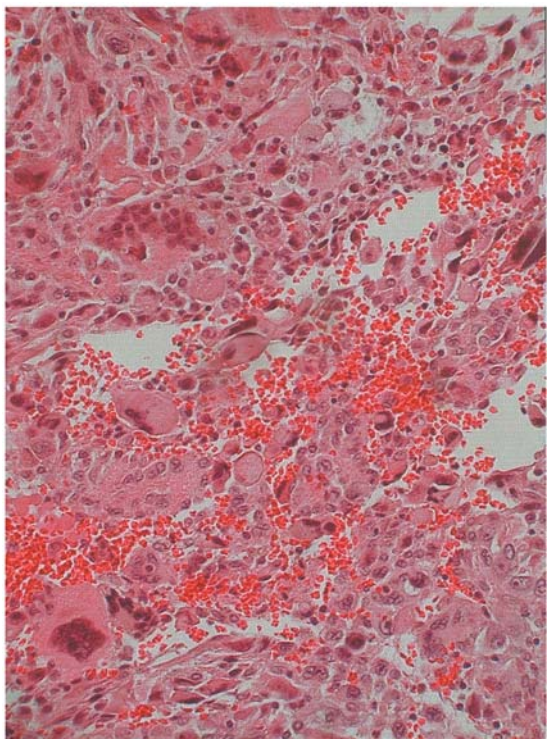

Figure 2. *Malignant fibrous histiocytoma of giant cell type. The presence of numerous multinucleated giant cells is accompanied by hemorrhagic necrosis (x200).*

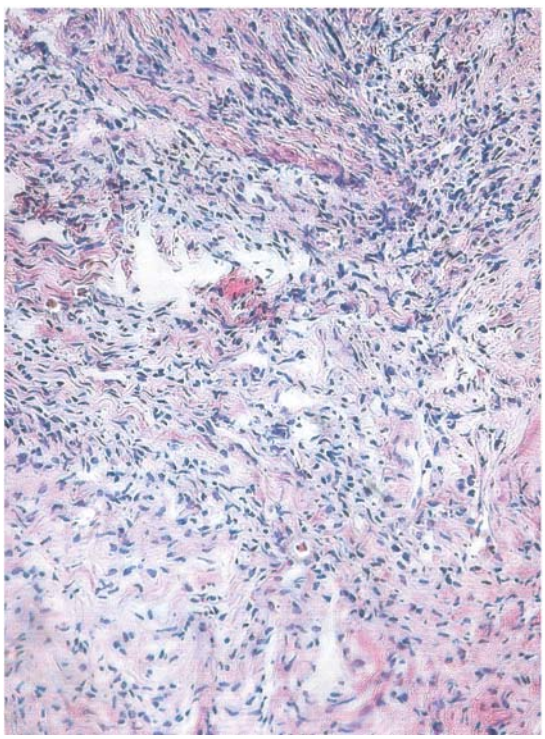

Figure 4. *Osteosarcoma of osteoblastic type. Spindle tumor cells with strong heteromorphism exhibit hyperplasia. (x200).*

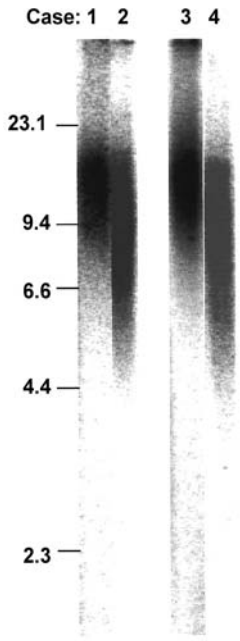

Figure 5. *Southern hybridization analysis of the telomere region in tumors paired with normal tissue DNA. Case 1: Normal muscle tissue sample (12.4 kb), Case 2: MFH sample (8.7 kb), Case 3: Normal muscle tissue sample (12.0 kb), Case 4: OS sample (8.4 kb).*

