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BRIEF REPORT

Molecular characterization of a thymic neuroblastoma in an adult associated with inappropriate antidiuretic hormone secretion syndrome

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Abstract

Neuroblastoma (NB) is extremely rare in adults, and anterior mediastinal location is even more unusual. We report a primary thymic NB in a 72-year-old male, who presented with the inappropriate antidiuretic hormone secretion (SIADH) syndrome. Molecular testing revealed copy number alterations of chromosome 3, i.e., loss of whole 3p and partial gain of distal 3q, including gain of copies of the *PIK3CA* gene. To the best of our knowledge, only five mediastinal NB cases in adults have been reported with genetic evaluation. One case showed loss of 3p material with *SETD2* gene mutation and gain of *PIK3CA* gene, similar to our case. As thymic NB is extremely rare, report of more genetically characterized cases should help to delineate their pathobiology and shed light on possible mechanisms involved in the associated SIADH syndrome.

Keywords NB · Thymus · SIADH · *PIK3CA* gene

Introduction

NB typically occurs in children and younger adults and is an extremely rare tumor in middle age and elderly. In addition, anterior mediastinal NB is rather rare [1] with 26 reported cases to date [2]. However, most of them have not been genetically characterized. We describe a thymic NB associated with SIADH and its genetic findings.

Material and methods

Immunohistochemistry

Tissue specimens were formalin-fixed and processed for histopathological evaluation. Immunohistochemistry (IHC) was performed on 4-µm sections cut from paraffin blocks. Slides were stained on the BenchMark ULTRA IHC/ISH automatic staining platform (Ventana Medical Systems) with Pankeratin AE1/AE3, CK8/18, EMA, synaptophysin, chromogranin, TdT, TTF1, p63, CD5, S100, CD117, desmin, SALL4, calcitonin, PTH, ALK, STAT6, NUT, ARID1A, SMARCB1, SMARCA4, and CD99.

Genetic testing—FISH

As a first approach to the initial diagnosis, and to verify whether some of the molecular markers characteristic of pediatric NB, such as 1p deletion, 1q gain, *ALK*, and *N-MYC* amplification, were present in this tumor, we performed FISH analysis on 3-µm thick, formalin-fixed,

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paraffin-embedded sections using probes for 1p36/1q25 regions (Vysis LSI 1p36/LSI 1q25 Dual-Color Probe, Abbott Molecular), *ALK* gene (Vysis *ALK* Break Apart FISH Probe, Abbott Molecular), and *N-MYC* gene (Vysis LSI *N-MYC* SpectrumGreen/CEP 2 SpectrumOrange Probes, Abbott Molecular) with standard protocols according to the manufacturer's instructions. Following chromosome comparative genomic hybridization (cCGH) analysis showing distal gains on chromosome 3q, we also performed FISH analysis for the *PI3KCA* gene (3q26.32) to assess gene copy number (ZytoLight ®SPEC *PIK3CA*/CEN 3 Dual Color Probe, Zytovision). Nuclei were counterstained with DAPI-Vectashield mounting solution (Vector). FISH signals were captured and analyzed using a Zeiss epifluorescence microscope coupled to Cytovision FISH Probe software (Cytovision version 7.4, Leica Biosystems, Richmond, VA, USA).

Genetic testing—cCGH

For cCGH analysis to determine the copy number profile of the tumor genome, nucleic acid DNA extraction from FFPE samples was performed by an automated method using the Maxwell® RSC DNA FFPE Kit in a Maxwell® RSC instrument (Promega, Madison, USA). The cCGH technique was performed according to the method of Kallioniemi et al. [3]. A Zeiss epifluorescence microscope coupled to Cytovision HR-CGH software (Cytovision version 7.4, Leica Biosystems, Richmond, VA, USA) was used for image analysis. A minimum of 15 metaphases were acquired for cCGH profiling. The ratio of green (test DNA) to red (reference DNA) fluorescence along the length of the chromosomes was calculated. This high-resolution version of cCGH uses dynamic standard reference intervals based on the systematic

