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【 CASE REPORT 】

Nuclear Protein in Testis Carcinoma of the Thorax

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Abstract:
Nuclear protein in testis (NUT) carcinoma (NUT-C) is an exceedingly rare and aggressive neoplasm. We herein report a case of a 57-year-old man with a rapidly progressing tumor of the thorax and left pleural effusion. The pathological features and immunohistochemical staining of specimens obtained by a transbronchial lung biopsy initially indicated poorly differentiated squamous cell carcinoma. However, given the clinical presentation along with the additional histopathologic features, NUT-C was considered. Immunohistochemical staining for NUT was positive in the pleural fluid cell block, confirming the diagnosis of NUT-C. This report indicates the utility of immunohistochemical staining for diagnosing NUT in the pleural fluid cell block.

Key words: nuclear protein in testis NUT carcinoma, NUT-C, immunohistochemical staining for NUT, pleural fluid cell block

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Introduction

Nuclear protein in testis (NUT) carcinoma (NUT-C) is a poorly differentiated tumor that is characterized by the rearrangement of the NUT gene on chromosome 15q14. It was first described in 1991 by Kubonishi et al. (1). as a highly malignant thymic and mediastinal carcinoma with t(15:19). The fusion of the NUT gene on chromosome 15 to bromodomain-containing protein 4 (BRD4-NUT) is a result of this translocation and was first identified in 2003 (2). NUT-C was first reported to arise in the mediastinum and other midline organs. However, other sites of origin, such as the parotid gland, pancreas, and lung, have also been reported (3-5). Because NUT-C is a rare and highly malignant carcinoma, it is registered by the Internal NUT Midline Carcinoma Registry (INMCR). Approximately 15 to 20 cases are registered annually.

NUT-C cases of the lung or the mediastinum are extremely rare (6, 7). It was reported by Sholl et al. (8). that 1 case among 166 consecutive in-house biopsies of lung carcinomas lacking glandular differentiation was identified as NUT-C. The new 2015 World Health Organization (WHO) Classification of Tumors recognizes the existence of NUT-C in the thorax, specifically the lungs and the mediastinum. In addition, it is also rare for NUT-C to be diagnosed from immunohistochemical staining for NUT in the pleural fluid cell block.

We herein report a 57-year-old man with NUT-C localized in the thorax who was diagnosed based on the findings of immunohistochemical staining for NUT in the pleural fluid cell block.

Case Report

A 57-year-old man, with no significant medical history, was referred to Yodogawa Christian Hospital with acute coughing with a history of approximately 1 month. He was a non-smoker and non-drinker. On a physical examination, his breath sounds were decreased on the left side. His performance status (PS) was 1 because of his coughing. His father had died of gastric cancer at 50 years of age.

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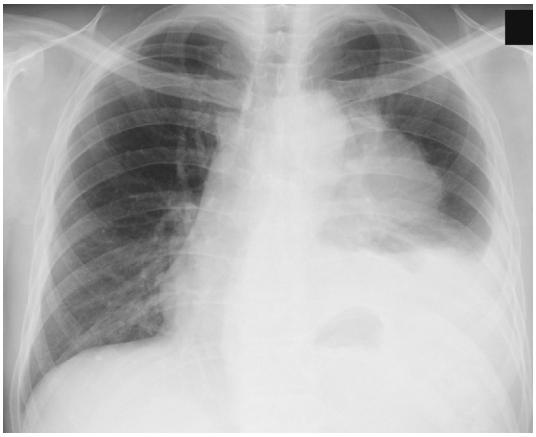

Figure 1. **Chest radiograph demonstrated left-sided pleural effusion and a mass in the left hilar area.**

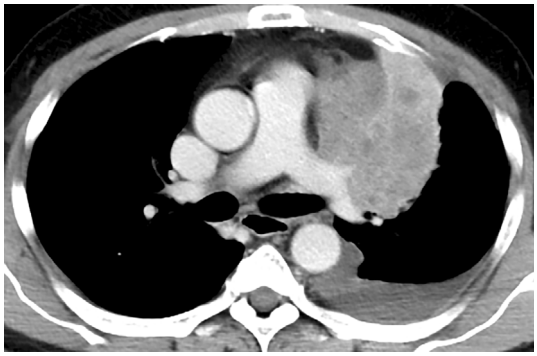

Figure 2. **CT of the chest showed a 9×9-cm mass in the upper lobe of the left lung that was confluent with hilar and mediastinal adenopathy and invading the left pulmonary artery.**

