Note: This article was posted on the Archives Web site as an Early Online Release. Early Online Release articles have been peer reviewed, copyedited, and reviewed by the authors. Additional changes or corrections may appear in these articles when they appear in a future print issue of the Archives. Early Online Release articles are citable by using the Digital Object Identifier (DOI), a unique number given to every article. The DOI will typically appear at the end of the abstract.

The DOI for this manuscript is doi: 10.5858/arpa.2021-0153-OA

The final published version of this manuscript will replace the Early Online Release version at the above DOI once it is available.
CIC-NUTM1 Sarcomas Affecting the Spine

A Subset of CIC-Rearranged Sarcomas Commonly Present in the Axial Skeleton

Shaomin Yang, MD, PhD; Lili Liu, MM; Yu Yan, MM; Liang Jiang, MD; Songbo Han, MD; Danhua Shen, MD; Bo Zhang, MD, PhD

Context.—Tumors harboring CIC-NUTM1 fusion are a newly recognized rare sarcoma, but the documented cases are still limited. It is unclear whether it is the same as classic CIC-DUX4 sarcoma in terms of its clinical, pathologic, and behavioral aspects.

Objective.—To further explore the clinicopathologic characteristics of CIC-NUTM1 sarcoma.

Design.—The cases were diagnosed based on immunophenotype, next-generation sequencing, and fluorescence in situ hybridization tests and compared with the reported CIC-NUTM1 sarcomas in the literature.

Results.—Three cases of CIC-NUTM1 sarcomas involving the spine in adults were described. They were 2 men and 1 woman, aged 38 to 61 years. Two tumors were located in thoracic vertebrae and 1 in a cervical vertebra. All were locally advanced lesions destroying the bone and soft tissues without spinal cord involvement or metastasis.

CIC-rearranged sarcomas have been acknowledged as a distinct entity and described in separate sections in the newest WHO classification of bone and soft tissue tumors (5th edition).1–5 Genetically, they are most commonly grounded by CIC-DUX4 fusion, which results from t(4;19) or t(10;19) translocation. CIC-NUTM1 malignancy was first recognized in 2016 from unclassified round cell sarcoma of the central nervous system.6,7 CIC-NUTM1 is thought to be a molecular variant of CIC sarcoma because both methylation and transcription profile studies demonstrated that it clustered together with CIC sarcoma but away from NUT carcinoma.

To date, the number of cases with CIC-NUTM1 fusion is still limited. A series of 6 cases reported by Le Loarer and colleagues8 was the only cohort described with clinicopathologic details. A predisposition for central nervous system in pediatric and young adult patients and overall poor prognosis were suggested. In addition to this small series, there have been a few sporadic case reports of this entity affecting locations outside of the brain, including the bone, soft tissue, and viscera.9,10 It is unclear whether CIC-NUTM1 sarcoma is the same as classic CIC-DUX4 sarcoma in terms of its clinical, pathologic, and behavioral aspects.

Here, we describe 3 new cases of CIC-NUTM1 sarcoma affecting the spine with dismal prognosis. In our cases combined with those in the literature,9,9 we find that sarcomas characterized by this fusion are prone to present at axial locations and affect bone. Awareness of these features is important for correct diagnostic and prognostic evaluation.

Materials and Methods

Patients

Cases 1 and 2 were identified by reviewing the files of the Department of Pathology, Peking University Third Hospital (Beijing, China), between 2012 and 2020. Cases with the following features were first screened by NUT immunostaining: (1) at least focal round cell morphology, (2) comprehensive immunostaining not indicative of tumor differentiation, and (3) available fluorescence in situ hybridization (FISH) or polymerase chain reaction data negative for EWSR1, FUS, SS18, and so on. Second, NUT staining–positive cases were further tested by FISH using CIC and NUTM1 probes, and by next-generation sequencing. The third case was retrieved from the Department of Pathology, Peking University...
Abbreviations: C, cervical spine; CT, chemotherapy; DOD, died of disease; FISH, fluorescence in situ hybridization; MSI, microsatellite instability; MSS, microsatellite stable; NGS, next-generation sequencing; NUT, NUT protein; RT, radiotherapy; T, thoracic spine; TMB, tumor mutational burden.

