Published in final edited form as:

J Pediatr Hematol Oncol. 2016 January ; 38(1): e21–e25. doi:10.1097/MPH.0000000000000463.

Genomic Characterization of Poorly Differentiated Neuroendocrine Carcinoma in a Pediatric Patient

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Abstract

Primary neuroendocrine carcinomas (NEC) are rare tumors in children and young adults, resulting in a lack of standardized treatment approach. To refine the molecular taxonomy of these rare tumors, we performed whole exome sequencing in a pediatric patient with mediastinal NEC. We identified a somatic mutation in *HRAS* gene and LOH regions in *NF2*, *MYO18B* and *RUX3* genes. Additionally, a germline heterozygous somatic variant in *BRCA2* with LOH at that same position in the tumor tissue was also found. Our data provides valuable insight into the genomic landscape of this tumor, prompting further investigation of therapeutic targets.

Keywords

Whole exome sequencing; Neuroendocrine carcinoma; BRCA2 mutations

Introduction

Primary mediastinal neuroendocrine carcinomas (NEC) are rare entities, accounting for less than 5% of all mediastinal neoplasms[1]. NECs are typically found in middle-aged men and are rarely found in children[2]. While historically classified into four subtypes – typical carcinoid (type 1), atypical carcinoid (type 2), small cell carcinoma (type 3), and large cell carcinoma (type 4) – modifications in the 2010 WHO guidelines specify the term “neuroendocrine carcinoma” for poorly differentiated neoplasms (small cell carcinoma and large cell carcinoma) to emphasize their malignant clinical course[3].

Epidemiological studies surveying pediatric populations report an increasing incidence of neuroendocrine tumors over the past 30 years, particularly in gastrointestinal NEC, and diagnoses are more common in adolescents and young adults compared to children[4].

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Disclaimers: None

Nonetheless, this remains an exceedingly rare diagnosis, thereby providing clinicians with little basis for implementing an effective therapeutic regimen.

Here, we report the genomic profile of a poorly differentiated neuroendocrine small cell carcinoma of the mediastinum in a five-year-old girl by performing whole exome sequencing to better characterize the tumor and identify therapeutic targets for this rare entity. While we found several somatic mutations and loss of heterozygosity regions none of them suggested the option of targeted therapy. We also identified a germline heterozygous single nucleotide polymorphism (SNP) located in *BRCA2* with LOH at that locus in the tumor prompting us to use a PARP inhibitor with chemotherapy in this highly aggressive and fatal tumor.

Case presentation

A five year old previously healthy girl, presented to her pediatrician with a one week history of right neck pain, not associated with intercurrent illness, trauma, fever, cough, night sweats or weight loss. On physical examination, the patient had full range of motion of the neck without significant cervical adenopathy. The remainder of the physical exam was unremarkable. Given the unusual nature of the pain, a neck ultrasound and chest x-ray was obtained. While the ultrasound was unremarkable, the chest x-ray revealed a large anterior mediastinal mass with associated mediastinal adenopathy. Laboratory testing showed a normal complete blood count and no evidence of tumor lysis. Analysis of serum tumor markers revealed a normal α -fetoprotein (AFP) but a mildly elevated β -HCG of 14 mIU/ml (normal <5 mIU/ml for a non-pregnant female). A chest CT revealed a large lobulated anterior mediastinal mass measuring $5.7 \times 5.7 \times 4.3$ cm that displaced the superior vena cava posteriorly. Additional findings included a $2.4 \times 2.4 \times 2$ cm lobulated mass in the right middle lobe of the lung contiguous with the mediastinal mass as well as paratracheal mass, and a subcarinal adenopathy. CT guided biopsy of the mediastinal mass revealed a poorly differentiated small round blue cell tumor (Figure 1A). The tumor stained negative for lymphoma markers (CD3, TdT and CD20) and markers of other common childhood small round blue cell tumors (CD99, S100, p63, vimentin, desmin, WT1 and myogenin), such as Ewing's sarcoma and rhabdomyosarcoma. However, epithelial (CK7, EMA) and neuroendocrine (NSE, CD56, chromogranin, synaptophysin) markers were positive, supporting a diagnosis of poorly differentiated neuroendocrine small cell carcinoma (Figure 1B and C). Given the mildly elevated β -hCG, the possibility of a malignant transformation within a teratoma was entertained, although the limited biopsy samples did not reveal any teratomatous background.

