Original

Undifferentiated Pleomorphic Sarcoma of Soft Tissue with Multinucleated Giant Cells with Osteogenic Phenotypes: A Mimicker of Malignant Giant Cell Tumor of Soft Tissue

Shunichiro Matsuoka1*, Hiromasa Hasegawa1*, Sachie Koika1, Tsutomu Koyama1, Tetsu Takeda1, Kentaro Miura1, Takashi Eguchi1, Kazuotshi Hamanaka1, Munehisa Kito6, Jun Takahashi1, Toshiro Fukushima1, Tatsuo Koizumi1, Kimihiro Shimizu1 and Takeshi Uehara3

1) Division of General Thoracic Surgery, Department of Surgery, Shinshu University School of Medicine, Nagano, Japan
2) Hard Tissue Pathology Unit, Graduate School of Oral Medicine, Matsumoto Dental University, Nagano, Japan
3) Department of Laboratory Medicine, Shinshu University School of Medicine, Nagano, Japan
4) Department of Orthopaedic Surgery, Shinshu University School of Medicine, Nagano, Japan
5) Department of Hematology and Medical Oncology, Shinshu University School of Medicine, Nagano, Japan
6) Division of General Thoracic Surgery, Department of Surgery, Shinshu University School of Medicine, Nagano, Japan

Correspondence to: Dr. Shunichiro Matsuoka, Division of General Thoracic Surgery, Department of Surgery, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano, 3908621, Japan; Tel: +81263372657; Fax: +81263372721; E-mail: m-shunichiroh@shinshu-u.ac.jp

Abstract: This study aimed to summarize the clinicopathological findings and assess the immunophenotypes for undifferentiated pleomorphic sarcoma of soft tissue with multinucleated giant cells (UPS-ST-MGCs). We retrospectively identified five cases of UPS-ST-MGCs between 2010 and 2020, and evaluate histological and immunohistochemical findings using osteogenic markers, which were receptor activator of nuclear factor-kappa B ligand (RANKL), runt-related transcription factor 2 (RUNX2), and special AT-rich sequence-binding protein 2 (SATB2). Cases were divided into two types, based on the distribution of multinucleated giant cells (MGCs), as diffusely (MGCs diffuse type) or focally scattering (MGCs focal type). One out of five cases was classified as MGCs diffuse type and comprised relatively monotonous proliferation of atypical spindle cells widely expressing RANKL, RUNX2, and SATB2. This case showed aggressive clinicopathological features, such as a rapidly growing tumor with a high maximum standardized uptake value, high Ki-67 labeling index, and early postoperative recurrence, which can be called malignant giant cell tumor (GCT-ST). Conversely, the other four cases of the MGCs focal type were focally positive for RANKL, and negative for RUNX2 and SATB2, which appeared to be consistent with conventional features of undifferentiated pleomorphic sarcoma of soft tissue. Our results indicate that malignant GCT-ST can be included in UPS-ST-MGCs. Therefore, it is important to note its aggressive malignant characteristics and osteogenic differentiation. Osteogenic immunohistochemical examinations should be considered for UPS-ST-MGCs to confirm an accurate diagnosis and provide appropriate treatment.

Key words: Malignant giant cell tumor, Osteogenic phenotype, RANKL, Soft tissue, Undifferentiated pleomorphic sarcoma

Introduction

Giant cell tumor of soft tissue (GCT-ST) is a relatively rare primary neoplasm and has been described as a tumor of uncertain behavior in the 5th edition of the World Health Organization (WHO) classification1). So far, several studies have reported its aggressive behavior ranging from benign to very malignant2-5). In a study, the malignant behavior of GCG-STs has been found later to be included for giant cell tumors of malignant fibrous histiocytomas (MFH)6), which have been replaced by the term of undifferentiated pleomorphic sarcoma (UPS) in the 4th edition of the WHO classification7). The diagnosis of GCT-ST and UPS is still challenging; nevertheless, the number of studies regarding their genotypes and immunohistochemistry has gradually increased8-10). We experienced the case of a patient with a clinical aggressive malignant tumor, wherein it was histologically difficult to differentiate between GCT-ST and undifferentiated pleomorphic sarcoma of soft tissue with multinucleated giant cells (UPS-ST-MGCs). It is also possible that the diagnosis of these diseases has been confused, and that they have been misdiagnosed.

