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**ARTICLE**

# H3K27me3 deficiency defines a subset of dedifferentiated chondrosarcomas with characteristic clinicopathological features

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## Abstract

Dedifferentiated chondrosarcoma is a rare bone sarcoma, whose genetic background remains incompletely understood. Mutations in *SUZ12* or *EED*, which encode polycomb repressive complex 2 (PRC2) components, and resulting deficiency in H3K27me3 are characteristic features of the majority of malignant peripheral nerve sheath tumors. Here, we investigated H3K27me3 and PRC2 status in dedifferentiated chondrosarcoma. Among 19 evaluable dedifferentiated chondrosarcoma cases, six (32%) showed immunohistochemical loss of H3K27me3 only in the dedifferentiated component, whereas the well-differentiated component retained H3K27me3. H3K27me3-deficient dedifferentiated chondrosarcoma occurred in two men and four women with a median age of 66. All of these tumors affected bones of the upper half of the body, with the ribs being preferentially involved, which represented a significantly different distribution compared to that in the 13 H3K27me3-intact dedifferentiated chondrosarcomas. H3K27me3-deficient dedifferentiated chondrosarcomas were histologically different from H3K27me3-intact dedifferentiated chondrosarcomas, as the former invariably demonstrated dedifferentiated histology with a striking similarity to classic malignant peripheral nerve sheath tumor, comprising sweeping to swirling fascicles of relatively uniform spindle cells. Heterologous rhabdomyoblastic differentiation, the focal presence of grade 3 chondrosarcoma histology, and a cartilaginous component in the metastatic sites were exclusively seen in some cases of H3K27me3-deficient dedifferentiated chondrosarcoma. In all three H3K27me3-deficient dedifferentiated chondrosarcomas that contained focal grade 3 histology, dedifferentiated components did not juxtapose to the grade 3 areas but transitioned abruptly from the grade 1–2 components. Targeted next generation sequencing, which was successfully performed on four H3K27me3-deficient dedifferentiated chondrosarcomas, identified an *IDH2* mutation in one case and *COL2A1* truncations in three cases. The dedifferentiated areas of three cases harbored *SUZ12* or *EED* alterations, which were absent in the well-differentiated component, suggesting a role for PRC2 aberrations in dedifferentiation. H3K27me3 deficiency defines a novel subset of dedifferentiated chondrosarcoma that requires recognition because of its diagnostic and potential clinical implications.

## Introduction

Dedifferentiated chondrosarcoma is defined as a non-chondrogenic high-grade sarcoma associated with well-differentiated chondrosarcoma with abrupt transition [1, 2]. It is a rare bone sarcoma, accounting for ~10% of chondrosarcomas. Dedifferentiated chondrosarcoma typically occurs at an age of 50 or older (median, 58–61 years of age) with a predilection to the pelvic bone and proximal extremity, and is associated with dismal outcome with a high rate (90–100%) of distant metastasis and a reported 5-year survival of 24% [1, 3–6]. The classic clinical presentation of primary dedifferentiated chondrosarcoma is a slow-growing mass that suddenly undergoes rapid enlargement [1]; however, de novo dedifferentiation can also occur. Secondary dedifferentiation can take place as recurrent disease at the site where low-grade chondrosarcoma was previously resected. Histologically, the dedifferentiated area usually exhibits a histology resembling undifferentiated pleomorphic sarcoma or osteosarcoma, with rare

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examples showing differentiation to rhabdomyosarcoma, leiomyosarcoma, angiosarcoma, or even carcinoma [1, 3–11]. In approximately half of dedifferentiated chondrosarcomas, both well-differentiated and dedifferentiated components can be detected based on radiological examination [12].

Recent studies have identified several genetic abnormalities associated with chondrosarcomas. *IDH1, IDH2*, and/or *COL2A1* mutations are highly recurrent and specific for chondrosarcoma, being reported in 43–71% of cases [13–15]. These characteristic mutations have been reported in both well-differentiated and dedifferentiated chondrosarcoma, supporting the clonal relationship [13–15]. However, these studies did not involve longitudinal analysis and the mechanism associated with the dedifferentiation process remains unclear, with only a limited number of studies performed for further clarification [16–19].

Trimethylation of lysine 27 of histone 3 (H3K27me3) is a hallmark mechanism of transcriptional silencing, which is catalyzed by polycomb repressive complex 2 (PRC2) [20]. Inactivation of PRC2 therefore results in H3K27me3 deficiency, which is reliably detected immunohistochemically as a complete loss of H3K27me3 staining. PRC2 requires EZH1, EZH2, SUZ12, and EED as core subunits for its catalytic activity [21], and inactivating mutations in *SUZ12* or *EED* are a major cause of PRC2 dysfunction. In malignant peripheral nerve sheath tumors, mutations/deletions in *SUZ12* or *EED* [22, 23] contribute to H3K27me3 deficiency in 34–75% of cases, and immunohistochemical loss of H3K27me3 is proposed to be a specific marker. However, H3K27me3 deficiency involves a wider array of tumor entities including H3K27M-mutant glioma [24–27]. Recently, we demonstrated a role for H3K27me3 in a small subset (6%) of dedifferentiated liposarcomas, in which only the dedifferentiated components, and not the well-differentiated components, were deficient in H3K27me3 due to an *EED* mutation [28]. In this study, we aimed to determine if a similar mechanism might play a role in the dedifferentiation process of chondrosarcoma.