A chemotherapeutic regimen consisting of cisplatin, etoposide, and bleomycin was initiated, which has been used to treat both neuroendocrine carcinomas and germ cell tumors [5, 6]. She responded well to the therapy initially, with $> 50\%$ reduction in the overall size of the tumor. Following chemotherapy, she underwent resection of the residual mediastinal mass and a wedge resection of the right middle lobe. The pathology from both surgical sites revealed small cell carcinoma with lymphovascular invasion. Tumor cells were also seen in the retrocaval, paratracheal and subcarinal lymph nodes.

Following surgical resection, the patient received additional chemotherapy and local radiation therapy (4500 cGy) to treat microscopic residual disease at the surgical margins. The patient remained disease free for five months and then developed metastatic brain lesions, which were treated with gamma knife radiation surgery followed by additional chemotherapy consisting of temsirolimus, irinotecan and temozolomide [7]. The patient subsequently failed this salvage regimen as well, prompting us to investigate the genetic make-up of this tumor.

Given the rarity of this entity in pediatrics, we sought to genomically characterize this tumor using whole exome sequencing. Our aim was to understand the biology of the underlying malignancy, identify any inherited cancer risk associated germline lesions, and to investigate whether this tumor harbors therapeutic targets that could potentially be used to treat this highly aggressive neoplasm. Based on our findings and given the fact that BRCA1 and BRCA2 deficiency tumors are exquisitely sensitive to PARP (poly (ADP-ribose)-polymerase inhibition, we initiated veliparib (PARP inhibitor) 25 mg/m² twice daily along with temozolomide for 5 days on a compassionate basis when the tumor became refractory to all standard and salvage chemotherapy regimens. A single patient use treatment protocol was written which was approved by the FDA followed by institutional IRB approval. The patient did show brief initial favorable response in terms of symptomatic improvement in her respiratory symptoms and received this combination for 2 cycles; however, unfortunately due to advanced stage and rapid brain and spinal metastatic spread over a short span of about 2.5 months, further imaging was not pursued and she ultimately succumbed to the disease.

Materials and Methods

Written informed consent was obtained in accordance with the Helsinki protocol from the parents of the child reported here. Tumor tissue and blood samples were collected under our institutional review board-approved tissue procurement protocols for children with cancer.

Genomic DNA isolation

Tumor DNA was isolated from fresh frozen paraffin embedded (FFPE) tumor tissue using the Gentra Puregene Tissue Kit (Qiagen) following protocol guidelines. To filter out germline polymorphisms, genomic DNA was isolated from fresh peripheral blood (non-tumor tissue) using the Gentra Puregene Blood Kit (Qiagen). Briefly, buffy coat was prepared by centrifuging 5 ml of fresh whole blood at 2500 × g for 10 minutes at room temperature. Following centrifugation, the intermediate layer containing the buffy coat (containing concentrated leukocytes) was collected and utilized for subsequent DNA isolation. DNA was quantified using the nanodrop spectrophotometer and quality was verified by an Agilent 2100 Bioanalyzer (Palo Alto, CA).

Whole exome library preparation and validation

1 µg of genomic DNA from each sample (tumor and germline) was used for library preparation. Libraries were constructed using KAPA LTP library preparation kit (KAPA Biosystems). Exome capture was performed using SeqCap EZ library SR (Roche

Nimblegen, Madison, WI). Enriched exome libraries were sequenced on a HiSeq 2000 platform (Illumina). For downstream validation of identified somatic mutations, specific PCR primers were designed for the genomic sequences of the genes of interest. DNA was extracted from the tumor and germline samples as described above. Primers were designed within 200–300 bp of the variant site, and sequences were amplified by PCR. PCR products were purified using QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced using Sanger sequencing. Trace files were manually inspected for variation from the reference genome using the Mutation Surveyor program (Softgenetics). Primer sequences used were: BRCA2 forward primer- AGCAAACGCTGATGAATGTG and BRCA2 reverse primer-AGACCATTACAGGCCAAAG; HRAS forward primer- CTCACCTCTATAGTGGGGTCGTATT and HRAS reverse primers- GAGGAGCGATGACGGAATATAAG; CASC5 forward primer- CTTGCAGTGGGCCAAGACTG and CASC5 reverse primer- AAGGCATGAGCCATAGTGC; and ARHGAP23 forward primer- GAGAGCAGTGGGCCAAACTG and reverse primer-ATGTCCACGAGGCAGGAG.

