Targeted Next-Generation Sequencing Identifies Molecular and Genetic Events in Dedifferentiated Chondrosarcoma

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Context.—Dedifferentiated chondrosarcoma (DDCS) is a rare, highly aggressive mesenchymal tumor of the skeleton with reproducible pathologic, radiographic, and clinical features. A biphasic tumor that consists of conventional chondrosarcoma component with abrupt transition to a high-grade nonchondrogenic component defines the classic histopathology and radiographic findings of DDCS. Dedifferentiated chondrosarcoma confers high mortality typically from lung metastasis despite aggressive multimodal therapy.

Objective.—To determine the genetic and molecular events that drive progression from a conventional chondrosarcoma to high grade nonchondrogenic sarcoma.

Design.—We analyzed the genomic landscape of paired conventional and dedifferentiated components of 11 dedifferentiated chondrosarcoma using targeted next-generation DNA sequencing with immunohistochemical validation. Clinical, radiographic, and pathologic features of tumors were reviewed. Capture-based DNA sequencing targeting the coding regions of 479 cancer genes and select introns was performed.

Results.—The tumors arose in the femur (n = 4; 36%), scapula (n = 3; 27%), pelvis (n = 3; 27%), and humerus (n = 1; 9%) of 7 men (64%) and 4 women (36%; median age, 61 years). DNA was adequate for sequencing from all 11 dedifferentiated components (100%) and 9 paired conventional chondrosarcoma components (82%). All tumors (100%) harbored either IDH1 p.R132 or IDH2 p.R172S hotspot mutations. Seven tumors (64%) displayed COL2A1 alterations. TERT promoter mutations were present in 5 of 9 pairs (56%) and 2 (22%) additional unpaired dedifferentiated components. IDH1/2, COL2A1, and TERT mutations were identical in both components of the paired samples. Pathogenic missense or truncating mutations in TP53 and large-scale copy number alterations were more common in dedifferentiated components than in those of matched conventional components.

Conclusions.—The results support IDH1/2, COL2A1, and TERT promoter mutations being common in dedifferentiated chondrosarcoma and as likely early events in progression, whereas inactivating mutation of TP53 and high-level copy number alterations may be later events in the dedifferentiated phenotype.

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matched conventional dedifferentiated pairs using immuno-
histochemistry, methylation assays, and array compar-
ative genomic hybridization demonstrating alterations in
IDH1/2, p53, and retinoblastoma pathways in 50%, 59%,
and 85% of tumors, respectively.

The above findings are intriguing and have provided
insight into potential mechanisms but have been limited to
single cases and/or specific molecular targets. A study of the
genomic landscape of both components of DDCS may
provide a clearer picture of the mechanisms of tumorigen-
esis. Such data may also uncover potential diagnostic or
therapeutic targets in DDCS. In the present study, we used
targeted hybrid-capture DNA sequencing of nearly 500
therapeutic targets in DDCS. In the present study, we used
immunohistochemistry. The tumor components (ie, con-
centric sarcoma and dedifferentiated components of all tumors were
macrodissected and analyzed separately.

Materials and Methods

Patient Cohort and Tumor Samples

Eleven patients with DDCS were included in this study. All
tumor specimens were fixed in 10% neutral-buffered formalin
and embedded in paraffin. Pathologic review of all tumors was
conducted by an expert bone pathologist. Conventional chondro-
sarcoma and dedifferentiated components were scored independently.

Immunohistochemistry

Immunohistochemistry was performed on whole formalin-fixed,
paraffin-embedded tissue sections using the following antibodies:
IDH1-R132H mutant protein (clone H09, 1:500 dilution; DiaNova,
Hamburg, Germany) and p53 (clone DO-7, 1:100 dilution; Dako,
Glostrup, Denmark). Staining was performed on a Leica (Wetzlar,
Germany) Bond-III automated staining processor. Conventional and
dedifferentiated components were scored independently.