## Materials and methods

### Case selection

After receiving approval from the institutional review board (2014–089), 16 cases of dedifferentiated chondrosarcoma were retrieved from the pathology archive (1998–2017) of the National Cancer Center Hospital, Tokyo, Japan and the consultation archive of one of the authors (AY). An additional five cases were contributed from Komagome Hospital, Tokyo. The slides were reviewed along with clinical and radiological findings, and the diagnoses were confirmed. By

definition, all cases demonstrated well-differentiated chondrosarcoma and a high-grade non-cartilaginous component with abrupt transition.

## Immunohistochemistry

Well-differentiated and dedifferentiated components of each case were immunostained for H3K27me3. Four-micrometer-thick sections from the paraffin block of each specimen were routinely deparaffinized, and exposed to 3% hydrogen peroxide for 15 min. Preparations were pretreated by autoclaving in citrate buffer, incubated with a monoclonal H3K27me3 antibody (C36B11) for 1 h at room temperature, and subsequently labeled with the Envision system (Dako, Glostrup, Denmark). Diaminobenzidine was used as the chromogen, and hematoxylin as the counterstain. The complete loss of staining was considered significant for H3K27me3. Mosaic loss of staining in which 5–95% of immunonegative cells were dispersed amongst immunopositive cells was not considered loss of staining, because this pattern is nonspecific in terms of tumor type, as shown by our previous study [29]. When a tumor demonstrated a geographic area of complete loss of staining with a sharp border on a background showing intact or mosaic loss of staining, we categorized it as a geographic complete loss [29]. Nuclear staining of endothelial cells served as an internal positive control. H3K27me3 status in dedifferentiated chondrosarcoma was then correlated with clinicopathological data.

For H3K27me3-deficient dedifferentiated chondrosarcoma cases, we applied immunohistochemistry for H3K27M and SUZ12. H3K27M staining is an accepted surrogate for H3K27M mutations, a known cause of H3K27me3 deficiency, and diffuse nuclear staining was considered positive as reported previously [24, 27]. The complete loss of SUZ12 staining indicates *SUZ12* aberration [23], and the extent of SUZ12 nuclear staining was classified as 0–5% (complete loss), 5–95% (focally positive), and >95% (diffusely positive), in a background of intact endothelial staining as reported previously [28]. Myogenin, S100, and SOX10 staining was also performed in select cases. Results for other immunohistochemical markers were retrieved from the original pathology reports. Antibodies and staining protocols used in this study are summarized in Table 1.

## Targeted next-generation sequencing

Targeted next-generation sequencing was attempted for five cases of H3K27me3-deficient dedifferentiated chondrosarcoma. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) blocks using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). DNA was

**Table 1** Summary of the primary antibody used

| Antibody | Clone | Dilution | Pretreatment | Solution | LINKER | Source |
|---|---|---|---|---|---|---|
| H3K27me3 | C36B11 | 1:200 | Autoclaving | Citrate buffer | No | Cell Signaling Technology, Danvers, MA, USA |
| SUZ12 | ab126577 | 1:200 | Water bath | Targeted Retrieval Solution, pH 9 (Dako) | Yes | Abcam, Cambridge, UK |
| H3K27M | Polyclonal | 1:8000 | Autoclaving | Citrate buffer | Yes | Millipore, Billerica, MA, USA |
| Myogenin | F5D | 1:100 | Water bath | Targeted Retrieval Solution, pH 9 (Dako) | No | Dako, Glostrup, Denmark |
| S100 | Polyclonal | 1:2000 | Autoclaving | Targeted Retrieval Solution (Dako) | No | Dako |
| SOX10 | A-2 | 1:100 | Water bath | Targeted Retrieval Solution, pH 9 (Dako) | No | Santa Cruz Biotechnology, Dallas, TX, USA |

quantified using the NanoDrop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and Qubit (Thermo Fisher Scientific). In four cases, tumor DNA was separately extracted from well-differentiated and dedifferentiated components. The primary tumor of case 5 was also subjected to next generation sequencing. Target genes included *CDKN2A*, *CDK4*, *COL2A1*, *EED*, *EZH1*, *EZH2*, *HRAS*, *IDH1*, *IDH2*, *MDM2*, *MYC*, *NF1*, *PTEN*, *RB1*, *SUZ12*, and *TP53*. All reference sequences were based on the NCBI37/hg19 assembly of the human genome. This panel was designed using SureDesign (Agilent Technologies, Santa Clara, CA, USA) to capture all coding exons of the 16 genes. Sequencing libraries were prepared using SureSelect XT reagent (Agilent Technologies). Paired-end sequencing ($2 \times 150$ bp) was performed using a NextSeq sequencer (Illumina, San Diego, CA, USA). Mutations, gene amplifications, and homozygous deletions were detected using the in-house program cisCall Ver 5.1.0.