Laboratory investigation findings were significant for elevated lactate dehydrogenase (LDH) (393 U/L), squamous cell carcinoma-related antigen (SCC) (2.9 ng/mL), neuron-specific enolase (NSE) (26.1 ng/mL), and soluble interleukin-2 receptor (sIL-2R) (568 U/mL). Chest radiograph demonstrated left-sided pleural effusion and a mass in the left hilar area (Fig. 1). Computed tomography (CT) of the chest showed a 9×9-cm mass in the left upper lobe that was confluent with hilar and mediastinal adenopathy and invading the left pulmonary artery. In addition, there was right supraclavicular lymphadenopathy and pleural effusion (Fig. 2). Positron emission tomography (PET)/CT demonstrated marked fluorodeoxyglucose (FDG) avidity of the dominant lung lesion, as well as lymphadenopathy and pleural effusion. A transbronchial lung biopsy of the mass showed a poorly differentiated neoplasm comprising atypical cells with a monomorphic appearance and granular-to-coarse chromatin. Mitotic figures were slightly seen but not clearly, as apoptotic cells and degenerative cells are frequently seen with neutrophil infiltration (Fig. 3A and B). Immunohistochemical staining was positive for cytokeratin AE1/AE3 (CK AE1/AE3) and cytokeratin 5/6 (CK 5/6), and negative for thyroid transcription factor 1 (TTF-1), p40, leukocyte common antigen (LCA), and S-100 (Fig. 3C-E). Thus, the patient was diagnosed with poorly differentiated squamous cell lung carcinoma.

He received combination chemotherapy (cisplatin 80 mg/m^2 and docetaxel 60 mg/m^2, every 3 weeks). CT of the chest performed after two cycles of chemotherapy showed that the tumor size and pleural effusion had increased, and pericardial effusion emerged. Therefore, he received second-line chemotherapy (nivolumab 3 mg/kg, every 2 weeks).

We doubted the reliability of his diagnosis of poorly differentiated squamous cell carcinoma, because he was a young non-smoker, and we found its resistance to conventional chemotherapy unusual. Therefore, we planned to perform a histological examination a second time in consideration of other malignant tumors. Additional testing was scheduled with a pleural fluid cell block, as his poor condition did not allow for an invasive inspection, such as a transbronchial lung biopsy. The cytological findings in the pleural fluid showed loosely cohesive clusters and solitary monomorphic cells with irregular nuclear contours, granular-to-coarse chromatin, and prominent nucleoli (Fig. 3F). The pleural fluid cell block showed the same pathological features as the lung biopsy tissue. Given the clinical presentation, including a resistance to conventional chemotherapy and rapid growth, along with the histopathologic features, the possibility of NUT-C was suggested. Thus, immunohistochemical staining for NUT using NUT (C52B1) rabbit monoclonal antibody was performed with the cell block at Osaka Habikino Medical Center and Iwata City Hospital. The results were positive, confirming the diagnosis of NUT-C. Immunohistochemical staining for NUT in the lung biopsy tissue was also positive (Fig. 3G and H).

At the time of the definitive diagnosis, three cycles of nivolumab had already been administered. However, his disease progressed rapidly, and he died four months after the onset of the disease.

Discussion

NUT-C is a rare and aggressive cancer. In approximately 70% of cases, the NUT gene has been found to form a stable fusion oncogene with BRD4 on chromosome 19. The remaining cases have been fused to BRD-3 or other rare or uncharacterized fusion partners (2, 9).

The exact incidence and frequency of NUT-C are unknown. Although it was first recognized as occurring almost exclusively in young children, recent studies have reported a wider age group, including individuals older than 60 years of age (10). The risk factors of this malignancy remain unknown. Most patients with this tumor have little or no smoking history. In addition, Epstein-Barr virus and human papilloma virus have not been detected in patients with NUT-C.

