Isocitrate dehydrogenase mutation is frequently observed in giant cell tumor of bone

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Giant cell tumors of bone (GCTB) are benign and locally destructive tumors that include osteoclast-type multinuclear giant cells. No available treatment is definitively effective in curing GCTB, especially in surgically unresectable cases. Isocitrate dehydrogenase (IDH) mutations have been reported not only in gliomas and acute myeloid leukemias, but also in cartilaginous tumors and osteosarcomas. However, IDH mutations in GCTB have not been investigated. The IDH mutations are remarkably specific to arginine 132 (R132) in IDH1 and arginine 172 (R172) or arginine 140 (R140) in IDH2; IDH1/2 mutations are known to convert α-ketoglutarate to oncometabolite (R(-))-2-hydroxyglutarate. We recently reported that the most frequent IDH mutation in osteosarcomas is IDH2-R172S, which was detected by MsMab-1, a multispecific anti-IDH1/2 mAb. Herein, we newly report the IDH mutations in GCTB, which were stained by MsMab-1 in immunohistochemistry. DNA direct sequencing and subcloning identified IDH mutations of GCTB as IDH2-R172S (16 of 20; 80%). This is the first report to describe IDH mutations in GCTB, and MsMab-1 can be anticipated for use in immunohistochemical determination of IDH1/2 mutation-bearing GCTB.
Table 1. The characteristic of giant cell tumor patients used in immunohistochemical analysis by MsMab-1

| Patient no. | Age | Gender | Race | Sample class | Site     | MsMab-1 staining (Mesenchymal stromal cells) | IDH1 (R132) | IDH2 (R172) | Direct sequencing | Subcloning | IDH2 (H175) | H3F3A (K27, G34, K36) | H3F3B (G34, K36) |
|------------|-----|--------|------|--------------|----------|--------------------------------------------|-------------|-------------|-------------------|------------|---------------|---------------------|------------------|
| 1          | 32  | M      | Asian| Primary      | Tibia    | +++ +                                       | WT          | R172S       | –                 | –          | WT            | WT                  | WT                |
| 2          | 17  | F      | Asian| Primary      | Femur    | + +                                         | WT          | R172S       | –                 | –          | WT            | WT                  | WT                |
| 3          | 42  | F      | Asian| Primary      | Humerus  | ± +                                         | WT          | R172S (2/25: 8%) | –           | –          | WT            | WT                  | WT                |
| 4          | 60  | F      | Asian| Primary      | Maxilla  | ± +                                         | WT          | R172S (1/7: 14%) | –           | –          | WT            | WT                  | WT                |
| 5          | 24  | F      | Asian| Primary      | Humerus  | +++ +                                       | WT          | R172S       | –                 | –          | WT            | WT                  | WT                |
| 6          | 38  | F      | Asian| Primary      | Radius   | ++ +                                        | WT          | R172S (6/21: 29%) | –           | –          | WT            | WT                  | WT                |
| 7          | 34  | M      | Asian| Primary      | Tibia    | +++ +++                                     | WT          | R172S       | –                 | –          | WT            | WT                  | WT                |
| 8          | 45  | F      | Asian| Primary      | Tibia    | +++ +++                                     | WT          | R172S       | –                 | –          | WT            | WT                  | WT                |
| 9          | 33  | F      | Asian| Primary      | Femur    | +++ +                                       | WT          | R172S       | –                 | –          | H175Y         | WT                  | WT                |
| 10         | 40  | F      | Asian| Primary      | Radius   | ++ +                                        | WT          | R172S       | –                 | –          | WT            | WT                  | WT                |
| 11         | 33  | M      | Asian| Primary      | Humerus  | – –                                         | WT          | R172S       | –                 | –          | WT            | WT                  | WT                |
| 12         | 36  | F      | Asian| Primary      | Tibia    | +++ +                                       | WT          | R172S       | –                 | –          | WT            | WT                  | WT                |
| 13         | 28  | M      | Asian| Primary      | Clavicle | ++ +                                        | WT          | R172S       | –                 | –          | H175Y         | WT                  | WT                |
| 14         | 48  | F      | Asian| Primary      | Femur    | ++ +                                        | WT          | R172S       | –                 | –          | WT            | WT                  | WT                |
| 15         | 23  | M      | Asian| Primary      | Femur    | ± +                                         | WT          | R172S (0/38: 0%) | –           | –          | WT            | WT                  | WT                |
| 16         | 34  | M      | Asian| Primary      | Sacrum   | ± +                                         | WT          | R172S (0/42: 0%) | –           | –          | H175Y         | WT                  | WT                |
| 17         | 50  | M      | Asian| Primary      | Femur    | ++ +                                        | WT          | R172S       | –                 | –          | WT            | WT                  | WT                |
| 18         | 38  | F      | Asian| Primary      | Humerus  | ± +                                         | WT          | R172S (0/41: 0%) | –           | –          | H175Y         | WT                  | WT                |
| 19         | 47  | M      | Asian| Primary      | Tibia    | + +                                         | WT          | R172S       | –                 | –          | H175Y         | WT                  | WT                |
| 20         | 20  | M      | Asian| Primary      | Femur    | + +                                         | WT          | R172S       | –                 | –          | WT            | WT                  | WT                |

†, no staining; ±, <1%; +, 1–10%; ++, 10–50%; and ++++, >50%. ‡, no staining; +, weak; ++, medium; ++++, strong.
IDH2-R172S mutation in GCTB patients, which was detected by MsMab-1 mAb and direct DNA sequencing.

Materials and Methods

Immunohistochemical analyses. Tissue microarrays (BO2081; US Biomax, Rockville, MD, USA) were used in this study. Immunohistochemical analyses were carried out as described in Document S1.

