

Original Article

CD56 may be a more useful immunohistochemical marker than somatostatin receptor 2A for the diagnosis of phosphaturic mesenchymal tumors

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Abstract: Phosphaturic mesenchymal tumors (PMTs) are the most typical cause of tumor-induced osteomalacia (TIO) associated with mesenchymal neoplasms. Specifically, TIO is attributed to the production of phosphatonins, such as fibroblast growth factor 23 (FGF23), participating in the homeostasis of phosphate. Although immunohistochemistry (IHC) for FGF23 showed characteristic positive staining in PMTs, FGF23 antibodies that can be used for the reliable diagnosis of PMTs are hard to obtain in common pathology laboratories. Somatostatin receptor 2A (SSTR2A) has been previously proposed as an alternatively useful marker for the diagnosis of PMTs. However, SSTR2A is not commonly utilized in pathological laboratories. The CD56 marker is a useful alternative that is comparable to SSTR2A and is similar considering the sensitivity. Even in cases of PMTs originating in the bones, ethylenediaminetetraacetic acid-based decalcification for tissue processing does not seem to affect the IHC of CD56. As CD56 immunopositivity in mesenchymal tumors is limited, it also has some degree of specificity for PMTs. Thus, when PMTs are suspected, the use of CD56 is recommended.

Keywords: Phosphaturic mesenchymal tumor, immunohistochemistry, CD56, SSTR2A, FGF23

Introduction

Tumor-induced osteomalacia (TIO) is known to be a minor cause of osteomalacia [1]. Most cases of TIO are related to mesenchymal neoplasms, while TIO secondary to an epithelial neoplasm has been rarely documented, particularly in prostate carcinoma [2]. Although several mesenchymal neoplasms were thought to cause TIO, it has recently been revealed that phosphaturic mesenchymal tumors (PMTs), a histologically uniform neoplasm, are the main cause of TIO associated with mesenchymal neoplasms [3, 4].

Usually, PMTs occur in the soft tissues, bones, and sinonasal region [3]. They typically consist of bland, spindled to stellate cells, accompanied by a very well developed capillary network [3]. In some instances, larger vessels presenting as a “staghorn” pattern are observed. The matrix of PMTs typically calcifies in an unusually grungy or flocculent manner [3]. Osteoclast-like giant cells are sometimes observed.

Tumor-induced osteomalacia is attributable to the production of phosphatonins, hormones which impair the re-uptake of phosphate in the kidneys [5]. The most common phosphatonin is fibroblast growth factor 23 (FGF23), which is derived from the bones and participates in the homeostasis of phosphate [6].

Immunohistochemistry (IHC) for FGF23 is seemingly the optimal method for correctly diagnosing PMTs, but it can only be conducted in limited laboratories, because commercially available FGF23 antibodies do not have a satisfactory specificity for the reliable diagnosis of PMT [3, 7]. Because of its diffuse strong expression, IHC of somatostatin receptor 2A (SSTR2A) provides relatively reliable information for PMT diagnoses, providing high sensitivity but not enough specificity [7]. Moreover, because IHC of SSTR2A is used for managing neuroendocrine tumors, SSTR2A antibodies are more widely available in surgical pathology laboratories compared to FGF23 antibodies [7]. However, SSTR2A antibodies are not commonly stocked in surgical pathology laboratories, and

Table 1. Clinical findings including the serum fibroblast growth factor 23 (FGF23) level

| Case No. | Age (years) | Sex | FGF23 (pg/ml) | Location |
|----------|-------------|-----|---------------|-----------------------|
| 1 | 68 | F | 2236 | Right sciatic bone |
| 2 | 63 | M | 9165 | First lumbar vertebra |
| 3 | 61 | M | 225 | Dura mater |
| 4 | 40 | F | 162 | Right knee |
| 5 | 51 | M | 482 | Left sole |
| 6 | 58 | M | 3066 | Right femur |
| 7 | 58 | M | 98.9 | Left femur |
| 8 | 68 | M | 446 | Right humerus |
| 9 | 64 | F | 111 | Right ilium |
| 10 | 41 | F | 423 | Sphenoid bone |
| 11 | 77 | M | 242 | Left parotid gland |

M: male, F: female.

they tend to be prepared only in laboratories conducting detailed examinations in neuroendocrine pathology.

