Complete Necrosis of a Giant Cell Tumor with High Expression of PPARγ: A Case Report

AKIHIKO TAKEUCHI[1], NORIO YAMAMOTO[1], HIDEJI NISHIDA[1], HIROAKI KIMURA[1], HIROKO IKEDA[2] and HIROYUKI TSUCHIYA[1]

[1]*Department of Orthopedic Surgery, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan;* [2]*Section of Diagnostic Pathology, Kanazawa University Hospital, Kanazawa, Japan*

Abstract. *Giant cell tumor of the bone (GCTB) is a common primary benign tumor, but in some cases, it behaves aggressively, resulting in tumor recurrence. The standard treatment for GCT is thorough curettage with adjuvant treatment such as phenol, liquid nitrogen, high-speed burr, or methylmethacrylate cement. This article presents the case of a 30-year-old male with GCT of the right distal femur, which demonstrated a complete necrosis of GCTB. Interestingly, the specimen also showed adipocytic lineage, and strong expression of apoptotic markers by [terminal deoxynucleotidyl-transferase dUTP nick-end labelling (TUNEL) and caspase-3] and peroxisome proliferator-activated receptor gamma (PPARγ). To the Authors' knowledge, this is the first reported case of complete necrosis of GCTB concurrent with adipocytic lineage and high expression of PPARγ. PPARγ is a master regulator of fat differentiation. PPARγ possesses antitumor activity through suppression of tumor proliferation and invasion and induction of differentiation and apoptosis. Although we could not conclude on the exact cause of complete necrosis and high expression of PPARγ in this case, we focused on the medical history, where this patient took zaltoprofen (240 mg/day) for four weeks before the biopsy to alleviate his pain. Zaltoprofen is a propionic-acid derivative non-steroidal anti-inflammatory drug, and it is reported to act as a direct ligand for PPARγ. We speculated that one of the possible mechanisms of PPARγ activation in this case was induction by zaltoprofen, at least in part. Although further analysis using cultured tumor cells with ligands specific to the receptor is necessary, PPARγ may be a novel therapeutic target in GCTB.*

Correspondence to: Akihiko Takeuchi, MD, Ph.D., Department of Orthopaedic Surgery, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8641, Japan. Tel: +81 762652374, Fax: +81 762344261, e-mail: a_take@med.kanazawa-u.ac.jp

Key Words: Giant cell tumor of bone, peroxisome proliferator-activated receptor gamma (PPARγ), apoptosis.

Giant cell tumor (GCT) of the bone is a common primary benign tumor; however, it exhibits aggressive behavior and occasionally gives rise to pulmonary metastases (1). The standard treatment for GCT is thorough curettage with adjuvant treatment such as phenol, liquid nitrogen, high-speed burr, or methylmethacrylate cement (2-6) to reduce the local recurrence. The treatment for recurrent lesions is complicated and sometimes requires the sacrifice of adjacent joints (6). Although GCT is also known to exhibit necrosis with hemorrhage (7), complete necrosis is quite rare. There have been only a few reports on spontaneous necrosis of GCTs, and the underlying mechanism has not been fully- clarified (8-10).

Peroxisome proliferator-activated receptor-gamma (PPARγ) is a master regulator of fat differentiation (11). It is also expressed in various types of cancer and possesses antitumor activity through suppression of tumor proliferation and invasion and induction of differentiation and apoptosis. PPARγ ligands have been investigated and include synthetic ligands such as thiazolidinediones (TZDs), widely used to treat type-2 diabetes mellitus (12), and endogenous ligands, such as fatty acids and prostaglandin-D2 metabolite 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) (13). It has also been reported that some non-steroidal anti-inflammatory drugs (NSAIDs), including indomethacin, act as direct ligands for PPARγ (14). In some types of cancer-including liposarcoma (15), and cancer of the colon (16), breast (17), and prostate (18), targeted-therapy for PPARγ has been performed.