## Sanger sequencing

To validate the results of targeted next-generation sequencing, Sanger sequencing was performed in both well-differentiated and dedifferentiated components of case 1 using an ABI PRISM 3130xl Genetic Analyzer (Thermo Fisher Scientific). Tumor DNA was extracted from FFPE blocks and subjected to polymerase chain reaction (PCR) using a pair of primers (5′-ACCACAACTGTTACCTGTTAGT-3′ and 5′-TCACTA TGTTGCACATTAGGCA-3′) targeting the *EED* mutation site that was detected in the dedifferentiated component of case 1 by next-generation sequencing. The PCR products were purified using ExoSAP-IT (Thermo Fisher Scientific). The isolated PCR products were then sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). The sequence chromatogram files were examined and mutations were identified using Sequencher software (version 4.8; Gene Codes Corporation, Ann Arbor, MI, USA).

## Results

### One third of dedifferentiated chondrosarcomas are deficient in H3K27me3

H3K27me3 staining was interpretable in 19 cases, whereas staining failed in two cases. The well-differentiated chondrosarcoma component of 17 cases retained H3K27me3, whereas staining failed in one case and the remaining case had no specimen available. In dedifferentiated components, H3K27me3 was lost in six cases (32%), whereas it was retained in the remaining 13 cases. Among the six H3K27me3-deficient cases, five tumors showed global

staining loss, whereas one tumor (case 2) showed geographic (subclonal) loss of staining. Among six H3K27me3-deficient dedifferentiated chondrosarcomas, SUZ12 was diffusely positive in one case, focally positive in two cases, and lost in three cases (Supplementary Fig. 1). H3K27M was negative in all four H3K27me3-deficient cases tested.

## H3K27me3-deficient dedifferentiated chondrosarcomas are histologically distinct

Clinicopathological data of H3K27me3-deficient dedifferentiated chondrosarcoma are summarized in Table 2, and Table 3 compares them with those of H3K27me3-intact dedifferentiated chondrosarcoma. All six H3K27me3-deficient tumors showed well-differentiated chondrosarcoma and non-cartilaginous high-grade sarcoma with a sharp transition (Fig. 1). Well-differentiated components in all cases retained H3K27me3, whereas dedifferentiated components lost H3K27me3, with the staining transition being sharp when the border was available for staining (Fig. 2) The dedifferentiated components of all six cases showed similar histologies. They consisted of sweeping or swirling fascicular proliferation of spindle cells with relatively uniform hyperchromatic nuclei and scant cytoplasm, and three cases showed occasional alternating dense and hypodense zones with perivascular cellular accentuation. Geographic necrosis was common. The abovementioned pattern of histological features closely resembled the typical morphology of malignant peripheral nerve sheath tumor [30–32] (Fig. 3). Three and two cases contained a heterologous osteosarcomatous component and rhabdomyoblastic differentiation (immunopositive for myogenin), respectively. S100 and SOX10 were negative in the dedifferentiated component of the three cases tested. In three cases, the cartilaginous component contained a focal G3 histology. The metastatic tumors, which was examined in four cases, showed only a dedifferentiated component ($n = 2$), well-differentiated component ($n = 1$), or both components ($n = 1$). In contrast, dedifferentiated areas of H3K27me3-intact cases exhibited spindle to epithelioid cell proliferation with a more enhanced degree of nuclear pleomorphism (severe, nine cases; moderate, two cases; mild, two cases), and more abundant readily appreciable eosinophilic cytoplasm than their H3K27me3-deficient counterparts. Ten cases therefore resembled undifferentiated pleomorphic sarcoma (Fig. 4) and three cases additionally contained osteosarcomatous differentiation, whereas none of these cases showed morphologies similar to those of classic malignant peripheral nerve sheath tumor. None of the cases contained a G3 chondrosarcoma component. Myogenin was negative in all 13 cases tested.

