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Case Report

Primary renal NUT carcinoma identified by next-generation sequencing: a case report and literature review

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Abstract: Background: NUT carcinoma is a rare aggressive squamous cell carcinoma subtype genetically characterized by *NUTM1* rearrangements. NUT carcinoma can be easily misdiagnosed as an undifferentiated carcinoma or Ewing sarcoma due to its primitive differentiation. Case presentation: We report a case of renal-derived NUT carcinoma diagnosed as a malignant small round-cell tumor resembling Ewing sarcoma/primitive neuroectodermal tumor where the diagnosis was revised to NUT carcinoma with a characteristic NUTM1 rearrangement based on next-generation sequencing (NGS). The patient received a standard NUT carcinoma treatment after recurrence but died of first-line chemotherapy failure due to advanced neoplasm progression. Conclusion: Routine NUT immunohistochemistry staining, NGS, and/or fluorescent *in situ* hybridization for poorly differentiated carcinoma and sarcoma can help avoid misdiagnosis of NUT carcinoma-related tumors, allowing patients to benefit from bromodomain and extra-terminal motif inhibitor therapy.

Keywords: Carcinoma, diagnosis, kidney, sarcoma, NUTM1

Introduction

Nuclear protein of testis (NUT) midline carcinoma (NUT carcinoma) is a relatively rare, highly malignant tumor [1]. Approximately 70% of NUT carcinomas have a chromosome translocation, t (15;19) (q15;p13), which leads to the gene fusion of NUT midline carcinoma family member 1 (*NUTM1*) with bromodomain containing 4 (*BRD4*). In the remaining cases, NUTM1 fuse with bromodomain containing 3 (*BRD3*) and other non-BRD genes [2, 3].

NUT carcinoma generally exhibits primitive differentiation with an abrupt transition to squamous epithelial component. The lack of squamous epithelial component in nearly 50% of the cases might lead to a misdiagnosis of undifferentiated carcinoma or Ewing's carcinoma. With the rapid development of molecular pathology, next-generation sequencing (NGS) has emerged as a means to provide unique advantages for the diagnosis of poorly differentiated cancers that lack characteristic histological features. We report a case of primary renal NUT carcinoma that was identified by NGS to have a *NUTM1* rearrangement and present a review of the relevant literature.

Case report

The patient was a 41-year-old Chinese male with no remarkable medical history who visited a local hospital due to the persistent lumbago for 1 month. An enhanced computed tomography (CT) scan revealed a soft tissue density shadow 7.1 cm × 2.6 cm in the left kidney. The density was uneven with a local protrusion from the renal silhouette and was intensified upon contrast enhancement of the CT scan. Laparoscopic radical excision was performed approximately 2 months after the mass was first noticed.

Methods

The nephrectomy specimen was fixed in 10% buffered formalin and processed in a routine

manner. Immunohistochemistry was performed on the formalin-fixed, paraffin embedded tissue using the streptavidin biotin complex technique after microwave retrieval. Antibodies were as follows: cytokeratin (EP16, Zhongshanjinqiao, 1:100), CK7 (EP16, Zhongshanjinqiao, 1:100), CK20 (EP23, Zhongshanjinqiao, 1:100), P63 (MX013, Maxim), S-100 (15E2E2+4C4.9, Zhongshanjinqiao, 1:100), BCOR (C-10, Zhongshanjinqiao, 1:100), CD99 (1217, DAKO, 1:200), Fli-1 (MRQ-1,Celnovte-bio,1:200), NUT (EP20, Zhongshanjinqiao, 1:100), Ki67 (EP5, Zhongshanjinqiao, 1:200). The immunohistochemical stains were evaluated by two pathologists with consensus.

Fluorescence in situ hybridization (FISH) for the formalin-fixed, paraffin-embedded tumor sections was performed for the exclusion of Ewing/PNET. Break-apart probes were used to detect the breakpoint, in accordance with the instructions. After obtaining consent from the patient, the surgically resected FFPE tissues and peripheral blood were tested for YuanSu S™ 450 tumor-related genes at both the DNA and RNA level for further diagnosis. The testing was carried out by OrigiMed (Shanghai, China).