Results

The exome captured DNA was sequenced with an average coverage depth of 175X for the normal (germline) and 80× for the tumor (somatic) tissue. Reads were aligned to the hg19 genome build using the Burrows-Wheeler Alignment (BWA) tool. In the final mapping, 84% of the high-quality sequence reads mapped to the reference genome, with 72% of all uniquely mapped reads mapping within a region targeted by the NimbleGen SeqCap EZ library. Data obtained was used for the detection of somatic mutations and to identify loss of heterozygosity regions.

Somatic Sniper v1.0.2 and MuTect softwares were used to generate single-nucleotide variation (SNV) calls, using the matched tumor and germline samples with standard parameter settings. Tumor and germline variants were further filtered using SNP138 and 1000 genome database. All mutations were annotated with ANNOVAR and manually examined via Integrative Genomics Viewer (IGV) v2.1. Tumor specific non-synonymous variants called by SomaticSniper and MuTect are enumerated in Table 1 and Table 2 respectively. A total of 12 missense mutations were identified that were specifically found in the tumor specimen while absent in the germline DNA. Most of the mutations were predicted to have a deleterious effect on the protein structure by Polyphen-2 and/or SIFT. Three mutated genes, namely *HRAS*, *CASC5*, and *ARHGAP23*, were identified by both variant calling methods and thus were chosen for downstream validation by Sanger sequencing. While we were able to validate a heterozygous mutation in *HRAS* (Figure 2a), mutations were not detected in *CASC5* and *ARHGAP23* by Sanger sequencing in the tumor DNA. G:C>A:T transitions were the most common single base pair substitution, followed by A:T>G:C transitions.

Next, we analyzed the data for the loss of heterozygosity regions by utilizing heterozygous mutation calls from VarScan at each heterozygous position followed by application of the Circular Binary Segmentation (CBS) algorithm to tune the granularity of the LOH regions. We identified 3 LOH regions in this tumor which were not present in the patient's germline

sample. These included: 1) LOH region on chromosome 13 spanning from q12.1 to q14.1 which consisted of *BRCA2* in addition to several other genes. One of the areas of LOH in the *BRCA2* gene was located at the same site where a germline heterozygous mutation was noted, exon 10 position 32906729, and leading to the homozygous non-synonymous SNV N382H. This germline mutation and subsequent LOH in the tumor tissue was validated by PCR and Sanger sequencing (Figure 2b); 2) LOH region on chromosome 22 spanning from q11.21 to q12.3, which consisted of genes with tumor suppressor roles *NF2* and *MYO18B*; and 3) LOH region on chromosome 1 spanning from p36.11 to p36.12, consisting of *RUNX3* amongst others.

Discussion

Small cell carcinoma (SCC) is a particularly aggressive variant of neuro-endocrine carcinoma characterized by rapid, infiltrative growth and a dismal prognosis[8]. The diagnosis is made with histological examination and a characteristic phenotype, including positive stains for cytokeratin, chromogranin, and CD117 and negative stains for MIC-2, consistent with the pathological findings of this tumor. Gross total resection, when possible and chemotherapy remain important strategies for treatment. However, case studies report 0% disease-free survival at five years and mortality within ten years for poorly differentiated mediastinal NEC[2, 9].

Herein, we describe the genomic alterations in the mediastinal neuro-endocrine carcinoma in a pediatric patient who had an aggressive clinical course, in an attempt to identify therapeutically targetable lesions and to better classify these rare tumors by performing whole exome sequencing. Somatic mutations were found in *HRAS*, a member of the Ras oncogene superfamily of GTP-binding proteins, which helps control several key cell-cycle proteins. While aberrant RAS signaling has been reported in up to 20% of human tumors, *HRAS* mutations are found in less than 1% of human tumors[10]. Among the most common tumors associated with activating *HRAS* mutations are skin, head and neck, and urinary tract tumors[11]. The G12S missense mutation has been previously reported in a wide variety of tumors, including transitional cell carcinoma of the urinary bladder, actinic keratosis, cervical adenocarcinoma, anaplastic carcinoma, embryonal rhabdomyosarcoma, keratoacanthoma, and squamous cell carcinoma (Cosmic database). While the G12A missense mutation in *HRAS* has been identified previously in neuroendocrine carcinomas of the breast[12] and small intestine[13], the G12S missense *HRAS* mutation has not been previously reported in small cell neuroendocrine carcinoma of mediastinum. While therapeutic attempts to drug the RAS pathway has proved difficult in the past, targeting the downstream components of the pathway may be possible (e.g. MEK).[14]

