Whole genome transcriptome analysis in a case of a neonatal soft tissue sarcoma with YWHAE:NUTM2B fusion

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Abstract

Soft tissue sarcomas in neonates are rare and heterogeneous tumors. We report an aggressive neonatal undifferentiated round cell sarcoma with a YWHAE:NUTM2B fusion. The tumor was identified antenatally and the neonate underwent surgical resection at four days of age. Whole-genome and transcriptome sequencing of tumour and germline was undertaken to provide molecular characterization and elucidate possible novel therapies. In addition to molecular characterization of a YWHAE:NUTM2B fusion, RNA expression outliers were described. Targeted therapy was not pursued due to rapid clinical decline. Understanding the genomic profile of rare tumors remains important in the development of novel therapeutic strategies.

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Figures 2

Supplemental information file 1

Abbreviations key

CCSK	Clear Cell Sarcoma of the Kidney
MRI	Magnetic Resonance Imaging
POG	Personalized OncoGenomics
RTK	Receptor Tyrosine Kinases
YWHAE	Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Epsilon
URCS	Undifferentiated round cell sarcoma
WGTA	Whole Genome Transcriptome Analysis
SHH	Sonic Hedgehog
MAPK	Mitogen-Activated Protein Kinase
mTOR	mammalian Target Of Rapamycin

Abstract

Soft tissue sarcomas in neonates are rare and heterogeneous tumors. We report an aggressive neonatal undifferentiated round cell sarcoma with a YWHAE:NUTM2B fusion. The tumor was identified antenatally and the neonate underwent surgical resection at four days of age. Whole-genome and transcriptome sequencing of tumour and germline was undertaken to provide molecular characterization and elucidate possible novel therapies. In addition to molecular characterization of a YWHAE:NUTM2B fusion, RNA expression outliers were described. Targeted therapy was not pursued due to rapid clinical decline. Understanding the genomic profile of rare tumors remains important in the development of novel therapeutic strategies.

Introduction

Malignancies diagnosed in the neonatal period are rare and account for 2% of pediatric cancers.[1,2] Soft tissue sarcomas comprise 8-12% of all neonatal malignancies, with rhabdomyosarcoma being the most common histologic subtype (32.8%), followed by infantile fibrosarcoma (24.5%) and malignant rhabdoid tumor (14.2%).[3–5] Other neonatal non-rhabdomyosarcoma soft tissue sarcomas are less frequent.[3] We report a case of a neonate with a congenital undifferentiated round cell sarcoma (URCS) with an underlying YWHAE:NUTM2B fusion who had whole genome transcriptome analysis (WGTA) of tumour performed to characterize its molecular features. YWHAE:NUTM2B is a rare fusion gene described in pediatric clear cell sarcoma of the kidney (CCSK) and adult endometrial stromal tumours[6,7], but only three cases have been identified in URCS of infancy. These cases demonstrated aggressive behavior where two of three infants died within months of birth.[8,9]

Clinical History

A newborn male was delivered by elective caesarian section for a large back mass initially identified on routine antenatal ultrasound. Family history was non-contributory. Postnatal ultrasound showed a 13.0 cm exophytic soft tissue mass along the left upper back with features uncharacteristic of a vascular malformation or hemangioma. Magnetic resonance imaging (MRI) performed one day post-birth showed the mass arising from the left posterolateral chest wall with signal characteristics suggestive of intermixed enhancing vascular and fibrous or hemorrhagic tissue. A near total-surgical resection was performed four days post-birth with positive margins and lymphovascular invasion (Fig. 1).Whole-body MRI did not highlight distant metastatic disease. The family was offered adjuvant chemotherapy but opted for observation only, recognizing the likely poor prognosis. The infant developed distant metastasis and recurrence at the primary site, prompting two doses of Vincristine and one dose of Dactinomycin with no clinical response. Chemotherapy was ceased and supportive care was provided until his death from disseminated disease at 5 months-old. Post-mortem whole-body MRI (in lieu of an autopsy at the family's request) showed widespread metastatic disease, including tumor burden in the posterior fossa obstructing the ventricular system.