Results

Based on gross examination, the resected kidney specimen was 14.0 cm × 10.0 cm × 6.5 cm with a mass at the inferior pole being 7.0 cm × 5.7 cm. Cut section revealed a gray-white and gray-red surface with an unclear boundary. The mucosae of the renal pelvis and ureter were smooth. Microscopic examination revealed a diffuse, patchy infiltration of tumor cells which were poorly differentiated. The cells were round or oval shape and ranged in size from small to medium. The cytoplasm was relatively rich and eosinophilic with plasmacytoid and epithelioid morphologies. They had irregularly shaped nuclei, some of which were vesicular-like. Nucleoli and mitosis were both prominent. Focal patchy necrosis was present with perineural invasion in some foci. There was extensive vascularization of the tumor stroma (**Figure 1A**).

Immunohistochemistry demonstrated that scattered tomor cells positively express CK (**Figure 1B**), and were negative for other epithelial markers like CK7, CK20. CD99 was diffusely expressed, while p63 (**Figure 1C**) and FLI-1 were expressed scatteredly. Stains for desmin, S-100, BCOR, and other lymphoid markers were negative. The Ki-67 proliferation index in the region of interest reached 25%. Based on the following results, a diagnosis was made of a malignant small round-cell tumor with a high likelihood of Ewing sarcoma/primitive neuroectodermal tumor (PNET). However, fluorescent *in situ* hybridization (FISH) was negative since a Ewing sarcoma breakpoint region 1 (*EWSR1*) could not be detected. NGS revealed that the tumor harbored only one reported mutation at the DNA and RNA level, a *BRD4* exon 14-*NUTM1* exon 3 fusion (**Figure 2**) with the tumor mutational burden (TMB) 0.8 mutations/megabase and stable microsatellite status. Further immunohistochemical analysis demonstrated positive expression of NUT protein (**Figure 1D**). Based on these results, a diagnosis of primary renal NUT carcinoma was made.

Follow-up and treatment

During the 5-month post-operative follow up, imaging showed enlarged perirenal lymph nodes and multiple nodules in both lungs. Although there was a high probability of metastasis, aspiration biopsy was not performed due to the position of lung lesions. Based on the standard treatment for NUT carcinoma, a 4-week course of vincristine, doxorubicin, cyclophosphamide/ifosfamide, and etoposide (VDC/IE) chemotherapy was initiated. The patient presented with extensive peritoneal dissemination and ultimately died of disease progression with the overall survival from the time of diagnosis being eight months.

Review of literature on ectopic thymoma

To date, under 1000 total NUT carcinoma cases have been reported, the first of which was from Kubonishi et al. in 1991 [3]. Primary renal NUT carcinoma is extremely rare. Our literature search identified reports of only five other cases of primary renal NUT carcinoma [4-7]. The clinicopathologic features of the current patient and those of the previously reported five cases are summarized in **Table 1**.

Discussion

NUT carcinoma is a rare, highly malignant tumor of unclear origin that is often considered a poorly differentiated squamous cell carcino-

