Clinicopathological and histological analysis of secondary malignant giant cell tumors of bone without radiotherapy

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Abstract. Giant cell tumor of bone (GCTB) is an intermediate bone tumor that rarely undergoes malignant transformation. Secondary malignant GCTB (SMGCTB) is defined as a lesion in which high-grade sarcoma occurs at the site of previously treated GCTB. The present study retrospectively reviewed the medical records of patients with GCTB treated at Okayama University Hospital between April 1986 and April 2020. The clinicopathological and histological features of patients with SMGCTB without prior radiotherapy were investigated. A total of three patients (4%) with SMGCTB were detected, and the tumor sites were the distal ulna, distal femur and sacrum. Two of the patients had been treated with curettage and bone graft, and one had been treated with denosumab. In all cases, the lesions were made up of two components, the conventional GCTB component and the malignant component. The Ki67 labeling index was higher in the malignant components of SMGCTB and metastatic lesions compared with that in primary and recurrent conventional GCTB, or the conventional GCTB component of SMGCTB. Moreover, p53 expression was higher in these same components in patients who underwent curettage and bone grafting; however, there was no difference in the patient that received denosumab treatment. In this patient, clinical cancer genomic profiling revealed loss of *CDKN2A*, *CDKN2B* and *MTAP* expression. All three patients developed distant metastasis. The patients with SMGCTB in the ulna and femur died 13 and 54 months after detection of malignant transformation, respectively. The patient with SMGCTB in the sacrum received carbon-ion radiotherapy to the sacrum and pazopanib; the treatment was effective and the patient was alive at the last follow-up 3 years later. In conclusion, p53 may be associated with malignant transformation in GCTB. Future studies should investigate the association of between denosumab treatment and malignant transformation, as well as molecular targeted therapy to improve the clinical outcomes of SMGCTB.

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Introduction

Giant cell tumor of bone (GCTB) is an intermediate bone tumor occurring in the sacrum or other vertebral bones, as well as the epiphysis of long bones, including the distal femur, proximal tibia, distal radius, and proximal humerus (1-3). Moreover, GCTB is relatively rare, accounting for about 3-5% of all primary bone tumors and is most common in patients 20-40 years of age (1-3). GCTB is composed of two types of cells: multinucleated giant cells expressing receptor activator of nuclear factor-kB (RANK) and neoplastic mononuclear stromal cells expressing RANK ligand (RANKL) (4). The interactions between these two cell types induce bone destruction.

Treatment of GCTB often includes curettage and adjuvants such as argon, phenol, alcohol, or polymethylmethacrylate. A bone graft is used to overcome the bone defect. Other procedures, such as radiotherapy (RT) and embolization may be employed in cases where surgery is not possible. However, it is locally aggressive, and recurrence is observed in 15-50% of cases, usually within 3 years after treatment (1-3). Some clinical trials have shown that denosumab, a monoclonal antibody inhibitor of RANKL, is effective in patients with recurrent or unresectable giant cell tumors, although recent studies have demonstrated that it may increase the risk of recurrence (5-7).

Rarely, GCTB undergoes transformation into a malignant tumor, becoming primary or secondary malignant GCTB (SMGCTB) (8-11). Primary malignant GCTB (PMGCTB) is defined as a lesion in which a high-grade sarcoma component appears simultaneously next to the conventional GCTB component at the time of first presentation. SMGCTB is defined as a lesion in which a high-grade sarcoma component occurs at the site of previously treated GCTB. Most MGCTBs are secondary and occur after RT, multiple local recurrences after surgery for GCTB, or late local recurrence (8). The incidence of MGCTB,

PMGCTB, and SMGCTB was reported to be 1.1-11.3, 0.5-9.7, and 1.3-5%, respectively among GCTB patients (8). Moreover, because most cases of SMGCTB occur post-RT, they should be classified as radiation-induced sarcomas. True spontaneous SMGCTB following surgery without prior RT treatment is extremely uncommon with an incidence rate of 0.5-1.5% among GCTB cases and has been reported in less than 150 cases (9-31). Furthermore, recent studies have reported the occurrence of SMGCTB following denosumab treatment, with a total of less than 20 cases (6,7,32-40).

Notably, p53 has been proposed to be a cause of SMGCTB development, and it has been reported that p53 is overexpressed in these tumors (41,42). However, there are no reports on continuous changes in p53 expression at the time of primary cancer, recurrence, malignant change, or metastasis.

Surgical resection is the definitive management for resectable SMGCTB (8). However, while resection with wide margins is an achievable goal for SMGCTB located in the limbs, it is more challenging for sacrum or vertebral lesions due to their anatomical complexity (28). Furthermore, chemotherapy is performed for advanced SMGCTB, but only limited data is available regarding the role of chemotherapy in SMGCTB due to its rarity (8). Clinical outcomes of SMGCTB are poor, with a distant metastasis rate of 33-80%, a 5-year disease-free survival rate of 32%, and a 5-year overall survival rate of 40% (8,16,20,30,31). However, these reports include patients treated with various modalities, including RT in most cases, and only a few reports have assessed the clinical outcomes of SMGCTB (30,31). Moreover, evaluation of clinical cancer genomic profiling and the effects of molecular targeted therapy and proton ion therapy have not been reported.

Therefore, we investigated the clinicopathologic and histologic features of SMGCTB in patients not previously treated with RT. We specifically asked the following questions: i) How about the rate of SMGCTB in patients treated with surgery or denosumab? ii) What are the changes of the immunohistochemical features and expression of p53 and Ki67, including primary, recurrence, malignant change, and metastasis? iii) What is the role of clinical cancer genomic profiling and heavy iron treatment and molecular targeted therapy? Furthermore, we reviewed the clinical features of SMGCTB in patients not previously treated with RT and the clinical effects of denosumab.

Patients and methods

Patients. We retrospectively evaluated the medical records of 75 patients with pathologically proven GCTB treated at Okayama University Hospital (Okayama, Japan) between March 1986 and August 2020. The inclusion criterion was a pathologically proven diagnosis of sarcoma. Patients excluded were those followed up for less than one year after surgery, patients with PMGCTB, or treated in other institution. Additionally, 48 patients underwent curettage, and 19 patients underwent resection. None of the patients received RT. We examined the rate of SMGCTB in these patients.

Imaging. Plain X-ray, computed tomography (CT), and magnetic resonance (MR) imaging (MRI) were utilized for initial examination in all cases. CT (Discovery CT750 HD, GE) images, obtained at 120 kV and with a slice thickness of 5 mm, were viewed in the axial, sagittal, and coronal planes. Results of MRI (MEGNETOM Prisma, Siemens) consisted of T1-weighted images, and T2-weighted images were obtained in the axial, sagittal, and coronal planes. In two cases of SMGCTB, we utilized 2-deoxy-2[^{18}F] fluoro-D-glucose positron emission tomography-computed tomography (FDG PET-CT) (Biograph 16; Siemens Medical Solution USA, Knoxville, TN, USA) at a diagnostic imaging center adjacent to our institution. After fasting for at least 5 h, the patients received an intravenous injection of 3.7 MBq/kg 18F-FDG. PET image acquisition was started 90 min after injection of 18F-FDG, with the patient in a relaxed supine position. First, a total-body low-dose CT scan for the calculation of attenuation correction was performed, using a standardized protocol involving 120 kV, auto mA mode, rotation time of 0.5 sec, pitch of 0.8, section thickness of 3 mm, and scan field from the head to the mid-thigh level. Thereafter, PET imaging consisting of 6-8 bed positions with 2.4 min per position over the same region was performed. The PET images were reconstructed with an ordered-subset expectation maximization iterative reconstruction algorithm. Integrated, co-registered PET/CT images were obtained using a workstation that enables image fusion and analysis (syngo. via; Siemens Medical Solution USA).

Pathologic findings of SMGCTB

Preparation for histologic evaluation. All cases were immediately fixed in 4% paraformaldehyde for 12 h then decalcified in 10% ethylenediaminetetraacetic acid at 4˚C for 14 days. The tissue was routinely embedded in paraffin, and five thick serial sections were prepared. The sections were subjected to hematoxylin-eosin (HE) and immunohistochemistry (IHC) staining.