GCT-ST, which is morphologically identical to a giant cell tumor of the bone (GCT-B), consists of osteoclast-like multinucleated giant cells (MGCs) and mononucleated cells. Currently, several immunohistochemical markers, which were associated with osteogenesis, have been shown to be useful in evaluating GCT-ST11). On the other hand, UPS is defined as an exclusive neoplasm that shows no discernible microscopic evidence of any specific differentiation. However, there are no studies regarding osteogenic immunohistochemical examination for UPS-ST-MGCs.

In this study, we retrospectively investigated cases of UPS-ST-MGCs using immunohistochemical examination to clarify whether these cases showed the osteogenic differentiation, and we evaluated the usefulness of differentiating between a diagnosis of GGT-ST and of UPS-ST-MGCs.
Materials and Methods
We reviewed the database of patients who underwent surgical resection from the Pathology Department of Shinshu University Hospital between 2010 and 2020, and identified five patients that were diagnosed with UPS-ST-MGCs out of 50 cases of UPS-ST (HH and TU). All clinical data associated with the patients were collected from the individual electronic medical records. This study was approved by the Ethics Committee of Shinshu University (approval number 5163), and the study was conducted according to the principles of the Declaration of Helsinki (version 2008).

Immunohistochemical staining
Three µm thick sections were prepared from formalin-fixed paraffin-embedded tissue block followed by deparaffinization and hydration for immunohistochemistry. The antibodies used were obtained from the following sources: Histone H3.3 G34W (HG34W (clone RM263, RevMAB Biosciences USA, Inc., South San Francisco, CA, USA), receptor activator of nuclear factor-kappa B ligand (RANKL) (Bioss Antibodies Inc., Woburn, MA, USA), runt-related transcription factor 2 (RUNX2) (Novus Biological, LLC, Littleton, CO, USA), special AT-rich sequence-binding protein 2 (SATB2) (clone SATBA4B10, Santa Cruz Biotechnology, Inc., Princeton, NJ, USA), and Ki-67 (clone MIB-1, BioGenex Laboratories, Fremont, CA, USA). Antigen retrieval was performed using a pressure cooker for 30 min in a target retrieval solution, with a pH of 9.0 (Agilent Technologies, Santa Clara, CA, USA) for HG34W, RUNX2, and SATB2, and in a target retrieval solution of citrate, with a pH of 6.0 (Agilent Technologies) for Ki-67, respectively. For RANKL, sections were digested using 0.125% trypsin (Nichirei Biosciences Inc., Tokyo, Japan) for 20 min at room temperature. HG34W (1:400) and Ki-67 (1:100) antibodies were incubated at room temperature for 60 min, and RUNX2 (1:200), RANKL (1:500), and SATB2 (1:500) were incubated at 4°C for 24 h. Nichirei MAX-PO Multi (Nichirei Biosciences Inc.) was used as a secondary antibody and was applied at room temperature for 30 min. After visualization using 3,3’-diaminobenzidine tetrahydrochloride (Agilent Technologies), sections were counterstained with hematoxylin. Negative control slides were processed with phosphate-buffered saline instead of primary antibodies.