In this study, we present the case of a 30-year-old man with GCT of the right distal femur, which showed complete necrosis and high expression of PPARγ. The patient was informed that data of his case would be submitted for publication, and gave his consent.

Case Report

A 30-year-old man was referred to our University Hospital with pain in the right knee of two months duration without any history of associated trauma. Physical examination revealed no soft tissue swelling or mass around the knee joint,

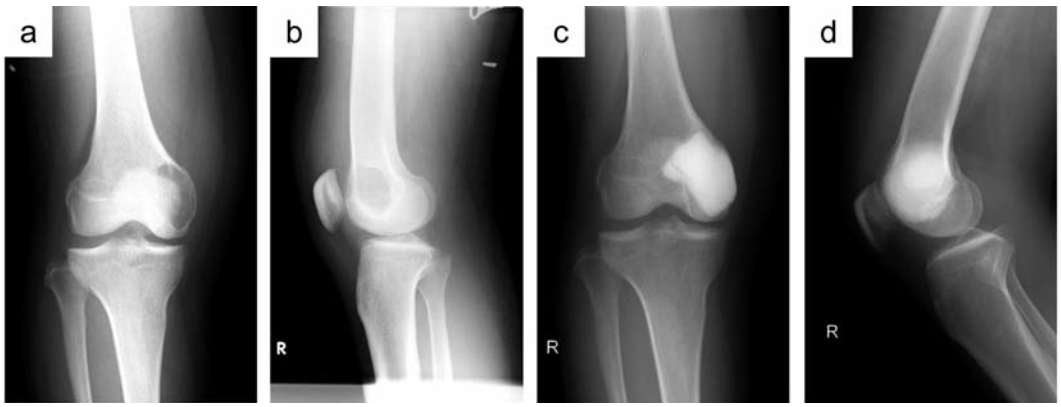

Figure 1. *Anteroposterior (AP) (a) and lateral (b) radiographs of the patient at presentation, revealing an osteolytic lesion of the right distal femur. AP (c) and lateral (d) radiographs taken 24 months after surgery, showing stable artificial bone graft without evidence of tumor recurrence.*