right proximal tibia revealed abnormal findings (Figure 3). An open biopsy was performed and a pathological diagnosis of osteosarcoma (osteoblastic type) was confirmed (Figure 4). CBDCA (100 mg/m^2), VP-16 (100 mg/m^2) and MTX (10 ~ 12 g/m^2) were administered for a total of four courses as neoadjuvant chemotherapy. In July 2003, wide resection of the sarcoma and reconstruction with prosthesis were performed. For postoperative chemotherapy, CBDCA (100 mg/m^2), VP-16 (100 mg/m^2) and MTX (12 g/m^2) were administered in a total of six courses from August to December 2003 and, as of January 2005, no local recurrence or metastasis has been observed.

Materials and Methods

Tissue samples. Tumor samples were obtained from the patient by resection, and normal muscle samples were also obtained as healthy tissue samples under informed consent. After surgery, all samples were immediately frozen and stored at –80˚C until use.

Telomere length analysis by Southern blotting. Genomic DNA was isolated from frozen samples of tumor and paired normal muscles. For the analysis of telomere length, 2 μg of DNA were digested to completion with 10 units of Hinf 1, electrophoresed on 0.8% agarose gels and then blotted onto nitrocellulose filters. The filters were hybridized to a ^{32}P-labelled $(TTAGGG)_4$ probe, washed and then autoradiographed. The mean length of TRFs was estimated at peak position of hybridization signal using the Bioimage Analyzer (BAS 2000, FUJIFILM, Tokyo, Japan) and MacBass software (FUJIFILM).

Telomerase assay. Evaluation of telomerase activity was performed by the TRAP (telomeric repeat amplification protocol) assay. Briefly, frozen tumors and adjacent normal muscle samples of 50-100 mg were homogenized in 200 μl of CHAPS lysis buffer. After 25 minutes of incubation on ice, the lysates were centrifuged at 16,000 x g for 20 minutes at 4˚C, and the supernatant was frozen at –80˚C. The concentration of tumor protein was measured using the BCA Protein Assay Kit (Pierce Chemical Co., Rockford, IL, USA), and an aliquot containing 1 μg of tumor protein was used for each TRAP assay. The levels of telomerase activity were measured using the TRAPeze XL Telomerase Detection Kit (Intergen Co., NY, USA), which is a quantitative fluorescein-labelled PCR system, with the use of a PCR internal control. For the direct detection of fluorescein and sulphorhodamine, the PCR product was measured in a fluorescent plate reader to detect the levels of fluorescein and sulphorhodamine by using the appropriate excitation and emission filters. The telomerase activity was quantified by the ratio of the fluorescein intensity of the entire TRAP ladder to the sulphorhodamine intensity of the internal control after the correction of each fluorescent intensity for the negative control and the background, and was expressed as Total Product Generated (TPG) units.

Results

The telomere length of the two adjacent normal muscles was 12.4 kb and 12.0 kb, whereas those of the postradiation tumors were 8.7 kb (MFH) and 8.4 kb (OS) (Figure 5). The telomere lengths of the tumors were shorter than those of normal tissues (percentage of telomere reduction; MFH 70.2%, OS 70.0%). Telomerase activity was detectable in tumor samples, with detectable levels of 12.1 TPG (MFH) and 17.7 TPG (OS).

Discussion

Successful management of malignant neoplasms can be achieved by combinations of therapies such as irradiation, chemotherapy and surgery. On the other hand, the risk of secondary cancer is increased by the use of such methods to improve cancer patient prognosis. Postradiation sarcoma is a rare type of iatrogenic cancer (3). Amendola *et al.* estimated an incidence of 0.09-0.11% among cases after radiation therapy (4) and Tountas *et al.* reported that postradiation sarcoma of bone occurred in 0.02% of irradiated patients (5). Postradiation sarcoma is sometimes misdiagnosed because it is rare and, thus, it is necessary for clinicians to remain vigilant for this disease following radiotherapy (6).