Results

Clinical Summary
The characteristics of the patients in this study are presented in Table 1. Their mean age was 72 (range 52–86) years, and there were three male and two female patients. Diagnostic opportunity included a radiograph screening in one case and symptom due to expansion of the tumor swelling in four cases. The primary location of the tumor was the chest wall in two patients, the buttock in two patients, and the back in one patient (Fig.1). The median tumor size was 5 cm (range 4–12 cm). All patients underwent resection with a wide margin that included the tumors, and Case 1 underwent additional excision of the fifth to seventh ribs and wedge lung resection, as these organs had been invaded by the tumor. Although all patients underwent complete resection (R0), postoperative pathological findings revealed pleural dissemination on the resected lung in Case 1. Three patients developed tumor recurrence, and in Case 1 and Case 5, recurrence occurred early after surgery. The sites of recurrence were distant (pleural dissemination) in one case, and the tumor recurred in the lungs in two cases. Case 4 involved surgery, radiotherapy, and chemotherapy for lung metastasis. Two patients died of the tumor within a year after surgery, and one patient died of another disease. The other two patients were still alive at the end of the study period. The follow-up time ranged from 9 months to 106 months.

Histopathological findings
All five selected patients had MGCs with a varying number of nuclei. The five cases could be divided into two types based on the distribution of the MGCs diffusely (MGCs diffuse type) or focal scattering of the MGCs (MGCs focal type), and these are summarized in Table 2. Only one patient with the diffuse type of MGCs (Case 1) had osteoclast-like MGCs diffusely scattered throughout the lesion without necrosis or a myxoid background (Fig. 2a). MGCs had an eosinophilic irregular cytoplasm containing numerous nuclei that were usually over 20. The background was proliferation of mononucleated cells showing short-spindle, oval, or round cytoplasm having atypical nuclei with an irregular contour, similar to nuclei of MGCs. Over 19 mitotic figures were seen per 10 high power fields (HPF) (Fig. 2b). According to the French Federation of Cancer Centers Sarcoma Group (FNCLCC) grading system, this case was evaluated as grade 3. The other four cases of MGCs focal type (Case 2 to 5) demonstrated some MGCs in a back- ing system, this case was evaluated as grade 3. The other four cases of MGCs focal type (Case 2 to 5) demonstrated some MGCs in a backing system, this case was evaluated as grade 3. The other four cases of MGCs focal type (Case 2 to 5) demonstrated some MGCs in a backing system, this case was evaluated as grade 3. The other four cases of MGCs focal type (Case 2 to 5) demonstrated some MGCs in a backing system, this case was evaluated as grade 3. The other four cases of MGCs focal type (Case 2 to 5) demonstrated some MGCs in a backing system, this case was evaluated as grade 3. The other four cases of MGCs focal type (Case 2 to 5) demonstrated some MGCs in a backing system, this case was evaluated as grade 3. The other four cases of MGCs focal type (Case 2 to 5) demonstrated some MGCs in a backing system, this case was evaluated as grade 3. The other four cases of MGCs focal type (Case 2 to 5) demonstrated some MGCs in a backing system, this case was evaluated as grade 3. The other four cases of MGCs focal type (Case 2 to 5) demonstrated some MGCs in a backing system, this case was evalua...
Figure 1. Case1: Chest radiography reveals a circumscribed mass in the right upper fields (a), which was not detected six months earlier (b). Contrast-enhanced computed tomography reveals a heterogeneous mass, 4 cm in size, at the right mediastinum without destruction of the adjacent bones (c). Magnetic resonance image reveals a mostly hypointensity mass with extension through only the intercostal muscles on T2-weighted image (d). 18-F fluorodeoxyglucose-position emission tomography reveals maximum standardized uptake value of 12.6 on the mass (e), and no other lesion was detected throughout the body (f).
Figure 2. In only one case of multinucleated giant cells (MGCs) diffuse type (case 1), MGCs are diffusely scattered throughout the lesion composed of short spindle, mononuclear cells (a). Over 20 nuclei are found in the multinucleated giant cells (b). In MGCs focal type, MGCs focally appear as fascicular (c) or myxoid (d) proliferation of spindle cells. MGCs mostly harbor some nuclei less than 10 (e). Not only foreign body-type giant cells but also bizarre giant cells can be seen (f). Scale bars: 100 µm in a, c and d; 20 µm in b, e and f.