## Clinical characteristics of H3K27me3-deficient dedifferentiated chondrosarcomas

H3K27me3-deficient dedifferentiated chondrosarcomas occurred in two men and four women with a median age of 66 years. The primary tumor sites of H3K27me3-deficient cohort were the rib ($n = 4$), humerus ($n = 1$), and thoracic vertebra ($n = 1$). One case (case 5) presented with secondary (metachronous) dedifferentiation in the metastatic tumor 20 years after the first curettage treatment, whereas the other five cases showed primary (synchronous) dedifferentiation at the time of initial surgery or biopsy. Two primary tumors had a 7-year history of a slow-growing mass prior to initial histological examination. By radiological and/or gross examination, three cases showed a cortex-expanding intraosseous cartilaginous component with an extraosseous non-cartilaginous component. One patient had a family history of multiple osteochondromas; however, whether dedifferentiated chondrosarcoma arose from osteochondroma was unclear. The age and sex of these six patients were not different from those of 13 patients with H3K27me3-intact dedifferentiated chondrosarcoma. However, the primary tumor sites were significantly different, because they were restricted to the upper half of the body (above the waist) in all cases, whereas the lower half of the body was involved in eight of 13 H3K27me3-intact examples ($P = 0.018$, Fisher's exact test). In particular, four of six H3K27me3-deficient dedifferentiated chondrosarcomas arose from the rib, whereas only one of 13 H3K27me3-intact cases involved the rib ($P = 0.017$, Fisher's exact test). The Kaplan–Meier method and log-rank test revealed no significant differences between four H3K27me3-deficient dedifferentiated chondrosarcomas and nine H3K27me3-intact dedifferentiated chondrosarcomas for which complete resection was performed in terms of overall and disease-free survival ($P = 0.304$ and $0.755$, respectively; Supplementary Fig. 2).

## H3K27me3 deficiency in dedifferentiated chondrosarcoma is related to SUZ12/EED mutation

The results of genetic analyses are summarized in Table 2. A *SUZ12/EED* aberration was observed only in dedifferentiated (H3K27me3-deficient) components as follows: *EED* W263* in case 1, *SUZ12* R196fs*4 in case 4, and *SUZ12* exon 5 deletion in case 5. No aberrations in *SUZ12* and *EED* were detected in the dedifferentiated area of case 2. Notably, *SUZ12/EED* aberrations corresponded to the results of SUZ12 immunohistochemistry, as *SUZ12*-mutant cases exhibited complete loss of SUZ12 expression; one *EED*-mutant case showed partial staining for SUZ12, and one case that was diffusely positive for SUZ12 did not

**Table 2** Summary of H3K27me3-deficient dedifferentiated chondrosarcoma

| Clinicopathological findings | | | | | | Immunohistochemistry | | Genetics | Chemotherapy/ radiotherapy | Clinical course | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case | Age/sex | Primary site | Component | Histology | Heterol. diff. | H3K27me3 | SUZ12 | | | Local Rec | Distant Met | Outcome |
| 1 | 65/F | Lt 8th rib | WD | G1,2,3 | — | Ret | Focal | Insuff, $EED$ wildtype (1) | Yes/ No | No | Lung (3 m) | DOD (11 m) |
| | | | DD | MPNST-like | RMS | Lost | | $EED$ W263* (1), $CDKN2A$ homodel | | | | |
| 2 | 77/F | Lt humerus | WD | G1,2,3 | — | Ret | Focal | $IDH2$ R172S, $COL2A1$ G924fs*10 | No/ No | No | Lung (2 y 1 m) | NED (2 y 6 m) |
| | | | DD | MPNST-like | OS | Lost | Diffuse | $IDH2$ R172S, $TP53$ V216M | | | | |
| 3 | 67/M | Lt 7th rib | WD | G1,2 | — | Ret | ND | ND | No/ No | NA (2) | Lung (1 m) | DOD (5 m) |
| | | | DD | MPNST-like | No | Lost | Focal | Insuff | | | | |
| 4 | 62/F | Rt rib | WD | G2 | — | Ret | ND | $COL2A1$ P212fs*27 | Yes/ No | No | Lung (2 y 5 m) | AWD (4 y) |
| | | | DD | MPNST-like | No | Lost | Lost | $SUZ12$ R196fs*4, $NF1$ G629R, $TP53$ P153fs*28 (few) | | | | |
| 5 | 70/F | Thoracic vertebra | WD (Pri) | G1,2 | — | ND | ND | $COL2A1$ W1224fs*19 (few), $CDKN2A$ Y129* (few) | No/No | No | Liver (20 y) | AWD (21 y) |
| | | | WD (Met) | G2,3 | — | Ret | Focal | $COL2A1$ W1224fs*19, $CDKN2A$ homodel | | | | |
| | | | DD (Met) | MPNST-like | RMS, OS | Lost | Lost | $COL2A1$ W1224fs*19, $CDKN2A$ homodel, $SUZ12$ exon 5 deletion | | | | |
| 6 | 51/M (3) | Lt 7th rib | WD | G1 | — | ND | ND | ND | Yes/ No | NA (4) | No | DOD (5 m) |
| | | | DD | MPNST-like | OS | Lost | Lost | ND | | | | |

Although the outcome was expressed by the time elapsed from the initial resection or biopsy in this table, the time from dedifferentiation was used for survival analysis for case 5. Only the first metastatic site was recorded

(1) Validated by Sanger sequencing

(2) Not applicable, because this patient did not undergo resection

(3) This patient had a history of multiple osteochondromas

(4) Not applicable, because this patient underwent incomplete resection

*AWD* alive with disease, *DD* dedifferentiated, *DOD* died of disease, *F* female, *G* grade, *Heterol. diff.* heterologous differentiation, *Homodel* homodeletion, *Insuff* insufficient for next-generation sequencing, *Lt* left, *M* male, *Met* metastasis, *MPNST* malignant peripheral nerve sheath tumor, *NA* not applicable, *ND* not done, *NED* no evidence of disease, *OS* osteosarcoma, *Pri* primary, *Ret* retained, *RMS* rhabdomyosarcoma, *Rt* right, *WD* well differentiated