Radiation-induced sarcomas reportedly require high doses of radiation (3, 7). There are several reports

indicating that the sarcoma is generated by irradiation of 40 Gy or more, with no appearance of sarcoma after doses of 30 Gy or less. Tountas *et al.* reported a secondary osteosarcoma after treating a primary malignant neoplasm with 40 to 70 Gy (5), and Murray *et al.* reported that no postradiation sarcoma patient received less than 30 Gy (8). Our patient received irradiation at 40 Gy and 60 Gy in the left and right tibias and, thus, our observations are in agreement with these reports.

Some reports have suggested that higher doses of radiotherapy are associated with a shorter latency (9,10). In contrast, other reports were inconclusive regarding the relationship between latency and dose (1). In the present case, the latent period was shorter by four years after 40-Gy irradiation than after 60-Gy irradiation. This contradicts the notion that larger doses of radiation result in shorter periods of time before developing sarcoma. Furthermore, in the present patient, MFH took eight years and seven months and OS took twelve years and eight months to develop after the start of the radiotherapy. Hence, there was an approximately four-year difference in the latent period. According to previous reports, there is no clear difference in the latent periods between MFH and OS (3, 9).

If treatment of cancer does not necessarily require radiotherapy, irradiation should be avoided wherever possible. However, prognosis in many cancer patients is improved by radiotherapy. For patients who have undergone irradiation, regular observation of the irradiated area is necessary. If pain, swelling and/or local heat develop in an irradiated area, postradiation sarcoma should be considered and the area should be subjected to MRI scanning.

The chemotherapeutic agents used in the treatment of cancer have been shown to be oncogenic in animals (11), and it is evident that many of these agents increase the expected damage to tissue when given in conjunction with radiotherapy. The risk of radiation-induced sarcoma increases with the use of high-dose chemotherapy, and the latent period to secondary tumors becomes shorter in cases treated with combined therapy (12, 13). The present patient had leukemia as the underlying disease and required potent chemotherapy. Therefore, it is also necessary to consider the likelihood of postradiation sarcoma when radiotherapy is performed on patients with underlying diseases requiring chemotherapy.

Many telomere-related studies have been conducted on human carcinomas for diagnostic and/or prognostic value. Telomerase activity has been reported in 80-90% of carcinomas, and in some types of carcinomas high telomerase activity has been cited as a marker of tumor aggressiveness (14-18). In addition, a considerable shortening of telomeres has been found in many cancer cells, despite the expression of telomerase (15-18, 19). In contrast, almost no benign tumors express telomerase activity (19). Postradiation sarcoma is a very aggressive tumor and the prognosis for patients is generally poor (9, 10). In our study, telomere reduction was observed in tumor samples and high telomerase activity was detected. These results reflect the aggressive nature of postradiation sarcomas. Treatment for secondary sarcomas is based on surgical resection and additional therapies, such as chemotherapy, may also be necessary. In the present case, no metastasis or local recurrence was observed, probably due to early diagnosis, chemotherapy and wide resection.

An extremely rare case of postradiation malignant fibrous histiocytoma and osteosarcoma occurring in the bilateral tibia of the same patient, following secondary radiation therapy for leukemia-related osteolytic lesions, with telomere reductions and high telomerase activities, was reported here. It is necessary to consider the occurrence of postradiation sarcoma when irradiation of 40 Gy or more is used. In addition, even when the exposure dose is relatively small, sarcoma may exhibit short latencies and, thus, long-term observation of all irradiated fields is necessary. However, even if sarcoma occurs after irradiation in more than two fields in a single patient, improvements in prognosis are anticipated by appropriate chemotherapies and wide resection.

References

1 Hatfield PM and Schulz MD: Postirradiation sarcoma. Including 5 cases after X-ray therapy of breast carcinoma. Radiology *96*: 593-602, 1970.

2 Mark RJ, Poen J, Tran LM, Fu YS, Selch MT and Parker RG: Postirradiation sarcomas. A single-institution study and review of the literature. Cancer *73*: 2653-2662, 1994.

