Primary cardiac CIC-rearranged undifferentiated sarcoma in an infant

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Funding source
Special Fund of The Pediatric Medical Coordinated Development Center of Beijing Hospitals Authority [XTCX201806]; Beijing Hospitals Authority Clinical medicine Development of Special Funding Support [XMLX202121]; Beijing Advanced Innovation Center for Big Data-Based Precision Medicine, Beihang University & Capital Medical University, Beijing [BHME-201904].

Received: 10 September, 2020
Accepted: 7 December, 2020

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. 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 1F).

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 |
| Viscera1                    | 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,2 Specht et al,2 Owosho et al,10 Le Guellec et al,9 and Mangray et al8) were counted only once. One case with brain metastasis in the research of Specht et al2 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 EWSRI 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 revealed two breakpoints on chr19 (42697321 and 42799319, GRCh37/hg19 assembly), and they were included in the same intrachromosomal translocation (ITX). One of these two breakpoints (site 42799319 on chr19) 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 intrachromosomal 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 chr19) 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 EWSRI 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 telmeric 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. Furthermore, non-DUX4 fusion partners, such as FOXO4, LEUTX, NUTM1 or NUTM2A, have also been reported. 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. 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. In rare cases, neoplastic cells adopt an epithelioid morphology with occasionally rhabdoid-like cytoplasm or a clear cell change in cytoplasm. 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. 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. 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.2 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.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Zhang M, Yang Y, Guan X, Yao X, Guo Y, He L. Primary cardiac CIC-rearranged undifferentiated sarcoma in an infant. Pediatr Investig. 2021;00:1-5. https://doi.org/10.1002/ped4.12264