Table 2. Immunohistological summary of five patients

| Case | FNCLCC | Mitosis | Necrosis | Strifom | Myxoid | Mononuclear cell feature | Giant cell distribution | Giant cell nuclei | Ki-67 | HG43W | RANKL | RUNX2 | SATB2 |
|------|--------|---------|----------|---------|--------|-------------------------|-----------------------|------------------|-------|-------|-------|-------|-------|
| 1    | G3     | 3       | 0        | -       | -      | short spindle           | diffuse               | >20   | 40%   | -     | +d    | +d    | +f    |
| 2    | G3     | 2       | 2        | +       | -      | spindle/bizzare         | focal                 | <10   | 30%   | -     | +f    | -     | -     |
| 3    | G2     | 1       | 0        | +       | +      | spindle/bizzare         | focal                 | <10   | 20%   | -     | +f    | -     | -     |
| 4    | G2     | 2       | 0        | +       | -      | spindle/bizzare         | focal                 | <10   | 15%   | -     | +f    | -     | -     |
| 5    | G3     | 3       | 1        | -       | -      | spindle/bizzare         | focal                 | <10   | 20%   | -     | +f    | -     | -     |

FNCLCC: French Federation of Cancer Centers Sarcoma Group system; f: focal; d: diffuse; HG43W: Histone 3.3 G34W (H3F3A G34W); RANKL: receptor activator of nuclear factor-kappa B ligand; RUNX2: runt-related transcription factor 2; SATB2: special AT-rich sequence-binding protein 2.
Immunohistochemistry

Immunohistochemical findings were different between the case with MGCs diffuse type and the MGCs focal type, and this is summarized in Table 2. All tumor cells had high Ki-67 labeling index ranging from 15% to 40%, and were completely negative for HG34W. In the MGCs diffuse type (Case 1), most mononuclear cells and MGCs were diffusely cytoplasmic-positive for RANKL (Fig. 3a1) but focally positive in the other cases with MGCs focal type (Fig. 3a2). The mononucleated cells of Case 1 had a nuclear-positive reaction against RUNX2 (Fig. 3b1) and SATB2 (Fig. 3c1) in wide and partial areas, respectively. However, cases of MGCs focal type were completely negative for RUNX2 (Fig. 3b2) and SATB2 (Fig. 3c2).

Discussion

In this study, UPS-ST-MGCs accounted for 10% of UPS-ST, and this seems to be relatively rare. The immunohistochemical examination demonstrated that one of the cases showed RUNX and STAB2, which represent osteogenic differentiation. Therefore, the case should be reclassified to GCT-ST because UPS is an exclusive neoplasm without any specific differentiation. Interestingly, its clinicopathological features included an aggressive malignant course, which was consistent with the characteristics of UPS. Our results indicate that a case of GCT-ST with

Figure 3. Immunohistochemically, one case of MGCs diffuse type (case 1) demonstrates diffuse positive reaction against RANKL (a1) and RUNX2 (b1), and focal positivity for SATB2 (c1). The others are focally positive for RANKL (a2) but completely negative for RUNX2 (b2) and SATB2 (c2). All scale bars: 50 µm
high malignant potential can be called malignant GCT-ST.

The WHO classification recognizes GCT-ST as a tumor of uncertain behavior based on previous reports. Folpe et al. first proposed that GCT-ST had a low malignant potential due to its frequent clinical recurrence, unknown metastatic potential, and mild to moderate nucleated atypia. Later, Oliveria et al. reported that GCT-ST had a wide spectrum from benign to malignant outcome. In the same year, Connell et al. distinguished between benign GCT-ST and malignant GCT-ST, which was defined as a distinct entity from giant cell type of MFH (UPS). Therefore, GCT-ST appears to have the potential to exhibit varying degrees of aggressive malignant features.

