Clinical Sequencing of High-Grade Undifferentiated Sarcomas: A Case Series and Report of an Aggressive Primary Cardiac Tumor With Multiple Oncogenic Drivers

Chandan Kumar-Sinha, PhD^{1,2}; Bailey Anderson, MPH³; Amer Heider, MD²; Josh N. Vo, PhD^{1,4}; Dan R. Robinson, PhD^{1,2}; Yi-Mi Wu, PhD^{1,2}; Arul M. Chinnaiyan, MD, PhD^{1,2,5,6}; and Rajen Mody, MD, MS^{1,3,5}

INTRODUCTION

Undifferentiated sarcomas (UDS) present with no defined histologic or genetic features and represent 20% to 25% of all soft tissue sarcomas.¹⁻³ Here, we describe a case series of 13 UDS tumors analyzed through the integrative clinical sequencing (ICS) program MI_Oncoseq at the University of Michigan. We identified “actionable” aberrations, including diagnostic gene fusions and therapeutically targetable aberrations, in a majority of cases. One unusually aggressive case of cardiac UDS in a 3-year-old girl is described, in which tumor sequencing identified multiple actionable alterations, and gene expression signature⁴⁻⁷ suggested a tumor of neuroectoderm/neural crest origin.

MATERIALS AND METHODS

Patients were enrolled in institutional review board (IRB)-approved studies at the University of Michigan (IRB Nos. HUM00046018, HUM00067928, and HUM00056496). All patients and/or patients’ parents provided informed consent. Samples were processed per the methodologies described previously using standard protocols in our Clinical Laboratory Improvement Amendments-compliant sequencing laboratory as described previously.^{4,7,8}

Gene expression data compiled from St Jude Cloud^{9,10} and in-house MI_Oncoseq data set⁷ for a total of 16 pediatric melanoma (MiOncoSeq [n = 8], PECAN [n = 8]), 217 neuroblastoma (MiOncoSeq [n = 57], PECAN [n = 160]), and 74 pediatric rhabdomyosarcoma (MiOncoSeq [n = 29], PECAN [n = 45]) cases were used to perform principal component analysis (PCA) for log-transformed gene expression (FPKM) of selected markers: neuroblastoma (*NCAM1*, *B4GALNT1*, *PHOX2A*, *PHOX2B*, *TH*, *DDC*, *DBH*, *GAP43*, *CHRNA3*)¹¹; rhabdomyosarcoma (*MYOD1*, *DES*, *FOXO1*, *PAX3*, *PAX7*, *FGFR4*, *MYOG*)^{12,13}; and melanoma (*MLANA*, *SOX10*, *S100A1*, *S100B*)^{13,14} (Data Supplement).

RESULTS

High-grade UDS cases included in the study are summarized in Table 1. Five of the 13 patients

harbored loss of tumor suppressor genes, *RB1*, *ATRX*, and *TP53*, typically seen across sarcomas.¹⁵ Eight patients showed molecular aberrations with potential implications for diagnosis and/or treatment (defined as “actionable”⁷), and 4 patients received treatments matched to their tumor’s genotype. One case initially diagnosed as a mesenchymal undifferentiated round cell sarcoma from a primary mass in the left kidney of a 45-year-old male (TP_2329) showed the presence of *NAB2*-*STAT6* gene fusion and associated high expression of *NAB2*, *NAB1*, and *IGF2*, providing definitive diagnosis of a solitary fibrous tumor.^{16,17} Another case of UDS of a right thigh mass from a 23-year-old female (TP_2023) showed the presence of *CIC*-*CITED1* gene fusion. *CIC*-rearranged sarcomas define a new class of “Ewing-like” small round cell tumors,^{1,18} with *CIC* fusions involving diverse 3’ partner genes including *DUX4*/*DUX4L*¹⁹⁻²¹ and others.^{22,23} Here we report a new fusion partner, with almost full length *CIC* (break inside the last exon, 21) fused in frame to *CITED1* (break inside exons 4 and 5, including the *CITED* domain). Importantly, *CIC*-*CITED1* fusion was accompanied by outlier expression of *ETS* family genes^{19,21,24} and *CCNE1*²⁵ as noted for *CIC*-rearranged sarcomas (Data Supplement).

