CASE REPORT

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Primary cardiac *CIC*-rearranged undifferentiated sarcoma in an infant

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ABSTRACT

Introduction: Cardiac neoplasms are particularly rare in children, and the majority of these tumors are benign. Approximately 10% of cardiac neoplasms are malignant, including soft tissue sarcomas and lymphomas. Cardiac tumors could also be metastases. Primitive *EWSR1*-negative round or spindle cell undifferentiated sarcoma harboring *CIC* gene translocation is a highly aggressive malignancy mainly occurring in soft tissues. However, it has not yet been described in the heart.

Case presentation: We report a sarcoma that arose from the right ventricle in a 1-year-old girl. Histologically, it was composed of closely arranged small round or oval undifferentiated cells with fibrovascular separation, hyaline degeneration, and geographical necrosis. Immunohistochemically, the neoplastic cells exhibited focal membrane positivity for CD99 and diffuse positivity for WT1 and ETV4. Fluorescent *in situ* hybridization analysis showed *EWSR1*-negative but *CIC*-positive split signals. The breakpoint was also confirmed by whole genome sequencing.

Conclusion: Based on morphological, immunohistochemical and molecular findings, this cardiac mass was diagnosed as *CIC*-rearranged sarcoma.

KEYWORDS
Cardiac, Sarcoma, *CIC*, Child

INTRODUCTION

CIC-rearranged sarcomas are a recently defined group of *EWSR1*-negative Ewing-like primitive round- or spindle-cell sarcomas that commonly harbor translocations between the human homolog of the Drosophila capicua (*CIC*) gene on chromosome 19q13 and the double-homeobox 4 (*DUX4*) gene on chromosome 4q35 or 10q26.3.[1] Rare cases harbor variant translocations between the *CIC* gene and the *FOXO4* gene on chromosome Xq13, *LEUTX* gene on 19q13.2, *NUTM1* gene on 15q14 or *NUTM2A* gene on 10q22.[1-6] Although most of these tumors primarily occur in soft tissue, a few cases which originated in the brain, bone, and viscera, including the lung, stomach, small intestine, colon, prostate, spermatic cord and kidney, have also been reported (Table 1).

Tumors rarely develop in heart, and only 10% of these are malignant in children. Here, we report a rare case of *CIC*-rearranged sarcoma arising from the heart and describe the presenting symptoms, pathological features, and molecular genetics of the tumor.

CASE REPORT

This study was approved by the Ethical Committee of Beijing Children's Hospital. Informed consent was obtained from the patient's parents.

The patient was a 1-year and 7-day-old girl at diagnosis who presented with a poor appetite for 12 days, shortness of breath for 3 days and aggravation for 1 day. Echocardiography showed pericardial effusion and a solid mass occupying the right ventricle. She was diagnosed with grade IV cardiac function and underwent heart surgery. After opening the pericardium, a mass with a diameter of nearly 10 cm was observed at the right anterior ventricle. The mass enveloped the aorta and pulmonary artery. There was no obvious boundary between the mass and right ventricular outflow tract or pulmonary artery root. The mass was 5 cm × 6 cm in size and was resected for pathological examination.

Histological examination revealed that the tumor was composed of closely arranged small round or oval undifferentiated cells in the form of nests with fibrovascular separation, hyaline degeneration and geographical necrosis (Figure 1A). The tumor cells had clear cytoplasm, fine chromatin, small nucleoli and vague boundaries. Some cells had a lateralized nucleus. Cell atypia was evident, and mitotic figures were easily observed (Figure 1B).