Other ways to confirm the diagnosis of PMTs are reverse transcription polymerase chain reaction (RT-PCR) and chromogenic in situ hybridization (CISH) for *FGF23* mRNA [8]. While RT-PCR for *FGF23* mRNA is highly sensitive, *FGF23* mRNA can be detected in non-PMTs originating in the bones [8]. This is probably due to low-level *FGF23* mRNA expression in even non-neoplastic bone tissue [9], and is thus confusing when applied to PMTs originating in the bones. Though it is not commonly available, CISH for *FGF23* mRNA is a sensitive and specific method [8].

Therefore, we conducted a search for the identification of commonly available IHC markers in surgical pathology laboratories, which are especially useful for the diagnosis of PMTs when dealing with limited sample amounts such as biopsy specimens.

Materials and methods

Study population

The computerized pathological database of The University of Tokyo Hospital was searched between 2000 and 2015 to identify cases of PMTs. Seventeen cases were identified, and formalin-fixed, paraffin-embedded blocks were available in 14 cases. Three cases were not used in this study because the amount of the samples was not enough for this study. Clinical information for these 11 cases was obtained

from the computerized medical record system. Curettage was performed for cases developing in the bones, and enucleation was performed for cases developing in the soft tissue.

Enzyme-linked immunosorbent assay

Serum FGF23 levels were measured using an enzyme-linked immunosorbent assay (ELISA) that recognizes only full-length FGF23 (Intact FGF23 ELISA, Kainos Laboratories, Tokyo, Japan). The normal range of serum FGF23 using this measurement is 0 to 71 pg/ml. Both preoperative and postoperative serum FGF23 levels were measured.

Histopathological evaluation

Each operative specimen was fixed in 10% buffered-formalin and then embedded in paraffin. Decalcification using an ethylenediaminetetraacetic acid (EDTA)-based reagent (10% EDTA-2Na; Muto Pure Chemicals, Tokyo, Japan) was performed for specimens obtained from bones, for approximately 1 week before the process of paraffin-embedding. Sections of 4- μ m-thick were obtained from paraffin-embedded blocks from all samples and stained with hematoxylin and eosin.

Immunohistochemistry

Sections of 4- μ m-thick were obtained from paraffin-embedded blocks. IHC for CD56, synaptophysin, chromogranin A, SSTR2A, SSTR5, CD34, and S100 protein were performed using primary antibodies against CD56 (clone 1B6, 1:50; Novocastra Laboratories, Newcastle, UK), synaptophysin (A0010, 1:100; Dako, Glostrup, Denmark), chromogranin A (A0430, 1:200; Dako), SSTR2A (ab134152, 1:100; Epitomics, Burlingame, CA), SSTR5 (sc-25679, 1:100; Santa Cruz Biotechnologies, Santa Cruz, CA), CD34 (QBEnd 10, 1:100; Dako), and S100 protein (polyclonal, 1:1000, Dako), respectively. IHC was conducted using a BenchMark XT Autoimmune Stainer (Roche Ventana Medical Systems Inc., Tokyo, Japan) and an I-View DAB detection kit (Roche Ventana Medical Systems Inc.). The proportion of immunopositive tumor cells was evaluated, and when it was difficult to find immunonegative tumor cells, the proportion was expressed as >95%. The intensity of