Three UDS tumors showed high tumor mutation burden. These cases included a 34-year-old male (MO_1991) and a 60-year-old male (MO_2207) with lung nodules and a 44-year-old female (TP_2102) with tumor in the right arm. Interestingly, MO_1991 also showed the presence of *BRAF* p.V600E mutation (and mutations in *TP53*, *PIK3CA*, and *PTEN*) and a strong signature of ultraviolet-induced mutations, suggestive of skin origin. Using the cancer of unknown primary identification⁴ algorithm supported a melanoma diagnosis (Data Supplement). These observations led to a change in the diagnosis to “sarcomatoid melanoma,” which was followed up by treatment with the checkpoint blockade inhibitor pembrolizumab. The third case of a 44-year-old female (TP_2102) with hypermutation in the tumor of the right arm was recorded as sarcomatoid/sarcoma (Data Supplement).

ASSOCIATED CONTENT

Appendix

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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TABLE 1. Summary of Salient Molecular Aberrations Noted in UDS Analyzed Through MI_Oncoseq

Patient ID	Age (years), Sex; Biopsy Site	Notable Aberrations	Potential Therapies/Clinical Trials	Treatment Summary	Outcome
MO_1554	50, F; groin	Complex copy-number profile; SNVs: RB1 exon1 splice site mutation; LOH by UPD; outlier expression of FGFR1	FGFR inhibitor?	Radiation, FGF plus PD-1 inhibitor, chemotherapy	3 months SD on targeted treatment; deceased
MO_1824	62, M; lung	ATRX p.W1634* stopgain; homozygous deletion: PTEN, RB1, TP53; amplification: MYC, CCNE1; outlier expression: CCNE1, RSP02, WSP1, FAT4	CHK1 inhibitor, WNT pathway/porcupine inhibitor	Chemotherapy, PD-1 inhibitor	Deceased
MO_1936	51, M; lung	TP53 homozygous deletion		Chemotherapy, PD-1 inhibitor, surgery, mTOR inhibitor	Deceased
TP_2153	60, F; uterus	TP53 p.Y163C; recurrent, LOH by UPD; RB1 copy loss and RB1-DCLK2 gene fusion		Chemotherapy, radiation	Unknown
MO_1839	41, M; lung	CUL4A splice acceptor (exon 17); outlier expression: FGF7, FGF5; high expression: PDGFRB, VEGFB, VEGFC, FGFR1		Chemotherapy, radiation, pazopanib, PD-1 inhibitor	Deceased
TP_2329	45, M; kidney	NAB2-STAT6 (and STAT6-NAB2) gene fusion	Diagnosis: SFT	Surgery	Alive; NED
TP_2023	23, F; thigh	CIC-CITED1 gene fusion	Diagnosis: CIC rearranged sarcoma	Chemotherapy, ponatinib, pazopanib	Deceased
MO_2188	70, M; chest	MDM2, CDK4 amplification (chr12); outlier expression	MDM2 inhibitors (TP53 is wild type) CDK4 inhibitors (RB1 is wild type)	Surgery, radiation	Unknown
MO_2207	60, M; lung	Hypermethylation: TP53 p.R248Q, LOH; PIK3CA p.N345K, hotspot, LOH; RB1 p.Q689* stopgain, LOH; B2M p.X120YextX?; TERF1 p.R147* stopgain	PD-1/PD-L1 blockade immunotherapy PI3K/AKT/mTOR pathway inhibitors	Chemotherapy plus PDGFRa inhibitor, surgery	Deceased
TP_2102	44, F; arm	Hypermethylation; TP53 p.Q167H, LOH by copy loss; BRAF cryptic fusion	PD-1/PD-L1 blockade immunotherapy	Notch inhibitor, radiation, chemotherapy	Deceased
MO_2114	50, F; liver	TSC1 p.E423* stopgain, LOH; CDKN2A deletion; NF2 fusion, copy loss	PI3K/AKT/mTOR pathway inhibitors CDK4 inhibitors (RB1 is wild type)	Everolimus	Deceased
MO_1991	34, M; lung	Hypermethylation (ultraviolet signature); BRAF p.V600E, TP53 p.V218G, LOH by UPD, PIK3CA p.R4_P104 in frame deletion (also amplification), PTEN p.A12dup, in frame insertion, IRS1 p.Arg302fs insertion	Diagnosis: sarcomatoid melanoma; PD-1/PD-L1 blockade immunotherapy BRAF/MEK inhibitor, PI3K/AKT/mTOR pathway inhibitors	PD-1 inhibitor, radiation, dabrafenib	Deceased
PO_3253	3, F; heart	MYCN amplification, NRAS p.Q61R, PIK3CA p.Q546R and p.R93Q, ERBB3 p.V104L, p.T389K and p.L924I, ARID1A p.S1558fs deletion; outlier expression of MYCN, FOXD3, DDX1, SOX10	ERBB2/ERBB3 inhibitors, PI3K/AKT/mTOR pathway inhibitors MEK inhibitors, BRD inhibitors	Chemotherapy	Rapid clinical decline; deceased