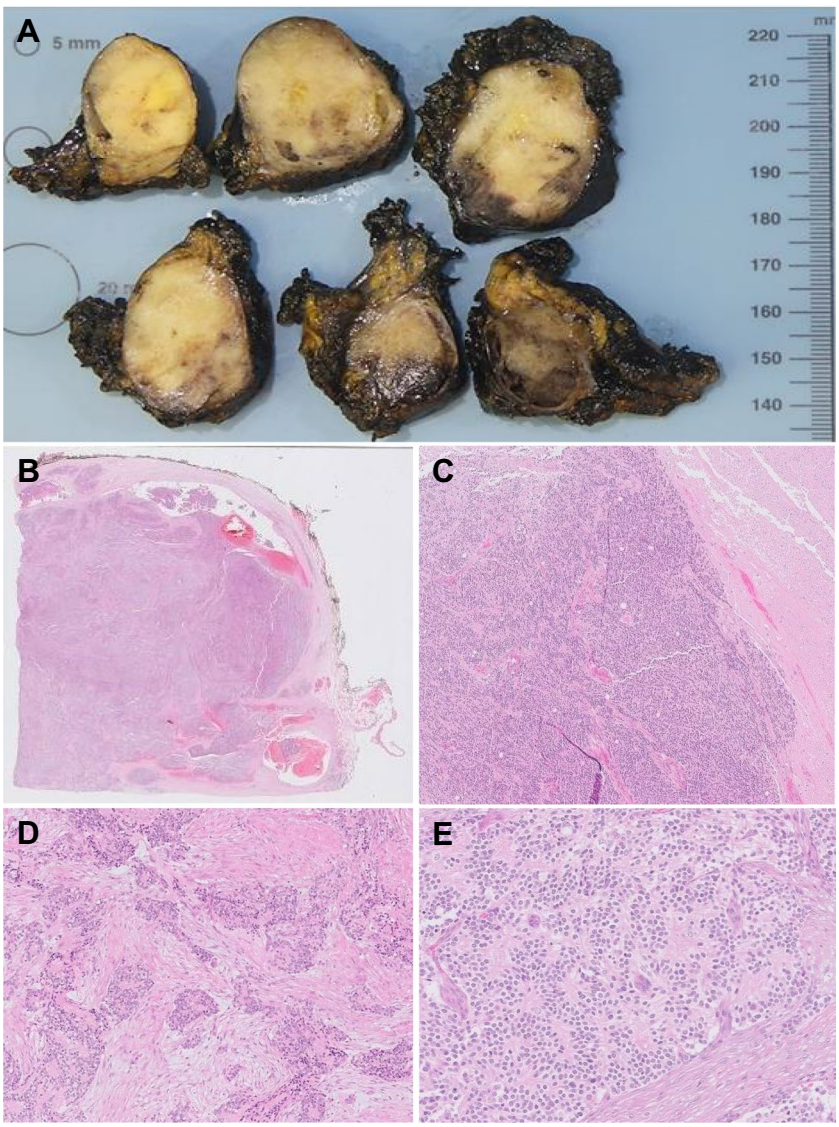

Fig. 1 Macroscopic evaluation: thinly encapsulated, white-tan, solid, and fleshy tumor with focal hemorrhage (**A**). Hematoxylin–eosin-stained sections showed a low-power view of the tumor with a lobular pattern of growth and hemorrhagic foci (**B**). Necrosis was evident in several lobules (**C**). Tumor composed of small round blue cells suspended in a fine, fibrillary, eosinophilic matrix with intervening fibrovascular septae (**D**). Rosette-like formations (**E**)

variation seen in normal samples. If the dynamic standard reference intervals do not overlap the confidence limits, the software interprets this as either a loss (red) or gain (green) in the test DNA. Heterochromatic regions in chromosomes 1, 9, 16, and Y and the p arms of the acrocentric chromosomes were excluded from the analysis.

Genetic testing—fusions

Molecular testing to assess gene fusions has been performed using the TruSight RNA fusion panel, which covers 507 fusion-associated genes (Illumina) as described previously [4].

Genetic testing—mutations

Molecular analysis for mutation detection has been carried out using the Ampliseq Focus panel (Illumina) on a Miseq platform (Illumina) with DNA extracted from FFPE samples as described above [4].