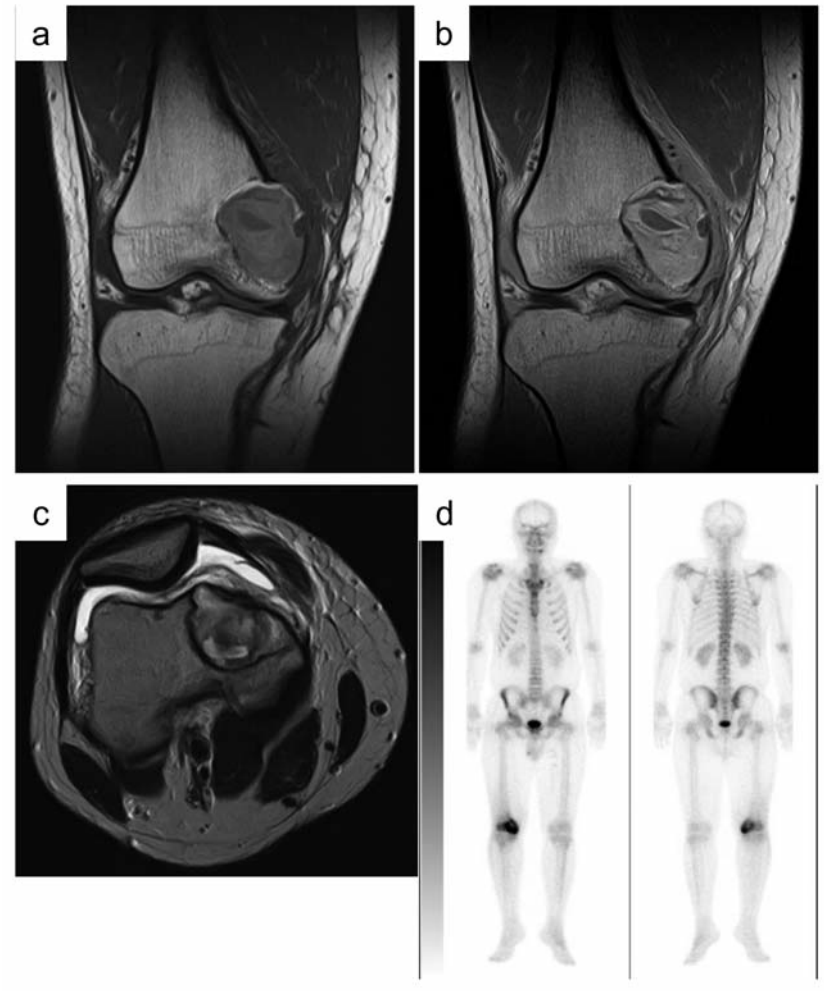

Figure 2. *a: Coronal T1-weighted magnetic resonance imaging (MRI) (TR 675 ms, TE 15.7 ms) scan revealing mixed low- and iso-intensity. b: T2-Weighted MRI (TR 4500 ms, TE 106 ms) images showing areas of mixed low- and high-intensities. c: Axial T2-weighted MRI (TR 4500 ms, TE 106 ms) showing areas of mixed low and high-intensity. No abnormal soft tissue mass can be seen. d: Bone scintigram (^{99}m-technetium) showing areas of high accumulation in the right distal femur.*

despite his limp. A plain radiograph showed an osteolytic lesion around the right distal femur (Figure 1a and b). Magnetic resonance images (MRI) of the lesion showed mixed low- and iso-intensities on T1-weighted images (Figure 2a) and mixed iso- and high-intensities on T2-weighted images (Figure 2b). There was no abnormal soft tissue mass (Figure 2c). Technetium-^{99}m scintigraphy revealed an area of strong accumulation in the right distal femur (Figure 2d). The patient had taken zaltoprofen (240 mg/day) for four weeks before the biopsy to alleviate his pain.

An open biopsy was performed, and the lesion was diagnosed as necrotic GCT with fatty change (Figure 3a and b), which was strongly-positive for CD68 (Figure 3c), a marker of monocyte–macrophage lineage cells. Two-thirds of the tumor cells were necrotic. Three weeks after the biopsy, thorough curettage was performed using a high-speed burr, adjuvant phenol, and artificial bone paste (α-tricalcium phosphate; BIOPEX, Mitsubishi Materials Corporation, Tokyo, Japan). In the permanent section, no viable tumor cells were seen after surgery. The patient was permitted to place partial weight on the right leg one week after surgery. Full weight-bearing was permitted one month after surgery. The patient was free of disease for 24 months after surgery. Although the artificial bone had not been replaced by newly formed bone, incorporation with the host bone was achieved (Figure 1c and d). The patient also showed normal functionality of his right knee joint.

To evaluate the cause of total necrosis, we performed terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay and immunohistochemical analysis for caspase-3. A specimen obtained from another patient (59-year-old female) was used as a control. A cell death kit (Roche, Mannheim, Germany) and antibody to active caspase-3 (Promega, Madison, WI, USA) were used according to the manufacturers' instructions to detect the TUNEL reaction and active caspase-3. Nuclear staining was performed using 4',6'-diamidino-2-phenylindole (DAPI) (Vectashield; Vector Laboratories, Inc., Burlingame, CA, USA), and a fluorescence microscope (BZ-9000; Keyence, Osaka, Japan) was used to obtain images. A very strong TUNEL response (Figure 4a and b) and caspase-3-positive cells (Figure 4c and d) were detected, whereas no positive cells were detected in the control (Figure 5a-d). We also investigated fatty de-generation, in which PPARγ is a key factor. Expression of PPARγ in the tumor specimen was examined. A mouse monoclonal antibody against PPARγ (1:250; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and rat anti-mouse immunoglobulin-G fluorescein isothiocyanate (1:400 dilution; eBioscience, San Diego, CA, USA), as the secondary antibody were used for detection. Strong expression of PPARγ was observed (Figure 4e and f) in the specimen of this patient, whereas no positive cells were observed in the control (Figure 5e and f).

