Case Report

Development of high-grade osteosarcoma in a patient with recurrent giant cell tumor of the ischium while receiving treatment with denosumab

Shinji Tsukamoto\textsuperscript{1,*}, Alberto Righi\textsuperscript{2}, Daniel Vanel\textsuperscript{2}, Kanya Honoki\textsuperscript{1}, Davide Maria Donati\textsuperscript{3}, and Costantino Errani\textsuperscript{3}

\textsuperscript{1}Department of Orthopaedic Surgery, Nara Medical University, Kashihara, Nara, Japan, \textsuperscript{2}Department of Pathology, Rizzoli Institute, and \textsuperscript{3}Department of Orthopaedic Oncology, Rizzoli Institute, Bologna, Italy

*For reprints and all correspondence: Shinji Tsukamoto, Department of Orthopaedic Surgery, Nara Medical University, 840, Shijo-cho, Kashihara-city, Nara 634-8521, Japan. E-mail: shinji104@mail.goo.ne.jp

Received 25 April 2017; Editorial Decision 17 July 2017; Accepted 18 July 2017

Abstract

Malignant transformation of giant cell tumor of bone (GCTB) without radiotherapy exposure is exceptionally rare, occurring in less than 1% of GCTBs. The safety and efficacy of denosumab in patients with GCTB was recently reported. We herein report a case of a benign recurrent GCTB with an H3F3A mutation that underwent secondary malignant transformation during treatment with denosumab. A 29-year-old woman underwent curettage of a GCTB of the left ischium in 2005. Ten years after the first surgery, the GCTB recurred locally. We started treatment with denosumab. During the first 5 months of treatment, we observed a demarcated area of osteosclerosis in the recurrent lesion, and the patient’s clinical condition improved. At 6 months, however, the patient developed pain, and a rapidly growing mass was detected by computed tomography. An incisional biopsy was performed. Histologic analysis showed a high-grade osteosarcoma. The patient developed lung metastases and died soon after beginning chemotherapy. The mechanism of sarcomatous transformation of GCTB during denosumab therapy is unclear. These findings suggest that the scientific community should be aware of the possible malignant transformation of GCTB during denosumab treatment.

Key words: giant cell tumor of bone, malignancy in giant cell tumor of bone, osteosarcoma, denosumab

Introduction

Giant cell tumor of bone (GCTB) is a benign but locally aggressive primary bone neoplasm (1). The tumor usually arises in the metaepiphyseal region of long bones, predominantly the distal femur and proximal tibia, but it can occur anywhere throughout the entire skeleton (2). Although generally considered benign, GCTB can rarely metastasize despite maintaining a benign histology (3). Even more uncommonly, GCTB has the potential to transform into a true malignant tumor (3). The standard treatment is surgery with as near complete removal of the tumor possible without major morbidity (4). This is usually accomplished by detailed curettage, although resection or even amputation may be required for tumors with extensive bone destruction (4).

Denosumab is a fully humanized monoclonal antibody that specifically binds to and inhibits receptor activator of nuclear factor kappa-B ligand (RANKL) activation of receptor activator of nuclear factor kappa-B (RANK), thereby inhibiting osteoclastogenesis and osteoclast-mediated bone destruction (5). An ongoing open-label, Phase II study has reported the safety and efficacy of denosumab in more than 280 patients with complicated GCTB (6). Based on these data, the Food and Drug Administration approved denosumab for the treatment of unresectable GCTB or cases in which surgery is
likely to result in severe morbidity (3). However, five cases of malignant transformation of GCTB without prior radiation exposure during denosumab therapy have been reported (5–8).

We herein present the sixth case of progression of a GCTB to a conventional osteosarcoma during denosumab treatment. The diagnosis of GCTB and osteosarcoma was molecularly confirmed. This study was approved by our hospital’s institutional ethics board and registered with ClinicalTrials.gov (identifier NCT02996734).

Report of the case

A 25-year-old woman presented to another institution because of left hip pain in 2001. A lytic mass was found in her left ischium. Initial imaging was unavailable. The lytic mass was thought to be a simple bone cyst because of her chronic and slight pain, and she underwent a follow-up examination with imaging. The lesion size did not change until 2005 and increased slightly in 2006.

Figure 1. Preoperative anteroposterior radiograph showed a lytic, expansile mass in the left ischium.