Immunohistochemistry. Following antigen retrieval in a cooker heating for 1 or 8 min in 0.01 M Dako Target Retrieval Solution (pH 9; cat. no. S2367; Agilent Technologies, Inc. USA), 5-μm sections were blocked with 10% normal serum (Vector Laboratories, Inc. USA) for 20 min at room temperature and incubated with primary antibodies, including ki67 (cat. no. A0047; 1:50; DAKO, USA), p53 (M7001; 1:50; DAKO, USA) overnight at 4˚C. Signals were enhanced using the avidin-biotin complex method (Vector Lab, Burlingame, CA, USA). Color development was performed using 3,3'-diaminobenzidine (Histofine, Nichirei, Tokyo, Japan), and the staining results were observed with an optical microscope (BX53, Olympus, Tokyo, Japan).

Quantification and statistical analysis. To compare the tissue characteristics throughout each time of GCTB progression in the same patient, cell counting was performed in each area. After counterstaining with hematoxylin, the sections were examined microscopically at x400 magnification. Five areas were chosen randomly in each sample, one hundred cells were counted in each area, and the percentage of positive cells was calculated and compared among the groups. Counting was performed by a pathologist specializing in tumor evaluation. All statistical analyses were conducted using GraphPad Prism 9.1.1. Repeated measures ANOVA followed by Sidak's

Table I. Patient characteristics.

Case	Gender	Age at Presentation, years	Location	Campanacci classification	Initial treatment	Time to SMGCTB, years	Treatment	Recurrence	Metastases	Outcome
1	Male	61	Ulna	3	Surgery	7	Surgery	+	Lung and bone	Dead
2	Male	23	Femur	2	Surgery	3	Surgery and chemotherapy	-	Lung	Dead
3	Female	23	Sacrum	3	Embolization	10	Carbon-ion radiotherapy	-	Lung and heart	Alive

multiple comparison post hoc test was used to compare differences among more than two groups where necessary. Differences were considered significant at $P<0.05$. Data are presented as mean ± SD.

Clinical cancer genomic profiling. One patient (Case 3; patient with stage IIA sacral MGCTB) underwent clinical cancer genomic profiling using the FoundationOne Medicine (Cambridge, MA, USA; https://www.foundationmedicine.com/) platform to identify potential targetable molecular aberrations. The FoundationOne®CDx assay is the first and only comprehensive companion diagnostic for all solid tumors approved by the Ministry of Health, Labor, and Welfare in Japan (43). This assay evaluates 309 genes involved in substitutions, insertions/deletions, copy number (CN) alterations, as well as introns of 36 genes related to rearrangements. Genomic DNA was isolated from the formalin-fixed paraffin-embedded tissue samples (excised specimen of the sacrum), and its purity and concentration were determined.

Brief literature review. We performed a literature review to identify other published cases and describe the clinical characteristics of SMGCTB.Searches through the PubMed database were conducted through December 2021, identifying the reports published till April 2021. Keywords employed in the search process included: giant cell tumor, malignancy, and secondary malignant giant cell tumor. The literature search was further limited to articles published in English. We also searched 'related articles' of included studies suggested by PubMed.

Results

The frequency of SMGCTB. Eighteen patients experienced local recurrence, and twenty-two patients had received denosumab among 75 patients. Moreover, we investigated SMGCTB in patients without prior RT treatment and detected three patients (4%) with SMGCTB. The patient characteristics are summarized in Table I. Two of the three cases of SMGCTB occurred after surgery and one of the cases occurred after denosumab treatment. There were two men and one woman. The tumors were located in the distal ulna (case 1), distal femur (case 2), and sacrum (case 3). The follow-up periods were 12, 4, and 13 years. Moreover, for initial treatment of GCTB, the patient with the tumor in the distal ulna underwent tumor resection, the patient with the tumor in the distal femur underwent curettage and artificial bone graft, and the patient with the tumor in the sacrum underwent conservative embolization followed by denosumab and resection.

Pathologic finding

Histologic findings of primary and recurrent lesion of conventional GCTB. In all cases, the primary tumors were mainly composed of a proliferation of ovular or short, spindle-shaped stromal cells with evenly scattered, osteoclast-type giant cells, diagnosed as conventional GCTB. The tissue pattern was monogenous, and no necrosis was observed. Additionally, mitotic figures were not found in the tumor cells (Fig. 1A and B). The recurrent lesions had a similar pattern to that of the primary specimen (Fig. 1C). Moreover, the multinuclear giant cells were sparsely spread around the ovoid/round or spindle mononuclear stromal cells (Fig. 1D). Additionally, woven bone was observed and was considered to be the remaining artificial bone (Fig. 1E). Finally, no necrotic area or atypical findings were observed.

Histologic findings of lesion of malignant transformation. In all cases, the lesions of malignant transformation were composed of two components (Fig. 2A). One was a conventional GCTB component (Fig. 2B), and the other was a malignant component (Fig. 2C). In conventional GCTB component is composed of a proliferation of mononuclear, oval, or short, spindle-shaped stromal cells with evenly scattered, multi-nucleolus giant cells. Mitotic figures were not found in these tumor cells (Fig. 2B). In the fibrosarcoma component, there were interlacing fascicular pattern of proliferation of spindle-shaped tumor cells in a collagen background (Fig. 2C). Tumor cells were highly atypical and had hyperchromatic nuclei and atypical mitosis could also be found. Giant cells were banished. A wide necrotic section was observed in the fibrosarcomatous area, where it was possible to find cells that had more eosinophilic

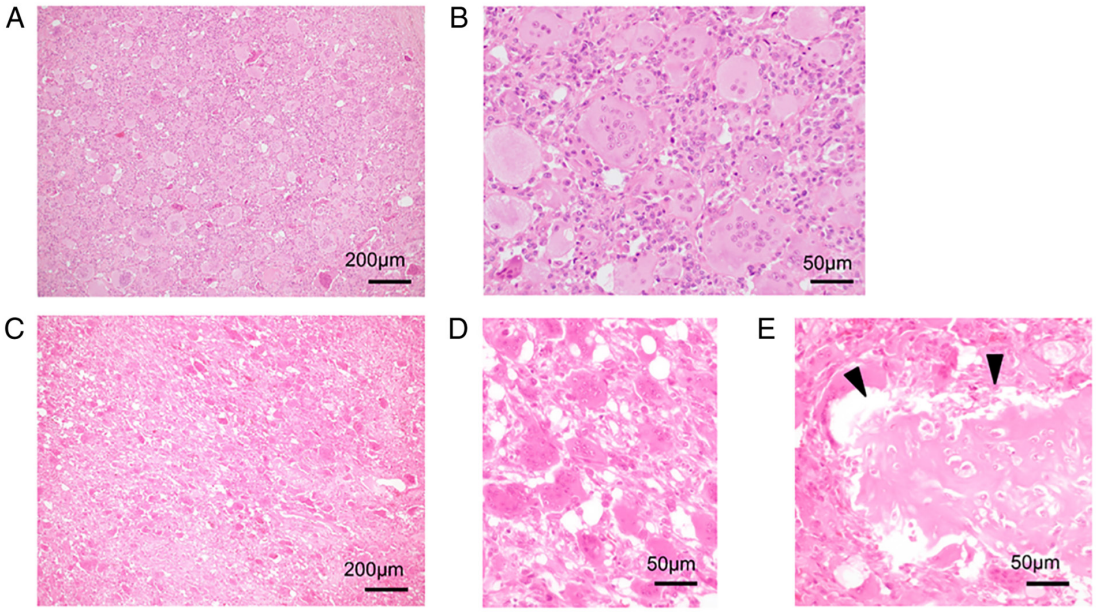

Figure 1. Histologic evaluation of (A and B) primary and (C-E) recurrent lesions of conventional giant cell tumor of bone. (A) Low-power image of HE staining. Bar: 200 μm. (B) High-power image of HE staining. Multi-nucleolus giant cells were observed. Stromal cells were oval to short spindle-shaped. Bar: 50 μm. (C) Low-power image of HE staining. Bar: 200 μm. (D and E) High-power image of HE staining. Tissue findings were similar to those of the primary lesion. Woven bone was observed (arrowhead). Bar: 50 μm. HE, hematoxylin and eosin.