Abbreviations: F, female; FGF, fibroblast growth factor; LOH, loss of heterozygosity; M, male; NED, no evidence of disease; PD-1, programmed cell death-1; PD-L1, programmed cell death 1 ligand 1 (CD274); SD, stable disease; SFT, solitary fibrous tumor; SNV, single nucleotide variant; UDS, undifferentiated sarcomas; UPD, uniparental disomy.

CASE REPORT OF AN UNUSUALLY AGGRESSIVE HIGH-GRADE UNDIFFERENTIATED ATRIAL SARCOMA

Clinical History

A 3-year-old female presented with a recent history of fevers, vomiting, facial dropping, and confusion (PO_3253). Electrocardiogram revealed right atrial enlargement; ECG and chest computed tomography showed a large right atrial tumor. The patient underwent a subtotal excision of the right atrial tumor and adjacent right atrial wall, repair of atrial septal defect, and tricuspid valve repair. Pathology of the mass was consistent with high-grade UDS with spindle cell, round cell, and focal epithelioid features. A positron emission tomography (PET) scan showed a hypermetabolic mass in the right atrium, extension of metabolic activity into the right anterior mediastinum, a hypermetabolic mass in the left atrium, and metabolic evidence for fluorodeoxyglucose-avid osseous metastases. Approximately 2 weeks later, in preparation for beginning chemotherapy, a second PET scan showed rapid disease progression including increased metabolic activity at the known disease sites, new lung nodules, and new, intense bilateral adrenal uptake. The patient then started treatment according to ARST0332, arm D, consisting of doxorubicin and ifosfamide. After completing the first cycle of therapy, the patient experienced encephalopathy and eventual

significant mental deterioration characterized by increasing hypertonia of her extremities and nonresponsiveness. Brain magnetic resonance imaging showed numerous embolic strokes (some present since diagnosis) and multiple areas of hemorrhage. Unfortunately, because of her declining condition and no additional viable interventions, the patient was transitioned to comfort care and expired approximately 2 months after original presentation. Because of the aggressive nature of the patient's disease presentation, she was enrolled in our ICS (paired normal/tumor DNA sequencing and tumor transcriptome sequencing) study and received mandatory pre-enrollment genetic counseling in which no family history of cancer was noted.

Pathology

The histologic sections showed high-grade infiltrative malignant neoplasm with necrosis and hemorrhage involving both atrial samples (Fig 1A). The tumor had multiple patterns, with spindle cell, round cell, and focal epithelioid features (Fig 1B). Nuclear pleomorphism and numerous mitotic figures were observed (Fig 1C). The malignancy was negative for rhabdomyosarcoma and angiosarcoma markers including desmin, myogenin, CD31, and CD34. The sarcoma was positive for SOX-10 and PgP9.5 (Fig 1D), favoring a UDS diagnosis over malignant peripheral nerve sheath tumor.

FIG 1. Histopathology of the high-grade undifferentiated sarcoma, round cell tumor from the left atrial mass of PO_3253. (A) Hematoxylin and eosin (H&E) staining, $\times 4$. (B) H&E staining, $\times 10$. (C) H&E staining, $\times 40$. (D) Immunohistochemical staining with SOX10 antibody.

FIG 2. Copy number profile of PO_3253 on the basis of targeted exome capture of 1,704 genes. Overall diploid genome with a copy gain of chromosome 1q and focal amplification of MYCN. LOH, loss of heterozygosity.