**Table 3** Clinicopathological comparison between H3K27me3-deficient and H3K27me3-intact dedifferentiated chondrosarcomas

| | H3K27me3-deficient DDCS ($N = 6$) | H3K27me3-intact DDCS ($N = 13$) |
|---|---|---|
| Sex (male:female) | 2:4 | 8:5 |
| Median age in years (range) | 66 (51–77) | 69 (42–90) |
| Primary site ($N$) | Rib (4), humerus (1), thoracic vertebra (1) | Rib (1), humerus (3), phalanx of the hand (1), femur (5), pelvic bone (2), tibia (1) |
| Histology ($N$) | MPNST-like (6) | UPS-like (10), OS-like (3) |
| Heterologous rhabdomyoblastic differentiation | 2/6 | 0/13 |
| Focal G3 component | 3/6 | 0/13 |
| Outcome ($N$) | DOD (3), AWD (2), NED (1) | DOD (7), AWD (2), NED (4) |

Outcome was measured after a follow-up length of 1–152 months from surgery or initial biopsy for sarcomas that demonstrated dedifferentiated histology

*AWD* alive with disease, *DDCS* dedifferentiated chondrosarcoma, *DOD*, died of disease, *MPNST* malignant peripheral nerve sheath tumor, *NED* no evidence of disease, *OS* osteosarcoma, *UPS* undifferentiated pleomorphic sarcoma
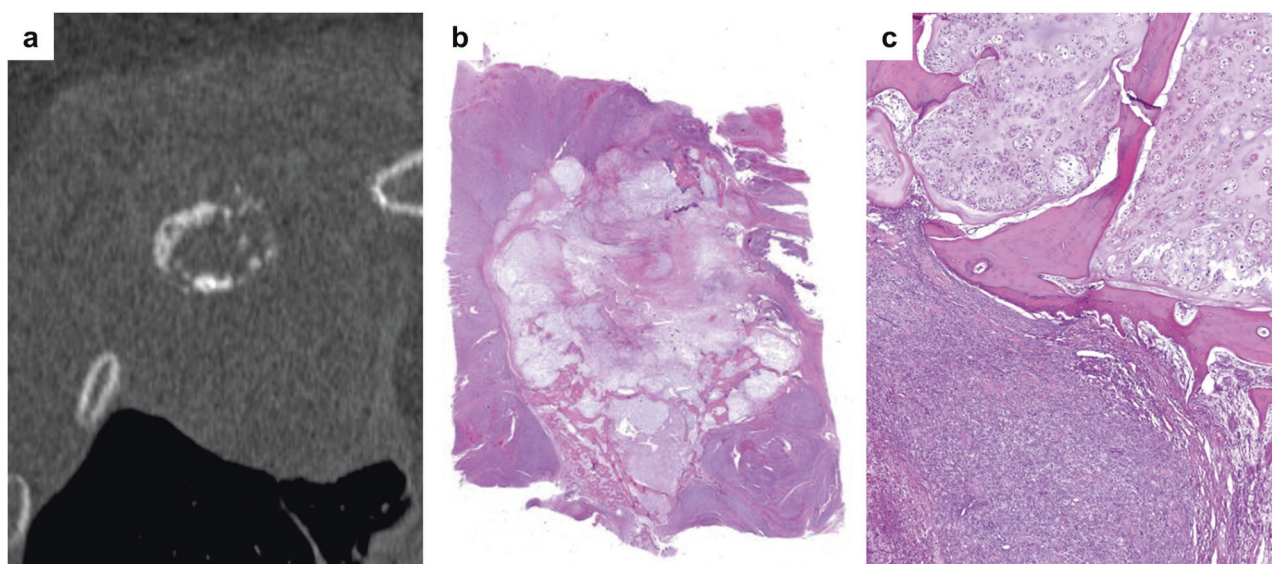
**Fig. 1** H3K27me3-deficient dedifferentiated chondrosarcomas showed a classic biphasic pattern. Based on computed tomography (**a**), one tumor of the rib (case 4) expanded and destructed the cortex, with a large soft tissue component. Histologically, well-differentiated cartilaginous tumor involved the bone, which was surrounded by an extraosseous dedifferentiated component (**b**, H&E). The transition between the two elements was sharp (**c**, H&E)

harbor *SUZ12/EED* mutations. To validate next generation sequencing results, Sanger sequencing was performed on case 1, which confirmed the *EED* W263* mutation in the dedifferentiated component. The well-differentiated component of this case, which was not subjected to next generation sequencing owing to insufficient DNA, did not harbor the same *EED* mutation as determined by Sanger sequencing. There were no mutations or deletions in *EZH1* and *EZH2* in the four cases tested. Other mutations identified included *IDH2* R172S in both the well-differentiated and dedifferentiated component of case 2. A *COL2A1* truncating mutation was detected in at least one of the components from three cases. An *NF1* G629R was detected in the dedifferentiated component of case 4. A homozygous *CDKN2A* deletion was detected in cases 1 and 5.