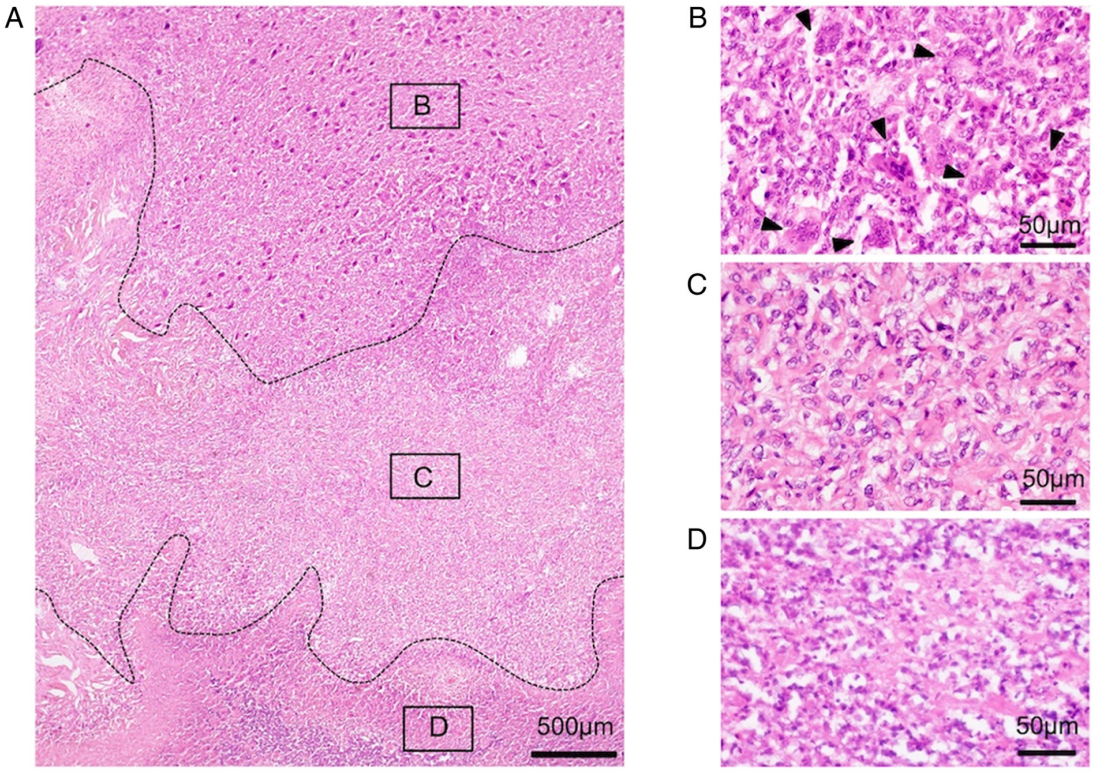

Figure 2. Histologic evaluation of malignant transformation. (A) Low-power image of HE staining. Tissue was divided into three parts; B, benign area; C, malignant area; and D, necrotic area. Bar: 500 μm. (B) High-power image of HE staining in benign area. Multi-nucleolus giant cells were observed (arrowheads). Stromal cells were oval to short spindle-shaped. Bar: 50 μm. (C) High-power image of HE staining in malignant area. Atypical spindle tumor cells were observed, and giant cells were banished. Bar: 50 μm. (D) High-power image of HE staining in necrotic area. Bar: 50 μm. HE, hematoxylin and eosin.

cytoplasm with irregular distribution, or flocculation of chromatin and fragmentation, or disappearance of nuclear (Fig. 2D). In the recurrent SMGCTB, fibrosarcomatous tissue was observed similar to that of the first specimen of malignant transformation (Fig. 3A and B). In addition, osteoid formation was observed in some areas (Fig. 3C). These findings were indicative of osteosarcoma. In the osteosarcoma component, the cells were highly anaplastic and pleomorphic with large

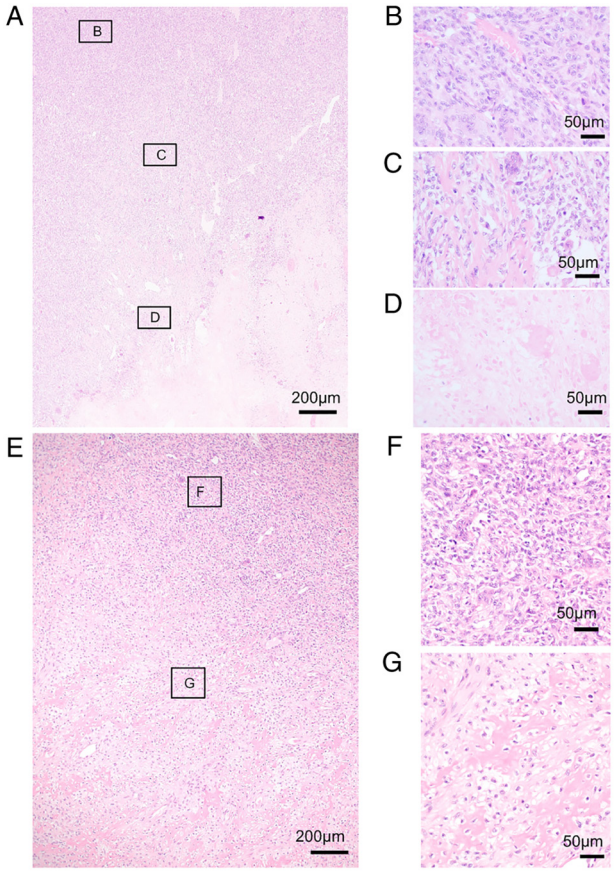

Figure 3. Histologic evaluation of recurrent and metastatic lesions of secondary malignant giant cell tumor. (A-D) Recurrent lesion. (A) Low-power image of HE staining. Bar: 500 μm. (B-D) High-power image of HE staining. Atypical spindle tumor cells proliferated densely, and small number of giant cells were observed. Bar: 50 μm. Placement of (B-D) was shown as squares in (A). (E-G) Metastatic lesion. (E) Low-power image of HE staining. Bar: 500 μm. (F and G) High-power image of HE staining. Atypical spindle tumor cells and woven bone were observed, and number of giant cells decreased. Placement of (F and G) are shown as squares in (E). Bar: 50 μm. HE, hematoxylin and eosin.

hyperchromatic nuclei. Moreover, wide necrosis was observed in the specimen of recurrence of SMGCTB (Fig. 3D). No benign components were found. The specimens from lung metastasis were similar to those of recurrent SMGCTB (Fig. 3E) displaying a combination of fibrosarcomatous tissue (Fig. 3F) and osteosarcomatous tissue (Fig. 3G).

Immunohistochemical evaluation. To investigate the changes of malignant transformation within the same tumor, we performed Ki67 and p53 immunostaining. The Ki67 labeling index increased according to malignant transformation. Histologically, the Ki67 labeling index was low in primary and recurrent conventional GCTB (Fig. 4A and B) and the conventional GCTB component of SMGCTB (Fig. 4C). Meanwhile, it increased in the malignant component of SMGCTB (Fig. 4D) and recurrent and metastatic lesions (Fig. 4E and F). For example, the benign and malignant areas of case 1 displayed significantly different Ki67 labeling indices (Fig. 5). In the remaining two cases of SMGCTB, the index also increased significantly according to malignant changes (Fig. 5; Case 2 and 3, Fig. S1).

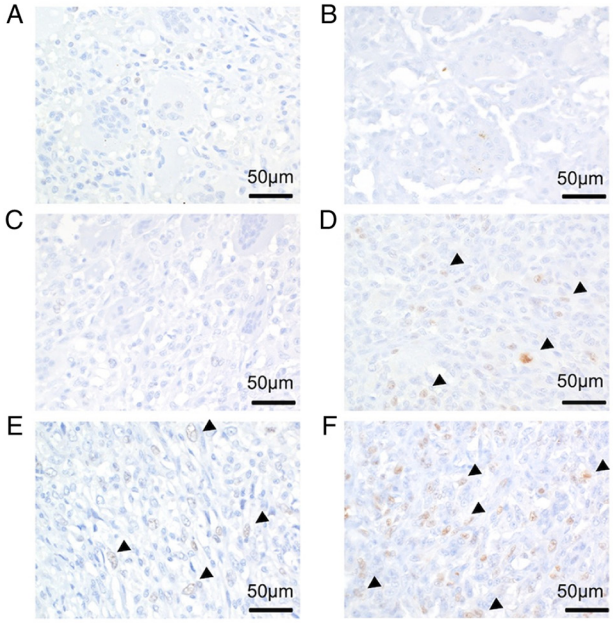

Figure 4. Expression of Ki67 in case 1. (A) Primary lesion. (B) Recurrent lesion. (C) Conventional GCTB component of SMGCTB. (D) Malignant component of SMGCTB. (E) Recurrent lesion of SMGCTB. (F) Metastatic lesion of SMGCTB. Bar: 50 μm. Arrowheads, Ki67 positive cells.

