Case Report

Transcriptomic profiling of a late recurrent nuclear protein in testis carcinoma of the lung 14 years after the initial operation: a case report

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Background: Nuclear protein in testis (NUT) carcinoma (NC) of the lung is a rare cancer that occurs mainly in young adolescents and adults. NC is genetically characterized by *NUTM1* rearrangements, which usually take the form of *BRD4-NUT* fusions. The prognosis for NC is dismal, and treatment with conventional chemotherapeutic regimens is ineffective.

Case Description: We herein describe the case of a 53-year-old woman with recurrent NC of the lung 14 years after surgery for nasal cavity cancer. Chest computed tomography revealed a 5.5-cm tumor in the lower lobe of the left lung. We completely resected the recurrent lung NC via thoracotomy. Immunohistochemistry (IHC) of the lung and nasal cavity cancers showed diffuse strong expression of NUT. RNA-seq of the lung NC revealed *NUTM1* rearrangement, with a fusion of *BRD4* exon 10 to *NUTM1* exon 4. This breakpoint has never been reported before. In addition, IHC revealed elevated expression of parathyroid hormone-like hormone in the lung NC but not in the nasal cavity NC, indicating that the lung and nasal cavity NCs were metachronous multiple primary cancers.

Conclusions: We experienced a rare recurrence of lung NC 14 years after the initial surgery. The *BRD4-NUT* fusion consisted of a new breakpoint. Furthermore, the expression pattern of parathyroid hormone-like hormone (*PTHLH*) suggested that the NCs in the nasal cavity and lung may be metachronous multiple lung cancers. This extremely rare case highlighted the possibility of identifying less malignant NCs in patients with poorly differentiated tumors via fusion gene analysis and the need to develop more effective treatment strategies for this malignancy.

Keywords: *BRD4-NUT*; parathyroid hormone-like hormone (*PTHLH*); late recurrence; extraterminal domain; case report

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Introduction

Nuclear protein in testis (NUT) carcinoma (NC) of the lung is a rare malignancy that predominantly affects adolescents and young adults. NC occurs in the upper aerodigestive tract, particularly the sinonasal area. NC cases of the lung or mediastinum are extremely rare; only few cases have been reported to date (1). NC is characterized by the rearrangement of *NUT*. The *NUT* rearrangements usually form *BRD4-NUT* fusions, but *BRD3-NUT* or NUT-variant fusions are formed in a minority of cases (2,3). Treatment of NC with conventional chemotherapeutic regimens is ineffective, and the prognosis of patients with NC remains poor. Herein, we describe the case of a woman with recurrent NC of the lung 14 years after the removal of NC from the nasal cavity. The pathology of this rare disease and the relationship between the primary and recurrent tumors were investigated through RNA-seq and immunohistochemical analysis of the tumor tissue. We present this article in accordance with the CARE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-259/rc).

Case presentation

A 53-year-old woman was referred to Nagoya University Hospital for examination of a tumor in the left lung after its detection via computed tomography (CT) (*Figure 1*). The patient was generally in good condition and had a history of spinocerebellar degeneration and surgery for nasal cavity cancer 14 years ago, which existed on middle nasal meatus and was 3.2 cm in size. Chest CT revealed a 5.5-cm tumor in the lower lobe of the left lung. The left inferior pulmonary vein (PV) was obstructed by the tumor, but no intrapericardial invasion was detected (*Figure 1*). Fluorodeoxyglucose (FDG) positron emission tomography revealed high accumulation of FDG in the tumor (maximum standard uptake was 7.25) but no accumulation in the hilar and mediastinal lymph nodes or distant metastases. After bronchoscopy, the tumor was diagnosed as squamous cell carcinoma and left lower lobectomy was planned.

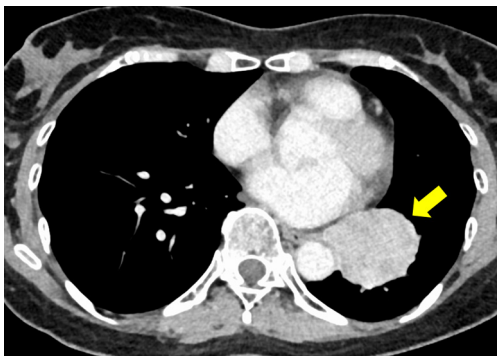

Figure 1 Preoperative chest computed tomography scan showing a 5.5-cm tumor in the left lower lobe (yellow arrow).