Case history

A 72-year-old male presented with an idiopathic SIADH and a remote history of carotid artery stenosis. The thorax CT imaging revealed an anterior mediastinal mass, $5 \times 3.8 \times 2.5$ cm. Given the concern for a thymoma, the patient underwent a thymectomy and an 84.1 g mass was removed.

Pathological and molecular findings

On gross examination, a $5.5 \times 5 \times 3.7$ cm lobulated and well-circumscribed mass was surrounded by thymic fat. Its cut surface was white-tan, solid, with areas of hemorrhage, necrosis, and a thin fibrous peripheral capsule (Fig. 1).

Histologically, the tumor had a complete fibrous capsule and focally extended through the capsule into the surrounding fatty tissue (Fig. 1). The tumor was composed of lobules separated by fibrovascular bands with diffuse sheets and Homer-Wright pseudorosettes surrounding delicate, eosinophilic neuropil. Areas of necrosis and/or hemorrhage were identified (Fig. 1). The neoplastic cells had indistinct cell borders, abundant eosinophilic cytoplasm, round nuclei, and variably condensed chromatin, predominantly with a finely granular ("salt and pepper") appearance. Rare larger cells with clear to eosinophilic cytoplasm were noted, but no mature ganglion cells or Schwannian stroma were seen. Occasional mitotic figures and karyorrhectic bodies were identified (mitosis-karyorrhexis index < 1%). The neoplastic cells were synaptophysin +, chromogranin + (focal), CD56 +, NSE +, TdT + (focal), PAX8 + (focal), TTF1-,

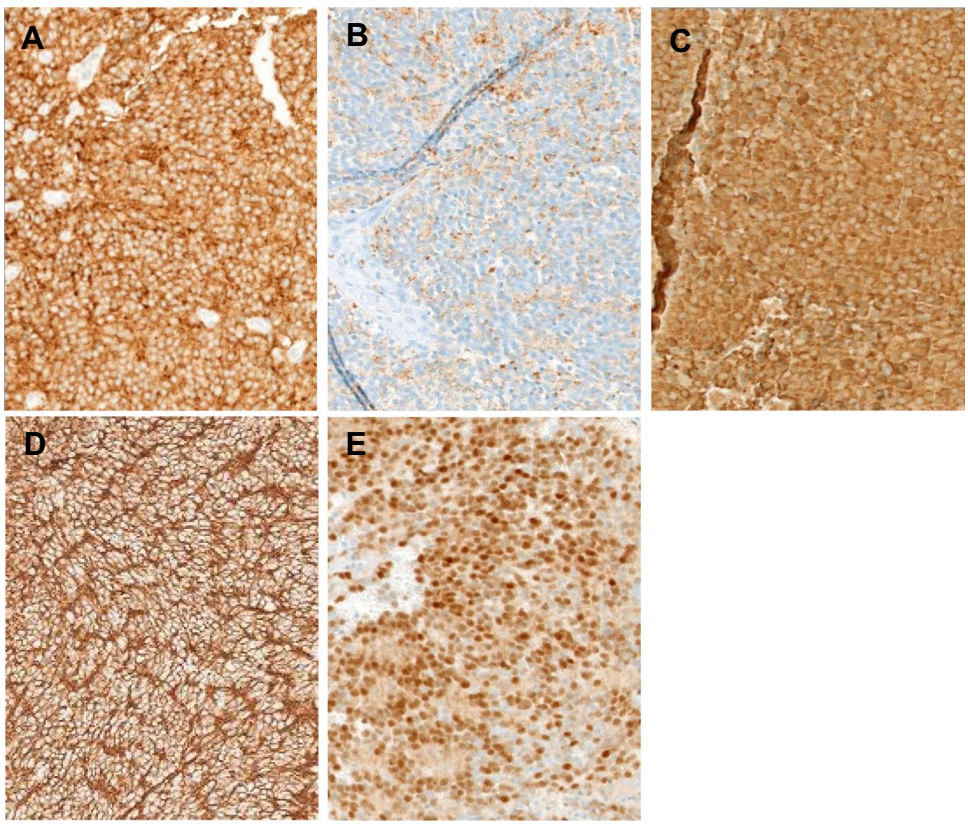

Fig. 2 Immunohistochemical staining showed the tumor positive for synaptophysin (**A**), chromogranin (weak) (**B**), neuron-specific enolase (**C**), CD56 (**D**), and TdT (**E**)