Figure 2. (a) Hematoxylin and eosin staining relative to the first curettage in 2006 revealed cellular proliferation arranged in irregular fascicles, mild nuclear pleomorphism without mitosis, scattered osteoclast-like giant cells (×200 magnification), and (b) reactive bone formation comprising immature woven bone and trabeculae with osteoblastic rimming (×100 magnification). (c) Immunohistochemical analysis showed strong nuclear positivity for p63 protein in the mononuclear neoplastic cells (×200 magnification).
Radiographs showed a lytic, expansile mass in the left ischium (Fig. 1). Computed tomography (CT) revealed destructive, expansile mass in the ramus of the ischium. Examination of a CT-guided biopsy specimen led to a diagnosis of GCTB. Therefore, the patient was referred to our institute in September 2006.

She had no history of major illness. Physical examination revealed left hip pain at maximum flexion and external and internal rotation. She experienced tenderness on her left ischium. Curettage was performed using a high-speed burr and followed by cement packing with phenol adjunct. The entire specimen obtained from the curettage procedure showed spindle and oval cell proliferation of fibroblasts and fibrohistiocytes. The cells were arranged in irregular fascicles. The cell size was variable, and nuclear pleomorphism with coarse, granular chromatin and clearly malignant features was absent. Scattered myxoid areas of variable dimensions were present. The osteoid or bone matrix was scanty. Small spaces lined by fibroblasts and fibrohistiocytes. The cells were arranged in irregular fascicles. The cell size was variable, and nuclear pleomorphism with coarse, granular chromatin and clearly malignant features was absent. Scattered myxoid areas of variable dimensions were present. The osteoid or bone matrix was scanty. Small spaces lined by fibroblasts and fibrohistiocytes.

These morphological features along with the absence of USP6 gene rearrangement (which is found in 60% of patients with aneurysmal bone cysts (9)) and the absence of MDM2 gene amplification (which is found in almost all patients with low-grade central osteosarcoma and in a small percentage of those with high-grade osteosarcoma) were consistent with the diagnosis of a giant cell lesion (10). However, the morphological features did not allow us to distinguish among a giant cell reparative granuloma, solid aneurysmal bone cyst or GCTB. We recently attempted to identify the driver mutation in the histone gene H3F3A, typical of GCTB, but the quality of extracted DNA was not sufficient to complete the test because the tissue blocks were very old. However, we evaluated the immunohistochemical expression of p63 protein, which showed strong nuclear positivity in mononuclear neoplastic cells, supporting the diagnosis of GCTB (Fig. 2c) (11).

Postoperatively, the patient did well. However, she redeveloped left hip pain in February 2015. CT showed osteolytic lesions on the lateral side of the cement (Fig. 3) and in the body of the ischium. A CT-guided biopsy of the lytic lesion of the body of the ischium was performed in June 2015. Histologically, we observed the same morphological features of the previous samples in association with the immunohistochemical nuclear expression of p63 protein in mononuclear cells (Fig. 4a). The only morphological difference was the increase in the number of osteoclast-like giant cells (Fig. 4b-d). Using this specimen, we repeated the molecular analysis for identification of MDM2 and USP6 rearrangements, but the results were still negative. Unlike in the previous samples, the molecular analysis revealed a driver mutation in the histone gene H3F3A. In particular, direct sequencing for the presence of H3F3A in the coding region between codons 1 and 42, including the hot spot codons (28, 35 and 37), was performed on DNA extracted from formalin-fixed, paraffin-embedded tissue and a p.Gly35Trp (p.G35W; NP_002098.1) mutation in the H3F3A gene was found (Fig. 4c). The diagnosis of GCTB was therefore confirmed.

