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Teaching case

NSD3-NUT-expressing midline carcinoma of the lung: First characterization of primary cancer tissue

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ABSTRACT

Background: Nuclear protein in testis (NUT) midline carcinoma (NMC) is a rare, aggressive malignancy. Only two pediatric and three adult cases of pulmonary NMCs have been documented. In more than two-thirds of NMC cases, a gene fusion between *NUT* and *BRD4* or *BRD3* has been documented; other fusions are rare.
Case presentation: A 36-year-old woman was admitted because of a rapidly progressing tumor of the lung with metastases to the breast and bone. A biopsy from the lung tumor revealed an undifferentiated neoplasm exhibiting round to oval nuclei with vesicular chromatin, prominent nucleoli, and scant cytoplasm. Immunohistochemical staining demonstrated focal EMA, cytokeratin AE1/AE3, cytokeratin CAM 5.2, p63, CD138, and vimentin positivity. Finally, the nuclear staining pattern for NUT confirmed a histopathological diagnosis of NMC. A 5′- rapid amplification of the cDNA end (RACE) procedure successfully identified the partner of the *NUT* translocation as *NSD3*, a recently discovered partner. Fluorescence *in situ* hybridization confirmed the *NSD3-NUT* gene rearrangement, whereas a *BRD3/4-NUT* fusion gene was not detected.
Conclusion: We herein describe the first case of an NSD3-NUT-expressing NMC of the lung. The further accumulation of variant NMCs should provide clues to the establishment of new individualized therapy for NMCs.

Introduction

Overall, malignant tumors of the lung that present with an undifferentiated morphology pose significant diagnostic challenges to attending surgical pathologists, especially in cases where a limited amount of biopsy material is available [20] and the immunohistochemical results are ambiguous or atypical [21]. Among these malignant neoplasms, nuclear protein in testis (NUT) midline carcinoma (NMC) is a recently recognized entity that is characterized by undifferentiated morphological features and immunoreactivity to NUT. This disease entity is notorious for its poor prognosis [1,16].

NMCs of the lung have been reported, but they are extremely rare; only 5 cases, including 2 pediatric [20] and 3 adult [7,21] cases, have been documented. Currently, the diagnosis of NMC depends on the identification of a genetic change (a rearrangement involving the *NUT* locus at 15q14) that generates a specific fusion transcript with a member of the bromodomain-containing protein (BRD) family, such as *BRD4* located on chromosome 19p13.1. In approximately two-thirds of NMCs [6,8,9], *BRD4-NUT* is specifically detected. Less commonly, NMC can harbor a different rearrangement involving *NUT* [9]; that is, a subset (approximately 25%) of these cases exhibit a *BRD3-NUT* rearrangement. In the remaining cases, the genes involved in *NUT* rearrangement are unknown [9]. For example, fluorescence *in situ* hybridization (FISH) has demonstrated a *BRD4-NUT* rearrangement in 2 pediatric cases of NMC of the lung [20]. In the remaining 3 adult cases of NMC arising in the lung, although *NUT* rearrangements were suspected because of break-aparts of the *NUT* locus in the cancer cells, the partner gene, supposed to be fused with *NUT*, was not identified

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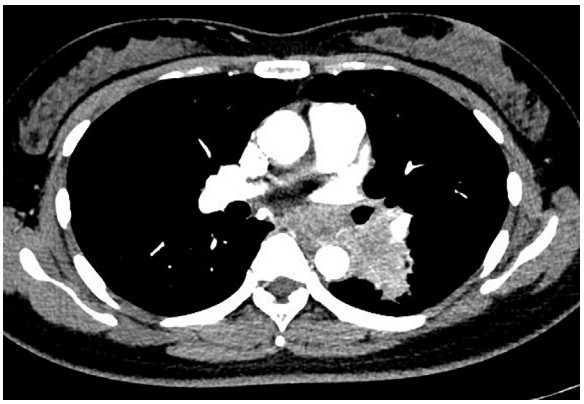

Fig. 1. Enhanced computed tomography scan performed at the time of hospitalization revealing a mass in the left lung.

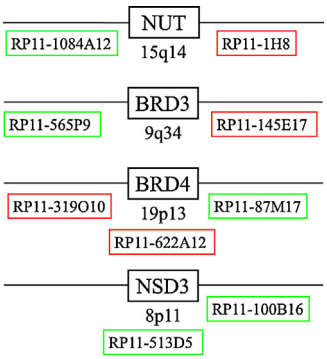

Fig. 2. Chromosomal positions of FISH probes flanking or including the genes of interest (*NUT*, *BRD3*, *BRD4*, and *NSD3*).

[7,21]. To date, no "variant NMC" of the lung harboring a fusion other than *BRD3/4-NUT* has been reported.

Recently, a variant NMC cell line (1221) was established, for the first time, from the discarded lung tumor tissue of a poorly differentiated squamous cell carcinoma of the mediastinum that had metastasized to the lung [10]. Using this cell line, a novel *NSD3* (*nuclear receptor binding SET domain 3*)-*NUT* fusion oncogene, which is both necessary and sufficient for the blockade of the differentiation and maintenance of proliferation in NMC cells, has been demonstrated [10]. Thus, NSD3 is a potential therapeutic target in NMC and a clinical example exhibiting this situation at a primary site has been sought.