Interestingly, we identified a heterozygous germline variant in *BRCA2*, which is a common non-synonymous (minor allele frequency >0.05) SNP in *BRCA2*. *BRCA2* is vital for repairing DNA double-strand breaks and maintaining genomic integrity. Deleterious *BRCA2* mutations are commonly associated with breast and ovarian cancers. Additional risks conferred by germline *BRCA2* mutations include increased incidences of pancreatic, prostate, and uveal melanoma. While earlier studies suggested homozygous N372H SNPs were associated with an increased risk of ovarian and breast cancer particularly among

young, pre-menopausal women, later more robust studies have refuted both associations[15–17]. To our knowledge, no associations between N372H and neuroendocrine carcinomas have been reported. LOH of *BRCA2* is frequently found in breast, ovarian, and various other tumors, and is generally associated with germline *BRCA2* mutations. In this patient we identified a rare germline non-silent polymorphism in *BRCA2* at the same position of LOH in the tumor tissue. Additionally, we found LOH of *NF2* and *MYO18B* which are known to have tumor suppressor roles.

Given the chemosensitization effect of PARP inhibitors in the setting of BRCA deficient tumors through synthetic lethality, and the promising results in combination with temozolomide in metastatic breast cancer patients who are carriers of *BRCA1/2* mutations[18], we used veliparib (PARP inhibitor) in this patient. We observed symptomatic improvement but given the advanced stage of the disease we could not document an objective response radiographically since the parents transitioned the child to hospice given the widespread CNS disease. Our study provides valuable insights into the genomic make-up of this class of very rare tumors prompting further investigation to identify therapeutic targets.

Conclusion

Small cell neuroendocrine carcinomas remain exceedingly rare tumors in the pediatric population. Moreover, the lack of molecular characterization of this family of tumors even in adults makes it diagnostically challenging and difficult to treat. In this report, we characterized the genome of a small cell neuroendocrine tumor presenting as a mediastinal mass in a five year old female patient by performing whole exome sequencing. Several unique lesions indicate that this tumor is a distinct entity, including LOH in *BRCA2* and *NF2* in tumor as well as a somatic mutation in *HRAS*. Sequencing additional samples might be helpful in further characterizing this tumor type to identify novel therapeutic targets to treat this rare entity.

Acknowledgments

This work was supported by the NYU Cancer Institute Cancer Center Support Grant (5 P30 CA016087), NYU genome technology core and Pediatric Cancer Foundation grant.

References

1. Macchiarini P, Ostertag H. Uncommon primary mediastinal tumours. *The lancet oncology*. 2004; 5(2):107–118. [PubMed: 14761815]
2. Chaer R, et al. Primary neuroendocrine tumors of the thymus. *The Annals of thoracic surgery*. 2002; 74(5):1733–1740. [PubMed: 12440652]
3. Klimstra DS. Pathology reporting of neuroendocrine tumors: essential elements for accurate diagnosis, classification, and staging. *Seminars in oncology*. 2013; 40(1):23–36. [PubMed: 23391110]
4. Yao JC, et al. One hundred years after “carcinoid”: epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol*. 2008; 26(18):3063–72. [PubMed: 18565894]
5. Gershenson DM, et al. Treatment of malignant germ cell tumors of the ovary with bleomycin, etoposide, and cisplatin. *J Clin Oncol*. 1990; 8(4):715–20. [PubMed: 1690272]

6. Fjallskog ML, et al. Treatment with cisplatin and etoposide in patients with neuroendocrine tumors. *Cancer*. 2001; 92(5):1101–7. [PubMed: 11571721]
7. Bagatell R, et al. Phase 1 trial of temsirolimus in combination with irinotecan and temozolomide in children, adolescents and young adults with relapsed or refractory solid tumors: a Children's Oncology Group Study. *Pediatr Blood Cancer*. 2014; 61(5):833–9. [PubMed: 24249672]
8. Travis WD, et al. Survival analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid. *Am J Surg Pathol*. 1998; 22(8):934–44. [PubMed: 9706973]
9. Moran CA. Primary neuroendocrine carcinomas of the mediastinum: review of current criteria for histopathologic diagnosis and classification. *Semin Diagn Pathol*. 2005; 22(3):223–9. [PubMed: 16711403]
10. Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer*. 2003; 3(1): 11–22. [PubMed: 12509763]
11. Calderaro J, et al. PI3K/AKT pathway activation in bladder carcinogenesis. *Int J Cancer*. 2014; 134(8):1776–84. [PubMed: 24122582]
12. Ang D, et al. Novel Mutations in Neuroendocrine Carcinoma of the Breast: Possible Therapeutic Targets. *Diagn Mol Pathol*. 2014
13. Bank MS, et al. The genomic landscape of small intestine neuroendocrine tumors. *J Clin Invest*. 2013; 123(6):2502–8. [PubMed: 23676460]
14. Akinleye A, et al. MEK and the inhibitors: from bench to bedside. *J Hematol Oncol*. 2013; 6:27. [PubMed: 23587417]
15. Auranen A, et al. BRCA2 Arg372His polymorphism and epithelial ovarian cancer risk. *Int J Cancer*. 2003; 103(3):427–30. [PubMed: 12471628]
16. Healey CS, et al. A common variant in BRCA2 is associated with both breast cancer risk and prenatal viability. *Nat Genet*. 2000; 26(3):362–4. [PubMed: 11062481]
17. Spurdle AB, et al. The BRCA2 372 HH genotype is associated with risk of breast cancer in Australian women under age 60 years. *Cancer Epidemiol Biomarkers Prev*. 2002; 11(4):413–6. [PubMed: 11927503]
18. Curtin NJ, et al. Novel poly(ADP-ribose) polymerase-1 inhibitor, AG14361, restores sensitivity to temozolomide in mismatch repair-deficient cells. *Clin Cancer Res*. 2004; 10(3):881–9. [PubMed: 14871963]