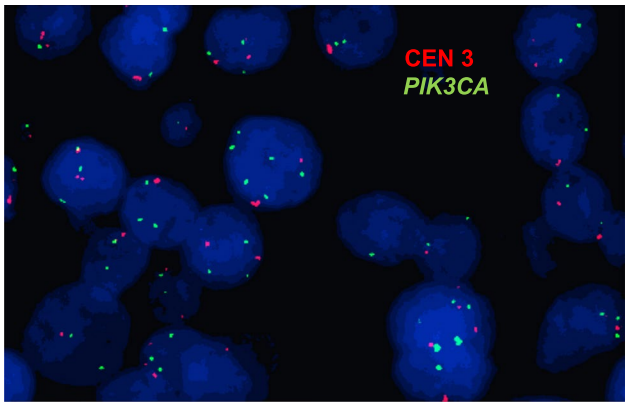

Fig. 3 FISH analysis with a copy number probe for *PIK3CA* gene (green) and a centromeric probe for chromosome 3 (red) showing *PIK3CA* gain of copies

p63-, CD5-, S100-, AE1/AE3-, CK8/18-, EMA-, CD117-, desmin-, SALL4-, calcitonin-, PTH-, CD99-, ALK-, STAT6-, NUT-, and ARID1A-, with retained expression of SMARCB1 and SMARCA4 (Fig. 2).

FISH analysis with probes to assess 1p deletion, 1q gain, *ALK*, and *N-MYC* amplification did not reveal any alterations. FISH analysis for the *PIK3CA* gene using a copy number probe showed gain of 1 copy in 60%, gain of 2 copies in 4%, and gain of 4 copies in 4% of the evaluated nuclei (Fig. 3).

The cCGH revealed an abnormal copy number profile with gain of chromosomal regions 3q26.3-q29 and loss of chromosomal regions 3p26-p11.2, 3q24-q25, 6q16-q27, and 11q14-q23 (Fig. 4).

Molecular analysis with the RNA fusion panel was negative without detectable gene fusions, and the Focus panel did not reveal *ALK* or *PIK3CA* gene mutations.

On the basis of these findings, a poorly differentiated thymic NB with associated SIADH was diagnosed. At 12-month follow-up, the patient is disease-free.

Discussion

Mediastinal neurogenic tumors are predominantly located in the posterior mediastinum of pediatric patients. NB arising in adult patients (> 60 years old) are extremely rare, and molecular characterization has been limited in the literature. Clinically, our patient presented with idiopathic SIADH, a rare but documented paraneoplastic syndrome associated with NB. SIADH in this context is typically due to ectopic production of antidiuretic hormone by the tumor. Including the current case, 41% of the anterior mediastinal NBs have been associated with SIADH [2]. Notably, this syndrome is not present in pediatric NB but is well documented in olfactory NB [5].

Diagnosis of thymic NB is a challenge, since in adults, the neurogenic tumors are usually located in the posterior mediastinum, and other tumor types including lymphoma, thymoma, and carcinoma are far more common in the anterior mediastinum. The differential diagnosis of this tumor results