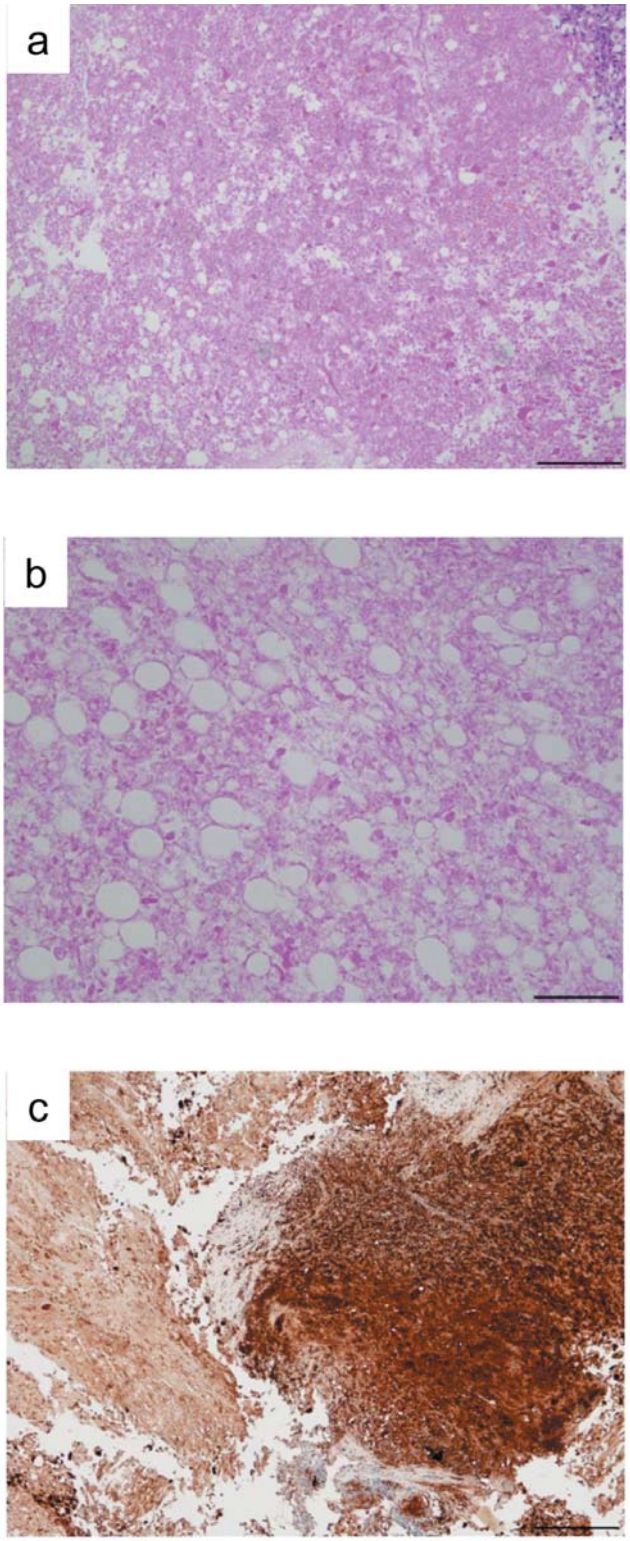

Figure 3. *Histological biopsy analysis revealing a necrotic giant cell tumor (a) with fatty change (b) (Hematoxylin and eosin stain, the scale bar indicates 100 μm.). c: Immunohistochemisty for macrophage-associated antigen (CD68) in a biopsy specimen. Strong expression of CD68 can be seen. The scale bar indicates 100 μm.*