Sequencing Results

An overall diploid genome with a high-level focal amplification of MYCN was detected, accompanied by an outlier expression of MYCN (Fig 2). In addition, a total of 7 somatic missense mutations were detected including hotspot activating mutations in *NRAS*, *ERBB3*, and *PIK3CA* (Table 2). MYCN amplification represents the most common driver aberration in neuroblastoma,²⁶ as well as in subsets of medulloblastoma, retinoblastoma, and others.²⁷⁻³⁰ Interestingly, in cBioportal summarizing 158 nonredundant studies, including 42,027 cancer samples, no sarcoma cases showing copy number aberrations in MYCN were noted (Data Supplement). The *NRAS* p.Q61R activating mutation is noted in approximately 20% of melanomas,³¹⁻³⁴ in 20% to 30% of thyroid carcinoma,³⁵ and in small but significant numbers of other cancer types including bladder urothelial carcinoma, malignant peripheral nerve sheath tumor, and neuroblastoma.³⁶ *PIK3CA* activating mutations have been noted in 4% to 5% of melanoma.³² *ERBB3* (*HER3*) p.V104L (p.T389K and p.L924I)³⁷ and *PIK3CA* p.R93Q (and p.Q546R)³⁸⁻⁴⁰ represent additional oncogenic

mutations seen in diverse solid cancers. The presence of concomitant hotspot driver mutations in 3 oncogenes, *NRAS*, *PIK3CA*, and *ERBB3*, is extremely rare, particularly considering the young age of this patient, and likely accounts for the remarkably aggressive and swiftly fatal course of the disease.

Gene Expression Signature Suggestive of a Neuroectodermal/Neural Crest Origin of this Tumor

RNAseq data from the tumor sample showed outlier expression of MYCN, *NBAS*, *FOXD3*, *DDX1*, *SOX10*, *S100B*, *PAX3* (Fig 3), supporting a neuroectodermal/neural crest lineage of the tumor cells. The transcription factor forkhead box D3 (*FOXD3*) is associated with the development of neural crest cells, downregulated in the neural crest-derived neuroblastoma,⁴¹ even as it promotes the progression of melanoma, another neural crest-derived tumor, through upregulation of *PAX3*,⁴² which also shows outlier expression in this case. *SOX10*, a nuclear transcription factor, is a sensitive, specific marker for melanoma⁴³; however, no expression of the other melanoma markers *MLANA* and *TYR*

TABLE 2. Somatic Mutations Detected in PO_3253

Gene	Location	Protein Change	Effect	Exon	Variant Reads (tumor)	Variant Reads (normal)	Copy Number	Expression (FPKM)
<i>NRAS</i>	chr1:115256529	p.Q61R	Missense	3	412 of 886 (47)	0 of 845 (0)	2	7.0
<i>PIK3CA</i>	chr3:178936095	p.Q546R	Missense	10	358 of 768 (47)	0 of 592 (0)	2	11.5
<i>PIK3CA</i>	chr3:178916891	p.R93Q	Missense	2	539 of 1,194 (45)	0 of 1,136 (0)	2	11.5
<i>ERBB3</i>	chr12:56486587	p.T389K	Missense	10	267 of 581 (46)	0 of 560 (0)	2	117.3
<i>ERBB3</i>	chr12:56478854	p.V104L	Missense	3	359 of 795 (45)	0 of 756 (0)	2	117.3
<i>ERBB3</i>	chr12:56492620	p.L924I	Missense	23	255 of 521 (49)	0 of 519 (0)	2	117.3
<i>ARID1A</i>	chr1:27101386	p.S1558fs	Frameshift	18	14 of 209 (7)	0 of 199 (0)	2	80.0

NOTE. Data are presented as No. of total (%).

Abbreviation: FPKM, fragments/Kb/million reads.

FIG 3. Expression profile of selected genes as indicated in PO_3253 (red), in the context of gene expression in our pan-cancer compendium comprising approximately 3,000 samples arranged in ascending order (see Appendix text for additional details).

at best supports a de-differentiated melanoma.^{3,44} DDX1 is coamplified with MYCN and shows outlier expression, with conflicting reports on its association with survival in MYCN-amplified neuroblastoma.^{45,46} In an attempt to resolve the diagnosis of this tumor on the basis of its gene expression profile, we performed a PCA using distinct markers of neuroblastoma, rhabdomyosarcoma, and melanoma, and as seen in Fig 4, the PO_3253 expression profile falls into the indeterminate region where the 3 tumor types are indistinct. This supports the histopathologic assessment of this tumor as of neuroectodermal/neural crest origin with no specific tissue type assignment.