Immunohistochemistry for IDH1-R132H was scored as positive if
50% or more of the cells showed strong nuclear immunoreactivity.20

Targeted Next-Generation Sequencing

Targeted next-generation sequencing (NGS) was performed
with the UCSFS00 Cancer Panel (University of California, San
Francisco) as previously described.21,22 Genomic DNA was
extracted from formalin-fixed, paraffin-embedded blocks of tumor
tissue from 11 paired conventional chondrosarcoma and dedif-
ferentiated components using the QIAamp DNA FFPE Tissue Kit
(Qiagen, Germantown, Maryland). Capture-based next-genera-
tion DNA sequencing was performed with an assay that targets all
coding exons of 479 cancer-related genes, select introns, and
upstream regulatory regions of 47 genes to enable detection of
structural variants including gene fusions, and DNA segments at
regular intervals along each chromosome to enable genome-wide
copy number and zygosity analysis, with a total sequencing
footprint of 1.4 Mb (Supplemental Table 1, see supplemental
digital content, containing 5 tables at https://meridian.allenpress.
com/aplm in the August 2021 table of contents). Multiplex library
preparation was performed with the KAPA Hyper Prep kit
(Roche, Santa Clara, California) according to the manufacturer’s
specifications using 250 ng of sample DNA. Hybrid capture of
pooled libraries was performed with the custom oligonucleotide
library SeqCap EZ Choice (Nimblegen, Madison, Wisconsin).
Captured libraries were sequenced as paired-end 100-base pair
(bp) reads on a HiSeq 2500 instrument (Illumina, San Diego,
California). Sequence reads were mapped to the reference human
genome build GRCh37 (hg19) with the Burrows-Wheeler aligner
software. Recalibration and deduplication of reads was performed
with the Genome Analysis Toolkit (Broad Institute, Cambridge,
Massachusetts). Coverage and sequencing statistics were deter-
mined with Picard CalculateHsMetrics and Picard CollectInsert-
SizeMetrics (Broad Institute). Single-nucleotide variant and small
insertion and deletion mutation calling was performed with
FreeBayes, Unified Genotyper, and pindel (Broad Institute). Large
insertion and deletion and structural alteration calling was
performed with Annovar. Single-nucleotide variants, insertions
deletions, and structural variants were visualized and verified
with an Integrated Genome Viewer (Broad Institute). Genome-
wide copy number analysis was performed with the CNVkit and
visualized with Nexus Copy Number (BioDiscovery, El Segundo,
California). All molecular data were evaluated by an expert
molecular pathologist.

Results

Clinicopathologic Features

The clinicopathologic features are summarized in the
Table. Eleven DDCS were included in this study (7 [64%]
from men, 4 from women [36%]). Median age at diagnosis
was 61 years (range, 36–70 years). Tumors were approxi-
mately evenly distributed between the appendicular skele-
ton (n = 6, 55%) and the axial skeleton (n = 5, 45%) and
averaged 10.6 cm at maximum dimension (range, 5–17.5

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| Age, y/Sex | Site          | Size, cm | Dedifferentiated Histology | Neoadjuvant Therapy | Adjuvant Therapy | Metastasis | Outcome   | Follow-up, mo |
|-----------|--------------|----------|----------------------------|---------------------|------------------|------------|-----------|-------------|
| 61/M      | Acetabulum   | 5.5      | UPS                        | CTX                 | N/A              | +          | DOD       | 2           |
| 60/M      | Femur        | 10.5     | UPS                        | CTX                 | RXA, CTX         | +          | DOD       | 14          |
| 61/M      | Iliac        | 15.5     | UPS                        | CTX                 | CTX, CTX         | +          | DOD       | 25          |
| 48/F      | Femur        | 8.5      | UPS, osteosarcoma           | CTX                 | CTX              | +          | DOD       | 20          |
| 68/M      | Femur        | 17.5     | UPS                        | –                   | RTX              | +          | N/A       | 8           |
| 51/F      | Pubis        | 10.8     | UPS                        | –                   | RTX              | +          | DOD       | 14          |
| 70/F      | Femur        | 14.7     | UPS                        | –                   | –                | +          | DOD       | 7           |
| 35/F      | Scapula      | 5        | UPS                        | –                   | CTX              | +          | DOD       | 14          |
| 52/M      | Scapula      | 4.5      | UPS, osteosarcoma           | –                   | RTX              | +          | DOD       | 4           |
| 64/M      | Humerus      | 11.5     | UPS                        | –                   | RTX, CTX         | +          | DOD       | 10          |
| 62/M      | Scapula      | 14       | UPS, osteosarcoma           | –                   | RTX              | +          | NED       | 2           |