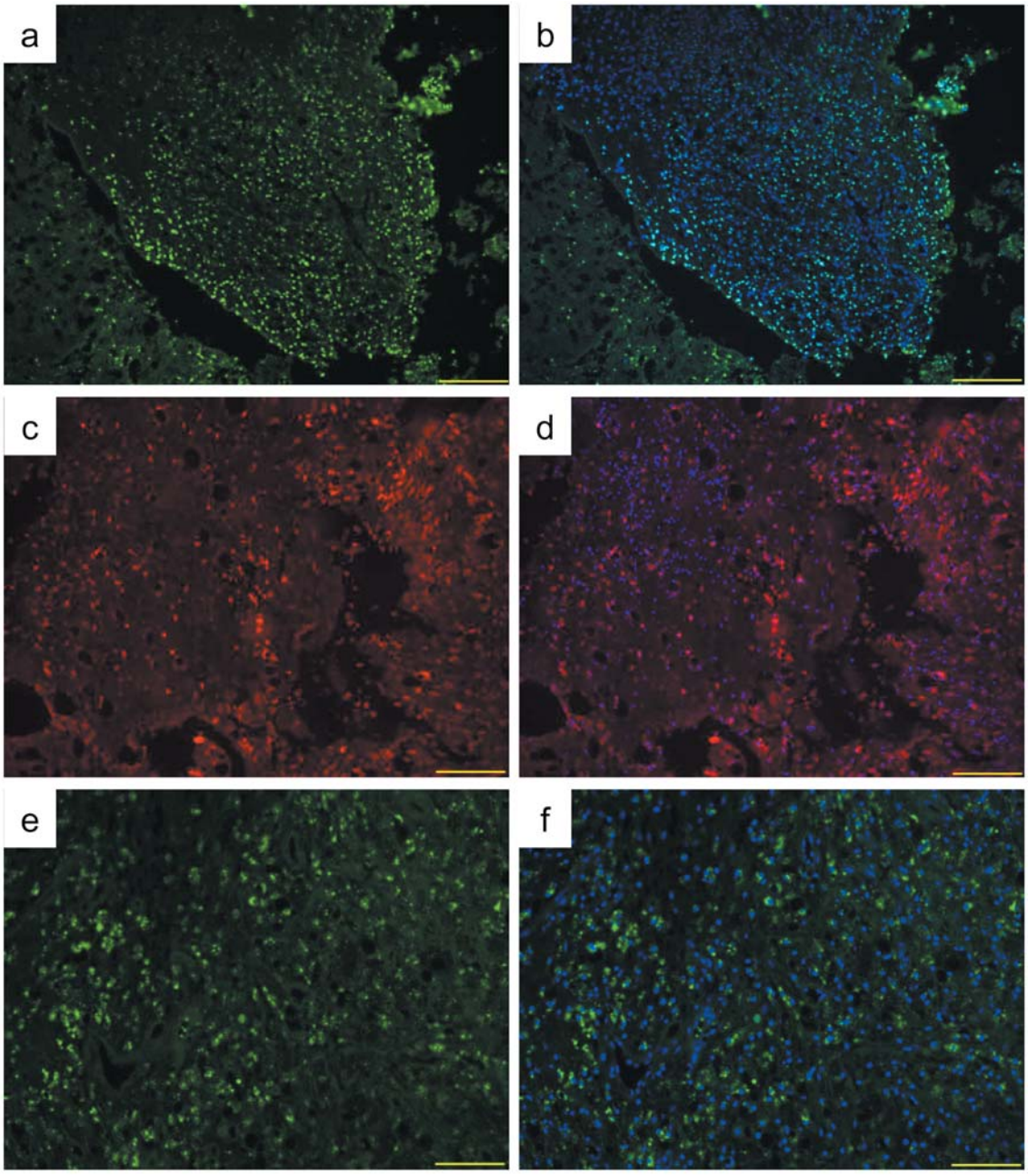

Figure 4. *Immunofluorescence confocal microsocopy. The expression patterns of terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL)-positive cells determined in the biopsy specimen (a); merged with 4',6-diamidino-2-phenylindole (DAPI) staining (b). The expression pattern of caspase-3-positive cells determined (c), merged with DAPI staining (d). The expression pattern of peroxisome proliferator-activated receptor gamma (PPARγ)-positive cells (e), merged with DAPI staining (f). The scale bar indicates 100 μm.*