Figure 1. Needle biopsy of the mediastinal mass

A. The hypercellular tumor consists of sheets of small poorly differentiated cells with scanty cytoplasm and round to ovoid, finely granular nuclei containing inconspicuous nuclei. Karyorrhectic debris is evident in the left side of the field. Other fields showed frequent mitoses. (Hematoxylin and eosin stain; 200×). Immunohistochemical evaluation of the tumor cells showed positive staining with **B.** cytokeratins (here AE1/AE3; 200×), and with **C.** neuroendocrine markers (here neuron-specific enolase; 200×).

Reference

Germline

Tumor

Reference

Germline

Tumor

Figure 2.

Sanger sequencing traces: **A.** A heterozygous mutation in *HRAS* detected in the tumor DNA at position 534289 (indicated in bold) but not in the germline DNA. **B.** Evidence of heterozygosity at position 32906729 in *BRCA2* (indicated in bold) in the germline sample but LOH in the tumor, which leads to dominate expression of this protein coding mutation, p.N382H. Top trace shows the reference sequence from hg19.

Table 1

Somatic mutations called by SomaticSniper

Gene	Chromosome	Position	Function	Nucleotide change	Protein Change	Allele Frequency	Polyphen-2 prediction	SIFT prediction	In COSMIC database?
HRAS	11	534289	Missense	c.G34A	p.G12S	0.39	Damaging	Damaging	Yes
CASC5	15	40947152	Missense	c.T6461C	p.V2154A	0.19	Damaging	Damaging	Yes
ARHGAP23	17	36633874	Missense	c.C2173T	p.R725C	0.28	Benign	Tolerated	Yes
KIRREL2	19	36352713	Missense	c.T1147C	p.S383P	0.20	Damaging	Damaging	Yes

Table 2

Somatic mutations called by MuText

Gene	Chromosome	Position	Function	Nucleotide change	Protein Change	Allele Frequency	Polyphen-2 prediction	SIFT prediction	In COSMIC database?
HRAS	11	534289	Missense	c.G34A	p.G12S	0.39	Damaging	Damaging	Yes
CASC5	15	40947152	Missense	c.T6461C	p.V2154A	0.18	Damaging	Damaging	Yes
TTC19	17	15907178	Missense	c.C175T	p.L59F	0.09	Damaging	Damaging	Yes
ARHGAP23	17	36633874	Missense	c.C2173T	p.R725C	0.32	Benign	Tolerated	Yes
PALM3	19	14165288	Missense	c.C1151A	p.T384K	0.09	Benign	Tolerated	Yes
SLC5A5	19	17984955	Missense	c.G366T	p.E122D	0.09	Benign	Tolerated	Yes
ZNF536	19	31040048	Missense	c.G3522T	p.K1174N	0.12	Benign	Damaging	Yes
PRKAG3	2	219691723	Missense	c.G1096A	p.A366T	0.12	Damaging	Tolerated	Yes
FASTKD5	20	3128534	Missense	c.C1183A	p.H395N	0.09	Damaging	Tolerated	Yes
SYNE1	6	152771893	Missense	c.C3283A	p.L1095I	0.08	Damaging	Tolerated	Yes
NRM	6	30658718	Missense	c.C34T	p.L12F	0.09	Damaging	Tolerated	Yes