In the present article, we describe the first case of an *NSD3-NUT* rearrangement identified in the primary tissue of an NMC of the lung; the NMC was diagnosed using immunohistochemistry with a highly sensitive and specific anti-NUT monoclonal antibody [11]. In addition, a *NSD3-NUT* fusion gene was successfully identified using 5′-rapid amplification of the cDNA end (RACE) and was validated using FISH.

Clinical summary

A 36-year-old woman sought medical advice because of a cough accompanied by wheezing with a 2-month duration. An enhanced computed tomography scan performed at the time of hospitalization revealed a tumor surrounding the proximal part of the left lower lobe bronchus with obstruction of the left bronchus B6 and extending to the middle mediastinum adjacent to the left hilum (Fig. 1), strongly indicating that the tumor was derived from the left lung rather than from the mediastinum. Metastatic lesions were revealed in the liver, breast, bones and lymph nodes. A biopsy was performed from the lung tumor and the lymph node.

The patient has been treated with chemoradiation therapy, including a regimen of cisplatin and docetaxel and radiotherapy for metastatic lesions, for 9 months since her diagnosis. The outcome was once partial response, however, eventually the status became disease progression at the point of 9 months after the diagnosis. The patient did not undergo targeted therapies.

Materials and methods

Histopathologic and immunohistochemical examination

The tissues were fixed in 10% buffered formalin and were embedded in paraffin after routine processing, followed by sectioning and staining with hematoxylin and eosin (H&E). Immunostaining was performed using DAKO autostainer universal staining system (DAKO, Glostrup, Denmark) with antibodies for the following antigens: CD5 (clone 4C7, 1:150 dilution, DAKO), CD30 (clone Ber-H2, 1:100, DAKO), CD45 (clone 2B11 + PD7/26, 1:150, DAKO), CD56 (clone CD564, 1:100, Leica Biosystems, Newcastle, United Kingdom), CD99 (clone 12E7, 1:100, DAKO), CD138/Syndecan-1 (clone MI15, 1:100, DAKO), CEA (poly, 1:600, DAKO), chromogranin A (poly, 1:100, DAKO), c-kit (poly, 1:100, DAKO), cytokeratin AE1/AE3 (clone AE1 + AE3, 1:100, DAKO), cytokeratin CAM5.2 (clone CAM5.2, prediluted by manufacturer, Becton, Dickinson and Company, CA, USA), desmin (clone D33, 1:100, DAKO), epithelial membrane antigen (EMA, clone E29, 1:150, DAKO), myogenin (clone F5D, 1:50, DAKO), NUT (C52B1, 1:25, Cell Signaling Technologies Inc., Danvers, MA, USA), p63 (clone 4A4, 1:100, Santa Cruz Biotechnology, Dallas, TX, USA), PLAP (clone 8A9, 1:100, Leica Biosystems), S-100 (poly, 1:1000, DAKO), synaptophysin (poly, 1:150, DAKO), TTF-1 (clone 8G7G3/1, 1:100, DAKO) and vimentin (clone V9, 1:200, DAKO).

FISH

The FISH procedure was performed as previously reported [17–19]. FISH probes were prepared from the Bacterial Artificial Chromosome (BAC) library. BAC probes flanking *NUT* (RP11-1084A12 and RP11-1H8), flanking *BRD3* (RP11-565P9 and RP11-145E17), flanking *BRD4* (RP11-319O10 and RP11-87M17), flanking *NSD3* (RP11-100B16), containing *BRD4* (RP11-622A12), and containing *NSD3* (RP11-513D5) were purchased from Advanced GenoTechs Co. (Tsukuba, Japan). All the probes were confirmed to be correct by hybridizing them to a metaphase human chromosome spread (data not shown). The RP11-1084A12, RP11-565P9, RP11-87M17, RP11-513D5 and RP11-100B16 probes were nick-translated using Green dUTP (2N32-50; Enzo Life Science, Farmingdale, NY, USA), and the RP11-1H8, RP11-145E17, RP11-319O10 and RP11-622A12 probes were nick-translated using Orange dUTP (2N33-50; Enzo Life Science). The colors and BAC probe RP numbers that were used are shown in Fig. 2. To detect the break-apart of *NUT*, *BRD3*, and *BRD4*, the RP11-1084A12, RP11-1H8; RP11-565P9, RP11-145E17; and RP11-319O10, RP11-87M17 probes were used, respectively. To detect the *BRD4-NUT* fusion, the combination of RP11-87M17 and RP11-1H8 was used. In addition, another combination of RP11-622A12 (containing BRD4) and RP11-1H8, the latter of which was nick-translated using Green dUTP (2N32-50) for this particular purpose, was used to detect the *BRD4-NUT* fusion for validation purposes. To detect the fusion of *BRD3-NUT*, the combination of RP11-1084A12 and RP11-145E17 was used. To detect *NSD3-NUT*, a mixture of RP11-513D5 (containing NSD3), RP11-100B16 (flank NSD3), and RP11-1H8 (flank NUT) was used.