## Discussion

In this study, we demonstrated that 32% of dedifferentiated chondrosarcoma cases tested were deficient for H3K27me3. This deficiency was restricted to the dedifferentiated component in all cases and was mainly associated with genetic aberrations in *EED* or *SUZ12*, suggesting a role for PRC2

**Fig. 2** The well-differentiated component of H3K27me3-deficient dedifferentiated chondrosarcoma retained H3K27me3 expression (**a**, H&E, **b**, H3K27me3 immunohistochemistry), whereas the dedifferentiated component exhibited complete loss of H3K27me3 (**c**, H&E, **d**, H3K27me3 immunohistochemistry; note strongly labeled vascular endothelial cells as internal positive control). The staining transition between the two components was sharp (**e**, H&E, **f**, H3K27me3 immunohistochemistry)
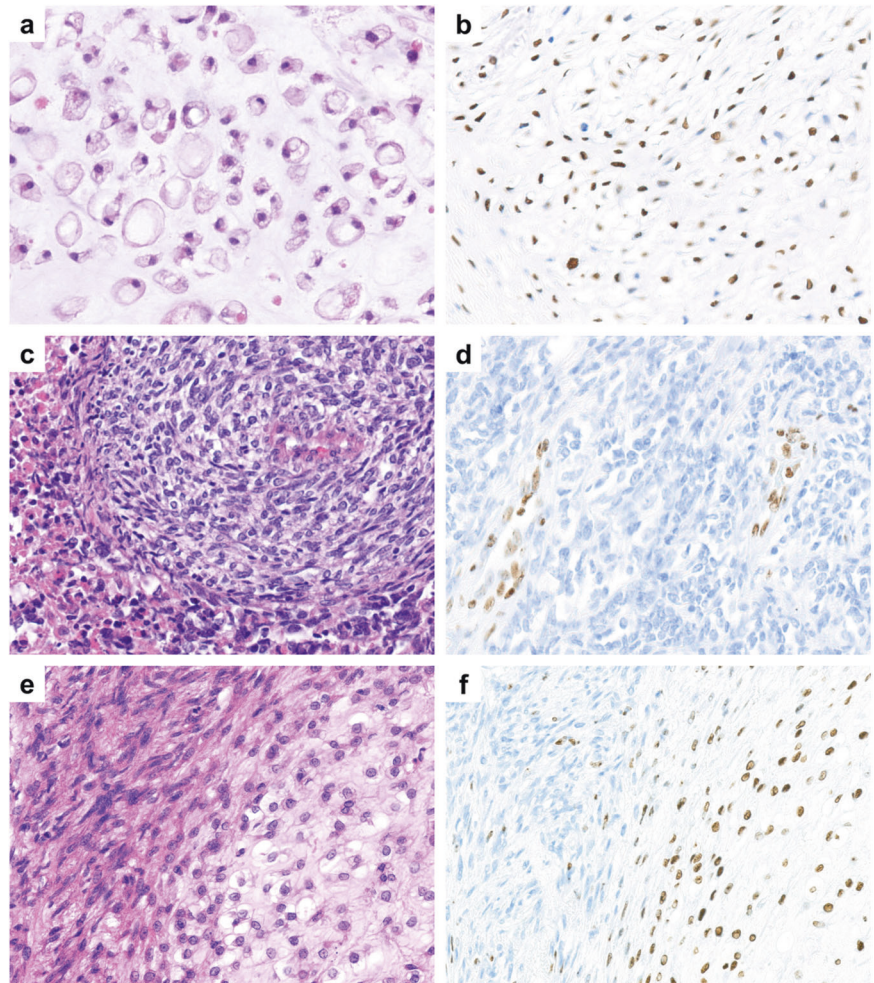


gene mutations and the resulting epigenetic dysfunction in the dedifferentiation of chondrosarcoma. One H3K27me3-deficient case did not harbor any detectable mutations in *SUZ12*, *EED*, *EZH1*, and *EZH2*; in addition, H3K27M was negative by immunohistochemistry, leaving the underlying mechanism for this deficiency unknown.