Direct DNA sequencing of IDH1, IDH2, H3F3A, and H3F3B. Genomic DNA extraction and PCR were carried out as described in Document S1.

Plasmid preparation, protein expression, and Western blot analyses. Osteosarcoma U-2 OS cells were transfected with appropriate amounts of plasmids as described in Document S1. The SDS-PAGE and Western blot analyses using MsMab-1 or anti-PA tag (NZ-1)(14-16) were carried out as described in Document S1.

Analysis of 2-HG production. Sample preparation and measurement by capillary electrophoresis time-of-flight mass spectrometry are described in Document S1.

Results

Immunohistochemical analysis by MsMab-1 against GCTB. We carried out immunohistochemistry against GCTB using a multispecific antimutated IDH1/2 mAb, MsMab-1. The characteristics of the GCTB patients are presented in Table 1. Typical staining patterns are shown in Figure 1. Both multinucleated osteoclast-like giant cells and mesenchymal fibroblast-like stromal cells were diffusely stained by MsMab-1 (Fig. 1a,b). In contrast, weak and focal staining of mesenchymal fibroblast-like stromal cells was observed in other samples (Table 1). Because MsMab-1 stained multinucleated giant cells in foreign-body granulomas (Fig. S1), multinucleated osteoclast-like giant cells in GCTB might be non-specifically stained by MsMab-1 (Fig. 1).

Mutational analyses in GCTB. Polymerase chain reaction was carried out using DNA samples obtained from tissue microarray. No IDH1 mutation was observed in 20 samples (Table 1). In contrast, 13 of 20 (65%) GCTB samples possessed IDH2 mutations. It is noteworthy that all 13 IDH2 mutations were of IDH2-R172S (AGG > AGT; Fig. 1d,e), which is also frequently observed in osteosarcomas and
chondrosarcomas.\(^{11,12}\) After subcloning of PCR products, 3 of 6 (50\%) GCTB samples were shown to possess IDH2-R172S (Fig. 2, Table 1). In total, 16 of 20 (80\%) GCTB samples were shown to possess IDH2-R172S (Table 1). In 5 of 20 (25\%) GCTB patients, IDH2-H175Y (CAT > TAT) mutations were detected (Fig. 3a, Table 1), although IDH2-H175Y mutation was not recognized by MsMab-1 in Western blot analyses (Fig. 3b). The U2 OS IDH2-R172S cells produced 99.4 \(\mu\)mol/L of oncometabolite 2-HG, whereas U2 OS IDH2-H175Y, U2 OS IDH2-WT, and U2 OS cells produced 1.7, 1.3, and 1.6 \(\mu\)mol/L of oncometabolite 2-HG, respectively (Fig. 3b).

Discussion

We recently reported that IDH mutations are observed in osteosarcomas.\(^{11,12}\) Herein, we investigated the IDH mutations in GCTB, because GCTB accounts for 5–20\% of all primary bone tumors in adults.\(^{1,2}\) Although both multinucleated osteoclast-like giant cells and mesenchymal fibroblast-like stromal cells were stained by MsMab-1 (Fig. 1), multinucleated osteoclast-like giant cells might be non-specifically stained by MsMab-1, because MsMab-1 also stained giant cells in foreign-body granulomas (Fig. S1). MsMab-1 can recognize over-expressed wild-type IDH1/2 in Western blot analyses;\(^{12}\) therefore, osteoclast-like giant cells in GCTB might overexpress wild-type IDH1/2 proteins. To clarify this issue, we should develop an anti-IDH2-R172S-specific mAb in the near future. We analyzed IDH mutations using 20 GCTB specimens with direct DNA sequencing (Table 1). Thirteen of 20 (65\%) GCTB samples possessed IDH2 mutations. Furthermore, PCR products of sample numbers 3, 4, and 6, included the IDH2-R172S mutation after subcloning, indicating that MsMab-1 indeed detected IDH2-R172S in those tissues (Table 1).\(^{12}\) The PCR products of sample numbers 15, 16, and 18 were not shown to possess IDH2-R172S mutation after subcloning; therefore, MsMab-1 reaction against these GCTB tissues might be non-specific, or little fraction of IDH2-R172S was included in these GCTB tissues. We will carefully check the MsMab-1 reaction in future immunohistochemical studies. Because the IDH2-H175Y mutation was not recognized by MsMab-1 in Western blot analyses (Fig. 3b), IDH2-H175Y is not relevant with MsMab-1 staining in immunohistochemistry. Furthermore, IDH2-H175Y did not produce oncometabolite 2-HG (Fig. 3b). We did not observe any clinical difference between IDH2 mutation-positive patients and IDH2 mutation-negative patients in this study; the number of patients should be increased to investigate the clinical importance of IDH2 mutation in GCTB in the future. We also investigated H3F3A and H3F3B mutations in the GCTB samples. However, we observed neither H3F3A mutations (K27, G34, K36) nor H3F3B mutations (G34, K36) in this study (Table 1, Figs S2, S3). We need further investigations to clarify the difference between this study and the previous one.\(^{7}\) Furthermore, anti-mutated H3F3A/H3F3B-specific mAbs could be useful for investigating H3F3A and H3F3B mutations in combination with anti-mutated IDH1/2 mAbs.\(^{14,15,17–23}\)

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Disclosure Statement

The authors have no conflicts of interest.

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**Supporting Information**

Additional supporting information may be found in the online version of this article:

**Fig. S1.** Immunohistochemical analysis by MsMab-1 against inflammatory tissues.

**Fig. S2.** Mutational analysis of H3F3A in giant cell tumor of bone.

**Fig. S3.** Mutational analysis of H3F3B in giant cell tumor of bone.

**Data S1.** Detailed materials and methods.