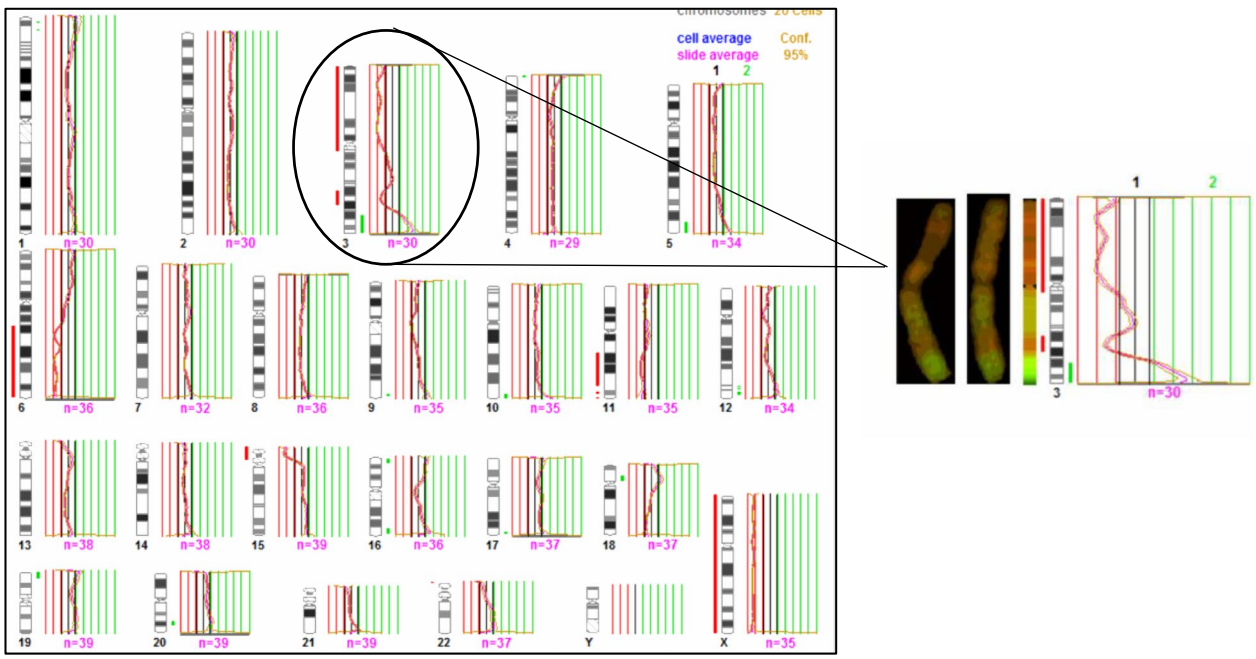

Fig. 4 cCGH profile showing gain of chromosomal regions 3q26.3-q29 and loss of chromosomal regions 3p26-p11.2, 3q24-q25, 6q16-q27, and 11q14-q23 and detail view of chromosome 3 alterations

Table 1 Clinical, histologic, immunophenotypic, and genetic features of primary anterior mediastinum NB reported cases with genetic evaluation (including the current case)

Report	No. of cases	Age (year)/sex	Primary site	SIADH	Size (cm)	Immunohistochemistry	Molecular	Follow-up
Ohtaki et al. [7]	1	64/M	SM	-	5 cm	Positive: NSE, SYN, CG, CD56. Negative: CK5/6, CK AE1/AE3, CD99, S100, EMA	*MCYN* amplified (FISH)	AWD, lymph node metastasis at 20 moths
Rogowitz et al. [8]	1	86/M	AM	+	5.2 cm	Positive: CD56, SYN, CG. Negative: GFAP, CK AE1/AE3, CD99	Negative *EWSR1* break-apart (FISH)	ANED at 11 months
Wiesel et al. [9]	1	62/M	Thymus	-	7.5 cm	Positive: NSE, SYN, CG. Negative: CK AE1/AE3, CD99	*MYCN* non-amplified (tumor sequencing)	ANED at 6 months
Kennedy et al. [10]	1	83/M	Thymus	+	3.6 cm	Positive: CD56, CG, SYN (weak), NSE, ALK (weak), CD99, TdT and TTF1 (variable), SOX10 (rare), S100 (rare), GFAP. Negative: panCK, Cam5.2, EMA, CK5/6, p40, NKX3.1, WT1, NF, desmin, NF	NGS and copy number analysis: diploid, 3q LOH, partial 3p loss (including *SETD2*, *MYD88*, *CTNNB1*, M*ITF*, *FOXP1* genes). No *MYCN* or *ALK* amplification. Negative *ESWR1* break-apart (FISH)	ANED at 13 months
Collins et al. [2]	1	71/M	Thymus	-	5.2 cm	Positive: SYN, CG, TdT, NKX2.2, GFAP (patchy). Negative: TTF1, CK20, CD99	Negative *ESWR1* break-apart and isochromosome 12p (FISH)	ANED at 13 months
Current case	1	72/M	Thymus	+	5 cm	Positive: SYN, CG (patchy) TdT, PAX8 (patchy), ALK (weak), NSE, CD56. Negative: TTF1, calcitonin, S100, AE1/AE3, desmin, CD99	By FISH, no *MYCN* or *ALK* amplification, gain of copies of *PIK3CA* gene. By cCGH, gain of chromosome distal 3q and losses of whole chromosome 3p, partial 3q, distal 6q, partial 11q. By NGS-Fusion panel, no detectable gene fusions	ANED at 12 months