DISCUSSION

ICS analysis of UDS tumors in this study provided diagnostic insights and identified aberrations with therapeutic implications in 10 of 13 patients (Table 1). The 3 patients found to harbor high tumor mutation burden could potentially benefit from immune checkpoint blockade immunotherapy, whereas the patient with *BRAF* p.V600E in MO_1991 could benefit from BRAF or mitogen-activated protein kinase (MEK) inhibitor therapies. Similarly, MO_2188 with amplification of MDM2 and CDK4 on chr12, accompanied by outlier expression of these genes, could be considered for MDM2 or CDK4/6 inhibitors. In the case of the pediatric atrial cardiac tumor (PO_3253), MYCN amplification and outlier expression, NRAS p.Q61R, ERBB3 p.V104L, and PIK3CA p.R93Q mutations represent multiple therapeutic options, including bromodomain and extra-terminal motif inhibitors, MEK inhibitors or phosphoinositide 3-kinase inhibitors or combination therapies.

AFFILIATIONS

¹Michigan Center for Translational Pathology, University of Michigan, Ann Arbor, MI

²Department of Pathology, University of Michigan, Ann Arbor, MI

³Department of Pediatrics, University of Michigan, Ann Arbor, MI

⁴Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI

⁵Rogel Cancer Center, University of Michigan, Ann Arbor, MI

⁶Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI

CORRESPONDING AUTHOR

Rajen Mody, MD, MS, Division of Pediatric Hematology Oncology, Pediatric Developmental Therapeutics and Precision Oncology Program, Michigan Medicine, D4207 Medical Professional Building, PO Box 5718, Ann Arbor, MI 48109; Twitter: @RajenMody; e-mail: rmody@umich.edu.

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AUTHOR CONTRIBUTIONS

Conception and design: Chandan Kumar-Sinha, Amer Heider, Dan R. Robinson, Yi-Mi Wu, Rajen Mody

FIG 4. Principal component analysis for log-transformed gene expression in FPKM of selected markers (Materials and Methods). MEL, pediatric melanoma; NBL, neuroblastoma; RHB, pediatric rhabdomyosarcoma.

PO_3253 represents an exceedingly rare primary malignant cardiac tumor, with a majority of these malignant tumors classified as sarcomas and a small subset reported as UDS.⁴⁷⁻⁵¹ Overall, our sequencing findings suggest that this case of the pediatric primary cardiac high-grade UDS had a molecular profile of neuroectoderm/neural crest–derived tumors, even as no definitive assignment to melanoma or neuroblastoma could be made.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Bailey Anderson

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Arul M. Chinnaiyan

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APPENDIX

Additional Details of Cases With Diagnostic Molecular Findings

TP_2329. In this 45-year-old male, the primary tumor and the sequenced sample were both located in the kidney. The initial diagnosis was mesenchymal undifferentiated round cell sarcoma, which was defined as solitary fibrous tumor (SFT) on the basis of the presence of NAB2-STAT6 gene fusion. Dedifferentiated SFTs have been observed in a wide range of locations, most commonly in the retroperitoneum and deep soft tissues (Olson NJ, et al: Arch Pathol Lab Med 142:761-766, 2018). We did note 1 report of dedifferentiated SFT in kidney as well (Magro G, et al: APMIS 116:1020-1025, 2008), although it seems exceedingly rare, making the fusion-based diagnosis as reported here, definitive.

TP_2023. In this 23-year-old female, the primary tumor and the sequenced sample were both located in the right thigh. The biopsy consisted of skeletal muscle focally infiltrated by poorly differentiated malignant neoplasm composed of rounded cells, which was diagnosed as undifferentiated soft tissue sarcoma, negative for immunostaining with CD99, desmin (DES), AE1/AE3, CAM 5.2, EMA, LCA, S100, and CD34. The detection of CIC-CITED1 gene fusion in this tumor provided the novel molecular category among undifferentiated round cell (non-Ewing) sarcomas (Doyle LA: Cancer 120:1763-1774, 2014). CIC-rearranged sarcomas define a new class of “Ewing Like” small round cell tumors (Doyle LA: Cancer 120:1763-1774, 2014; Yoshida A, et al: J Surg Pathol 40:313-323, 2016), with CIC fusions involving diverse 3' partner genes including *DUX4*/*DUX4L* (Kawamura-Saito M, et al: Human molecular genetics 15:2125-2137, 2006; Italiano A, et al: Chromosomes Cancer 51:207-218, 2012; Smith SC, et al: Mod Pathol 28:57-68, 2015), *FOXO4* (Sugita S, et al: Am J Surg Pathol 38:1571-1576, 2014), and *LEUTX* (Huang SC, et al: Am J Surg Pathol 40:645-655, 2016), etc.