Immunohistochemically, the tumor cells exhibited focal membranous positivity for CD99 (Figure 1C), diffuse positivity for WT1 (Figure 1D), TLE1 (Figure 1E), ETV4
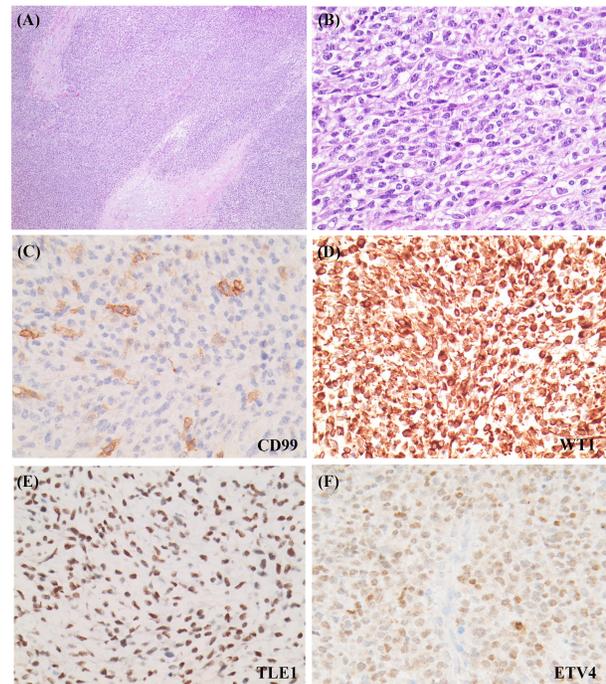

FIGURE 1 Morphological and immunohistochemical features of the sarcoma. (A) H&E sections showed that the tumor was composed of closely arranged small round or spindle undifferentiated cells in the form of nests with fibrovascular separation, hyaline degeneration, and necrosis (100×). (B) At high-power magnification, the tumor cells had clear cytoplasm, fine chromatin, and small nucleoli. Cell atypia was obvious (400×). (C) CD99 staining exhibited focal membrane positivity. (D) WT1 staining exhibited diffuse strong positivity. (E) Tumor cells showed positive TLE1 nuclear staining. (F) ETV4 staining exhibited diffuse positivity.

TABLE 1 Summary of the primary sites of the selected previously reported *CIC*-rearranged tumors[†]

Primary locations	Number of cases	Age (years)	Gender (male/female)	References
Soft tissue of head and neck	25	25 (6–66)	13/12	2, 4, 9–12, 14, 15, 17
Soft tissue of trunk	46	23.5 (7–73)	20/26	1–4, 9, 11–15, 17–19
Soft tissue of limbs	55	33 (5–81)	28/27	1, 2, 4, 9, 11–14, 17–19
Bone	7	36 (19–71)	5/2	2, 9, 12–14
Viscera[‡]	15	33 (13–83)	5/10	2–4, 8, 9, 11, 13, 14, 18, 20
Brain	5	15 (3–64)	2/3	3, 11, 17

Data are shown as *n* or median (range). [†]Repeated cases from more than one study (Italiano et al,[18] Specht et al,[2] Owosho et al,[10] Le Guellec et al,[9] and Mangray et al[8]) were counted only once. One case with brain metastasis in the research of Specht et al[2] was excluded due to a lack of the primary location. [‡]Viscera sites include the stomach, small intestine, colon, lung, kidney, spermatic cord and prostate.

(Figure 1F), SMA and FLI1 and partial positivity for Cyclin D1. They were negative for Myogenin, MyoD1, ERG, Desmin, CD34, CK, Caldesmon, S-100, BCOR, HMB45 and MUC4. The Ki67 index was about 80%.

Molecular analyses with fluorescent *in situ* hybridization (FISH) showed normal fused *EWSR1* gene signals (Figure 2A) but aberrant positive break apart signals of the *CIC* gene (79 out of 100 evaluated tumor cell nuclei, Figure 2B). However, the *CIC-DUX4* (4q35) fusion probe (LBP, Guangzhou, Guangdong, China) failed to detect positive signals (Figure 2C).

