Human Pathology (2017) **65**, 239–246

ELSEVIER

Human PATHOLOGY

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Original contribution

Diagnostic utility of *IDH1/2* mutations to distinguish dedifferentiated chondrosarcoma from undifferentiated pleomorphic sarcoma of bone☆

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Received 31 March 2017; revised 1 May 2017; accepted 10 May 2017

Keywords:
IDH1;
IDH2;
Dedifferentiated chondrosarcoma;
Undifferentiated pleomorphic sarcoma (UPS) of bone

Summary Histologically, it is nearly impossible to distinguish the dedifferentiated component of dedifferentiated chondrosarcoma from undifferentiated pleomorphic sarcoma (UPS) of bone when the low-grade cartilaginous component is absent. Previous studies have revealed that isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* mutations are present in a significant number of cartilaginous tumors including most conventional chondrosarcomas and dedifferentiated chondrosarcomas. These mutations have not been studied in UPSs of bone. We sought to investigate whether an *IDH1* or *IDH2* mutation signature could be used as a clinically diagnostic marker for the distinction of dedifferentiated component of chondrosarcoma from UPS of bone. Sixty-eight bone tumor cases, including 31 conventional chondrosarcomas, 23 dedifferentiated chondrosarcomas, and 14 UPSs of bone, were collected for *IDH1/2* mutation analysis either using the Qiagen *IDH1/2* RGQ PCR Kit or using whole-exome sequencing. *IDH1/2* mutations were detected in 87% (20/23) of dedifferentiated chondrosarcomas and 30% (6/20) of conventional chondrosarcomas. No mutations were detected in the *IDH1/2* codon 132 or codon 172 among 14 UPSs of bone. Identification of *IDH1* or *IDH2* mutations supports the diagnosis of dedifferentiated chondrosarcoma rather than UPS of bone while also providing some insight into the pathogenesis of these 2 lesions.

1. Introduction

Chondrosarcoma, a malignant cartilaginous tumor, is the second most common primary malignancy of bone after osteosarcoma, often arising from the pelvis, femur, or humerus. Tumors are classified as conventional chondrosarcoma when

☆ Competing interests: The authors declare no conflict of interest.

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http://dx.doi.org/10.1016/j.humpath.2017.05.015
0046-8177/

the histology resembles nonneoplastic cartilage, as opposed to dedifferentiated chondrosarcoma containing areas of high-grade sarcoma. The most common morphologic pattern of the dedifferentiated component is undifferentiated pleomorphic sarcoma (UPS), so-called malignant fibrous histiocytoma. Diagnosis of dedifferentiated chondrosarcoma relies on identifying both the well-differentiated cartilaginous and dedifferentiated components [1]. UPS of bone is commonly located in the lower extremities, particularly the femur followed by tibia and pelvis. Morphologically, tumors are composed of atypical spindled and pleomorphic cells, which may be arranged in a storiform, fascicular or haphazard pattern with marked nuclear pleomorphism, mitoses, and necrosis [2,3]. Importantly, these tumors lack chondroid and osteoid matrix deposition.

Although dedifferentiated chondrosarcoma and UPS of bone may share clinical presentations, including arising in similar anatomical locations, dedifferentiated chondrosarcoma is extremely aggressive with a dismal prognosis. Most patients with dedifferentiated chondrosarcoma die within 2 years of initial presentation. The 5-year survival rate is 19% based on a large study of 52 cases but ranges from 7.1% to 24% in studies throughout the literature [4,5]. With the lack of convincing evidence of the benefit of chemotherapy, complete surgical excision is the initial recommended treatment. UPS of bone is also aggressive. However, neoadjuvant or adjuvant chemotherapy is beneficial with the improved 5-year survival rate up to 59% (median survival time, 63 months) [3,6]. In fact, neoadjuvant chemotherapy using an osteosarcoma treatment protocol is gradually becoming the standard of care particularly for high-grade resectable UPS of bone [3]. Therefore, differentiating these 2 entities is of paramount importance. However, because there is significant morphologic overlap between UPS of bone and the dedifferentiated component of dedifferentiated chondrosarcoma, distinction often relies on the presence or absence of a low-grade cartilaginous component, which may not be present with biopsy or limited sampling. To date, there are no reliable ancillary tests, such as immunohistochemistry, cytogenetics, or molecular studies, to improve diagnostic specificity.