In case 1, the p53 expression level increased with malignant transformation. Specifically, p53 expression was very low in primary and recurrent (Fig. 6A and B) conventional GCTB. However, p53 expression level increased at malignant area of third surgery specimen of case 1 (Fig. 6D), although it was low in conventional GCT area in the same specimen (Fig. 6C). Interestingly, p53 expression pattern changed according to malignant transformation in the same specimen. And high expression levels were maintained in recurrent and metastatic lesions (Fig. 6E and F) of SMGCTB. Additionally, cell count quantification revealed that p53 expression levels were significantly different before and after malignant transformation (Fig. 7). Of particular importance was the significant change of p53 expression in MT (GCTB component) and MT (malignant component) in case 1, even though they were in the same sample. And after malignant transformation in case 2, p53 expression levels were significantly increased (Fig. S2A-C). However, no significant change in p53 expression was observed in patient 3 who received denosumab treatment (Fig. S2D-H).

Survival. All three patients with SMGCTB experienced local recurrence after 1, 1.5, or 36 months (Fig. 8). All patients developed distant metastasis to the lungs. Two patients also exhibited bone metastasis, and one patient developed heart metastasis. Two patients died 13 and 54 months after being found to have malignant transformation. One patient was alive at the last follow-up 3 years later.

Case report

Case 1. The patient was a 61-year-old man with GCTB in the left distal ulna. At first presentation, plain radiographs demonstrated a lytic lesion in the distal ulna (Fig. 9A). CT revealed an expanded, thinned, and partially discontinuous cortex (Fig. 9B). MRI revealed a large lesion of the distal

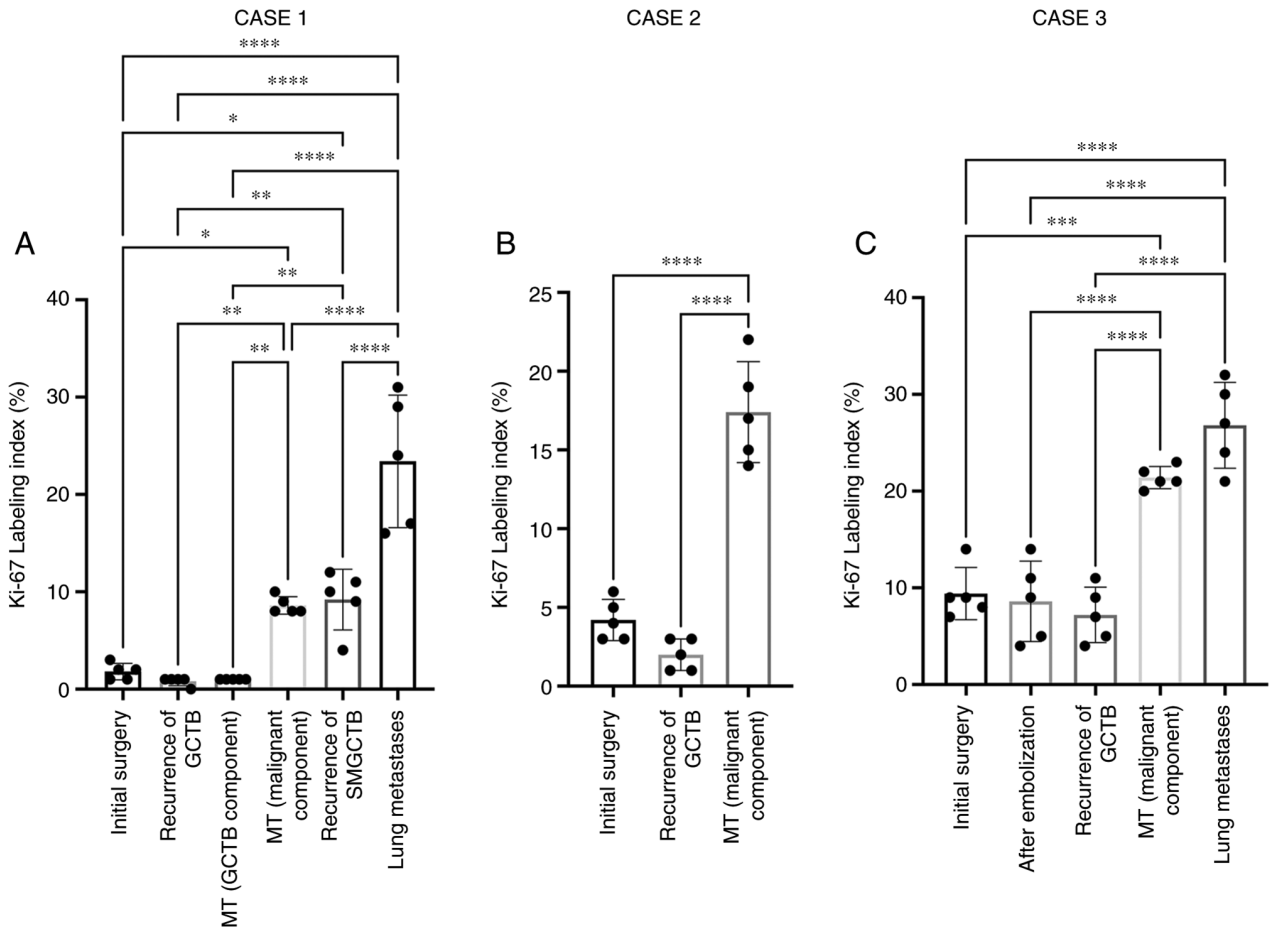

Figure 5. Comparison of Ki67 expression level in three cases. (A) Case 1. (B) Case 2. (C) Case 3. All data are shown as mean ± SD. Statistical analyses were performed using repeated measures ANOVA followed by Sidak's multiple comparison post hoc test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

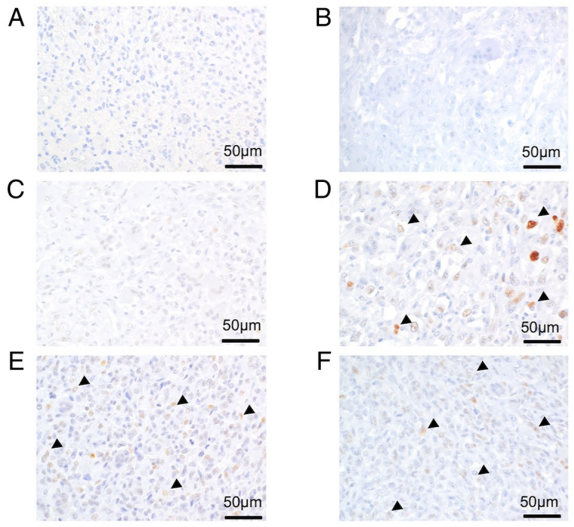

Figure 6. Expression of p53 in case 1. (A) Primary lesion. (B) Recurrent lesion. (C) Conventional GCTB component of SMGCTB. (D) Malignant component of SMGCTB. (E) Recurrent lesion of SMGCTB. (F) Metastatic lesion of SMGCTB. Bar: 50 μm. Arrowheads, p53 positive cells.

ulna with low intensity on T1-weighted images (Fig. 9C) and high intensity on T2-weighted images (Fig. 9D). An open biopsy revealed conventional GCTB. The tumor was marginally resected. Eleven months post-operation, local recurrence was observed in the forearm. MRI revealed a mass with low intensity on T1-weighted images (Fig. 9E) and high intensity on T2-weighted images (Fig. 9F) and uniform enhancement on gadolinium-enhanced images (Fig. 9G). The lesion was resected and histologically determined to be conventional GCTB. Seven years after the primary surgery, MRI revealed a large mass in the left forearm with low intensity on T1-weighted images (Fig. 9H) and high intensity on T2-weighted images (Fig. 9I) and heterogeneous enhancement on gadolinium-enhanced images (Fig. 9J). The lesion was resected and histologically determined to be malignant transformation of GCTB. Ten years after the primary surgery, MRI revealed the same findings as those from 7 years post-operation (Fig. 9K-M). Forearm amputation was performed, and the lesion was histologically diagnosed as recurrent SMGCTB. Subsequent FDG PET-CT demonstrated a nodular opacity with high FDG uptake in the lung, suggesting metastasis (Fig. 9N). The patient underwent metastasectomy of the lung metastasis. After 4 months, chest CT revealed multiple nodular opacities in both lungs, suggesting metastasis. The patient refused treatment and died 11 months later.