SM superior mediastinum, *AM* anterior mediastinum, *ANED* alive with no evidence of disease, *AWD* alive with disease

from the combination of morphologic features with immunohistochemical staining and molecular analysis, which is essential for final diagnosis. Furthermore, considering the morphology, the differential diagnosis included neuroendocrine tumor (absence of cytokeratin and TTF1), thymoma (negative for cytokeratin and p63), unusual metastatic olfactory NB (lack of S100 + sustentacular cells), Ewing sarcoma (absence of membranous CD99 staining and absence of *EWSR1* rearrangement), lymphoma (CD45, CD3, and CD4 negative), and rhabdomyosarcoma (MyoD1/myogenin and desmin negative).

The literature about the outcome of NB in elderly patients is scant. In younger adults, the prognosis is poor, but among the 21 case reports in patients more than 60 years old, only 1 patient died of disease [2, 6]. This finding suggests that despite the same morphology, the behavior is completely different, which might be explained by the molecular analyses.

The few molecular alterations documented are limited to five cases (Table 1): two *EWSR1* gene negative cases by FISH, one with amplification of *N-MYC* gene by FISH, one without amplification of *N-MYC* gene by tumor sequencing, and a fifth case, recently reported, with more detailed molecular characterization by NGS panels with *SETD2* gene mutation and copy number alterations on chromosome 3: loss of partial 3p including deletion of *SETD2*, *MYD88*, *CTNNB1*, *MITF*, and *FOXP1* genes; copy number neutral loss of heterozygosity of 3q; and gain of *PIK3CA* gene at 3q26.

The genetic findings in our case, particularly the loss of chromosome 3p arm and gain of regions 3q26.3-q29 including the *PIK3CA* gene, are similar to the previously reported case by Kennedy et al. [10]. Loss of chromosome 3p is a common finding in pediatric NB [1, 11]. The gain of the 3q26.3-q29 region, which includes the *PIK3CA* gene, is particularly notable given the role of *PIK3CA* in the PI3K/AKT/mTOR pathway, a critical signaling pathway for cell growth and survival [12]. *PIK3CA* mutations and amplifications are common in many human cancers, including colorectal, breast, brain, liver, gastric, and lung cancers [13], and represent potential therapeutic targets. The presence of *PIK3CA* gain in our case suggests that inhibitors targeting the PI3K/AKT/mTOR pathway may be a viable therapeutic option, although further research is needed to validate this approach in thymic NB. As gene fusions have been linked to the presence of ectopic hormone production in neuroendocrine tumors [14], we performed targeted RNA fusion testing using a large panel containing 507 cancer-related genes, but no fusion was detected.

In conclusion, this case of primary thymic NB with SIADH and significant genetic alterations provides valuable insights into the molecular characterization of this rare tumor. The findings of chromosome 3p loss and 3q gain, particularly involving the *PIK3CA* gene, suggest potential pathways for targeted therapy. Future studies should focus on comprehensive genetic profiling of thymic NB to further elucidate its pathogenesis and identify novel therapeutic targets.

Author contribution Sofia Lérias, Rafael Cabrera, Leonor Ruivo, Ana Saramago, Carmo Martins: conception and design of the work; acquisition, analysis, and interpretation of data; drafting the MS and revising it critically for important intellectual content and scientific integrity. Robert Stoehr, Abbas Agaimy: acquisition, analysis, and interpretation of data; reading and revising the MS critically for important intellectual content and scientific integrity. All authors: read and approved the final manuscript.

Data availability The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Declarations

Ethical approval Samples were used in accordance with ethical guidelines for the use of retrospective tissue samples provided by the local ethics committee of the Friedrich-Alexander University Erlangen-Nuremberg (ethics committee statements 24.01.2005 and 18.01.2012).

Conflict of interest AA is editor-in-Chief of Virchows Archiv. The authors declare no competing interests.

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