The histological features of NUT-C range from entirely undifferentiated carcinomas to carcinomas with prominent squamous differentiation. Thus, the differential diagnosis is

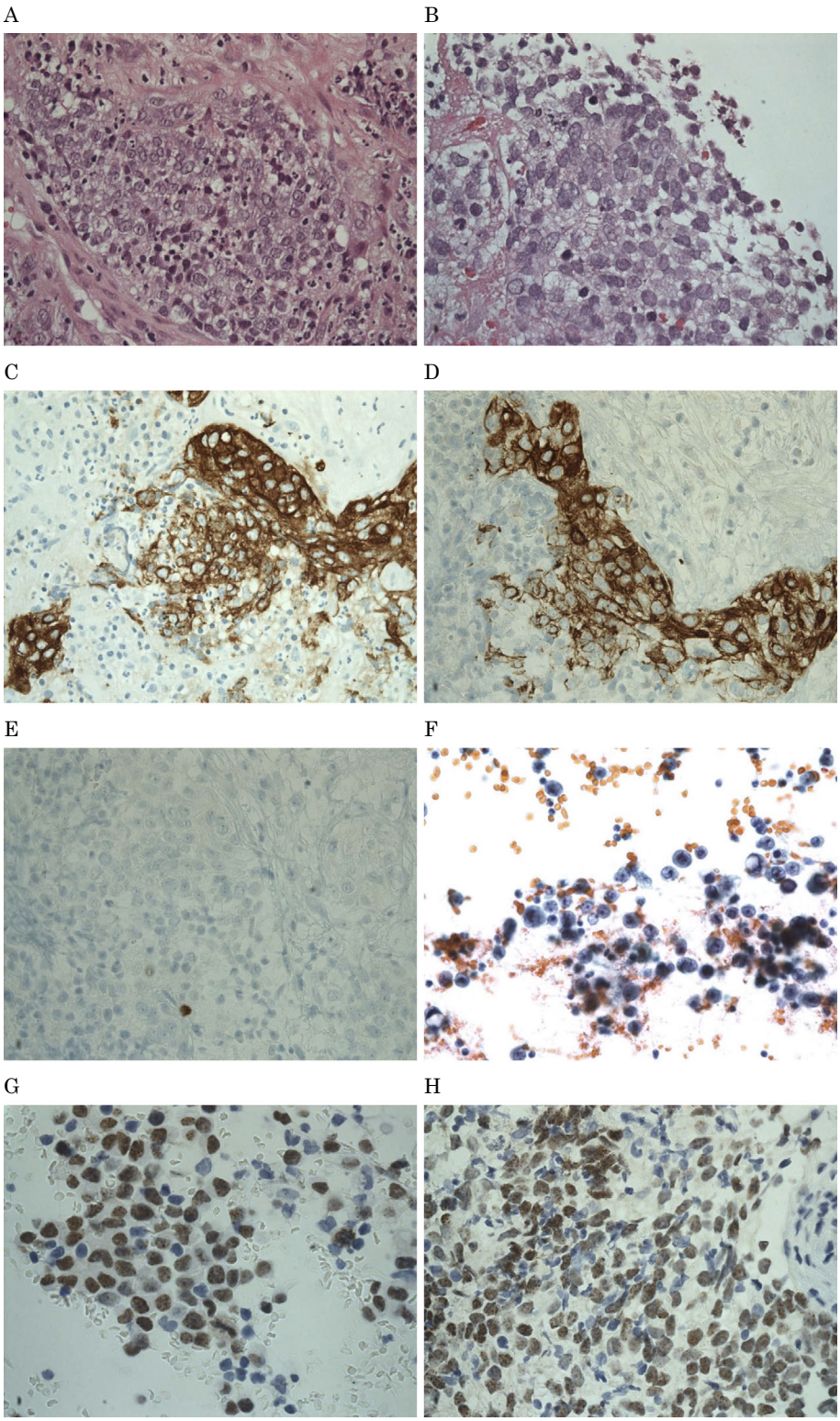

Figure 3. **On Hematoxylin and Eosin staining, a transbronchial lung biopsy of the mass showed a poorly differentiated neoplasm comprising atypical cells with a monomorphic appearance and granular-to-coarse chromatin. Mitotic figures were slightly seen but not clearly. (A ×40, B ×63). The neoplastic cells displayed diffuse moderate immunoreactivity for CK AE1/AE3 (C ×40), and CK 5/6 (D ×40). Immunohistochemical staining was negative for p40 (E ×40). On Papanicolaou-staining, the pleural effusion showed loosely cohesive clusters and solitary monomorphic cells with irregular nuclear contours, granular-to-coarse chromatin, and prominent nucleoli (F ×400). The immunoreaction for NUT was positive in the tumor cells of the pleural fluid cell block (G ×63) and biopsied specimen (H ×63). CK: cytokeratin**

broad, including poorly differentiated squamous cell carcinoma, small cell carcinoma, round cell sarcoma, high grade lymphoma, germinoma, and any other malignant tumor. The cytological features of NUT-C show a highly cellular, predominantly noncohesive pattern of relatively small cells with a round nucleus, scant cytoplasm, irregular nuclear contours, variably prominent nucleoli, and identifiable mitotic figures (11).

The tumor is usually diagnosed by fluorescence *in situ* hybridization (FISH) for NUT rearrangements or by demonstrating >50% nuclear immunohistochemical staining for a NUT monoclonal antibody. The NUT monoclonal antibody has demonstrated a specificity of 100%, sensitivity of 87%, and positive predictive value of almost 100% (12, 13) and can now facilitate the rapid and cost-effective diagnosis of NUT-C.

Furthermore, the findings in the cell block material can support the diagnosis of NUT-C, including immunohistochemical staining for NUT (14, 15). We were able to diagnose NUT-C based on the findings of immunohistochemical staining for NUT in the pleural fluid cell block. Even if tumor tissue cannot be collected due to the severity of a patient's condition or the lack of examination facilities, NUT-C can still be diagnosed as long as body cavity fluid is available for sampling.