People’s Hospital (Beijing, China), in a retrospective study of a panel of undifferentiated sarcomas using high-throughput RNA screening.

The clinical and follow-up information was collected from medical data and by inquiry. This study was approved by the Ethics Board at Peking University Health Science Center.

**Histopathologic and Immunohistochemical Analysis**

All hematoxylin and eosin–stained slides were reviewed by soft tissue pathologists. Comprehensive immunohistochemistry was also reviewed or supplemented. Representative tissue sections were stained with primary antibodies against NUT (1:500; polyclonal, ab122649, Abcam), ETV4 (1:300; polyclonal, AF0435, Affinity), CD99 (1:60; monoclonal, ZM0296, ZSGB-BIO), and others. Appropriate positive and negative controls were applied.

**Fluorescence In Situ Hybridization**

Fluorescence in situ hybridization analyses were performed on 4-μm-thick paraffin sections using dual-color break-apart probes for NUTM1 at 15q14 (CAT. F.01264, Guangzhou LBP) and CIC at 19q13.2 (CAT. F.01219, Guangzhou LBP) according to standard protocols in our laboratory.

**Targeted Next-Generation Sequencing**

Formalin-fixed, paraffin-embedded tumor samples and matched normal tissues were subjected to targeted next-generation sequencing analysis.

Total DNA was extracted using the AllPrep DNA/RNA Mini Kit (Qiagen) and RNA was extracted using the RNA extraction kit (Beckman Coulter) following the manufacturers’ protocols. All samples passed the quality criteria. DNA library construction was performed using the KAPA Hyper Prep Kit (KAPA Biosystems), and RNA libraries were prepared with approximately 10 ng complementary DNA, which was reverse transcribed from 350 ng total RNA using the KAPA Hyper Prep Kit. Further, the libraries were sequenced on NovaSeq using NovaSeq 6000 Reagent Kit V1.5 (300 cycles) by paired-end sequencing (2 × 150 bp). All coding exons from 638 cancer-related genes and parts of introns from 63 genes frequently rearranged in cancer were detected by the next-generation sequencing panel. Genomic alterations in DNA, including single-nucleotide variations, short and long insertions/deletions, copy number variations, and gene rearrangements/fusions, were assessed, and gene rearrangements/fusions in RNA were also analyzed.

**RESULTS**

**Clinical Findings**

The clinical features of the cases are summarized in Table 1. All 3 were adult individuals, aged 38, 52, and 61 years (mean, 50 years). Two were men. Two lesions were located in thoracic vertebrae, and the other was located in a cervical vertebra. On imaging, all tumors demonstrated poorly defined osteolytic lesions with infiltration into surrounding soft tissue but without involvement of the spinal cord parenchyma (Figure 1). Radiographic workup did not demonstrate evidence of metastatic disease at presentation. One patient received radiotherapy after core biopsy because of the anatomic location of the tumor. The other patient was treated by surgery with R1 margins combined with postoperative radiation and chemotherapy. The third patient underwent surgical resection with R1 margins without additional treatment. All patients died of locoregional progression, at 8, 9, and 15 months after presentation.

The previously reported CIC-NUTM1 fusion sarcomas with detailed clinicopathologic features are summarized in Table 2.

**Histologic Findings**

The tumors were initially diagnosed as myoepithelioma (1 case) and undifferentiated round cell sarcoma (2 cases).

| Table 1. Clinicopathologic and Molecular Findings of Spinal CIC-NUTM1 Cases in Current Study |
|-----------------|-----------------|-----------------|-----------------|
| Case No. | 1 | 2 | 3 |
| Age, y/sex | 61/M | 38/M | 52/F |
| Imaging findings | C6-C7 accessory destruction with the formation of soft tissue mass | T7-T8 vertebral destruction extending into adjacent ribs and soft tissue | T11-T12 vertebral destruction extending into adjacent ribs and soft tissue |
| Metastatic disease at diagnosis | None | None | None |
| Treatment | RT | Surgical resection; adjuvant RT and CT | Surgical resection |
| Follow-up | DOD at 8 mo | DOD at 15 mo | DOD at 9 mo |
| Histopathology | | | |
| Pattern | Lobulated | Solid and lobulated | Solid and lobulated |
| Myxoid stroma | Diffuse | Multifocal | Multifocal |
| Cells | Small to medium | Small to medium | Small to medium |
| Immunostaining | | | |
| NUT | Diffuse nuclear + | Diffuse nuclear + | Diffuse nuclear + |
| Other markers | CD99+ (membrane) | CD99+ (dotlike, cytoplasm) | CD99+ (membrane) |
| CIC | Break | Break | Break |
| NUTM1 | Break | Break | Break |
| NGS | CIC exon 16–NUTM1 exon 5 | CIC exon 17–NUTM1 exon 6 | CIC exon 17–NUTM1 exon 6 |
| TMB, muts/Mb | 2.8 (low) | 0 (low) | 1.1 (low) |
| MSI | MSS | MSS | MSS |