Abbreviations: –, absent; +, present; CTX, chemotherapy; DOD, died of disease; N/A, not available; NED, no evidence of disease; RTX, radiotherapy; UPS, undifferentiated pleomorphic sarcoma.
The most common presenting symptom was localized pain. One patient presented with a pathologic fracture of the distal femur. All 11 tumors (100%) exhibited “classic” biphasic histomorphology of a conventional (grade 1 of 3) chondrosarcoma sharply abutting a high-grade sarcoma composed of spindled and/or pleomorphic cells (Figure 1, C and D). None of the cases (0%) had transitional areas or higher-grade (grades 2 or 3) chondrosarcoma areas. Three cases (27%) contained small foci of osteosarcoma in the high-grade component (Figure 1, G and H), whereas the remainder (n = 8; 73%) were undifferentiated pleomorphic sarcomas based on a combination of morphology and immunophenotype.

Figure 1. Representative radiologic and pathologic findings of dedifferentiated chondrosarcoma. Case 10, sagittal (A) and axial (B) computed tomography scans of shoulder demonstrating destructive tumor with cartilage-matrix calcifications (arrows) and uncalcified tumor with transcortical spread (arrowheads) corresponding to chondrogenic (C) and undifferentiated spindle cell (D) components. Case 4, anterior-posterior radiograph of the right femur (E) displays areas of cartilage-matrix-type calcifications (arrows) and lytic, uncalcified areas with cortical destruction (arrowheads). Corresponding coronal section of the resection specimen (F) shows pale blue cartilage (arrow) and fleshy nonchondrogenic area (arrowhead). A pathologic fracture, sustained after the radiograph was taken, is also present. The hole in the metaphysis was the location of an external fixator pin. This tumor consisted of conventional chondrosarcoma (G) and high-grade sarcoma locally producing an osteoid (H) (hematoxylin-eosin, original magnification ×200 [C, D, G, and H]).

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Surgical resection of the tumors resulted in positive margins in 6 (55%) of the 11 tumors. Two patients (18%) received adjuvant chemotherapy while 6 (55%) received adjuvant radiotherapy. Ten of the 11 patients (91%) developed metastatic disease. One patient (9%) was lost to follow-up. Of the remaining 10 patients who had follow-up, 9 (90%) died of disease (average follow-up, 11 months; range, 2–24 months).

Targeted Next-Generation Sequencing Results

DNA was adequate for sequencing from 9 tumors (82%) with paired conventional chondrosarcoma and dedifferentiated sarcoma components. In the remaining 2 tumors (18%), only the high-grade sarcoma component yielded DNA for evaluation with the matching conventional chondrosarcoma components failing to yield sufficient DNA despite multiple attempts at purification from different areas of the tumor. One of those cases (case 10) did have a small amount of conventional chondrosarcoma for immunohistochemical evaluation. The mean (SD) target sequencing coverage for this cohort was 524 (162) unique reads per target interval (Supplemental Table 2). Recurrent pathogenic alterations, as well as copy number changes, are summarized in Figure 2, with representative copy number plots in Figure 3, A and B. Variant details, including genomic coordinates and mutant allele frequencies, are provided in Supplemental Tables 3 and 4. Specific copy number alterations, including focal amplifications and deletions, are detailed in Supplemental Table 5.