Case 2. The patient was a 25-year-old man with GCTB in the left distal femur. Open biopsy revealed conventional GCTB, and curettage and bone grafting were performed. At the 18-month

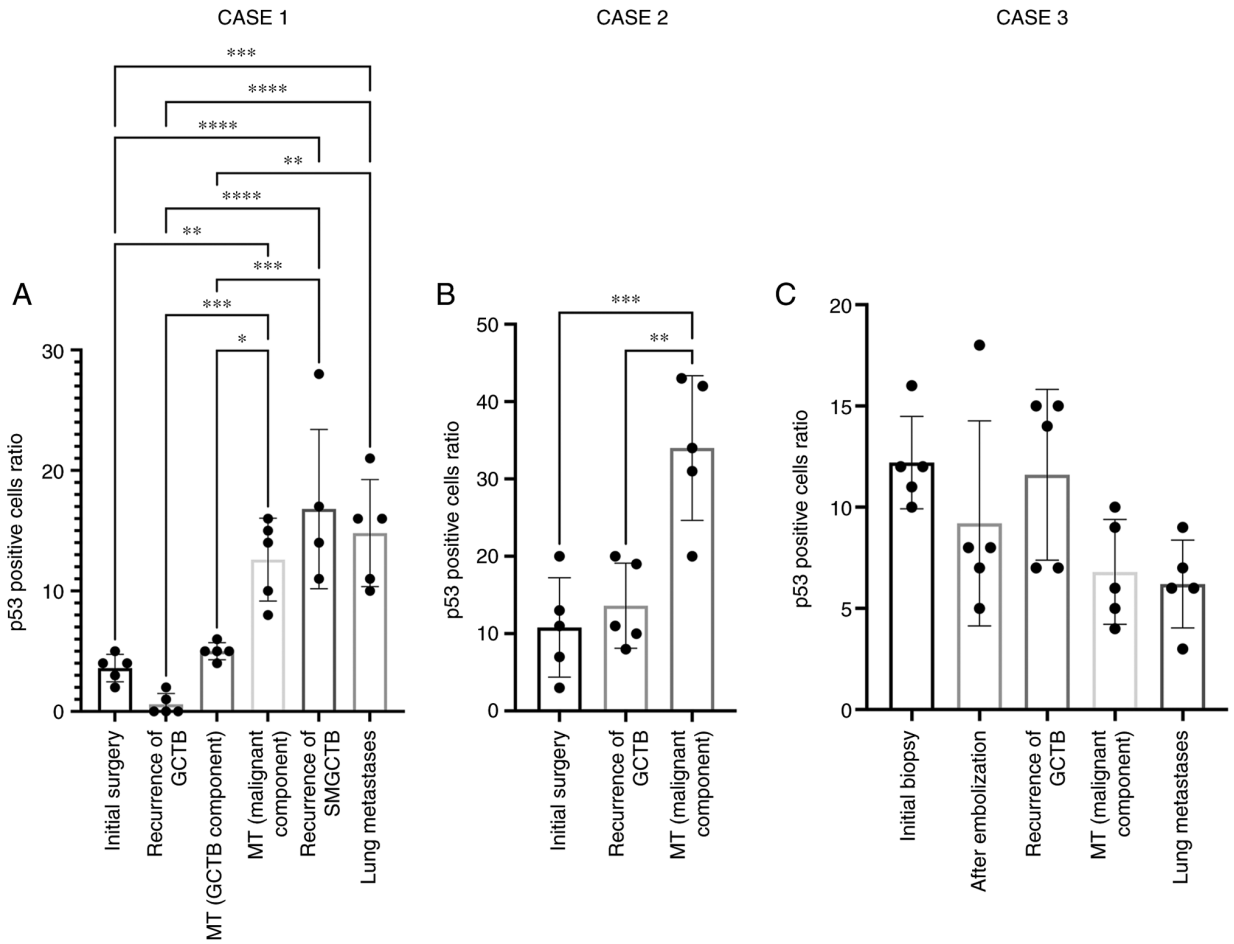

Figure 7. Comparison of p53 expression level in three cases. (A) Case 1. (B) Case 2. (C) Case 3. All data are shown as mean ± SD. Statistical analyses were performed using repeated measures ANOVA followed by Sidak's multiple comparison post hoc test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

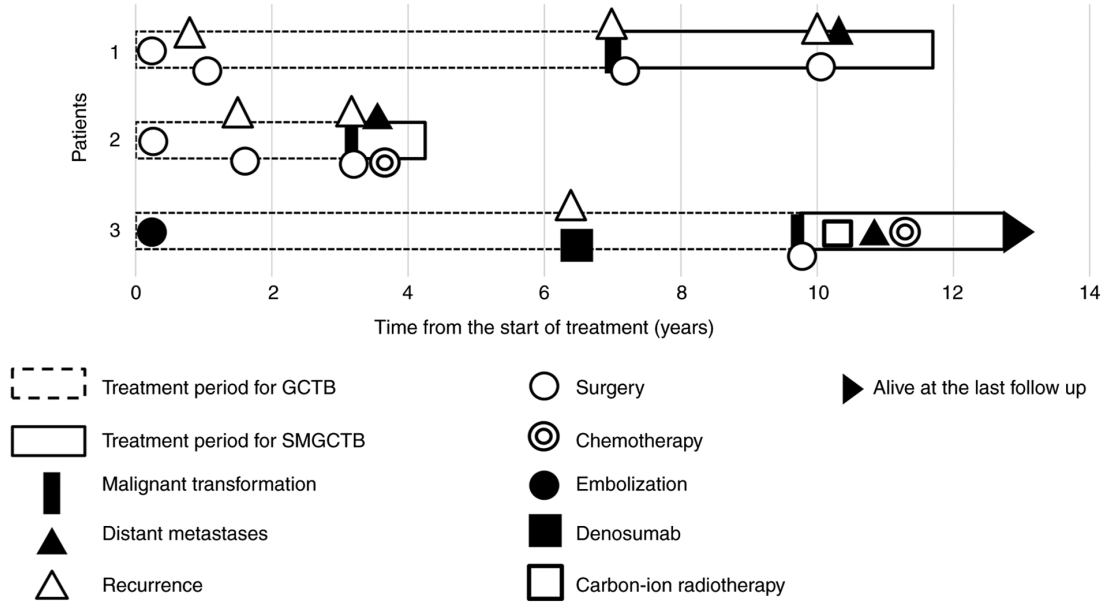

Figure 8. Swimmer plot. Clinical course of Case 1-3 of SMGCTB is shown. GCTB, giant cell tumor of bone; SMGCTB, secondary malignant GCTB.

follow-up, local recurrence was revealed. Curettage and bone grafting were performed, and the lesion was histologically determined to be conventional GCTB. Three years after the primary surgery, CT revealed a lytic lesion at the site of operation. The lesion was curetted and revealed to be SMGCTB. Subsequent chest CT revealed multiple lung metastasis. The