Methods

For full methodology, see Supplemental Information. Histologic slides were prepared by standard techniques and immunohistochemistry performed on a Ventana BenchMark XT Autostainer. DNA and RNA sequencing was performed on frozen tumour tissue (DNA and RNA) and peripheral blood (DNA-only) using the MGISEQ-2000RS Sequencer. Bioinformatic analysis was performed using methods previously described by our group.[10]

Results

4.1 Pathology

The tumor had sheets of atypical cells separated by thick fibrous septae (Fig. 2A,B). Rare areas of clear cell change were identified (Fig. 2C). Mitotic activity was prominent. Geographic necrosis, apoptotic debris, dystrophic calcifications, foci of hemorrhage and hemosiderin deposition were present. Immunohistochemistry was consistent with the diagnosis (Fig 2D). Electron microscopy demonstrated primitive intracellular junctions and rare foci of basal lamina formation, suggesting an element of epithelial differentiation. Rare primary cilia and abundant flocculent extracellular material were identified. A Nanostring-based fusion panel assay identified the YWHAE:NUTM2B fusion.

Molecular

WGTA data revealed somatic alterations in the tumour genome. Whole genome mutation burden was low at 0.4 mutations/million bases. Somatic copy number changes were found in a focal region of chromosome 17 and part of chromosome 10 long arm (Fig. 2E). WGTA data revealed 46 structural variants, including a t(10;17)(q22;p13) translocation resulting in fusion between exon 5 of *YWHAE* and exon 2 of*NUTM2B* (Fig. 2F). No relevant cancer predisposition germline findings from 98 reviewed predisposition genes were reported, suggesting this tumor was a sporadic event, and YWHAE:NUTM2B fusion was likely a driver.

RNA-seq data revealed multiple expression aberrations indicated from high expression percentile and overexpression when compared to the TARGET CCSK cohort (TARGET_CCSK), and GTEx normal tissues (GTEx_average), respectively. High outlier expression percentile and overexpression pathways comprised receptor tyrosine kinases (RTKs) including *NTRK3* , *RET* and *KIT* , and IGF signaling genes, namely *IGF2* and *IGF1R* . Genes in MAPK and PI3K/mTor pathway, *NRAS, BRAF, MAPK1, AKT1* and *mTOR,* showed high expression percentile compared to TARGET_CCSK. *SHH, SMO, GLI1/2/3,* transcription factors of SHH pathway and *HES4* from NOTCH pathway demonstrated high expression percentile and overexpression. WGTA demonstrated high outlier expression percentile of cell cycle regulators in phases G1, S and M, indicating heavy dysregulation: *CCND1* , *CCND2* , *CDK6* , *CCNE2* ,*CCNA2* , *CCNB1* and *CDK1* .

BCOR was overexpressed compared to GTEx_average. When compared to Personalized OncoGenomics (POG) Programs and TCGA sarcomas,*BCOR* was in the 100th percentile; however,*BCOR* showed average expression within TARGET CCSK cases, likely due to it being generally overexpressed in CCSK with either mutually exclusive changes, YWHAE:NUT2MB fusion or *BCOR* internal duplications.[11]

EZH2 , a gene part of polycomb repressive complex 2, had overexpression and high outlier percentile when compared to GTEx_average. *TERC* and *TERT* telomerases both showed high expression percentile and overexpression.

Discussion

Three other cases of YWHAE:NUTM2B fusion in URCS of infancy are reported, and all were aggressive tumors unresponsive to conventional chemotherapy.[8,9] Our case demonstrated similar clinical and pathological features.[8] As such, this infant was enrolled into the POG trial (NCT02155621) to describe the molecular landscape and identify any therapeutically actionable variants not discovered during routine work up. Targeted therapy was not pursued due to rapid clinical decline.

Based on WGTA there was limited evidence to support a targeted approach as most alterations were based on increased RNA expression data. We found mTOR transcription factors were overexpressed with high outlier expression profile. Previous literature and experience have clinically targeted mTOR in pediatric vascular tumors due to administration ease and limited side effects. Use of mTOR inhibitors had previously reduced tumor growth both *in vivo* and *in vitro* in infantile hemangiomas and angiosarcomas.[12,13] mTOR inhibitors are also proven efficacious in children with Kaposiform hemangioendothelioma, where loss of tumor suppressors PTEN and TSC2 leads to abnormally activated mTOR pathway and mTOR-inhibited suppressed tumor growth.[14–17] An mTOR inhibitor could have been an option in this infant and might be considered in future URCS cases.