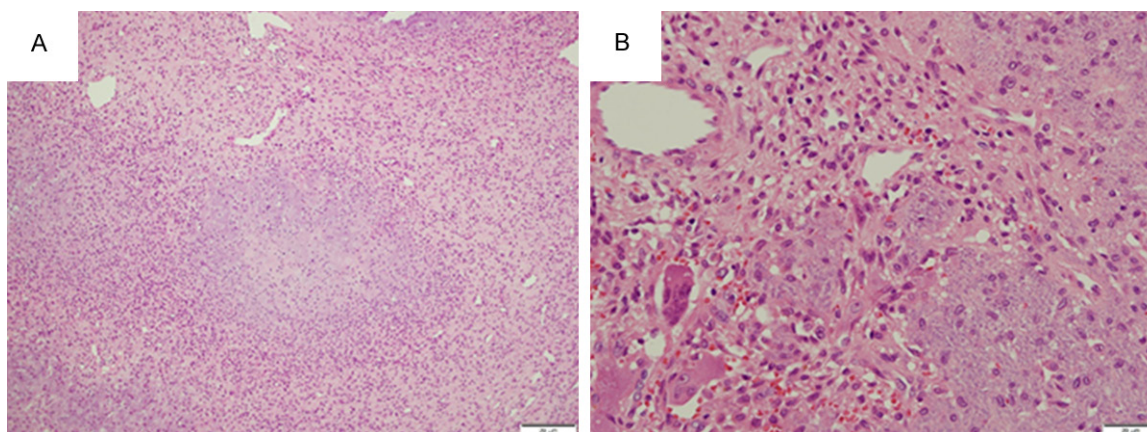


Figure 1. Histological findings. A. Case 5. Monotonous proliferation of tumor cells within a background of prominent microvessels is observed. Characteristic grungy or flocculent calcification is present at the center of the field ($\times 40$, bar: 200 μm). B. Case 5. Osteoclast-like giant cells are observed near the grungy or flocculent calcification. Tumor cells are spindle-shaped with scant eosinophilic cytoplasm and bland nuclei without prominent nucleoli ($\times 400$, bar: 20 μm).

Table 2. Immunohistochemical findings for CD56 and somatostatin receptor 2A (SSTR2A)

| Case No. | Decalcification | CD56 | | SSTR2A | |
|----------|-----------------|------------|-----------|------------|-----------|
| | | Proportion | Intensity | Proportion | Intensity |
| 1 | + | 80% | 3+ | 50% | 1+ |
| 2 | + | 80% | 3+ | 90% | 3+ |
| 3 | - | >95% | 3+ | >95% | 3+ |
| 4 | + | >95% | 3+ | >95% | 3+ |
| 5 | - | 30% | 1+ | 30% | 1+ |
| 6 | + | 90% | 3+ | 90% | 3+ |
| 7 | + | 70% | 3+ | 80% | 3+ |
| 8 | + | 90% | 3+ | 80% | 3+ |
| 9 | + | 80% | 3+ | 50% | 1+ |
| 10 | + | >95% | 3+ | >95% | 3+ |
| 11 | - | 70% | 2+ | 70% | 2+ |

immunostaining was evaluated by using four scores as follows: If immunopositivity of tumor cells was strong in low-power view ($\times 40$), the intensity was scored as 3+; if it was weak in low-power view but obviously stained in high-power view ($\times 400$), the intensity was scored as 1+; if the intensity was the median between 1+ and 3+, the intensity was 2+; and if tumor cells were not immunostained, the intensity was scored as 0.

Ethics

This study was approved by the appropriate ethics committees and governance boards.

Results

Clinical findings

The characteristics of the patients are summarized in **Table 1**. The mean patient age was 59 years (ranging from 40 to 77 years). The male to female ratio was 7:4. Eight cases (73%) arose from the bones and three cases (27%) from soft tissues. The mean preoperative serum FGF23 level was 225.3 pg/ml (ranging from 98.9 pg/ml to 9165 pg/ml). Postoperatively, serum FGF23 of all the patients decreased variably. None of the tumors had a malignant course.

Histological findings

Common histological findings in all cases were characterized by a monotonous proliferation of tumor cells within a background of prominent microvessels. The characteristic grungy or flocculent calcification was patchily observed in seven cases (64%, **Figure 1A**), and osteoclast-like giant cells were present near this calcification. Tumor cells were spindle-shaped with scant eosinophilic cytoplasm and bland nuclei without prominent nucleoli (**Figure 1B**). Another characteristic finding, staghorn vessels, was observed in nine cases (82%). Myxoid change and nuclear palisading were not observed.