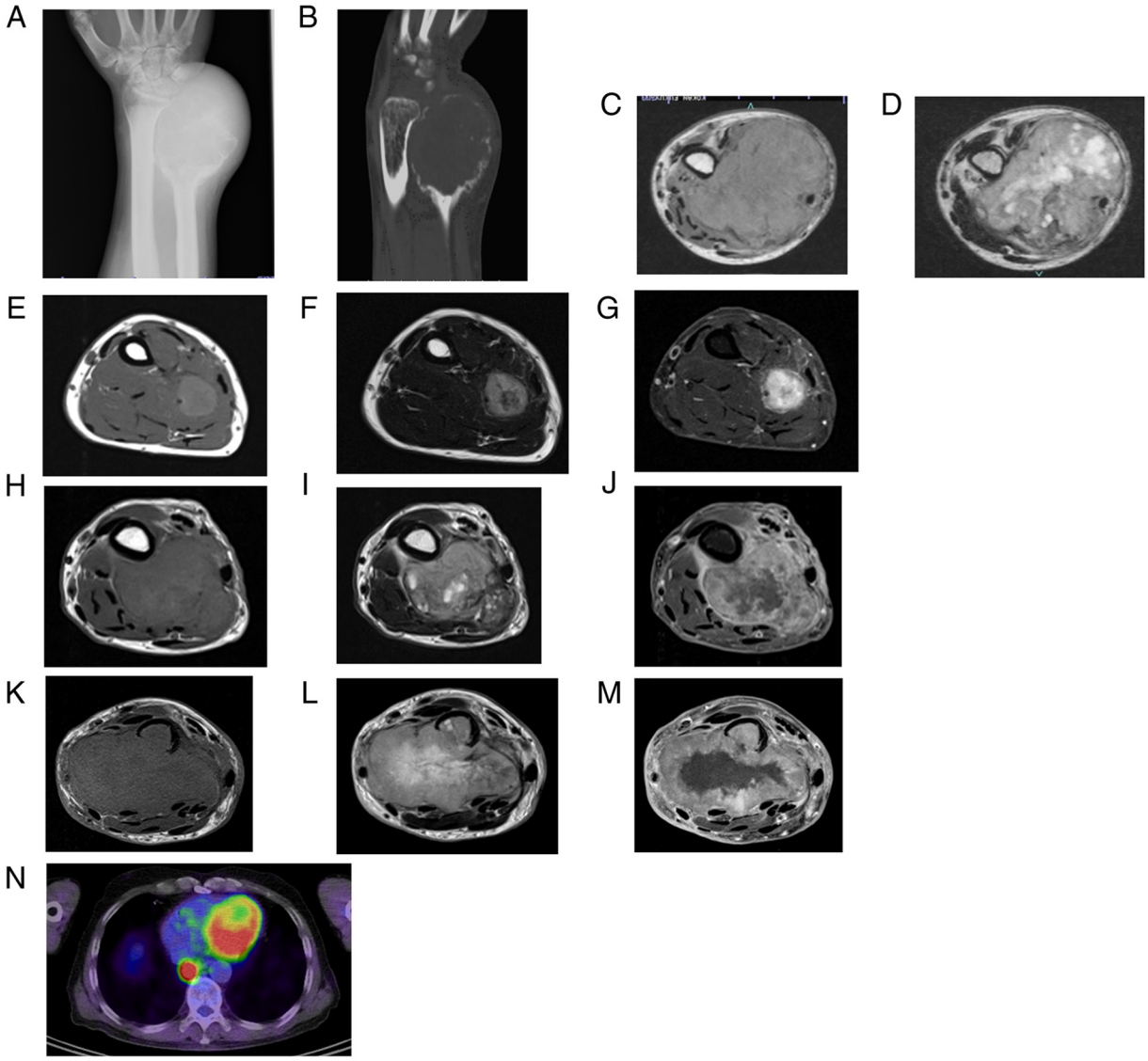

Figure 9. Radiologic findings of Case 1. (A) Plain radiographs and (B) CT demonstrating lytic lesion in the distal ulna at first presentation. (C) The tumor was low-to-intermediate intensity on T1-weighted images and (D) heterogeneously high intensity on T2-weighted images. MRI of local recurrence of GCTB with (E) low intensity on T1-weighted images, (F) high intensity on T2-weighted images and (G) uniform enhancement on gadolinium-enhanced MR images. MRI of SMGCTB with (H) low intensity on T1-weighted images, (I) high intensity on T2-weighted images and (J) uniform enhancement on gadolinium-enhanced MR images. Local recurrence of SMGCTB with (K) low intensity on T1-weighted images, (L) high intensity on T2-weighted images and (M) heterogeneous enhancement on gadolinium-enhanced MR images. (N) PET-CT demonstrating a nodular opacity with high FDG uptake in the lung, suggestive of metastasis.

patient underwent below-knee amputation. He also received adjuvant chemotherapy with high-dose methotrexate (10 g/m^2), cisplatin (120 mg/m^2), adriamycin (60 mg/m^2), and ifosfamide (14 g/m^2). However, this treatment had no effect and the patient died 13 months after the development of SMGCTB.

Case 3. A 24-year-old woman was referred to our institution for further examination after undergoing minimal curettage for conventional GCTB at another hospital. Radiographs (Fig. 10A) and CT (Fig. 10B and C) showed a large mass in the sacrum displaying cortical destruction and extensive soft tissue involvement. MRI revealed a large lesion in the sacrum with low intensity on T1-weighted images and high intensity on T2-weighted images (Fig. 10D and E). Intra-arterial embolization was performed using femoral access to selectively embolize the main arteries feeding the tumor. Post-embolization, the patient experienced no recurrence, with intermittent complaints of pain. MRI revealed tumor shrinkage (Fig. 10F). Additionally, the tumor showed stable disease throughout the next 6 years. However, 6.5 years after the initial embolization, follow-up MRI revealed enlargement of the soft tissue mass adjacent to the sacrum, which caused suspicion of recurrence (Fig. 10G). CT-guided biopsy confirmed the presence of recurrent benign GCTB. The patient was started on subcutaneous denosumab (120 mg monthly). A reduction in tumor size was observed, and imaging over the next 2 years showed stable disease (Fig. 10H). However, a follow-up MRI scan revealed enlargement of the soft tissue mass adjacent to the sacrum with low intensity on T1-weighted images and high intensity on T2-weighted images (Fig. 10I and J), suggestive of regrowth. CT-guided biopsy confirmed the presence of benign GCTB. Denosumab therapy was discontinued and the lesion gradually enlarged and excised, revealing

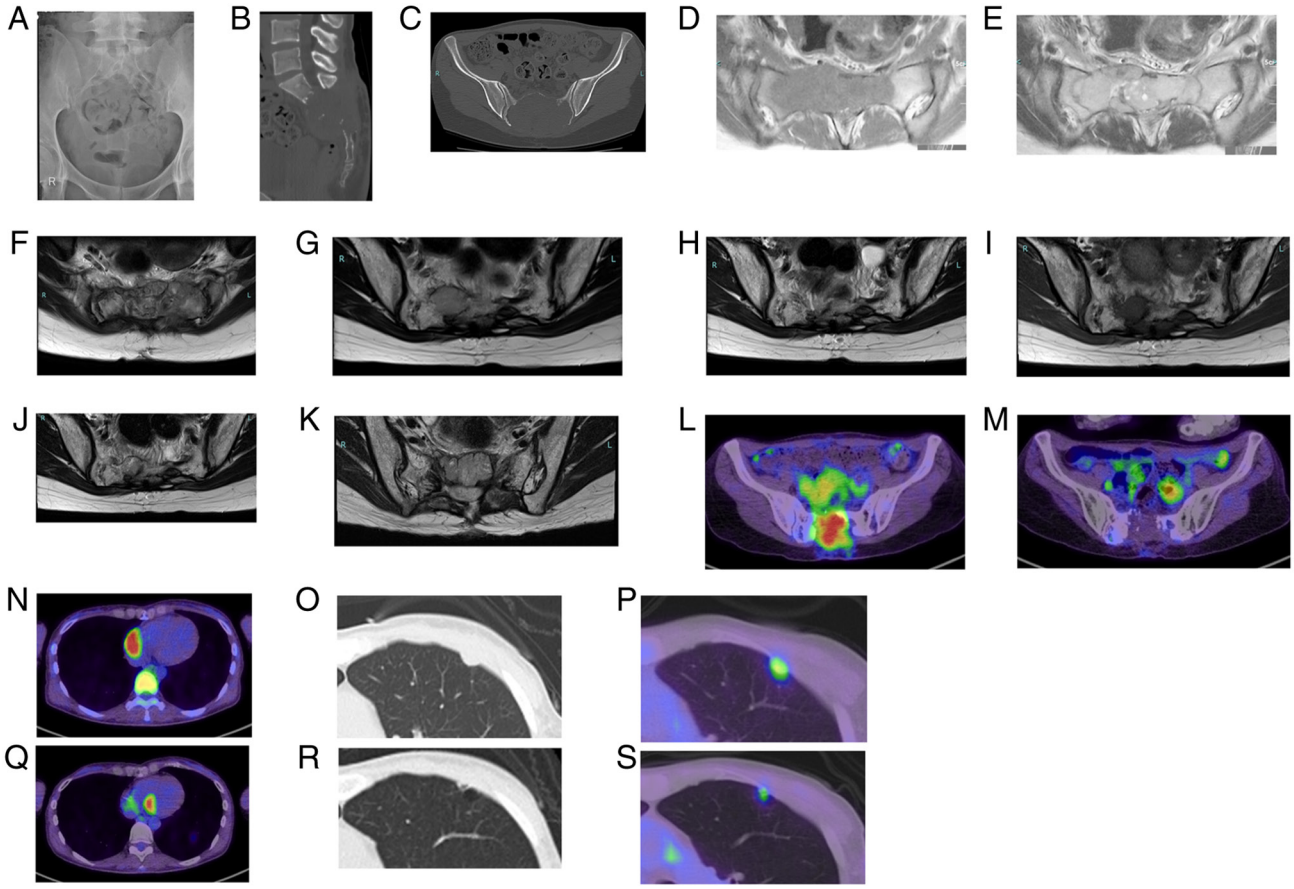

Figure 10. Radiologic findings of Case 3. (A) Plain radiographs and (B and C) CT demonstrating lytic lesion in the distal ulna at first presentation. The tumor was (D) low-to-intermediate intensity on T1-weighted images and (E) heterogeneously high intensity on T2-weighted images. (F) MRI showed tumor shrinkage after selective embolization. (G) MRI of the recurrent lesion after selective embolization. (H) MRI of tumor reduction by denosumab. (I and J) MRI of the regrowth of the tumor during denosumab therapy, which was revealed to be malignant. (K) MRI and (L) PET-CT of secondary malignant giant cell tumor of bone recurrence. (M) PET-CT after 3 years of carbon-ion RT. PET-CT and CT demonstrating a metastasis in the right atrium and the lung (N-P) before and (Q-S) after RT and chemotherapy.