Point mutations in the isocitrate dehydrogenase 1 (*IDH1*) or *IDH2* gene were originally detected in gliomas and secondary glioblastomas [7], and later identified in a variety of malignancies including some de novo acute myeloid leukemias [8,9], myelodysplastic disorders [10], cholangiocarcinoma [11], thyroid carcinoma [12], prostate carcinoma, B-cell lymphoma, and colorectal carcinomas [13]. Further studies have shown that most chondrosarcomas, including dedifferentiated chondrosarcomas, harbor mutations in the metabolic enzyme *IDH1* or *IDH2* gene, whereas osteosarcomas including chondroblastic, fibroblastic, osteoblastic subtypes, and so on, lack these genetic abnormalities [14-16]. IDH is an important homodimeric enzyme in the citric acid cycle (the Krebs cycle) and normally catalyzes the decarboxylation of isocitrate to α-ketoglutarate (α-KG). IDH1 and IDH2 use NADP+ as a cofactor and are located in the cytoplasm (IDH1), peroxisomes (IDH1), and mitochondria (IDH2) [17]. Mutations almost always affect a single amino acid residue at arginine-132 in IDH1 or ariginine-172 in IDH2. Most mutations occur in *IDH1* [14,15]. All reported *IDH1* and *IDH2* mutations are heterozygous, with cancer cells retaining 1 wild-type copy of the relevant *IDH1* or *IDH2* allele. These mutations lead to a reduced capacity to convert isocitrate to α-KG and a gain of function to convert α-KG to δ-2-hydroxyglutarate (D-2-HG), an oncometabolite [18]. Typically, mutations in *IDH1* or *IDH2* are mutually exclusive [18]. Biochemical analysis revealed a >100-fold increase in D-2-HG in human glioma samples with *IDH1* mutations, whereas D-2-HG is present at very low levels in normal tissue [19]. The pathogenesis by D-2-HG has been under extensive study [17,19-21].

That *IDH1/2* mutations are present in dedifferentiated chondrosarcoma and have not been studied in UPS of bone prompted us to evaluate whether an *IDH1* and *IDH2* mutation signature could be used as a molecular diagnostic marker to distinguish these 2 lesions, as well as give insight into the pathogenesis of these 2 lesions and provide useful information for clinical management.

2. Materials and methods

2.1. Ethics statement

This study was approved by the institutional review board of Indiana University, Mayo Clinic, and University of Alabama at Birmingham. Resected specimens were submitted for routine histologic diagnoses, and the remaining tissues were used for research. There was no compromise of the patient's privacy.

2.2. Collection of tumor specimens

After searching pathology information system databases, cases of conventional chondrosarcoma and dedifferentiated chondrosarcoma were collected from Indiana University, Mayo Clinic, and the University of Alabama at Birmingham. Samples of primary UPSs of bone were collected from the Musculoskeletal Pathology Department at the Royal Orthopaedic Hospital, Birmingham, United Kingdom. A significant number of conventional chondrosarcoma tissues were decalcified due to the presence of bone fragments. For a subset of dedifferentiated chondrosarcomas, both the conventional cartilaginous and the dedifferentiated components were available for mutation analysis from the same lesion. For each case, 1 or 2 hematoxylin and eosin (H&E)–stained slides with tumor cells were selected by one pathologist at each institution and the corresponding formalin-fixed, paraffin-embedded tissue blocks were retrieved. Ten unstained sections (10 μm in thickness per section) were cut from each tissue block and mounted on uncharged slides (1 section per slide). Areas with lesional cells on the unstained sections (at least 6-7 sections) were circled by comparing with the corresponding H&E-stained slides and removed for DNA extraction. Necrotic tissue and surrounding paraffin were avoided.