Discussion

As far as we are aware, this is the first reported case of massive apoptosis with fat differentiation of bone GCT strongly expressing PPARγ. The initial MRI and bone scintigraphy before treatment showed no apparent necrosis, although MRI demonstrated small hemorrhagic foci. More interestingly, at the time of biopsy, one-third of the tumor cells were still viable; however, the specimen obtained during curettage performed three weeks after biopsy showed complete necrosis. GCT occasionally exhibits spontaneous hemorrhage and necrosis (7), but we found only two cases reporting complete necrosis of

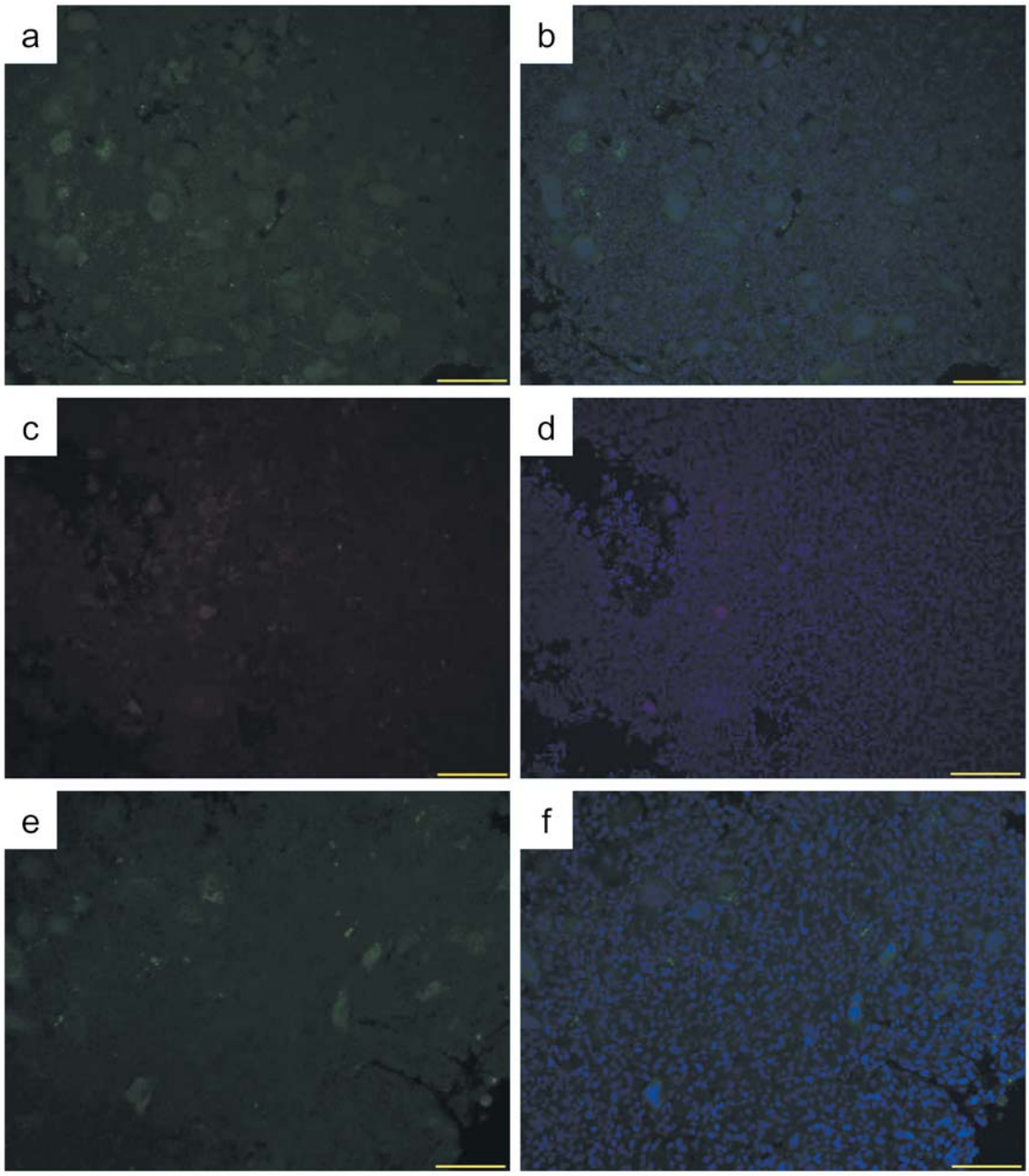

Figure 5. *Immunofluorescence confocal microsocopy of control. No terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL)-positive cells can be seen in the control case (a); image merged with 4',6-diamidino-2-phenylindole (DAPI) staining (b). Caspase-3-positive cells cannot be seen in the control case (c); image merged with DAPI staining (d). PPARγ-positive cells cannot be seen in the control case (e); image merged with DAPI staining (f). The scale bar indicates 100 μm.*

GCTs (8, 9). In both cases, the causes were unknown. Immunohistochemical analyses showed a very strong TUNEL response (Figure 4a and b) and caspase-3-positive cells (Figure 4c and d), which meant that massive apoptosis was induced in this patient. Also of note in the present case was the presence of fatty degeneration in the tumor. To our knowledge, there have been no reports of fatty degeneration in GCT. We considered the possibility of fat differentiation not fatty degeneration. These findings (apoptosis and fat differentiation) suggested that PPARγ may play a role in the underlying pathology. Strong expression of PPARγ was observed (Figure 4e and f) in the specimen of this patient. PPARγ is a key transcriptional factor involved in fat differentiation (10). It possesses antitumor activity through suppression of tumor