F-18 fluorodeoxyglucose positron emission tomography/CT showed increased fluorodeoxyglucose uptake in the left ischium and in no other locations. Resection of the tumor after decreasing its size by embolization and denosumab therapy was planned because the downstaging capacity of denosumab was recently described in an interim analysis of an open-label Phase II trial (6). Embolization of the lesion was performed in July 2015. However, the patient’s pain remained unchanged, and we thus began administration of denosumab 2 weeks after embolization. Subcutaneous denosumab (120 mg) was administered once a week for the first month, then once a month for 6 months. Chest CT showed no metastasis immediately before starting denosumab. One month after embolization and 2 weeks after beginning denosumab treatment, pelvic CT showed an increase in the size of the osteolytic lesion and sclerotic bone formation (Fig. 5). The pain around her left hip diminished 1 month after beginning denosumab administration. After 5 months of continuous denosumab treatment, CT showed a clearly demarcated region of osteosclerosis of the recurrent lesion in the left ischium, and the patient had no pain in November 2015 (Fig. 6). However, in December 2015, 6 months after beginning denosumab treatment, she developed pain in the left hip. A CT scan revealed enlargement of the poorly defined blastic mass (Fig. 7). The denosumab was discontinued. Chest CT showed multiple nodules suspicious of metastasis in both lungs. Incisonal biopsy of the left ischium revealed fields of high-grade fibroblastic and osteoblastic osteosarcoma alongside areas of GCTB associated with morphological features after denosumab treatment (Fig. 8a). In particular, the post-denosumab treatment tumor areas showed pronounced differences from the pretreatment samples, which mainly consisted of a lack of osteoclast-like giant cells, the presence of spindle cells arranged in a storiform pattern, and variable production of abundant fibrillary extracellular osteoid-like matrix organized in trabecular structures or a honeycomb pattern (Fig. 8b). Conversely, we observed areas of high-grade fibroblastic and osteoblastic osteosarcoma in which the mononuclear cells had become larger, were mainly round or round to oval, and had hyperchromatic nuclei that sometimes exhibited prominent nucleoli with a high nuclear/cytoplasmic ratio and atypical mitotic figures associated with osteoid matrix production (Fig. 8c). Different from the previous specimens, immunohistochemical analysis showed that p63 protein was absent in the nucleus of these malignant areas. No USP6 gene rearrangement or MDM2 gene amplification was identified in both areas of this sample by fluorescence in situ hybridization. Mutation analysis
Figure 4. (a) The nuclei of the mononuclear cells showed immunoreactivity for p63 protein (×200 magnification). (b-d) An increase in the number of osteoclast-like giant cells associated with mononuclear cell proliferation was evident in the sample relative to a biopsy in June 2015 (×200 magnification). (e) Direct sequencing for the presence of H3F3A in the coding region between codons 1 and 42a showed a p.Gly35Trp (p.G35W; NP_002098.1) mutation.
to identify a driver mutation in the histone gene H3F3A was also negative. A diagnosis of malignant transformation of GCTB was made. The patient received chemotherapy in another institute but died in June 2016.

Discussion

Malignant transformation in a previously histologically typical GCTB with no record of previous radiotherapy is exceptionally uncommon, occurring in less than 1% of patients with GCTB (12). Sarcomas arising in benign GCTB usually contain areas of benign giant cell tumors, and a biopsy may not initially reveal the malignant tumor (13). Our patient was diagnosed with recurrent GCTB by CT-guided needle biopsy, and we observed osteosclerosis of the recurrent lesion with clinical improvement under denosumab therapy. Based on these results, it seems unlikely that the malignant component was present initially and misdiagnosed because conventional high-grade osteosarcomas generally do not respond to denosumab therapy except for giant cell-rich osteosarcomas (14). Therefore, our patient appeared to have developed an osteosarcoma in a previously benign, recurrent GCTB while undergoing denosumab therapy.

The diagnosis of giant cell-containing lesions of bone is challenging (15). Cleven et al. (15) recently reported that H3F3A mutation analysis appears to be a highly specific, although less sensitive, diagnostic tool for the distinction of GCTB from other giant cell-containing tumors. Agaram et al. (9) reported that fluorescence in

situ hybridization for USP6 break-apart is a useful ancillary tool in the diagnosis of primary aneurysmal bone cysts and can help to distinguish them from giant cell reparative granulomas and other morphologically similar lesions. We were able to achieve an accurate diagnosis using these genetic analyses.

Thomas et al. (5) reported the first open-label Phase II study showing clinical benefits of denosumab treatment in 37 patients with GCTB. However, 2 of the 37 patients developed malignant transformation. One patient developed a high-grade sarcoma in the upper extremity during denosumab treatment. The other developed a malignant GCTB with metastases to the lung 8 months after discontinuing denosumab; this patient’s condition was considered unrelated to treatment.

Chawla et al. (6) reported an open-label Phase II study showing the clinical benefits of denosumab treatment in 282 patients with GCTB. Three had new primary malignancies: two had sarcomas (one was retrospectively suspected to be present at baseline, and the other was thought to be a malignant transformation), and one had thyroid cancer with a high-grade sarcoma in the lesion (attributed to previous radiation therapy).

Rutkowski et al. (16) reported an open-label Phase II study showing clinical benefits of denosumab treatment in 222 patients with GCTB. Four patients developed malignant GCTB transformation: two radiation-associated sarcomatous transformations at 4 and 6 years after radiotherapy, respectively, and two pelvic or sacral GCTB lesions that progressed under denosumab after 257 days of exposure. In these latter two cases, the investigators believed that a diagnosis of primary malignant GCTB was missed by sampling error at the time of the initial core biopsy. However, the report contained no description of the response to denosumab therapy in the early treatment period. In these three trials, the diagnosis was not confirmed by molecular analysis, and the authors did not show the morphology of the apparent primary GCTB that became sarcoma after denosumab therapy.