H3K27me3-deficient dedifferentiated chondrosarcoma demonstrated characteristic histological features. The most distinctive was its striking histological resemblance to classic malignant peripheral nerve sheath tumor [30–32], in contrast to H3K27me3-intact dedifferentiated chondrosarcoma. This similarity was so clear that we initially wondered if these six cases might actually represent a bone primary PRC2-mutant malignant peripheral nerve sheath tumor with heterologous cartilaginous differentiation. However, we excluded that hypothesis because the cartilaginous components of these cases always retained H3K27me3, unlike all 13 malignant peripheral nerve sheath tumors with heterologous differentiation from our archive, in which the heterologous element invariably showed H3K27me3 deficiency (data not shown). In addition, some

of these cases showed classic clinico-radiological and gross characteristics of dedifferentiated chondrosarcoma including long preoperative clinical courses and cortex-expanding intraosseous cartilaginous components. Further, based on targeted next generation sequencing, they harbored specific *IDH2* R172S (1/4) and *COL2A1* truncations (3/4), in addition to *SUZ12/EED* aberrations. Lastly, an *NF1* mutation, a nearly universal signature of malignant peripheral nerve sheath tumor, was identified in only one of four cases tested, and the significance of the *NF1* missense mutation in this single case was unclear because it was absent in the corresponding well-differentiated component. Although these findings supported the present classification as chondrosarcoma, phenotypic similarities between H3K27me3-deficient dedifferentiated chondrosarcoma and malignant peripheral nerve sheath tumor could pose diagnostic challenges in practice, because small biopsies from dedifferentiated chondrosarcoma might contain only dedifferentiated elements. Radiological correlation is thus mandatory, and the coexisting expansile lytic lesion with calcification and typical magnet resonance findings should

Fig. 3 Dedifferentiated histology of H3K27me3-deficient dedifferentiated chondrosarcomas resembled malignant peripheral nerve sheath tumors. They consisted of sweeping to swirling fascicular proliferation of spindle cells with uniform hyperchromatic nuclei and scant cytoplasm (a–c, e). Alternating dense and hypodense zones with perivascular cellular accentuation was occasionally seen (d). Necrosis was common (a). Rhabdomyoblastic differentiation was observed in two cases (f, inset myogenin immunohistochemistry)
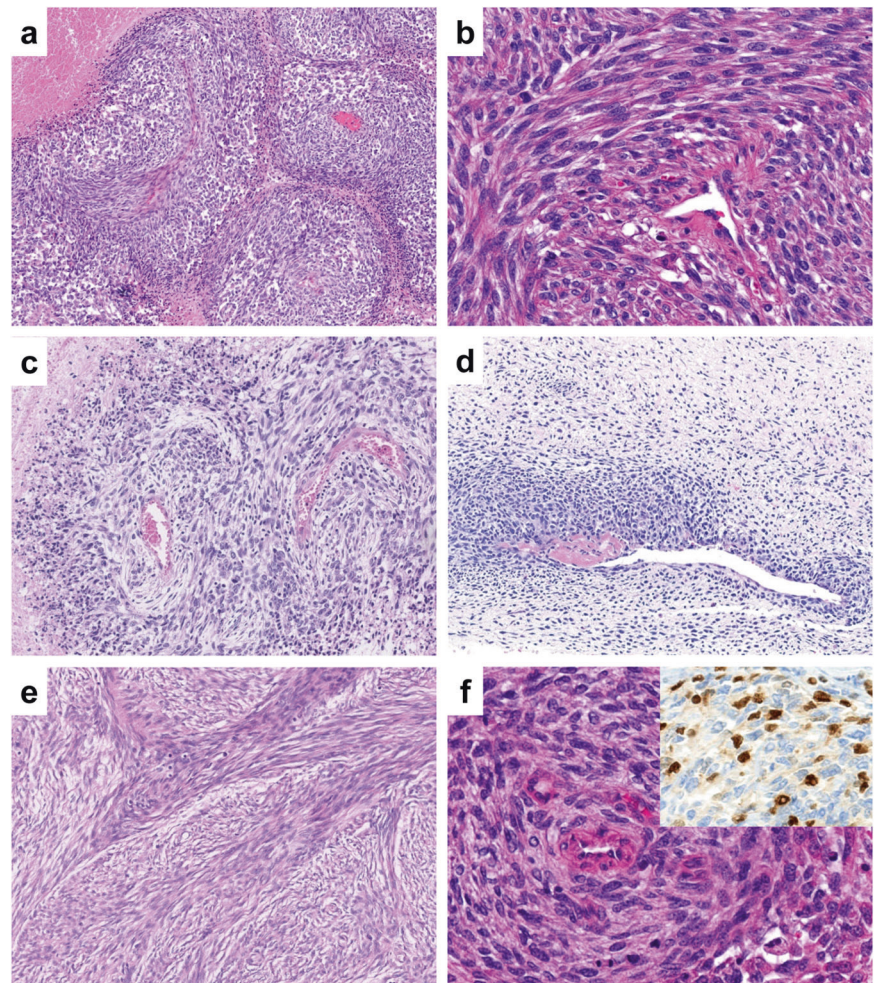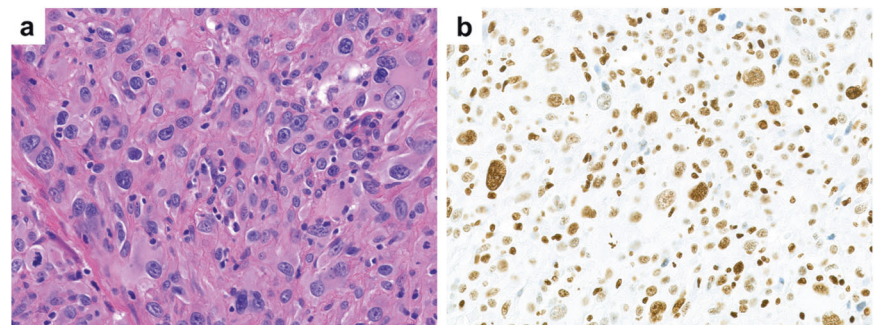