malignant transformation to osteosarcoma. Recurrence was noted 1.5 months later (Fig. 10K). PET-CT demonstrated high FDG uptake in the tumor (SUVmax, 7.0) (Fig. 10L). Carbon-ion RT was performed, and local control had been obtained for >3 years at the time of last follow-up (Fig. 10M). Nine months after the diagnosis of malignant transformation, CT revealed multiple lung metastasis. Subsequent PET-CT demonstrated a mass in the right atrium (Fig. 10N) and nodular opacities in bilateral lungs through high FDG uptake (Fig. 10O and P), suggesting metastasis. She underwent stereotactic radiosurgery of the right atrium and received pazopanib (800 mg). The treatment was effective, and tumor shrinkage and decreased FDG uptake were observed in the metastatic lesions of right atrium (Fig. 10Q) and lung (Fig. 10R and S). She received clinical cancer genomic profiling utilizing the excised specimen of the sacrum and the following CN alterations were detected: *CDKN2A* (CN=0), *CDKN2B* (CN=0), and *MTAP* (CN=0). The microsatellite instability status was stable, and the tumor mutation burden score was calculated to be 1.26. One lung metastasis regrowth was excised. The patient continued pazopanib treatment, and the other lesions were found to have remained stable at the last follow-up (3 years after the development of malignant transformation).

Discussion

The occurrence rate of SMGCTB after surgery among GCTB patients without prior RT treatment was reported to be 0.5-1.5% (8,18). We showed an occurrence rate of 4%. We performed a brief literature review using the PubMed database to identify other published cases and describe the clinical characteristics of SMGCTB (8-31). Less than 150 cases were included, and the clinical characteristics of these cases are summarized in Table SI. Furthermore, Picci *et al* reported that malignant transformation develops at a median of 22 years (range: 7-28) after primary surgery (24). Moreover, Bertoni *et al* reported a median latent period of 19 years (range: 7-28) (20). However, Liu *et al* reported a shorter median latent period of only 5.1 years (range: 0.5-25.6) (30). Additionally, Tsukamoto *et al* reported a longer interval from the last surgery to local recurrence with malignant transformation (median 15.2 years) than from the last surgery to conventional GCTB (median 1.3 years) (31). They also reported that multivariate analysis showed local recurrence to be an independent risk factor for unfavorable malignant transformation. In line with these studies, two of the three patients in the current study experienced recurrence of conventional GCTB, and the interval from the start of primary treatment to the

development of malignant transformation was 3 and 7 years in these two patients. Therefore, tumor specimens should be carefully inspected for malignant transformation in patients with late recurrences and a history of multiple recurrences.

Recently, several reports have described cases of malignant transformation of GCTB during or after denosumab therapy (6,7). We performed a brief literature review using the PubMed database to identify other published cases and describe the clinical characteristics of SMGCTB treated with denosumab. Less than 20 cases were included, and the clinical characteristics of these cases are summarized in Table SII (6,7,18-23). Almost all of these patients developed SMGCTB 6 months to 4 years after starting denosumab therapy. In a phase II study evaluating the clinical benefits of monthly denosumab treatment in 37 patients with recurrent or unresectable GCTB, Thomas *et al* reported two patients who developed malignant transformation: one patient during denosumab treatment and the other 8 months after discontinuing denosumab (6). Chawla *et al* also reported five cases (1%) of SMGCTB in a phase II study showing the clinical benefits of denosumab treatment in 532 patients with GCTB (7). In the current study, 1 of 22 patients (4.5%) treated with denosumab developed SMGCTB. GCTB is characterized by stromal cells expressing RANKL and osteoclast-like giant cells expressing RANK (4,5). Denosumab binds to RANKL, substantially reducing or eliminating osteoclast-like giant cells (4,5). RANKL also plays an important role in lymphocyte differentiation and upregulates nuclear factor IB, a transcription factor that reduces susceptibility to nuclear oncogenes. Thus, inhibition of RANKL could increase the risk of malignancy through immunosuppression and increase susceptibility to nuclear oncogenes (32). However, patients treated with denosumab therapy likely have a history of multiple recurrences and long-term treatments, which can lead to a higher baseline risk for malignant transformation. Thus, additional controlled studies and long-term follow-up are needed before drawing definitive conclusions regarding the direct correlation between denosumab and malignant transformation.

Histologically, we found that lesions of malignant transformation were constructed by two components: the conventional GCTB component and the malignant component with features of osteosarcoma. This finding indicates that primary GCTB replaces the malignant component after transformation over time. Additionally, we found that the Ki67 labeling index increased according to malignant transformation. The Ki67 labeling index was low in primary and recurrent conventional GCTB and in the conventional GCTB component of SMGCTB. Meanwhile, the Ki67 index increased in all malignant areas, especially metastatic lesions. Ki67 is a nuclear protein found in proliferating cells, and its index is usually high in aggressive tumors; thus, it is regarded as a poor prognostic factor (44). In this study, we found that Ki67 labeling index increased with each event, such as malignant transformation and metastasis, suggesting that the tumor acquires a greater ability to proliferate.

Recently, several studies had been revealed the possible mechanism of malignant transformation of GCTB without previous RT (18,41-44). In these studies, mutations or LOH of *p53* as well as p53 overexpression were found in malignant cases, though no p53 mutation nor overexpression was shown in the primary GCT (18,41-44). Okubo *et al* investigated the *p53* mutations and expression of p53 in samples of SMGCTB and conventional GCTB in two patients. They found mutations of *p53* and p53 overexpression in both patients with SMGCTB who received curettage for primary GCTB. However, no p53 mutation nor overexpression was shown in the primary GCT (42). Similarly, Oda *et al* reported a case of SMGCTB in which point mutation of *p53* and p53 nuclear accumulation was observed in the atypical stromal cells of the SMGCTB, whereas no *p53* mutation and no p53 nuclear accumulation was observed in stromal cells in the primary GCT (18). More recently, Ishihara *et al* performed next generation sequencing (NGS) and immunohistochemical analysis of SMGCTB. NGS of two SMGCTB revealed pathogenic mutations in TP53 and several other genes in both patients (45). Furthermore, three of four SMGCTB were immunohistochemically positive for p53. These results suggested that *p53* alteration may play an important role in the malignant progression of GCTB. Interestingly, we found that p53 expression significantly increased when lesions underwent malignant transformation in patients who underwent curettage and bone graft. On the other hand, p53 expression was low in the primary and recurrent samples. However, high expression of p53 was also maintained in metastatic lesions. Numerous cases have been reported the secondary malignancy of bone and soft tissue tumor associated with mutation of p53 (46). Furthermore, the association between p53 mutations and tumorigenesis has been widely investigated in many malignancies (47,48). Molecular mechanisms of mutant p53 include; i) Mutant p53 interacts with DNA directly using mutant p53 binding elements or other regions on the DNA to regulate transcription. ii) Mutant p53 enhances transcription by forming a complex with transcription factors that can include transcriptional cofactors and other proteins. iii) Mutant p53 decreases transcription by binding transcription factors and/or transcriptional cofactors and other proteins, sometimes preventing their binding to DNA. iv) Mutant p53 interacts with other proteins, not directly involved in transcriptional regulation, and enhances or blocks their function (47,48). It has been found that p53 may play a role in the malignant transformation of GCTB in patients who undergo curettage. However, p53 overexpression was not observed in the patient who received denosumab treatment. In this patient, we performed clinical cancer genomic profiling using the FoundationOne®CDx assay containing 309 genes to identify potential driver genes of SMGCTB specific for denosumab treatment. We detected copy number alterations in *CDKN2A* (CN=0), *CDKN2B* (CN=0), and *MTAP* (CN=0). *CDKN2A* encodes two unrelated tumor suppressor proteins, p16INK4a and p14ARF, whereas *CDKN2B* encodes the tumor suppressor protein, p15INK4b (49). Both p15INK4b and p16INK4a inhibit CDK4 and CDK6, thereby maintaining the growth-suppressive activity of the Rb tumor suppressor. Therefore, the loss of p15INK4b and p16INK4a leads to dysregulation of the CDK4/6-cyclin-Rb pathway and loss of cell cycle control (49). Moreover, the tumor suppressive functions of p14ARF involve stabilization and activation of p53 via MDM2 inhibition. The loss of *CDKN2A* and *CDKN2B* can lead to tumorigenesis through uncontrolled proliferation. Finally, the mechanism of malignant transformation induced by denosumab can be different from that of due to surgery. The

correlation between denosumab and malignant transformation should be investigated in additional controlled studies with long-term follow-up.