Abbreviations: C, cervical spine; CT, chemotherapy; DOD, died of disease; FISH, fluorescence in situ hybridization; MSI, microsatellite instability; MSS, microsatellite stable; NGS, next-generation sequencing; NUT, NUT protein; RT, radiotherapy; T, thoracic spine; TMB, tumor mutational burden.
tumors showed certain recurrent histopathologic features. Under low power, a multinodular contour separated by thick fibrocollagenous septa was apparent in 1 case (Figure 2, A). The tumor cells in the other 2 cases proliferated in sheets, but a vague lobulated architecture could be appreciated on the tumor periphery (Figure 2, B). Myxoid stroma was obvious in all tumors, diffusely or multifocally, with the formation of a mucous pool (Figure 2, C and D). The tumor cells were small to intermediate in size. Nuclei were relatively uniform in round or irregular shape, with mild pleomorphism. The chromatin was fine to vesicular. The nucleoli were distinct and focally prominent (Figure 2, C and D). There were moderate amounts of eosinophilic or clear cytoplasm. Highly pleomorphic anaplastic cells, plasmacytoid/rhabdoid cells, multinuclear giant cells, and hyaline cartilage matrix were focally present in samples after chemotherapy in case 2 (Figure 2, E and F). Necrosis was absent or focally present, but massive or geographic necrosis was absent. Mitoses were infrequent in all cases (1–5/10 high-power fields).

**Immunohistochemical Findings**

The tumors showed NUT protein positivity with intense diffuse nuclear staining (Figure 3, A). ETV4 was diffusely positive in 1 case. CD99 was diffusely expressed, with 2 cases on the membrane (Figure 3, B) and the other in a paranuclear dotlike pattern (Figure 3, C). WT1(A-ter), calretinin, and SOX9 were focally stained. Staining of other markers was negative, including S100, SOX10, muscle markers (desmin, myogenin, myoD1), epithelial markers (CKpan, CK19, CK8/18, EMA), p63, p40, brachyury, CgA, Syn, NKX2.2, DOG1, and CD117. The Ki67 indexes for each case were 30%, 20%, and 20%.

**FISH and Next-Generation Sequencing Results**

Fluorescence in situ hybridization examination of the tumors showed CIC and NUTM1 gene break-apart signals (Figure 3, D and E). Copy number alteration was not observed. High-throughput DNA and RNA sequencing identified in-frame chimeric CIC-NUTM1 transcripts in all cases. The fusion positions were in exon 16 (1 case) or exon 17 (2 cases) in the CIC gene and exon 5 (1 case) or exon 6 (2 cases) in the NUTM1 gene (Table 1; Figure 4). The predicted CIC-NUTM1 fusion protein is composed of almost the entire CIC protein, as well as most functional domains of the NUT protein, including its nuclear localization signals and acid domains (AD1). Their tumor mutational burden was as low as 0, 1.1, and 2.8 muts/Mb, and microsatellite status was stable. No germline mutation was detected in the corresponding normal tissues.