GCT-ST composed of MGCS and mononucleated cells are morphologically identical to GCT-B but are genetically distinct. It has been shown that 70–90% of GCT-B harbor HG34W mutation, and this could serve as a useful diagnostic tool for GCT-B. In our study, all patients were negative for HG34W. In addition, the MGCS population has been shown to have phenotypic and functional features of osteoclast, whereas the mononucleated population includes osteoclast precursors, which are recruited from the circulation by chemotactic factors secreted by the mononuclear cells. The mononucleated cells express RANKL, which is an essential regulator of osteoclastogenesis in both GCT-B and GCT-ST. RUNX2 and STAB2 are osteogenic markers that expressed in both MGCS and mononucleated cells of GCT-ST and GCT-B. Case 1 showed that MGCS were distributed throughout the lesion of mononucleated cells that were positive for RANKL, RUNX2, and STAB2. Hence, diffuse expression of RANKL may be compatible with the broad distribution of MGCS. Furthermore, Case 1 had aggressive clinicopathological findings, such as a rapidly growing tumor with a high maximum standardized uptake value, the tumor involved the ribs and right lung with pleural dissemination, the tumor had a high Ki-67 labeling index, and there was early recurrence. These results indicate that the tumor in Case 1 included some kind of osteogenic differentiation with high malignant potential, which can be called malignant GCT-ST. Although complete resection is the primary treatment for GCT-ST, other treatment options include bisphosphonates and Denosumab, a human monoclonal antibody directed against RANKL.

In conclusion, our results indicate that malignant GCT-ST can be included in UPS-ST-MGCs. We also outlined its aggressive malignant characteristics along with the occurrence of osteogenic differentiation. Furthermore, osteogenic immunohistochemical examinations should be considered in cases of UPS-ST-MGCs, in order to make an accurate diagnosis and to provide appropriate treatment.

Acknowledgement

This study was supported by the Pathology Department of Shinshu University Hospital and we would like to thank Editage (www.editage.com) for English language editing.

Conflicts of Interest

The authors have declared that no COI exists.