proliferation and invasion and in induction of differentiation and apoptosis. PPARγ ligands have been investigated and include synthetic ligands such as TZDs (12) and 15d-PGJ2 (13). It has also been reported that some NSAIDs, including indomethacin, act as direct ligands for PPARγ (14). We then focused on the medical history of this patient. He was prescribed the NSAIDs zaltoprofen (240 mg/day) for four weeks before the biopsy to alleviate his pain. Zaltoprofen inhibits bradykinin-induced nociceptive responses more potently than other NSAIDs such as indomethacin (19). Yamazaki reported that zaltoprofen induced apoptosis in rheumatoid synovial cells through the activation of PPARγ (20). Although we could not conclude the exact cause of complete necrosis and high expression of PPARγ in this case, we speculated that one of the possible mechanism of PPARγ activation was induction by zaltoprofen, at least in part. In some types of cancer including liposarcoma (15), and cancer of the colon (16), breast (17), and prostate (18), targeted-therapy for PPARγ has been performed. These findings suggest that activation of PPARγ could be a novel therapeutic tool against GCT.

In conclusion, GCT of the distal femur exhibited massive apoptosis and fat differentiation with strong expression of PPARγ, which may have been induced completely or partially by zaltoprofen. Although further analysis using cultured tumor cells with ligands specific to the receptor is necessary, PPARγ may be a novel therapeutic target in bone GCT.

Disclosure Statement

The Authors have declared that no competing interests exist.

Acknowledgements

The Authors would like to acknowledge and thank Ms. Keiko Nagashima for her great contribution.