**DISCUSSION**

Herein, we present 3 new cases of sarcomas with CIC-NUTM1 fusions. Of interest, all 3 cases affected the vertebrae. This phenomenon drove us to review all CIC-NUTM1 reports in the literature. As summarized in Table 2, 11 cases with clinicopathologic details have been published to date, including the current 3 cases.8–10 As expected, these tumors presented a distinct anatomic tropism. That is, 8 of 11 cases (72.7%) affected either the vertebrae (5 cases) or skull base (3 cases), presenting as locally invasive masses infiltrating the bone and surrounding soft tissues. Brain involvement was described in only 2 tumors arising at temporal and occipital sites, and there was no evidence to indicate their primary site.8 The spinal cord and brain parenchyma were spared in most of the remaining cases (6 of 8). Only one tumor arising at the trigone of the lateral

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, y/Sex</th>
<th>Bone Involved</th>
<th>CNS Involved</th>
<th>Follow-up, mo</th>
<th>Source, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61/M</td>
<td>Yes (C7-C8)</td>
<td>No</td>
<td>DOD, 8</td>
<td>Current study</td>
</tr>
<tr>
<td>2</td>
<td>38/M</td>
<td>Yes (T7-T8)</td>
<td>No</td>
<td>DOD, 15</td>
<td>Current study</td>
</tr>
<tr>
<td>3</td>
<td>52/F</td>
<td>Yes (T11-T12)</td>
<td>No</td>
<td>DOD, 9</td>
<td>Current study</td>
</tr>
<tr>
<td>4</td>
<td>60/M</td>
<td>Yes (sphenoid)</td>
<td>No</td>
<td>Alive, 10</td>
<td>Schaefer et al, 2018</td>
</tr>
<tr>
<td>5</td>
<td>3/M</td>
<td>Yes (temporal)</td>
<td>Yes</td>
<td>DOD, 18</td>
<td>Le Loarer et al, 2019</td>
</tr>
<tr>
<td>6</td>
<td>5/M</td>
<td>Yes (occipital)</td>
<td>No</td>
<td>DOD, 14</td>
<td>Le Loarer et al, 2019</td>
</tr>
<tr>
<td>7</td>
<td>18/M</td>
<td>Yes (T9-T10)</td>
<td>No</td>
<td>Alive, 40</td>
<td>Le Loarer et al, 2019</td>
</tr>
<tr>
<td>9a</td>
<td>22/F</td>
<td>No (lateral ventricle)</td>
<td>Yes</td>
<td>DOD, 17</td>
<td>Le Loarer et al, 2019</td>
</tr>
<tr>
<td>10</td>
<td>27/M</td>
<td>No (lung primary)</td>
<td>No</td>
<td>DOD, 7</td>
<td>Le Loarer et al, 2019</td>
</tr>
<tr>
<td>11</td>
<td>13/F</td>
<td>No (renal primary)</td>
<td>No</td>
<td>Alive, 36</td>
<td>Mangray et al, 2018</td>
</tr>
</tbody>
</table>

Abbreviations: C, cervical vertebra; CNS, central nervous system; DOD, dead of disease; L, lumbar vertebra; T, thoracic vertebra.

*a Case restricted to the brain.
Figure 2. CIC-NUTM1 sarcoma shows a multinodular or lobulated growth pattern under low-power view. (A) The core biopsy sample of case 1. (B) The resection sample of case 2. Myxoid stroma with mucin pool formation is observed (C). Tumor cells arranged in small sheets and trabecular patterns in myxoid stroma create a myoepithelial tumor appearance (D). High-power magnification reveals monomorphic tumor cells with round or irregular nuclei, vesicular chromatin, distinct nucleoli, and moderate amounts of eosinophilic or clear cytoplasm (D and E). Some rhabdoid cells (E), focal hyperchromatism, and pleomorphism (F) were present in the specimen after chemotherapy (case 2) (hematoxylin-eosin, original magnifications ×40 [A and B], ×200 [C], and ×400 [D through F]).
ventricle was restricted to the central nervous system based on the description.8 The other 2 cases without axial bone involvement developed in the lung and kidney.9,10 No case was found to affect the extremities. These data indicated that CIC-NUTM1 sarcoma is predisposed to axial location. In addition, it commonly presents as an osseous tumor, in contrast to the classic CIC-DUX4 sarcoma, which develops mostly in soft tissue. The common involvement of bone has also been noted by Le Loarer and colleagues,8 although they emphasized the central nervous system predisposition. Unfortunately, the precise locations of the earliest identified CIC-NUTM1 cases in the series of Sturm et al7 are unknown. Their cases might be completely restricted to the brain, but the possibility of some cases arising from the skull bone cannot be ruled out. Our study reinforced the axial bone anatomic predisposition feature of this rare sarcoma.