**IDH1/2**—Mutations in *IDH1* or *IDH2* were identified in all 11 tumors (100%). The mutations in these genes were mutually exclusive: recurrent *IDH1* p.R132 or *IDH2* p.R172S hotspot mutations were identified in 9 (82%) and 2 (18%) tumors, respectively. For cases with paired conventional and high-grade components the same *IDH1* or *IDH2* mutations were identified in both components (9 of 9; 100%).

Figure 2. Summary of clinical, genomic, and immunohistochemical data of dedifferentiated chondrosarcoma. Genomic profiles highlighting recurrent alterations of 11 dedifferentiated chondrosarcomas. Each row represents a gene, and each column represents a paired conventional chondrosarcoma and dedifferentiated component of a tumor. Abbreviation: IHC, immunohistochemistry.
**DISCUSSION**

This study evaluated the genomic landscape in a group of well-characterized DDCSs by pairwise comparison of conventional chondrogenic and high-grade nonchondrogenic components by hybrid-capture next-generation sequencing. This study expands on the detailed relationships between matched components of DDCS showing both clonal relationships and potential targets unique to the dedifferentiated phenotype.

This study confirms that the IDH and COL2A1 genes are frequently mutated in DDCS. Previous studies have identified similar alterations in benign enchondroma, conventional chondrosarcoma, and DDCS, but not other bone tumors, suggesting that these events may be important for chondrogenic neoplasia. Previous studies have shown IDH mutations in 50% and 87% of dedifferentiated chondrosarcomas. In contrast, IDH mutations were identified in 100% of the cases (11 of 11) in the present series. The discrepancy could represent statistical variance or detection of low-allele frequency mutations by our methods.

The presence of mutually exclusive IDH1 or IDH2 mutations in both components of all tumors in the present study supports IDH mutations as being necessary for tumorigenesis in DDCS. It is also reassuring that the 2 cases (18%) with IDH1 R132H mutations (cases 9 and 10; Supplemental Table 3) showed immunostaining with the R132H-specific antibody. Mutations in the IDH genes are also potential therapeutic targets. Inactivating mutations in the gene encoding the extracellular matrix protein COL2A were less frequent than IDH mutations were but, similar to IDH, were identical in both components when present.

To our knowledge, this study is the first to show that TERT promoter mutations are common in both components of DDCS. The TERT gene encodes the reverse-transcriptase subunit of the telomerase enzyme. Recurrent C228T and C250T promoter mutations, which lead to TERT overexpression and telomerase activity, have been reported in a wide range of human cancers but, apart from myxoid liposarcoma, are rare in sarcomas. Nevertheless, some studies suggest a role for TERT in chondrosarcoma. For example, in chondrosarcoma cell lines, upregulation of telomerase activity resulted in increased cell proliferation and increased invasive activity. Sanger sequencing of hotspots in the TERT promoter have shown activating C228T mutations in chondrosarcoma, with promoter mutations.
significantly associated with increased histologic grade (present in 8% of grade 1 chondrosarcoma versus 46% of grade 3 chondrosarcoma) as well as worse metastasis-free and chondrosarcoma-specific survival. Most recently, Zhu et al. found TERT promoter mutations in 20% of dedifferentiated and high-grade conventional chondrosarcoma. However, in a separate study, Saito et al. found wild-type TERT promoters in 10 chondrosarcomas although the grade was not reported. Our finding of TERT promoter mutations conserved between conventional and dedifferentiated components of DDCS suggests that alterations in TERT gene expression may be an early event in tumorigenesis. Alternatively, conventional chondrosarcoma with TERT promoter alterations may be genetically predisposed to dedifferentiation, and identification of TERT promoter alteration in an otherwise low-grade conventional chondrosarcoma may warrant more-aggressive surveillance or treatment. Interestingly, all of the conventional components

Figure 3. Representative copy number alterations of a conventional (A) and matched high-grade (B) component from case 4.
Figure 4. Representative immunohistochemistry results in dedifferentiated chondrosarcoma. Immunohistochemistry for p53 in case 5 (A and B) demonstrated staining in only the dedifferentiated component (B). By comparison, case 8 (C and D) demonstrated p53 immunostaining in both conventional (C) and dedifferentiated (D) components. Case 10 showed expression of the IHD1 R132H protein in both conventional (E) and dedifferentiated (F) components (p53 immunostain, original magnification ×200 [A through D]; R132H immunostain, original magnification ×200 [E and F]).
in the current study were low grade (grade 1), arguing against a correlation between TERT promoter mutations and morphologic grade.