To investigate the potential fusion partners, we performed whole genome sequencing (WGS) using the Illumina platform on formalin-fixed and paraffin-embedded (FFPE) sections. The LUMPY pipeline[7] revealed two breakpoints on chr19 (42697321 and 42799319, GRCh37/hg19 assembly), and they were included in the same intra-chromosomal translocation (ITX). One of these two breakpoints (site 42799319 on ch19) was located in the 20th exon of the *CIC* gene. However, due to the short read length of Next-generation sequencing, no reads could be found to map between these two breakpoints. Other previously reported fusion partners (weather inter- or intra-chromosomal translocations), such as *DUX4L*, *FOXO4*, *LEUTX*, *NUTM1* or *NUTM2A*, were all confirmed to be negative (data not shown). Hence, only one breakpoint (site 42799319 on ch19) in the 20th exon of the *CIC* gene, which was believed to be an ITX but with an unknown fusion partner, was found (Figure 2D).
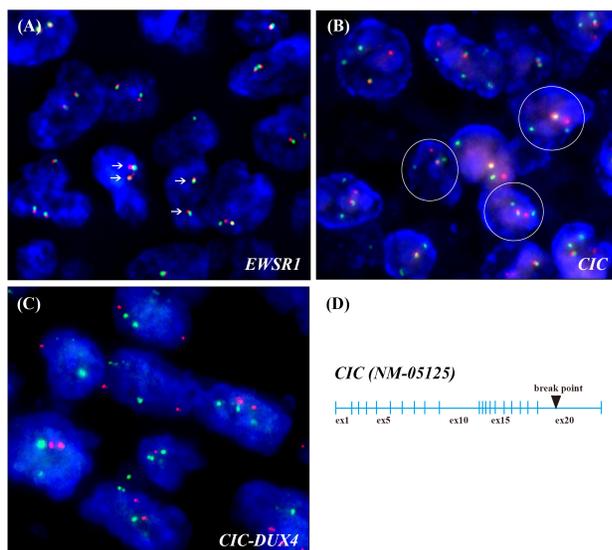

FIGURE 2 Molecular features of the sarcoma. (A) FISH with a dual-color break-apart *EWSR1* probe showed negative signals. Cells without rearrangement showed fused yellow or very close red-green signals (arrows). (B) FISH demonstrated translocation positive (circles) with splitting of the centromeric probe (red) and telemetric probe (green) of the *CIC* gene. (C) FISH with the *CIC-DUX4* fusion probe revealed negative signals. Typical cells showed separate red (*DUX4*) and green (*CIC*) but no fused signals. (D) Schematic diagram of the breakpoint from WGS. Ex, exon. WGS, whole-genome sequencing.

Finally, the diagnosis of an undifferentiated round cell sarcoma with *CIC* gene rearrangement was made based on morphological, immunohistochemical and molecular findings.

DISCUSSION

CIC-rearranged undifferentiated round cell sarcoma is also termed Ewing-like sarcoma because of its morphological and immunohistochemical seminaries with Ewing's sarcoma. However, it has completely distinct genetic characteristics. Ewing's sarcoma typically involves translocation between the *EWSR1* (22q12) or *FUS* (16p11.2) gene and ETS transcription factor family genes, whereas *CIC*-rearranged sarcoma generally involves translocation of the *CIC* gene with the *DUX4* or *DUX4L* gene.[1] Furthermore, non-*DUX4* fusion partners, such as *FOXO4*, *LEUTX*, *NUTM1* or *NUTM2A*, have also been reported.[3-6,8]

Histologically, *CIC*-rearranged sarcoma is similar to Ewing's sarcoma. It is composed of small- to medium-sized round or oval cells arranged in a nodular, lobulated, or lamellar pattern separated by fibrous connective tissue. Sometimes, tumor cell nests are arranged around blood vessels scattered in the background of geographical necrosis.[9,10] Slightly different from Ewing's sarcoma, *CIC*-rearranged sarcomas show significantly higher degrees of lobulation, nuclear pleomorphism, the prominence of nucleoli, spindle cell elements and myxoid changes compared with Ewing's sarcoma.[11] In rare cases, neoplastic cells adopt an epithelioid morphology with occasionally rhabdoid-like cytoplasm or a clear cell change in cytoplasm.[10-12] Additionally, the mitotic count is generally high. In this case study, the tumor was composed of closely arranged small round or oval undifferentiated cells in the form of nests with fibrovascular separation, hyaline degeneration, and geographical necrosis. The tumor cells had clear cytoplasm and small and prominent nucleoli. In addition, mitosis was common (Figure 1A, B). Therefore, the morphological features of this case were consistent with *CIC*-rearranged sarcoma.

Immunohistochemically, *CIC*-rearranged sarcoma also has several characteristics distinct from Ewing and other Ewing-like sarcomas, such as *BCOR*-rearranged sarcoma. CD99 usually shows weak to moderate positivity and partial staining, thereby lacking the strong and diffused membrane pattern observed in Ewing's sarcoma.[2,11,13,14] Other distinguishing immunohistochemical features include strong WT1, TLE1, ETV4 and DUX4 expression in *CIC*-rearranged sarcomas. In contrast, NKX2.2 is expressed in Ewing's sarcomas, and BCOR is expressed in *BCOR*-rearranged sarcoma.[2,9,11-15] Our case showed focal CD99 expression and diffuse positivity for WT1, TLE1 and ETV4 (Figure 1C–F). All of these features indicated *CIC*-rearranged sarcoma.