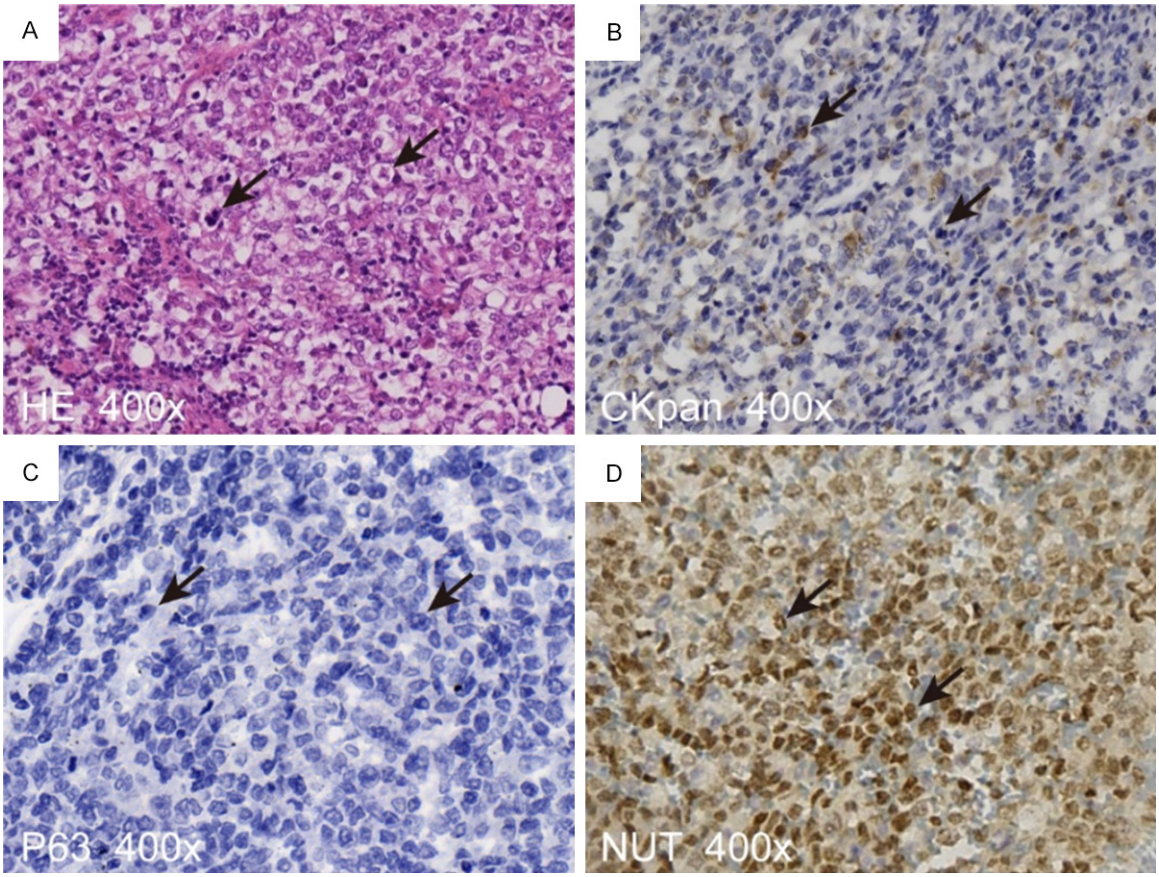

Figure 1. Morphology of surgically resected primary renal tumor tissue. A. Morphologic assessment of resected tumor specimen from the patient described in this report. The tissue sections were stained with hematoxylin and eosin (H&E) stain (400 × magnification). B-D. Immunohistochemical staining of tumor cells revealed diffuse positivity for pan-cytokeratin (CKpan), P63, and NUT.

ma whose clinicopathologic features are different from those of classical squamous cell carcinoma. NUT carcinomas can occur at any age (0-81.7 years); nevertheless, it primarily affects children, adolescents, and young adults. It typically develops at the midline with 50% of cases occurring in the aerodigestive tract and 41% occurring in the mediastinum [8]. It can also occur in parenchymal organs, including the urinary bladder, mammary gland, endometrium, kidney, and orbit, and is able to invade soft tissues and bone. However, confirmation of NUT mutations in tumors outside of the midline region has posed a new challenge that NUT fusions are no longer specific to the NUT carcinomas.

During the initial hospital visit by the patient described in the current case report, the tumor was limited to the kidney and imaging did not show significant abnormalities at any other sites. Accordingly, the kidney was considered to be the primary site.

Typical NUT carcinoma displays a patchy infiltration of small-to-medium-sized undifferentiated tumor components, low-to-moderate tumor cell cytoplasm volume, and active karyokinesis. Sudden triggering of squamous epithelial cell differentiation may occur in approximately half of the cases. In contrast to typical squamous cell carcinoma, which is characterized by rich polymorphism, the morphology of NUT carcinoma cells is relatively consistent with rare lesions *in situ* [9]. The current case of NUT carcinoma was similar to the five other reported cases in that all cells lacked squamous epithelial characteristics, and tumors demonstrated diffuse but strongly positive expression of CD99, which meant the tumor would also need differential diagnosis from Ewing sarcoma/PNET. EWSR1 was reportedly