Immunohistochemical staining for NUT can serve as an initial screening tool. Physicians and pathologists should recognize the possibility of NUT-C, especially in cases of young and non-smoking patients with rapidly progressing poorly differentiated carcinomas. Immunohistochemical staining for NUT is recommended in such cases.

The majority of patients with NUT-C of the thorax are treated with multimodal therapy including chemotherapy, radiation, and surgery. However, the outcome remains dismal. The median overall survival is 6.9 months, ranging from 3 days to 26 months (16). There have been no reports of NUT-C responding well to combination chemotherapy (cisplatin and docetaxel) or nivolumab, the treatments used in the present case. The effects of cytotoxic chemotherapy are limited. It may be impossible to cure or control NUT-C with cytotoxic chemotherapy alone. Two therapies that target the oncogenic mechanism of the dual bromodomains and the p300-binding portion of BRD4-NUT have emerged, including bromodomain inhibitors (BETis) and histone deacetylase inhibitors (HDACis), both of which may induce the differentiation and growth arrest of tumor cells (17, 18). Several phase 1 clinical trials of BETis (GSK525762 and OTX015/MK-8628) and HDACis (CUDC-907) are being carried out. These therapies are expected to improve the prognosis of NUT-C.

Conclusion

In summary, NUT-C remains an aggressive and usually fatal neoplasm. While the incidence remains low, the recent advent of immunohistochemical staining for NUT is expected to facilitate the diagnosis of NUT-C once it is clinically suspected. We were able to diagnose NUT-C based on immunochemical staining for NUT in the pleural fluid cell block. In addition, the present case showed that even if tumor tissue cannot be collected due to the severity of a patient's condition or the lack of examination facilities, NUT-C still be diagnosed as long as body cavity fluid is available for sampling. Immunohistochemical staining for NUT in the cell block may be useful if patients do not respond to chemotherapy directed towards the initial diagnosis.

The authors state that they have no Conflict of Interest (COI).

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