In most cases, the *CIC* gene is fused with the *DUX4* gene on chromosome 4q35.[2,4] We used a commercially available *CIC-DUX4* fusion gene probe to explore whether the t(4;19)(q35;q13.2) translocation had occurred. However, the result was negative (Figure 2C). We then performed WGS to identify potential fusion partners. WGS revealed one breakpoint in the 20th exon of the *CIC* gene as part of an ITX but with unclear fusion partner (Figure 2D). Exon 20 is the most common rupture region in *CIC*-rearrangement sarcomas.[16] RNA-sequencing, DNA methylation profiling, Genome Walking or even Nanopore Sequencing were considered to be applied to identify the fusion transcript. However only FFPE sections could be obtained, and the DNA/RNA extracted from these sample did not meet the quality requirements. Therefore, the ITX could not be verified so far.

Moreover, recurrent gain or trisomy of chromosome 8 and amplification of the *C-MYC* gene have been reported in *CIC* sarcomas.[1] However, we did not observe the gain of chromosome 8 or amplification of the *C-MYC* gene in this case (Figure S1).

A summary of published data showed that *CIC*-rearranged sarcoma had a relatively balanced ratio for both sexes (Table 1). Although it can affect patients of any age, this tumor mainly occurs in young adults with a median age of 28 years (Table 1). To our knowledge, this 1-year-old girl is one of the youngest patients reported to date.

Although Ewing's sarcomas primarily affect the skeleton, most *CIC*-rearranged tumors arise in soft tissues of the limbs, trunk or head and neck region. Primary occurrence at bones is rare. Visceral locations account for approximately 10% of reported cases, with the kidney being the most frequently attacked organ. The recently reported *CIC-NUTM1* variant appears to specifically target the brain.[3] In our case, the *CIC*-rearranged sarcoma occurring primarily in the heart.

Undifferentiated sarcomas of a cardiac origin are a rare entity among primary heart neoplasms. Their diagnosis is challenging because of the nonspecific clinical presentation and the rarity of cardiac sarcomas. Thus, a comprehensive examination, including routine pathological analyses and molecular genetic approaches, are recommended for an accurate diagnosis. Our case is the first report on *CIC*-translocation sarcoma of the heart. Hence, *CIC*-rearranged sarcoma should be considered as a potential differential diagnosis when facing undifferentiated sarcomas in this anatomic region.

CONSENT FOR PUBLICATION

Consent was obtained from the patient's parents.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

REFERENCES

1. Smith SC, Buehler D, Choi EY, McHugh JB, Rubin BP, Billings SD, et al. CIC-DUX sarcomas demonstrate frequent MYC amplification and ETS-family transcription factor expression. Mod Pathol. 2015;28:57-68.

2. Specht K, Sung YS, Zhang L, Richter GH, Fletcher CD, Antonescu CR. Distinct transcriptional signature and immunoprofile of *CIC-DUX4* fusion-positive round cell tumors compared to *EWSR1*-rearranged Ewing sarcomas: further evidence toward distinct pathologic entities. Genes Chromosomes Cancer. 2014;53:622-633.

3. Le Loarer F, Pissaloux D, Watson S, Godfraind C, Galmiche-Rolland L, Silva K, et al. Clinicopathologic features of *CIC-NUTM1* sarcomas, a new molecular variant of the family of *CIC*-fused sarcomas. Am J Surg Pathol. 2019;43:268-276.

4. Mantilla JG, Ricciotti RW, Chen E, Hoch BL, Liu YJ. Detecting disease-defining gene fusions in unclassified round cell sarcomas using anchored multiplex PCR/ targeted RNA next-generation sequencing-molecular and clinicopathological characterization of 16 cases. Genes Chromosomes Cancer. 2019;58:713-722.

5. Huang SC, Zhang L, Sung YS, Chen CL, Kao YC, Agaram NP, et al. Recurrent *CIC* gene abnormalities in angiosarcomas: A molecular study of 120 cases with concurrent investigation of *PLCG1*, *KDR*, *MYC*, and *FLT4* gene alterations. Am J Surg Pathol. 2016;40:645-655.