A
chr15
chr19
chr15:34,639,316-34,639,593
chr19:15,351,506-15,351,783
NUTM1
BRD4

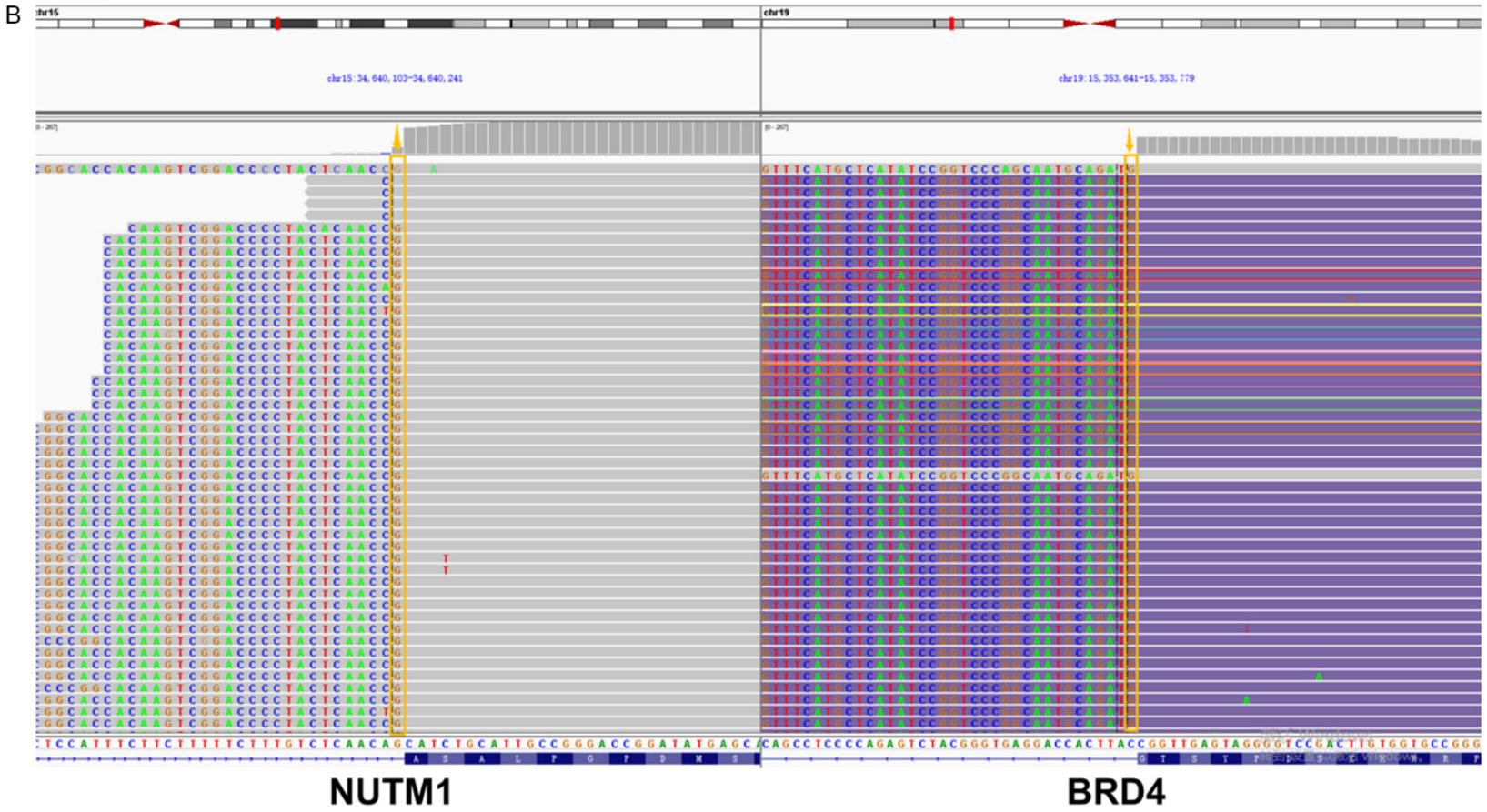

Figure 2. Integrative Genomics Viewer (IGV) screenshots of *BRD4* and *NUTM1* gene breakpoints detected by capture-based next-generation sequencing (NGS). Fusion of *BRD4-NUTM1* at the DNA (A) and RNA (B) levels according to by capture-based sequencing results.

Table 1. Clinicopathologic and genetic features of primary renal NUT carcinoma in 6 cases