Aponte-Tinao et al. (7) described a 20-year-old woman with a recurrent benign GCTB who developed a bone sarcoma while receiving denosumab treatment. The recurrent GCTB responded to denosumab until malignant transformation occurred, and the patient had received no previous radiation therapy, as in the present case. Broehm et al. (8) reported two cases of sarcomatous transformation of GCTB to osteosarcoma in patients receiving denosumab. Both GCTBs responded to denosumab until malignant transformation occurred, and neither patient had undergone previous radiation therapy. The latent periods between the diagnosis of benign GCTB and the diagnosis of sarcoma in the four patients receiving denosumab treatment were 5, 7, 13 and 10 years (mean, 8.8 years).
in the three above-mentioned case reports and the present case, respectively (7,8). Our institute previously reported that the latent periods between diagnosis of benign GCTB and diagnosis of secondary malignant GCTB were 16.0, 27.0, 16.3, 22.0, 27.7 and 7.3 years (mean, 19.4 years) in six patients who received neither radiotherapy nor denosumab treatment (13). The latent period between the diagnosis of GCTB and sarcoma was significantly shorter in patients who did than did not receive denosumab treatment ($P = 0.035$, analyzed using Student’s $t$-test for parametric analyses). In six cases of secondary malignancy in GCTB after previous radiotherapy, the interval between the start of radiotherapy and diagnosis of the malignancy was 1.7–15.0 years (mean, 8 years) (13). Conversely, in the three above-mentioned case reports and the present case, the interval between the start of denosumab and diagnosis of the malignancy was 0.5–2.5 years (mean, 1.2 years) (7,8). The interval between the start of denosumab and diagnosis of the malignancy is obviously shorter than the interval between the start of radiotherapy and the diagnosis.

Chen and Pu (17) recently performed a meta-analysis of randomized controlled trials showing the safety of denosumab versus zoledronic acid in patients with bone metastases. They found that a new primary malignancy occurred significantly more frequently in patients treated with denosumab than with zoledronic acid. However, the long-term safety of denosumab has not yet been assessed; long-term treatment surveillance is still ongoing (18).

The treatment effects of denosumab can be attributed to its actions against RANKL (6). GCTB is characterized by stromal cells expressing RANKL and osteoclast-like giant cells expressing RANK (6). Denosumab binds to RANKL, substantially reducing or eliminating osteoclast-like giant cells (6). Consequently, osteolysis is suppressed and the proliferative tumor stroma is replaced by nonproliferative, differentiated, densely woven new bone (19). Three possible mechanisms by which inhibition of RANKL may induce malignant transformation of GCTB are as follows. First, in osteosarcoma cells, RANKL upregulates the expression of the semaphorin 3A gene (20), and knockout of this gene induces abnormal bone and cartilage development (21). Therefore, inhibition of RANKL may induce abnormal differentiation of osteoblasts and result in osteosarcoma tumorigenesis via semaphorin 3A. Second, because RANKL upregulates nuclear factor IB, which is potentially implicated in cell morphology and reduced susceptibility to nuclear oncogenes (20,22), inhibition of RANKL may result in osteosarcoma tumorigenesis by
increasing susceptibility to nuclear oncogenes. Third, because the expression of RANKL plays an important role in B- and T-cell differentiation and dendritic cell survival, its inhibition could eventually increase the risk of infection or new malignancies due to immunosuppression (18,23,24).

In summary, we have reported a case involving a 39-year-old woman with a recurrent benign GCTB of the ischium showing an H3F3A mutation and in which an osteosarcoma developed during denosumab treatment. It is impossible for us to establish whether denosumab treatment contributed to the development of the osteosarcoma or whether this was coincidental. The findings in this case suggest that treating physicians should be aware of the possible association between malignant transformation and denosumab therapy and use denosumab with extreme caution because GCTB is a benign tumor and commonly occurs in young adults who have a long life expectancy. Further prospective studies should focus on the safety of denosumab therapy for treatment of patients with an unresectable GCTB.

Authors’ contributions
All authors had access to the data and played a role in the writing of the manuscript.

Conflict of interest statement
Alberto Righi is Amgen consultant for giant cell tumor about pathological revision of cases.

Funding
None.

References