To some extent, the axial predisposition of CIC-NUTM1 sarcoma could reflect the midline distribution of NUT carcinoma.11 It is possible that vertebral bone or peripheral soft tissues are susceptible to malignant transformation by CIC-NUTM1 owing to the stem or progenitor cells in the paraxial mesoderm.12 In addition, the experience of Ewing sarcoma has emphasized the importance of the cell context in which the mutation occurs, showing that mesenchymal stem cells are permissive cells required for the maintenance of EWSR1-FLI1 expression.13–15 A similar cell context could also be considered for CIC-NUTM1, which should be tolerable to the vertebral bone or peripheral soft tissues but lethal outside of this cellular context.

Wild-type CIC (capicua) acts as a tumor suppressor and belongs to the high-mobility group box family.16 CIC represses the transcription of target genes in tumor progression and metastasis. Its C1 domain is phosphorylated by the receptor tyrosine kinase (RTK) downstream effectors ERK and c-SRC, promoting its nuclear export and degradation and thus relieving target gene expression.17 In addition, diverse loss-of-function mutations in CIC have been discovered in various human cancers in recent years. However, in CIC-rearranged sarcoma, the addition of a C-terminal domain from DUX4 or NUTM1 seems to have gain-of-function effects, resulting in an obvious elevation of target gene transcription.18 The underlying mechanisms conferring CIC transformation from transcription repressor to activator remain to be elucidated. One commonality between the DUX4 and NUT proteins is their ability to

Figure 3. NUT protein is expressed in a diffuse and homogeneous nuclear pattern in CIC-NUTM1 sarcoma (A). CD99 reactivity is present with a diffuse and strong membranous pattern (B) or cytoplasmic dotlike pattern (C). Fluorescence in situ hybridization analysis using CIC (D) and NUTM1 (E) break-apart probes demonstrates split green and orange signals, indicating translocation (original magnifications ×400 [A through C] and ×1000 [D and E]).
recruit the histone acetyltransferase P300/CBP. In all of the cases reviewed here, CIC-NUTM1 retained exons 1 through 16 of the CIC gene, including the high-mobility-group box, and the NUTM1 gene after exon 6, consisting of most of the coding sequence of NUT. Although the normal function of wild-type NUT is unclear, in vivo and in vitro experiments suggest that its C-terminal AD1 domain is responsible for binding with p300. Therefore, it is possible that CIC-DUX4 and CIC-NUTM1 function in the same molecular pathway, causing tumorigenesis through an epigenetic rewiring mechanism (mediating chromatin hypoacetylation), and thus provide a rationale for drug design in these tumors. However, we also found 2 cases of sarcoma with round/spindle cell morphology bearing a CIC intergenic fusion (Danhua Shen, MD, unpublished data, April 22, 2021) with predicted loss of function, though their pathogenesis needs to be studied.

Expression of the aberrant CIC-NUT protein could be demonstrated by NUT immunostaining, mostly diffuse, occasional multifocal, coinciding with its localization and action in the nucleus. In addition, the staining pattern in CIC-NUTM1 sarcoma was homogenous, in contrast with the speckled pattern in NUT carcinomas. It is suggested that the CIC moiety might serve as a positioning function directing the chimeric protein to widespread CIC target genes and then transcriptionally activating them by the NUT moiety via recruitment of chromatin regulators.

Although CIC-NUTM1 tumors were originally thought to be predominant in childhood, an increasing number of reports document a wide distribution of ages, through adulthood. The patients in the current series were adults aged 38 to 61 years. Overall, CIC-NUTM1 sarcoma occurred across a broader age (3–61 years; mean, 22 years). Seven of 11 patients (63.6%) were older than 18 years. The demographic distribution is closer to that of conventional CIC-DUX4 fused sarcoma. On the other hand, male individuals (7 of 11; 63.6%) were predominantly affected (Table 2).

