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

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Key words
GCTB, giant cell tumor of bone, IDH mutations, isocitrate dehydrogenase 2, monoclonal antibody

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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.

Giant cell tumors of bone in adults in the USA and 20% in Asia.1,2 Although GCTB is generally benign, atypical GCTB may be associated with multiple local recurrences, multicentricity, and pulmonary metastases.3 Characterized by the presence of numerous multinucleated osteoclast-like giant cells, GCTB also includes mesenchymal fibroblast-like stromal cells and a mononuclear cell of myeloid lineage. Fibroblast-like stromal cells are considered to be responsible for the neoplastic character of the GCTB. The presence of telomeric associations, chromosomal aberrations, varied ploidy states, and gene amplifications have all been described within GCTB stromal cells.4,5 These stromal cells play an important role in the recruitment of tumor-associated myeloid lineage cells and formation of osteoclast-like giant cells.5 The stromal cells also induce osteoclastogenesis in vitro in coculture studies with osteoclasts, and produce several factors that are involved in the recruitment and induction of osteoclast differentiation and activation, including receptor activator of nuclear factor κB ligand, the master regulator of osteoclast differentiation.6,7 Isocitrate dehydrogenase (IDH) catalyzes the oxidative carboxylation of isocitrate to α-ketoglutarate.8 Mutated IDH1 and IDH2 convert α-ketoglutarate to oncometabolite R(-)-2-hydroxyglutarate (2-HG) in cytosol and mitochondria, respectively. Isocitrate dehydrogenase 1/2 mutations have been reported in gliomas,9 acute myeloid leukemias,10 cartilaginous tumors,11 osteosarcomas,12 Ollier disease,11 and Maffucci syndrome.11,13 The heterozygous IDH mutations are remarkably specific to a single codon in the conserved and functionally important arginine 132 residue (R132) of IDH1 and 172 residue (R172) of IDH2. We have established multispecific anti-IDH1/2 mAbs14,15 that are useful for diagnosis of IDH1/2 mutation-bearing tumors. Herein, we report the
Table 1. The characteristic of giant cell tumor patients used in immunohistochemical analysis by MsMab-1

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Gender</th>
<th>Race</th>
<th>Sample class</th>
<th>Site</th>
<th>MsMab-1 staining (Mesenchymal stromal cells)</th>
<th>IDH1 (R132)</th>
<th>IDH2 (R172)</th>
<th>Direct sequencing</th>
<th>Subcloning</th>
<th>IDH2 (H175)</th>
<th>H3F3A (K27, G34, K36)</th>
<th>H3F3B (G34, K36)</th>
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<td>1</td>
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<td>M</td>
<td>Asian</td>
<td>Primary</td>
<td>Tibia</td>
<td>+++</td>
<td>+</td>
<td>R172S</td>
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<td>2</td>
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<td>Femur</td>
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<td>R172S</td>
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<td>Humerus</td>
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<td>Maxilla</td>
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<td>Humerus</td>
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<td>Asian</td>
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<td>12</td>
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<td>Primary</td>
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<td>WT</td>
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<td>23</td>
<td>M</td>
<td>Asian</td>
<td>Primary</td>
<td>Femur</td>
<td>±</td>
<td>+</td>
<td>WT</td>
<td>R172S (0/38: 0%)</td>
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<td>16</td>
<td>34</td>
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<td>Asian</td>
<td>Primary</td>
<td>Sacrum</td>
<td>±</td>
<td>+</td>
<td>WT</td>
<td>R172S (0/42: 0%)</td>
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<td>17</td>
<td>50</td>
<td>M</td>
<td>Asian</td>
<td>Primary</td>
<td>Femur</td>
<td>++</td>
<td>+</td>
<td>WT</td>
<td>R172S</td>
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<td>Asian</td>
<td>Primary</td>
<td>Humerus</td>
<td>±</td>
<td>+</td>
<td>WT</td>
<td>R172S (0/41: 0%)</td>
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<td>19</td>
<td>47</td>
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<td>R172S</td>
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<td>R172S</td>
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†–, 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) 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

Fig. 1. Mutational analysis of isocitrate dehydrogenase 1/2 (IDH1/2) in giant cell tumor of bone. (a–c) Immunohistochemical analysis by MsMab-1, a multispecific anti-IDH1/2 mAb, against tissue microarray of giant cell tumor of bone. (d–f) DNA direct sequencing. (a, d) Sample no. 7; (b, e) no. 8; (c, f) no. 11.
Fig. 3. Isocitrate dehydrogenase 2 (IDH2)-H175Y (CAT > TAT) mutations were not detected by MsMab-1, a multispecific anti-IDH1-2 mAb. (a) DNA direct sequencing was carried out against bone sample no. 19. (b) Total cell lysate from U-2 OS osteosarcoma cells expressing IDH2 wild-type-PA tag (WT, lane 1) and IDH2 mutants (lane 2, IDH2-R172S-PA tag; lane 3, IDH2-H175Y-PA tag) were electrophoresed, and Western blotted using MsMab-1 or anti-PA tag (NZ-1). Production of oncometabolite R(-)-2-hydroxyglutarate (2-HG) was observed in IDH2-R172S, but not in IDH2-H175Y or IDH2-WT.

Discussion

We recently reported that IDH mutations are observed in osteosarcomas. Herein, we investigated the IDH mutations in chondrosarcomas. 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 µmol/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 µmol/L 2-HG, respectively (Fig. 3b).

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Disclosure Statement

The authors have no conflicts of interest.

References

IDH mutations in GCTB


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.