3 Huvos AG, Woodard HQ, Cahan WG, Higinbotham NL, Stewart FW, Butler A and Bretsky SS: Postradiation osteogenic sarcoma of bone and soft tissues. A clinicopathologic study of 66 patients. Cancer *55*: 1244-1255, 1985.

4 Amendola BE, Amendola MA, McClatchey KD and Miller CH Jr: Radiation-associated sarcoma: a review of 23 patients with postradiation sarcoma over a 50-year period. Am J Clin Oncol *12*: 411-415, 1989.

5 Tountas AA, Fornasier VL, Harwood AR and Leung PM: Postirradiation sarcoma of bone: a perspective. Cancer *43*: 182-187, 1979.

6 Kim RY, Weppelmann B, Salter MM and Brascho DJ: Skeletal metastases from cancer of the uterine cervix: frequency, patterns and radiotherapeutic significance. J Radiat Oncol Biol Phys *13*: 705-708, 1987.

7 Pettit VD, Chamness JT and Ackerman LV: Fibromatosis and fibrosarcoma following irradiation therapy. Cancer *7*: 149-158, 1954.

8 Murray EM, Werner D, Greeff EA and Taylor DA: Postradiation sarcomas: 20 cases and a literature review. Int J Radiat Oncol Biol Phys *45*: 951-961, 1999.

9 Laskin WB, Silverman TA and Enzinger FM: Postradiation soft tissue sarcomas. An analysis of 53 cases. Cancer *62*: 2330-2340, 1988.
10 Wiklund TA, Blomqvist CP, Raty J, Elomaa I, Rissanen P and Miettinen M: Postirradiation sarcoma. Analysis of nationwide cancer registry material. Cancer *68*: 524-531, 1991.
11 Harris CC: Immunosuppressive anticancer drugs in man: their oncogenic potential. Radiology *114*: 163-166, 1975.
12 Le Vu B, de Vathaire F, Shamsaldin A, Hawkins MM, Grimaud E, Hardiman C, Diallo I, Vassal G, Bessa E, Campbell S, Panis X, Daly-Schveitzer N, Lagrange JL, Zucker JM, Eschwege F, Chavaudra J and Lemerle J: Radiation dose, chemotherapy and risk of osteosarcoma after solid tumours during childhood. Int J Cancer *77*: 370-377, 1998.
13 Bechler JR, Robertson WW Jr, Meadows AT and Womer RB: Osteosarcoma as a second malignant neoplasm in children. J Bone Joint Surg Am *74*: 1079-1083, 1992.
14 Chadeneau C, Hay K, Hirte HW, Gallinger S and Bacchetti S: Telomerase activity associated with acquisition of malignancy in human colorectal cancer. Cancer Res *55*: 2533-2536, 1995.
15 Hiyama E, Hiyama K, Yokoyama T, Matsuura Y, Piatyszek MA and Shay JW: Correlating telomerase activity levels with human neuroblastoma outcomes. Nat Med *1*: 249-255,1995.
16 Hiyama E, Yokoyama T, Tatsumoto N, Hiyama K, Imamura Y, Murakami Y, Kodama T, Piatyszek MA, Shay JW and Matsuura Y: Telomerase activity in gastric cancer. Cancer Res *55*: 3258-3262, 1995.
17 Hiyama E, Kodama T, Shinbara K, Iwao T, Itoh M, Hiyama K, Shay JW, Matsuura Y and Yokoyama T: Telomerase activity is detected in pancreatic cancer but not in benign tumors. Cancer Res *57*: 326-331, 1997.
18 Hiyama E and Hiyama K: Clinical utility of telomerase in cancer. Oncogene *21*: 643-649, 2002.
19 Hiyama E, Gollahon L, Kataoka T, Kuroi K, Yokoyama T, Gazdar AF, Hiyama K, Piatyszek MA and Shay JW: Telomerase activity in human breast tumors. J Natl Cancer Inst *88*: 116-122, 1996.

Received February 17, 2005
Accepted May 17, 2005