There are numerous reports that 91-95% of GCTB harbour pathogenic *H3F3A* mutation, which may be a driver for tumorgenesis of GCTB (50,51). Gong L reported that *H3F3A* mutations was found in 95% of GCTB, including glycine 34 to tryptophan (G34W, 91%), glycine 34 to leucine (G34L, 2%), glycine 34 to valine (G34V, 1%), and glycine 34 to arginine (G34R, 1%) by DNA sequencing analysis (50). *H3F3A* mutation is characteristic of GCTB and shown to be useful for the diagnosis of GCTB and differential diagnosis from other bone tumors (50,51). Recently, several studies revealed the absence of *H3F3A* G34W mutation in malignant giant cell tumor of bone, though it was present in the associated giant cell tumor tissues (38,52). Yoshida *et al* investigated H3F3A G34W mutation in seven SMGCTB following surgery for conventional GCTB using a combination of immunohistochemical and molecular methods (Sanger sequencing and pyrosequencing or next generation sequencing) (52). They found that 5 of 7 patients had absence of *H3F3A* G34W mutation in malignant giant cell tumor of bone, though it was present in the associated giant cell tumor tissues. Potential interpretations for this discordant mutation status include: i) incidental coexistence of two genetically distinct independent tumors; ii) clonal replacement, with a minor population of preexisting H3F3A G34-wild-type clone in giant cell tumor of bone outgrowing an H3F3A-mutant clone; and iii) loss of H3F3A mutation during linear clonal evolution. Hasenfratz *et al* analysed the samples of 2 patients with H3F3A-mutated GCTBs before and after denosumab treatment by histomorphology, immunohistochemistry, and next generation panel sequencing (38). The initial GCTB in the biopsy and in the recurrence was H3F3A-mutated, while the sarcoma was negative for this mutation as shown by sequencing and immunohistochemical staining. Sequencing revealed a persisting H3F3A mutation in one patient while the other lost the H3F3A mutation after malignant transformation. They speculated that one explanation is a transformation of the H3F3A-negative mononuclear cells residing in the tumor after denosumab treatment. In this study, we did not find *H3F3A* G34W mutation in SMGCTB by cancer genomic profiling. Although we did not investigate the genomic profiling of the primary GCTB lesion, *H3F3A* G34W mutation in this patient can be lost during progression to malignant transformation.

Although surgery is the standard of care for extreme SMGCTB, its issue is limited for patients with SMGCTB of the spine or sacrum due to the complex anatomical structures and neurological dysfunction associated with surgery to these locations. Yin *et al* reported that three out of six SMGCTB cases demonstrated local recurrence in the spine (28). Postoperative RT was performed in two cases; both patients experienced recurrence, denying the effectiveness of RT (28). In our literature review, four cases utilized RT, however, no information regarding its effectiveness was reported (11,16,19,31). Recently, carbon-ion RT has been proven to be effective in patients with unresectable sarcoma of the sacrum (53). Additionally, we utilized carbon-ion RT in the patient with SMGCTB in the sacrum and achieved good and prolonged local control for >3 years. In this patient (case 3), conventional RT also proved to be effective for metastatic lesions. Thus, the role of RT, including carbon-ion RT for sacral SMGCTB should be further investigated.

Clinical outcomes of SMGCTB are poor, with local recurrence rates of 20-50% and distant metastasis rates of 22-80%. Liu *et al* reported that in a total of 20 cases of SMGCTB, local recurrence occurred in 4 patients (20%), and 16 patients (80%) developed metastasis [lung (all cases), brain (1 case), and bone (2 cases)] (30). They also reported that 14 patients died before the last follow-up, with a 5-year OS rate of 40%. In our review, most patients with metastasis had poor survival and died within 12 months. In this study, we also identified that all patients developed distant metastasis, including to the lung and bone, and two out of three patients died before the last follow-up.

There is no consensus regarding optimal treatment for SMGCTB due to its low incidence. Current treatment strategies include surgery alone or surgery combined with chemotherapy and RT. Moreover, Liu *et al* reported that local recurrence occurred in 7 of 9 cases with inadequate margins and in 5 of 24 cases with adequate margins (P=0.006) in patients with PMGCTB and SMGCTB (30). Thus, resection should be performed with wide margins. However, patients have often previously undergone multiple surgeries with curettage and bone grafting, which leads to difficulty in limb sparing. In some studies of various series of SMGCTB cases, the rate of amputation was as high as 33-66% (16,24,30,31). Our two patients with extremity SMGCTB also underwent amputation for local control.

There are limited data regarding the role of systemic treatment in patients with SMGCTB (15,17). The role of chemotherapy for SMGCTB is controversial, and there is still insufficient evidence of survival benefits (15,17). Some authors used high-dose methotrexate, cisplatin, doxorubicin (MAP), and ifosfamide, and other used cisplatin and doxorubicin (AP) regimen similar to those used to treat osteosarcoma (15,17,18). Based on our review, we could not conclude the utility of chemotherapy because there is very little information about the regimen and its effectiveness against SMGCTB; only two case reports have described the effect of chemotherapy (15,17). First, Hefti *et al* utilized an MAP regimen for a patient with SMGCTB in the tibia with lung metastasis, but the patient experienced progressive disease and died 9 months later (15). Second, Mori *et al* utilized four cycles of an AP regimen for localized SMGCTB in the tibia preoperatively, which resulted in shrinkage of the extraskeletal mass and ossification (17). The patient later underwent resection and prosthetic replacement and was disease-free at the last follow-up. In the current study, we used chemotherapy in two patients who had distant metastasis. One patient received MAP and ifosfamide. However, the treatment had no effect and the patient died 13 months after SMGCTB development. The other patient received pazopanib, a multi-kinase inhibitor, which was effective, and the patient was alive for >3 years after the development of SMGCTB. Although the histology of SMGCTB is similar to that of osteosarcoma, the effect of the MAP regimen, which is the standard chemotherapy for osteosarcoma, is limited in SMGCTB. Therefore, a new regimen for SMGCTB should be developed.

This study has several limitations. First, there was a small sample size of only three patients. However, this limitation is common in the study of patients with SMGCTB, because it

is an extremely rare malignancy. Second, we did not provide the figure of Western blotting. Since the GCTB and SMGCTB were bone tumors, we performed decalcification procedures. Decalcification methods employed hydrochloric acid to dissolve the calcium salts, which also might damage the protein of the samples. Then, we found that no band was detectable by samples from the formalin-fixed paraffin-embedded tissues. Another limitation is the inherent bias in the choice of treatment. Since standard treatment has not been established, we believe that multicenter studies will be necessary in the future.

In conclusion, high expression of p53 was found in SMGCTB, but not in conventional GCTB, and can be associated with tumorigenesis. The correlation between denosumab and malignant transformation should be investigated in additional controlled studies and long-term follow-up. The clinical behavior of SMGCTB is extremely aggressive, resulting in metastasis in all patients. New emerging treatments using molecular targeted therapy and carbon-ion RT should be further investigated to improve the clinical outcomes of SMGCTB.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

EN, HoK, HI and TO designed the study, and collected and analyzed data. EN and TK confirm the authenticity of all the raw data. TK, TF, HaK, TI and MF analyzed data. EN, TK and TF treated the patients presented in this manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This retrospective chart review study involving human participants was conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of Okayama University Hospital approved this study (approval no. K 2103-040).

Patient consent for publication

Written informed consent was obtained from each participant included in this study.

Competing interests

The authors declare that they have no competing interests.

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