CD56 in phosphaturic mesenchymal tumors

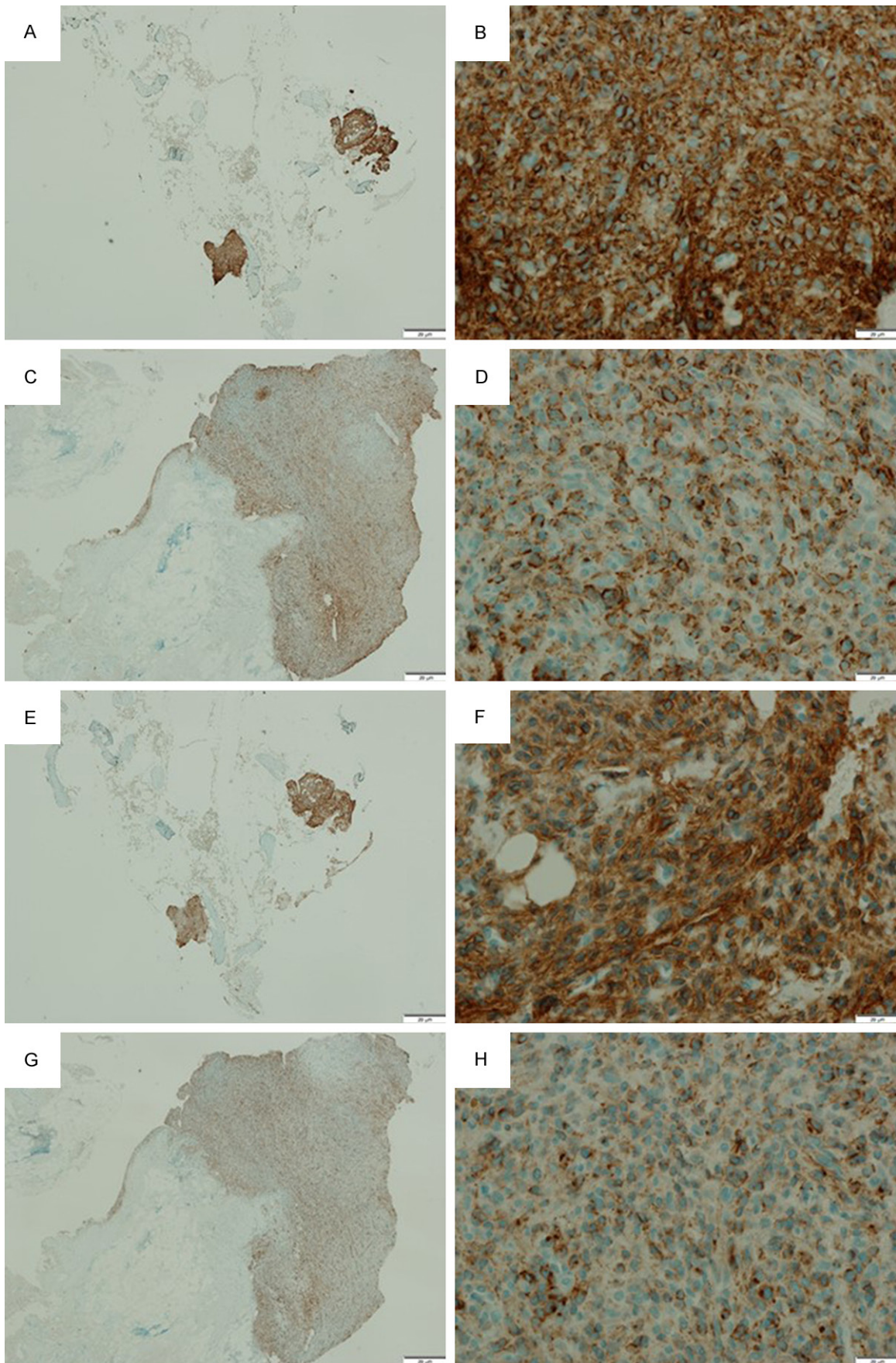


Figure 2. Immunohistochemical findings. (A) Case 10. In a decalcified specimen, nearly all the tumor cells (>95%) seem to be immunopositive for CD56 with strong (3+) intensity (×40, bar: 200 μm). (B) Higher magnification of (A). Immunonegative tumor cells are difficult to detect (×400, bar: 20 μm). (C) Case 11. In a non-decalcified specimen, more than half of the tumor cells seem to be immunopositive for CD56 with moderate (2+) intensity (×40, bar: 200 μm). (D) Higher magnification of (C). Approximately 70% of tumor cells are immunostained for CD56 (×400, bar: 20 μm). (E) Case 10. In a decalcified specimen, nearly all the tumor cells (>95%) seem to be immunopositive for somatostatin receptor 2A (SSTR2A) with strong (3+) intensity (×40, bar: 200 μm). (F) Higher magnification of (E). Immunonegative tumor cells are difficult to detect (×400, bar: 20 μm). (G) Case 11. In a non-decalcified specimen, more than half of the tumor cells seem to be immunopositive for SSTR2A with moderate (2+) intensity (×40, bar: 200 μm). (H) Higher magnification of (G). Approximately 70% of the tumor cells are immunostained for SSTR2A (×400, bar: 20 μm).