6. Lake JA, Donson AM, Prince E, Davies KD, Nellan A, Green AL, et al. Targeted fusion analysis can aid in the classification and treatment of pediatric glioma, ependymoma, and glioneuronal tumors. Pediatr Blood Cancer. 2020;67:e28028.

7. Layer RM, Chiang C, Quinlan AR, Hall IM. LUMPY: a probabilistic framework for structural variant discovery. Genome Biol. 2014;15:R84.

8. Mangray S, Kelly DR, LeGuellec S, Fridman E, Aggarwal S, Shago M, et al. Clinicopathologic features of a series of primary renal *CIC*-rearranged sarcomas with comprehensive molecular analysis. Am J Surg Pathol. 2018;42:1360-1369.

9. Le Guellec S, Velasco V, Pérot G, Watson S, Tirode F, Coindre JM. ETV4 is a useful marker for the diagnosis of *CIC*-rearranged undifferentiated round-cell sarcomas: A study of 127 cases including mimicking lesions. Mod Pathol. 2016;29:1523-1531.

10. Owosho AA, Estilo CL, Huryn JM, Zhang L, Fletcher CDM, Antonescu CR. Head and neck round cell sarcomas: a comparative clinicopathologic analysis of 2 molecular subsets: Ewing and *CIC*-rearranged sarcomas. Head Neck Pathol. 2017;11:450-459.

11. Yoshida A, Goto K, Kodaira M, Kobayashi E, Kawamoto H, Mori T, et al. *CIC*-rearranged sarcomas: A study of 20 cases and comparisons with Ewing sarcomas. Am J Surg Pathol. 2016;40:313-323.

12. Yamada Y, Kuda M, Kohashi K, Yamamoto H, Takemoto J, Ishii T, et al. Histological and immunohistochemical characteristics of undifferentiated small round cell sarcomas associated with *CIC-DUX4* and *BCOR-CCNB3* fusion genes. Virchows Arch. 2017;470:373-380.

13. Gambarotti M, Benini S, Gamberi G, Cocchi S, Palmerini E, Sbaraglia M, et al. *CIC-DUX4* fusion-positive round-cell sarcomas of soft tissue and bone: a single-institution

morphological and molecular analysis of seven cases. Histopathology. 2016;69:624-634.

14. Brčić I, Brodowicz T, Cerroni L, Kashofer K, Serbanescu GL, Kasseroler MT, et al. Undifferentiated round cell sarcomas with *CIC-DUX4* gene fusion: expanding the clinical spectrum. Pathology. 2020;52:236-242.

15. Siegele B, Roberts J, Black JO, Rudzinski E, Vargas SO, Galambos C. DUX4 immunohistochemistry is a highly sensitive and specific marker for *CIC-DUX4* fusion-positive round cell tumor. Am J Surg Pathol. 2017;41:423-429.

16. Yoshida A, Arai Y, Kobayashi E, Yonemori K, Ogura K, Hama N, et al. *CIC* break-apart fluorescence *in-situ* hybridization misses a subset of *CIC-DUX4* sarcomas: a clinicopathological and molecular study. Histopathology. 2017;71:461-469.

17. Kao YC, Sung YS, Chen CL, Zhang L, Dickson BC, Swanson D, et al. ETV transcriptional upregulation is more reliable than RNA sequencing algorithms and FISH in diagnosing round cell sarcomas with *CIC* gene rearrangements. Genes Chromosomes Cancer. 2017;56:501-510.

18. Italiano A, Sung YS, Zhang L, Singer S, Maki RG, Coindre JM, et al. High prevalence of *CIC* fusion with double-homeobox (DUX4) transcription factors in *EWSR1*-negative undifferentiated small blue round cell sarcomas. Genes Chromosomes Cancer. 2012;51:207-218.

19. Ko JS, Marusic Z, Azzato EM, Farkas DH, Van Arnam J, Seiwerth S, et al. Superficial sarcomas with *CIC* rearrangement are aggressive neoplasms: a series of eight cases. J Cutan Pathol. 2020;47:509-516.

20. Bremmer F, Fichtner A, Triefenbach R, Inniger R, Mayer P, Lukat L, et al. *CIC* fusion-positive sarcoma of the spermatic cord. Virchows Arch. 2019;474:253-257.

SUPPORTING INFORMATION

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