The histologic features of CIC-NUTM1 sarcomas are basically the same as those of classical CIC-DUX4 sarcoma. Most cases showed variable amounts of myxoid stroma, and vague lobulation or nodular structure was typically present. Tumor nuclei are usually monotonous, with vesicular chromatin and distinct nucleoli. CD99 was often diffusely expressed in a membranous pattern, but NKX2.2 negativity and the absence of EWSR1 rearrangement. The paranuclear dotlike staining of CD99 in one tumor in the current study has not been reported heretofore in CIC sarcomas, but it has been referenced in other small round cell neoplasms. Expression of PEA3 subfamily genes, notably ETV4, has been consistently observed in classic CIC sarcoma. However, ETV4 expression in CIC-NUTM1 is approximately 70%, which is not as consistent as in classic CIC sarcoma. Most importantly, the expression and distribution pattern of the NUT protein can be used as a surrogate marker for screening. NUT expression was not reported in conventional CIC-rearranged sarcoma or other sarcomas included in the differential diagnosis.

As FISH tests are mostly used in the current practice, and partner genes fused with CIC have not been studied in most studies, tumors harboring CIC-NUTM1 fusions are presumably underrecognized. In a large series containing 115 cases of CIC sarcoma, CIC-DUX4 fusion was detected in only 57% of cases using 3-color FISH combining CIC and DUX4 probes (located on 4q35 or 10q26). Furthermore, FOXO4 abnormalities were not revealed in the remaining cases. Kao and colleagues also documented 4 cases showing CIC-like histology and CIC FISH break-apart, but CIC fusion transcripts were not identified. In these instances, the presence of CIC-NUTM1 could not be determined without
the aid of more advanced technologies. Accordingly, we suggest that all sarcomas showing a CIC-like histology be screened by NUT and ETV4 immunostaining, with suspected cases subjected to molecular confirmation such as CIC and NUTM1 FISH or RNA sequencing.

One of the most important reasons for the significance of the accurate diagnosis of CIC-NUTM1 sarcoma is its association with poor survival. Compared with Ewing sarcoma, CIC-rearranged sarcoma generally displayed a more aggressive course. For CIC-NUTM1 sarcoma, an even worse prognosis than classical CIC sarcoma was demonstrated. The mean survival of CIC-NUTM1 patients in the series of Le Loarer et al. was 18.6 months, compared with 139 months for CIC-FOXO4 sarcoma. In the current study, all patients died within 15 months, although there was no evidence of metastasis. Their poor survival may be due to the complexity of the spine structure leading to difficulty of local control despite multimodality therapies including surgery, chemotherapy, and radiotherapy. One patient in the current study was treated with the Ewing sarcoma regimen of polyomycin + recombinant endostatin for 1 course but had to undergo surgery because of the rapid worsening of symptoms. Necrosis was not observed in the resected samples. He received further chemotherapy after surgery but with very limited benefit for the progression of local recurrence.

Aside from CIC-DUX4 and CIC-NUTM1 sarcomas with CIC-FOXO4, CIC-LEUTX, and NUTM2-CIC fusion have also been found.26–31 However, the incidence of the latter 3 fusions seems to be quite low. To date, CIC-FOXO4 or NUTM2-CIC fusion has been reported in only 1 or 2 isolated cases. Three cases of CIC-LEUTX fusion have been identified, but they were high-grade angiosarcomas with epithelioid and round cell morphology. Gathering experience from more cases is important to clarify the clinicopathologic features associated with these genetic changes.

In summary, our series improves the understanding of CIC-NUTM1 sarcoma, demonstrating a specific link between genetics and clinicopathologic features. This entity shows a unique predilection for axial bone and a more aggressive course and poorer outcome than classic CIC-rearranged sarcoma. However, because of the rarity of this tumor, definitive conclusions are hard to reach in the current small series, and more cases are needed to be accumulated for confirmation. Awareness of typical CIC morphology and judicious immunohistochemical workups including ETV4 and NUT proteins are crucial for detection. Further assessment by FISH and/or RNA sequencing is optimal to avoid missing cases.

References
2. Italiano A, Sung YS, Zhang L, et al. High prevalence of CIC fusion with double-homeobox (DUX4) transcription factors in EWSR1-negative undifferen-
20. Reynold N, Schwartz BE, Delvecchio M, et al. Oncogenic progression by sequestration of CBP/p300 in transcriptionally inactive hyperacetylated chroma-

Arch Pathol Lab Med