NO.	Age	Sex	Squamous epithelium	IHC		Gene	OS	Reference
				Positive	Negative			
1	43	Female	-	NUT	NA	BRD4	-	Justin.et al [3]
2	36	Female	-	CK7, P63	NA	BRD4	6 m	John.et al [4]
3	71	Female	-	CK, GFAP, NUT	P63, S100, Desmin, CgA, Syn	BRD4	-	Brendan.et al [5]
4	36	Female	-	CK, P63, NUT	S100, Desmin, CgA, Syn	BRD4	-	Brendan.et al [5]
5	27	Male	-	CAM5.2, CK8/18, EMA, CD99, NUT	CK7, S100, Desmin, CgA, Syn, TTF-1	BRD4	11 m	Zhu Y.et al [6]
6	41	Male	-	CK, P63, CD99, NUT	S100, Desmin, BCOR	BRD4	8 m	this case

fused with FLI1 in only 85% of cases [10]. Although the FISH results in our patientindicated that *EWSR1* escaped probe detection of gene breakage and translocation, this negative result could not completely rule out a diagnosis of Ewing sarcoma. Therefore, a diagnosis of NUT carcinoma was made only after NGS analysis.A review of the literature revealed that NUT carcinoma is often misdiagnosed as Ewing sarcoma/PNET [11]. Ewing sarcoma and other small round cell tumors are difficult to discriminate morphologically from NUT carcinoma when the latter consists of monomorphic small round cells. A characteristic feature of NUT carcinoma based on immunohistochemistry is that > 50% of tumor nuclei are positive for NUTM1, with a sensitivity and specificity of 87% and 100%, respectively. The diagnostic sensitivity can reach 100% combining immunohistochemistry with FISH [12].

While NUT immunohistochemistry can be used as a screening tool, definitive diagnosis of NUT carcinoma requires the molecular detection of *NUTM1* rearrangement/fusion. *NUTM1* has multiple fusion partners, two-thirds of which are *BRD4*, while the remaining are *BRD3* or *NSD3* [13]. The partner gene of NUTM1 of both the five primary renal NUT carcinomas from the literature and our current case all shows *BRD4*. Whether the partner gene is related to the primary site needs further investigation.

The rapidly increasing use of NGS has led to the discovery of additional fusion partners for *NUTM1*, including *CIC*, *BCORL1*, *MYXD1,* and *MGA* [6, 14, 15], which has also widened the range of tumor histology targets. A report by den Bakker *et al*. [16] described a parotid tumor as the primary site, which involved a *BRD4-NUTM1* fusion and cartilage-differentiated sarcoma component. Furthermore, of 26 cases of tumors with *NUTM1* rearrangements reported by Stevens *et al*. [17], one was a primary pulmonary tumor with an *MGA-NUTM1* fusion, which presented a myxoid chondrosarcoma morphology [15]. Underwood et al. [20] also presented an epithelioid hyalinizing sarcoma harboring the MGA-NUTM1 fusion in a acral site which corroborated the fact that NUTM1-rearranged soft tissue tumors represent a spectrum of heterogeneous morphologic entities. Therefore, tumors with *NUTM1* rearrangements are no longer specific for NUT carcinomas, but may also be present in NUTM1-fused sarcomas. The relationship between NUT sarcomas and the more common NUT carcinomas requires further investigation with some investigators suggesting that this type of tumor should be generically classified as a “NUT-related tumor” [5].

The clinical course of NUT carcinoma is naturally risk-prone and its prognosis is extremely poor. At the time of initial diagnosis, most patients already have distant metastases and respond poorly to traditional radiotherapy and chemotherapy with the median survival being only 6.7 months [18].

In recent years, the emergence of oral bromodomain and extra-terminal domain (BET) inhibitors targeting the BRD2, BRD3, and BRD4 proteins has been beneficial to the management of NUT carcinoma. BET inhibitors mimic the structure of acetylated histone lysine residues and thereby interrupt the interaction between BRD4 and actual acetylated histone lysine. This results in the attenuation of abnormal transcription and induces tumor cell differentiation. However, oral BET inhibitors may not be effective for patients without *BRD-NUTM1* fusions [19]. Detecting the NUTM1 rearrangement

might not be sufficient and identifying the fusion partner seems rather crucial for appropriate clinical management.

Conclusion

NGS not only is beneficial in the diagnosis of NUT carcinoma, but also allows for the use of more targeted therapies as a result of being able to assess the precise mutations of cancer-related genes. Our experience with the patient described in this report has led us to perform NUT immunohistochemistry on all poorly differentiated tumors, both carcinomas and sarcomas. This can then be followed by NGS and FISH for validation in order to avoid missing or incorrectly diagnosing *NUTM1* fusion-related tumors, thereby benefitting more patients.

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Written informed consent was obtained from the patient for the publication of this case report and any accompanying images. A copy of the consent form is available for review by the Editor of Diagnostic Pathology.

Disclosure of conflict of interest

None.

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