Reference

1. Qiliveira AM and Lee JC. Giant cell tumour of soft tissue. In: WHO Classification of Tumours Editorial Board. WHO Classification of Tumours, 5th Ed., Vol.3 Soft Tissue & Bone Tumours. Lyon: IARC, 2020, pp141-142
2. Guccion J and Enzinger F. Malignant giant cell tumor of soft parts. An analysis of 32 cases. Cancer 29: 1518-1529, 1972
3. Folpe AL, Morris RJ and Weiss SW. Soft tissue giant cell tumor of low malignant potential: a proposal for the reclassification of malignant giant cell tumor of soft parts. Mod Pathol 12: 894-902, 1999
4. Oliveira AM, Dei Tos AP, Fletcher CD and Nascimento AG. Primary giant cell tumor of soft tissues: a study of 22 cases. Am J Surg Pathol 24: 248-256, 2000
5. O’Connell IX, Wehrli BM, Nielson GP and Rosenberg AE. Giant cell tumors of soft tissue: a clinicopathologic study of 18 benign and malignant tumors. Am J Surg Pathol 24: 386-395, 2000
6. Jo VY and Fletcher CD. WHO classification of soft tissue tumours: an update based on the 2013 (4th) edition. Pathology 46: 95-104, 2014
7. Lau YS, Sabokbar A, Gibbons C, Giele H and Athanassou N. Phenotypic and molecular studies of giant-cell tumors of bone and soft tissue. Hum Pathol 36: 945-954, 2005
8. Lee J-C, Liang C-W and Fletcher CD. Giant cell tumor of soft tissue is genetically distinct from its bone counterpart. Mod Pathol 30: 728-733, 2017
9. Mancini I, Righi A, Gambarotti M, Picci P, Dei Tos AP, Billings SD, Simi L and Franchi A. Phenotypic and molecular differences between giant-cell tumour of soft tissue and its bone counterpart. Histopathology 71: 453-460, 2017
10. Nascimento AF and Raut CP. Diagnosis and management of pleomorphic sarcomas (so-called “MFH”) in adults. J Surg Oncol 97: 330-339, 2008
11. Chen S, Huang W, Luo P, Cai W, Yang L, Sun Z, Zheng B, Yan W and Wang C. Undifferentiated pleomorphic sarcoma: long-term follow-up from a large institution. Cancer Manag Res 11: 10001-10009, 2019
12. Behjati S, Tarpey PS, Presneau N, Scheipl S, Pillay N, Van Loo P, Wedge DC, Cooke SL, Gundem G, Davies H, Nik-Zainal S, Martin S, McLaren S, Goodie V, Robinson B, Butler A, Teague JW, Halai D, Khatri B, Myklebost O, Baumhoe R, Jundt G, Hamoudi R, TIrabosco R, Amary MF, Futeal PA, Stratton MR, Cameron PJ and Flanagan AM. Distinct H3F3A and H3F3B driver mutations define chondroblastoma and giant cell tumor of bone. Nat Genet 45: 1479-1482, 2013
13. Cleven AH, Hocker S, Briaire-de Bruijn I, Szhchai K, Clleton-Jansen AM and Bovée JV. Mutation analysis of H3F3A and H3F3B as a diagnostic tool for giant cell tumor of bone and chondroblastoma. Am J Surg Pathol 39: 1576-1583, 2015
14. Forsyth RG, De Bock G, Baele J, Taminiau AH, Uyttendaele D, Roels H, Pret MM and Hogendoorn PC. CD33+ CD14+ phenotype is characteristic of multinuclear osteoclast-like cells in giant cell tumor of bone. J Bone Miner Res 24: 70-77, 2009
15. Morgan T, Atkins GJ, Trivett MK, Johnson SA, Kansara M, Schlicht SL, Slavin JL, Simmons P, Dickinson I and Powell G. Molecular profiling of giant cell tumor of bone and the osteoclast-like localization of ligand for receptor activator of nuclear factor kappa B. Am J Pathol 167: 117-128, 2005
16. Atkins GJ, Kostakis P, Vincent C, Farrugia AN, Houchins JP, Findlay DM, Evdokiu A and Zannettino AC. RANK expression as a cell surface marker of human osteoclast precursors in peripheral blood, bone marrow, and giant cell tumors of bone. J Bone Miner Res 21: 1339-1349, 2006
17. Komori T. Regulation of osteoblast differentiation by transcription factors. J Cell Biochem 99: 1233-1239, 2006
18. Girolami I, Mancini I, Simoni A, Baldi GG, Simi L, Campanacci D, Beltrami G, Scoccianti G, D’Arienzo A, Capanna R and Franchi A. Denosumab treated giant cell tumour of bone: a morphological, immunohistochemical and molecular analysis of a series. J Clin Pathol 69: 240-247, 2016
19. Luengo-Alonso G, Mellado-Romero M, Shemesh S, Ramos-Pascua L and Pretell-Mazzini J. Denosumab treatment for giant-cell tumor of bone: a systematic review of the literature. Arch Orthop Trauma Surg 139: 1339-1349, 2019
20. Mokrani A, Guermazi F, Yahyaoui Y, Hmida L, Doghri R, Ayadi M, Khedija M, Letaief F, Chraiet N, Raies H, Mrad K and Mezlini A. Giant cell tumor of soft tissues: a case report and review of literature. J Cancer Sci Ther 9: 562-565, 2017
21. Nepucpan EB and Sacdalan DB. Giant cell tumor of soft tissue of the nasopharynx: A case report. Cancer Treat Res Commun 23: 100171, 2020