References

1 Dahlin DC: Caldwell Lecture. Giant cell tumor of bone: Highlights of 407 cases. Am J Roentgenol *144*: 955-960, 1985.
2 Marcove RC: A 17-year review of cryosurgery in the treatment of bone tumors. Clin Orthop Relat Res *163*: 231-234, 1982.
3 McDonald DJ, Sim FH, McLeod RA and Dahlin DC: Giant-cell tumor of bone. J Bone Joint Surg Am *68*: 235-242, 1986.
4 Campanacci M, Baldini N, Boriani S and Sudanese A: Giant-cell tumor of bone. J Bone Joint Surg Am *69*: 106-114, 1987.
5 Capanna R, Fabbri N and Bettelli G: Curettage of giant cell tumor of bone. The effect of surgical technique and adjuvants on local recurrence rate. Chir Organi Mov *75(1 Suppl)*: 206, 1990.
6 Takeuchi A, Tsuchiya H, Niu X, Ueda T, Jeon DG, Wang EH, Asavamongkolkul A, Kusuzaki K, Sakayama K and Kang YK: The prognostic factors of recurrent GCT: A cooperative study by the Eastern Asian Musculoskeletal Oncology Group. J Orthop Sci *16*: 196-202, 2011.
7 Yasko AW: Giant cell tumor of bone. Curr Oncol Rep *4*: 520-526, 2002.
8 Yao L, Mirra JM, Seeger LL and Eckardt JJ: Case report 715. Necrotic giant cell tumor of the femur. Skeletal Radiol *21*: 124-127, 1992.
9 Adams SC, Potter BK, Robinson PG and Temple HT: Giant cell tumor of the distal femur associated with complete tumor necrosis. Orthopedics *33*: 688, 2010.
10 Arnold RT, van Holsbeeck MT, Mayer TG, Mott MP and Koch SR: Best cases from the AFIP: Necrotic giant cell tumor of bone manifesting with pathologic fracture. Radiographics *31*: 93-98, 2011.
11 Rosen ED, Hsu CH, Wang X and Sakai S: C/EBPalpha induces adipogenesis through PPARgamma: A unified pathway. Genes Dev *16*: 22-26, 2002.
12 Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM and Kliewer SA: An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPARγ). J Biol Chem *270*: 12953-12956, 1995.
13 Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM and Evans RM: 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPARγ. Cell *83*: 803-812, 1995.
14 Lehmann JM, Lenhard JM, Oliver BB, Ringold GM and Kliewer SA: Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem *272*: 3406-3410, 1997.
15 Demetri GD, Fletcher CD, Mueller E, Sarraf P, Naujoks R, Campbell N, Spiegelman BM and Singer S: Induction of solid tumor differentiation by the peroxisome proliferator-activated receptor-gamma ligand troglitazone in patients with liposarcoma. Proc Natl Acad Sci USA *96*: 3951-3956, 1999.
16 Kulke MH, Demetri GD, Sharpless NE, Ryan DP, Shivdasani R, Clark JS, Spiegelman BM, Kim H, Mayer RJ and Fuchs CS: A phase II study of troglitazone, an activator of the PPARγ receptor, in patients with chemotherapy-resistant metastatic colorectal cancer. Cancer J *8*: 395-399, 2002.
17 Burstein HJ, Demetri GD, Mueller E, Sarraf P, Spiegelman BM and Winer EP: Use of the peroxisome proliferator-activated receptor (PPAR) gamma ligand troglitazone as treatment for refractory breast cancer: A phase II study. Breast Cancer Res Treat *79*: 391-397, 2003.
18 Hisatake JI, Ikezoe T, Carey M, Holden S, Tomoyasu S, Koeffler HP: Down-regulation of prostate-specific antigen expression by ligands for peroxisome proliferator-activated receptor gamma in human prostate cancer. Cancer Res *60*: 5494-5498, 2000.
19 Hirate K, Uchida A, Ogawa Y, Arai T and Yoda K: Zaltoprofen, a non-steroidal anti-inflammatory drug, inhibits bradykinin-induced pain responses without blocking bradykinin receptors. Neurosci Res *54*: 288-294, 2006.
20 Yamazaki R, Kusunoki N, Matsuzaki T, Hashimoto S and Kawai S: Nonsteroidal anti-inflammatory drugs induce apoptosis in association with activation of peroxisome proliferator-activated receptor gamma in rheumatoid synovial cells. J Pharmacol Exp Ther *302*: 18-25, 2002.

Received March 22, 2013
Revised April 14, 2013
Accepted April 17, 2013