The unique YWHAE fusion encodes a 14-3-3-ε protein which interacts with CDC25 phosphatases, RAF1, and IRS1, suggesting its role in diverse biochemical activities related to signal transduction (cell division and insulin sensitivity regulation). Since *IGF2* had high outlier expression in this tumor and correlated with *VEGF2* expression in infantile hemangioma, off-label use of a commercially available IGF-2 inhibitor with pediatric dosing, was considered.[18–22]Other targets included CDK4/CDK6 inhibitors due to increased expression of *CCND1/CyclinD1* and RTK inhibitors due to high outlier expression percentile for *NTRK3* , *RET* , and *KIT* .*NTRK3* overexpression is reported in undifferentiated sarcomas with *YWHAE/BCOR* genetic modifications but may be a less efficacious target in a setting of exclusive changes in RNA expression.[6,23–28]

Other possibilities with very low level of evidence based on RNA expression data included targeting *EZH2* ; *TERT* , which showed increased expression in CCSK; and *TERC* inhibition in clinical trials, reporting varied results in myelofibrosis, lung and breast cancer, and lymphoproliferative disorders (NCT02598661), (NCT01731951).[29–33]

Overall, a strongly supported targetable pathway was not identified in this infant's tumor, but we described more precisely the genomic profile of this entity allowing better understanding of the underlying molecular changes and reinforcing the value of a detailed oncogenomic approach to confirm conventional cytogenetic findings, detect additional genetic abnormalities, and suggest alternative therapeutic strategies. Further clinical and genomic studies are needed to understand how the YWHAE:NUTM2B fusion drives tumorigenesis and to develop more effective treatments.

Ethics statement

A guardian of the patient provided written informed consent to participate, and this study was approved by the University of British Columbia Research Ethics Board as part of the

Personalized OncoGenomics trial (NCT02155621) and represents part of the Personalized OncoGenomics trial NCT02155621.

Conflict of interest: The authors declare no competing interests.

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Author contribution: AL, JT, YS, DR and SC wrote the manuscript. AL and SC: collected clinical information. DR: performed radiology review. JT: provided pathological analysis. YS: performed bioinformatic analysis. DC, RR, RD and SC: provided patient treatment and clinical care. All authors read and approved the final manuscript.

Data availability statement: Data supporting the findings of this study are available from the corresponding author by request

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Figure Legends

FIGURE 1: Postnatal ultrasound (**A**) demonstrates a heterogeneous fusiform solid mass (M) arising along the left back. Calipers measure craniocaudal (1) and anteroposterior (2) dimensions. Highly echogenic region subjacent to the mass denotes air at the pleural surface (*black arrows*) and a portion of the spinal column is visible cranially (*white arrowheads*). **B** Sagittal fat-supressed T2-weighted MR image obtained at day 1 of life shows a large encapsulated mass arising from the back (*arrows*). Low signal material within the mass (*asterisk*) suggests a fibrous or hemorrhagic component, whereas the remainder of the mass is composed of higher signal tissue reflecting a larger cellular component. **C** Preoperative clinical photograph shows a large erythematous back mass with central skin ulceration (*asterisk*), the substance of which appears variably hemorrhagic and necrotic on subsequent photograph of the resected specimen (**D**).

FIGURE 2: Histological image of tumor at (**A**) 40x original magnification, (**B, C**) 200x original magnification showing tumor cells characterized by scanty cytoplasm, round nuclei with neuroendocrine-type chromatin, and 1-2 small nucleoli. A subset of tumor cells had increased clear cytoplasm (**C**). Tumor cells diffusely express BCOR based on IHC (**D** ; 200x original magnification). Additional markers such as BRG1, CD56, CD99 (cytoplasmic, granular), cyclin D1, INI1, pan-NTRK, TLE1, and vimentin showed strong diffuse expression; diffuse moderate expression of BCL2, CD117, and nestin (perinuclear dot pattern); focal expression of CD4 (perinuclear dot pattern) and SATB2, and weak focal expression of EMA and MYOD1 were also present. Pan cytokeratin (AE1/3), chromogranin, CD2, CD25, CD34, D2-40, desmin, GFAP, HMB-45, myeloperoxidase, PHOX2B, PLAP, S100, SALL4, and synaptophysin were negative. WGTA data showing somatic copy number changes in all chromosomes. Red regions indicate copy gain: green regions indicate copy loss (**E**). Schema of the YWHAE-NUT2MB fusion. B1:breakpoint 1. B2: breakpoint 2. Numbers in transcripts indicate exons (F).