Fig. 4 H3K27me3-intact dedifferentiated chondrosarcomas. The dedifferentiated component of these tumors demonstrated pleomorphic cell proliferation (a) and did not resemble the classic histology of malignant peripheral nerve sheath tumors. H3K27me3 immunohistochemical expression was retained (b)

be specifically sought. Malignant peripheral nerve sheath tumor rarely arises from the bone and we believe that the diagnosis of any primary osseous malignant peripheral nerve sheath tumor should be approached with caution. We have previously reported an H3K27me3-deficient malignant peripheral nerve sheath tumor of the femur, but that tumor had no evidence of a cartilaginous component after extensive sampling [33].

Another interesting histological aspect of H3K27me3-deficient dedifferentiated chondrosarcoma was the existence of a G3 chondrosarcoma component in some cases; in contrast, this was absent in all H3K27me3-intact cases examined. In addition, metastatic foci of H3K27me3-deficient dedifferentiated chondrosarcoma showed a chondrogenic component in a few cases for which such tissue was available, whereas metastases comprised non-chondrogenic components in all H3K27me3-intact cases. These observations might suggest more aggressive behavior of the cartilaginous component in H3K27me3-deficient dedifferentiated chondrosarcoma. Also notable was the

exclusive occurrence of heterologous rhabdomyoblastic differentiation in the H3K27me3-deficient cohort. In contrast, heterologous osteosarcomatous differentiation was observed in both cohorts.

H3K27me3-deficient dedifferentiated chondrosarcomas that focally contained a G3 chondrosarcoma component were readily distinguishable from the gradual progression of chondrosarcoma (high-grade chondrosarcoma with spindle cells) and chondroblastic osteosarcoma. Dedifferentiated chondrosarcoma was originally proposed by Dahlin et al. in 1971, as a combination of low-grade or "borderline" cartilaginous tumor and juxtaposed zones of anaplastic sarcoma with a sharp border [2]. Subsequent studies accepted the presence of G2, and rarely G3, histologies within the spectrum of dedifferentiated chondrosarcoma as long as the defining abrupt transition exists [1, 34, 35]. In all of our three cases with a G3 chondrosarcoma component, G3 histology was present distant from the non-cartilaginous proliferation with no direct transition between the two areas, with only grades 1–2 chondrosarcoma juxtaposing the non-cartilaginous element, thereby meeting the criteria of dedifferentiated chondrosarcoma.

The clinical features of H3K27me3-deficient dedifferentiated chondrosarcoma were somewhat characteristic as well. The primary tumor sites were restricted to the upper half of the body (above the waist) in all cases, with a clear predilection to the rib, and this distribution was significantly different from that of H3K27me3-intact cases. The clinical behavior of H3K27me3-deficient dedifferentiated chondrosarcoma was not different from that of H3K27me3-intact tumors based on this small series.

Interestingly, two H3K27me3-deficient dedifferentiated chondrosarcomas (cases 2 and 4) harbored a COL2A1 truncating mutation only in the cartilaginous component but not in dedifferentiated component, whereas the remaining case (case 5) harbored a COL2A1 mutation in both components. In the first two tumors, clinicopathological features readily excluded the possibility of collision tumors, and, therefore, this genetic discordance might suggest clonal diversification of a dedifferentiated component before the well-differentiated component acquired the COL2A1 mutation. Although the timing of acquisition of the COL2A1 mutation in chondrosarcoma has never been determined by longitudinal studies, these data might challenge the conventional linear understanding that well-differentiated chondrosarcoma dedifferentiates to obtain highly aggressive, non-chondrogenic properties, and might instead support the "monoclonal origin and early diversion model" [16].

H3K27me3 deficiency in dedifferentiated chondrosarcoma might provide a novel therapeutic opportunity. Recent studies showed that a mouse and cell line model of SUZ12-mutant malignant peripheral nerve sheath tumor showed upregulation of BRD4 and was effectively treated by a BET bromodomain inhibitor (JQ1) [36, 37]. The therapeutic effect of this inhibitor was also reported in H3K27M-mutant diffuse midline glioma [38], wherein the PRC2 function is inactivated and H3K27me3 is deficient [24, 26]. Whether H3K27me3-deficient dedifferentiated chondrosarcoma could be approached using a similar strategy is worth exploration.

In summary, approximately one-third of dedifferentiated chondrosarcomas are deficient in H3K27me3, most commonly via SUZ12 or EED mutations, and are associated with several clinicopathological features. This tumor subset requires recognition primarily because of its diagnostic significance, but future studies are necessary to better understand their potential clinical implications.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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