MO_1991. In this 34-year-old male, although the primary tumor was located on the left side of the neck, the sequenced sample was obtained from lung needle biopsy. The change in diagnosis of this patient to sarcomatoid melanoma, on the basis of molecular aberrations including ultraviolet signature in the hypermutated genome and the presence of BRAF p.V600E mutation, was perhaps most surprising and clinically actionable.

MI_Oncoseq. The MI_Oncoseq expression compendium (referred to in Fig 3) is a pan-cancer data set composed of adult and pediatric cancers and hematologic malignancies described in our previous publications encompassing more than 2,000 clinical cancer samples across 40 cancer types (Vo JN, et al: Cell 176:869-881.e13, 2019), with subsets including 500 cases of adult, advanced, metastatic solid cancers (Robinson DR, et al: Nature 548:297-303, 2012), 91 pediatric cases (Mody RJ, et al: JAMA 314:913-925, 2015), and 150

metastatic castrate resistant prostate cancers (Robinson D, et al: Cell 161:1215-1228, 2015). The total number of pediatric cases currently represented in this compendium includes more than 500 pan-cancer cases, including both hematologic and solid cancers (encompassing epithelial tumors, brain cancers, and bone and soft tissue tumors; these are described in a separate manuscript under preparation).

Additional Discussion of Primary Cardiac Tumors

In the pediatric population, primary cardiac undifferentiated sarcomas (UDS) is extraordinarily rare and is described only on the basis of small case series (Uzun O, et al: Orphanet J Rare Dis 2:11, 2007; Padalino MA, et al: Circulation 126:22-30, 2012; Sallee D, et al: Cardiol Young 9:155-162, 1999; Burke A, Virmani R: Cardiovasc Pathol 17:193-198, 2008). These tumors can be visualized through radiographic imaging; however, histopathologic review is essential for diagnosis and to develop a treatment plan. Histologically, these tumors reveal multiple patterns including storiform spindle cell, myxoid round cell, and epithelioid undifferentiated morphology. Irregular nuclei with pleomorphic and mitotic figures are easily recognized; necrosis is typically present (Tazelaar H, et al: WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. Lyon, France: IARC; 2015.). Immunohistochemical (IHC) profiles of UDS are typically noted to be positive for vimentin with high indices of Ki67 proliferation (40% to 60%), and negative for DES, CD31, CD34, CD99, HMB45, smooth muscle actin, and S-100 (Turhan N, et al: Cardiovasc Pathol 20:232-7, 2011), similar to our case. In other cases of cardiac UDS, histologic examinations revealed similar features, including spindle cells, irregular nucleoli, high mitotic activity, and IHC staining positive for vimentin (Orlandi A, et al: J Thor Cardiovasc Surg 124:192-193, 2002; Furukawa N, et al: J Cardiothorac Surg 7:96, 2012). PO_3253 tumor RNAseq data also shows high expression of vimentin and Ki67 and no expression of DES, and EMA (MUC-1; Fig 3). Cases reported in pediatric patients with primary cardiac UDS exhibited some histologic features similar to those noted in our case, including nuclear pleomorphism (Orlandi A, et al: J Thor Cardiovasc Surg 124:192-193, 2002; Collins CL, et al: J Pediatr Hematol Oncol 36:e237-e240, 2014).

Neural crest embryonic cells possess a multipotent differentiation capacity that generates diverse tissue lineages including melanocytes (melanoma), adrenal cells (neuroblastoma, pheochromocytoma), peripheral nervous system/Schwann cells (malignant peripheral nerve sheath tumor, ganglioneuroma, melanoma), and others (Maguire LH, et al: Dev Dynam 244:311-322, 2015; Dunder P, Ehrmann J: Stem Cells and Cancer Stem Cells, Volume 1:29-40, 2015; Adameyko I, et al: Cell 2009;139:366-379, 2009). During embryogenesis, cells derived from neural crest are known to migrate to the developing heart to form the cardiac ventricular and major vascular septum (Waldo K, et al: Dev Biol 196:129-44, 1998; Bergwerff M, et al: Circ Res 82:221-231, 1998). We speculate that the primary cardiac high-grade UDS noted here may be derived from such cells.