In a subset of cases, inactivating mutations of TP53 were identified only in the dedifferentiated components of DDCS. Prior studies have identified TP53 mutation and/or protein overexpression in DDCS, although only in a few tumors and without separate study of both components.10,15,16,31,32 The current study confirms that inactivating mutations in TP53 are common in DDCS and, in a subset of cases, the TP53 mutation is restricted to the dedifferentiated nonchondrogenic component. In the subset of cases in which TP53 mutations were present in both components, the alteration was subclonal in the chondrogenic components relative to the IDH1/2 alteration. The above findings are compatible with TP53 alterations occur late in tumorigenesis, potentially with a TP53-mutant subclone that progresses to the dedifferentiated component in DDCS. As mentioned with TERT promoter mutation, identification of TP53 mutation in an otherwise low-grade chondrosarcoma may indicate a tumor at increased risk of dedifferentiation. We also found excellent correlation between TP53 mutation status and protein expression by immunohistochemistry, validating the significance of the molecular data. Whether TP53 mutations and increased copy number alterations are drivers of progression remains to be determined. Because some DDCSs demonstrated a wild-type TERT promoter and/or TP53, neither of those mutations alone or in combination is necessary for the dedifferentiated phenotype. Additional studies are needed to unravel the complete landscape of molecular events that promote chondrosarcoma progression.

Previous studies have shown that numeric and structural genomic alterations are more common in DDCSs than they are in other types of chondrosarcomas.18,33 Here we report, based on NGS data, that large-scale copy number gains and losses are, in general, more common in the dedifferentiated components than they are in the matched conventional components of DDCS. In most cases, when large-scale changes were observed in the conventional chondrosarcoma component they were few and the paired dedifferentiated component demonstrated an increased number of such changes. Case 3 was exceptional in that it demonstrated copy number gains unique to the conventional chondrosarcoma component. The significance of that finding is unclear, but it suggests that those gains are not required for the dedifferentiated phenotype, perhaps superseded by the 2 TP53 mutations or other mutations unique to case 3. Nevertheless, in general, increased genomic instability appears to be a common event in DDCS and is more common in the dedifferentiated component.

We recognize a number of limitations of the present study. Foremost, this is a relatively small number of cases, 2 of which (18%) consist only of the dedifferentiated component. The few tumors available for study reflect the difficulty in obtaining adequate DNA from archival paraffin blocks, many of which were acid decalcified during processing. Gentler decalcification methods or avoiding decalcification entirely going forward should provide better-quality specimens in subsequent studies. Despite the small numbers, to our knowledge, our series is the largest NGS study of DDCS showing pairwise comparison of both components. We also acknowledge that our NGS array is limited to 500 genes, which, although targeting major pathways in carcinogenesis, does not interrogate the entire genome or reveal any epigenetic changes. For example, the YEATS2 locus was not contained in our array so we cannot exclude mutations in that gene as has been reported by some authors.33,34 Finally, all the DDCSs (11 of 11; 100%) reported here capture the genomic profile at a single time point of a biphasic tumor with the assumption that the 2 components of DDCS represent temporal progression of a conventional chondrosarcoma to a high-grade tumor. Such assumption is acceptable in many malignancies in which the temporal progression is well studied (eg, colon cancer) but it is less substantiated in bone sarcomas. Additional studies of metachronous DDCSs will be necessary to further explore that constraint.

In summary, the present study reveals molecular and genetic events in dedifferentiated chondrosarcoma, some of which may be important for progression toward a dedifferentiated, nonchondrogenic phenotype, with an NGS approach.

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