None of the cases showed histological atypia, indicative of malignancy.

Immunohistochemical findings

The IHC results for CD56 and SSTR2A are summarized in **Table 2**. The quality of immunostaining did not seem to be significantly affected by the EDTA-based decalcification. More than 70% of the tumor cells showed immunostaining for CD56, with an intensity score of 3+ (**Figure 2A, 2B**), except for one case (case 11) that showed an intensity score of 2+ (**Figure 2C, 2D**) and another case (case 5) that showed 30% positivity. Immunonegative tumor cells were absent in three cases (cases 3, 4, and 10). Moreover, SSTR2A showed a similar expression pattern to CD56 (**Figure 2E-H**), except for two cases (cases 1 and 9) that showed a relatively low proportion of immunopositive tumor cells (50%) with a relatively low intensity score (1+), compared with the CD56-positive tumor cells (80%) with an intensity score of 3+ in the aforementioned cases. Generally, CD56 immunostaining was equal to or better than SSTR2A regarding proportion and intensity, but in only two cases (cases 2 and 7), the proportion of immunopositive tumor cells was less than that of SSTR2A. The tumors were immunonegative for synaptophysin, chromogranin A, SSTR5, CD34, and S100 protein.

Discussion

In 2004, Folpe et al. demonstrated that most tumors related to T1O exhibit stereotypical histological features, recognizing PMTs as a distinct entity [3]. The differential diagnosis of PMTs is broad, which is suggested by the various original diagnoses of PMTs in the review by Folpe et al. [3]. Solitary fibrous tumors (SFTs) are the most difficult entity to differentiate from PMTs when grungy or flocculent calcification and osteoclast-like giant cells are inconspicuous, because PMTs exhibits proliferating small

blood vessels along with the staghorn vessel pattern reminiscent of SFTs [3]. Another difficult entity in the differential diagnosis is chondromyxoid fibroma, in which at least one third of cases show calcification of the chondromyxoid matrix, but without grungy or flocculent calcification [10]. Lesions such as non-ossifying fibromas and tenosynovial giant cell tumors that are composed primarily of spindled to ovoid cells and contain osteoclast-like giant cells are also included in the differential diagnosis [11, 12]. Furthermore, two cases reviewed by Folpe et al. were initially diagnosed as schwannomas [3]. All the cases in this study were preoperatively supposed to be PMTs, and confirmation of this diagnosis is the role of a pathologist.

Regarding IHC, it was proposed that the SSTR2A marker is an alternative to FGF23 because commercially available FGF23 antibodies are not reliable for the diagnosis of PMTs [3, 7]. However, SSTR2A is usually not available in common pathology laboratories. This study reveals that CD56, a commonly available marker, is generally not inferior to SSTR2A with respect to sensitivity. Concerning specificity, SSTR2A is immunostained in certain tumors other than PMTs [7]. Therefore, it is possible to make a diagnosis of a PMT using SSTR2A along with the recognition of its characteristic morphology. CD56 is also not a specific marker for PMTs. Amongst the benign mesenchymal tumors that are considered in the differential diagnosis of PMTs, CD56 is known to be immunopositive in schwannomas [13], but immunonegative in SFTs [14]. Along with the immunonegativity of PMTs for the S100 protein, schwannomas can be ruled out with careful morphological and IHC evaluation, even when CD56 positivity is observed. Hence, it seems that CD56 is not inferior to SSTR2A for excluding non-PMTs if it is used in combination with adequate IHC markers.

The lineage of differentiation of PMTs has not been elucidated [4]. CD56 and SSTR2A are immunostained in endocrine/neuroendocrine cells [15]. However, most pathologists do not regard PMTs as a neuroendocrine tumor [7]. Indeed, none of the cases was immunostained for synaptophysin and chromogranin A, which are two other markers of endocrine/neuroendocrine cells contained in their secretory granules. Further studies are required to identify the lineage specific IHC marker of PMTs.

In conclusion, CD56 was found to be a sensitive IHC marker comparable to SSTR2A. Even in cases originating in the bones, EDTA-based decalcification for tissue processing does not seem to affect IHC quality regarding CD56 and SSTR2A. As CD56 immunopositivity in mesenchymal tumors is limited, it also has some degree of specificity for PMTs. CD56 is more commonly available than SSTR2A, and its use is therefore recommended when the possibility of PMTs is suspected.

Disclosure of conflict of interest

None.

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