Surgery was performed via thoracotomy, with the patient under general anesthesia. The tumor was located in the left lower lobe and extended into the extrapericardial PV. The pulmonary ligament was cut and PV was secured and resected within the pericardial sac. The lower lobe pulmonary artery was dissected and cut with a surgical stapler. After dissection of the subcarinal lymph nodes, the lower lobe bronchus was divided with a stapler. The upper mediastinal lymph nodes were also dissected. The postoperative course was uneventful. The chest tube was removed on postoperative day 2, and the patient was discharged without complications on postoperative day 6. Postoperative systemic therapy was not administered because the lung cancer was completely resected and no metastasis was detected on pathological diagnosis. Two years after surgery, the patient developed putaminal hemorrhage, and CT revealed metastasis of NC to the chest wall. The patient died by suicide in the same year.

Histopathological examination revealed that the nasal

Highlight box

Key findings

- Transcriptome profiling of a resected case of late recurrent nuclear protein in testis (NUT) carcinoma (NC) of the lung demonstrated a *BRD4-NUT* fusion, which may have influenced the favorable prognosis of this patient.

What is known and what is new?

- NC of the lung is a rare cancer and the prognosis is dismal. This disease is genetically characterized by *NUTM1* rearrangements, which usually take the form of *BRD4-NUT* fusions.
- RNA-seq of the recurrent lung NC revealed *NUTM1* rearrangement, with fusion of *BRD4* exon 10 to *NUTM1* exon 4. This breakpoint has never been reported before. In addition, immunohistochemistry revealed elevated parathyroid hormone-like hormone in the recurrent lung NC but not in the nasal cavity NC, indicating that the lung and nasal cavity NCs were metachronous multiple primary cancers.

What is the implication, and what should change now?

- This rare case provides evidence for NC with a favorable prognosis and supports the utility of fusion gene analysis to predict prognosis.

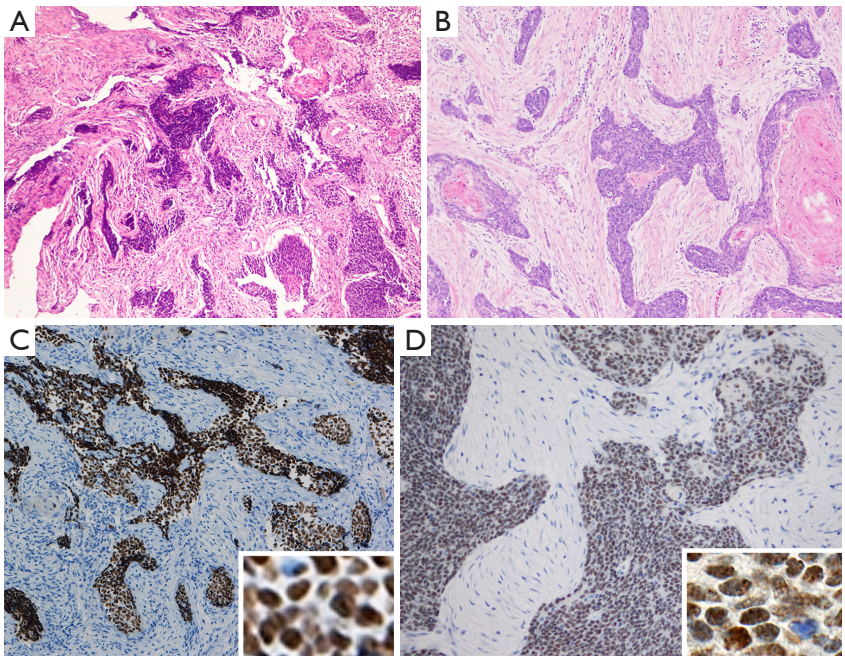

Figure 2 Histopathologic characteristics of the initial and recurrent tumors. Hematoxylin and eosin staining of the NUT carcinoma in the nasal cavity (A, ×40) and the lung (B, ×40). Both cancers formed solid alveoli of poorly differentiated cells. Immunohistochemistry for NUT in the nasal cavity (C, ×40 and ×400) and lung (D, ×40 and ×400) cancers demonstrated diffuse nuclear staining. NUT, nuclear protein in testis.

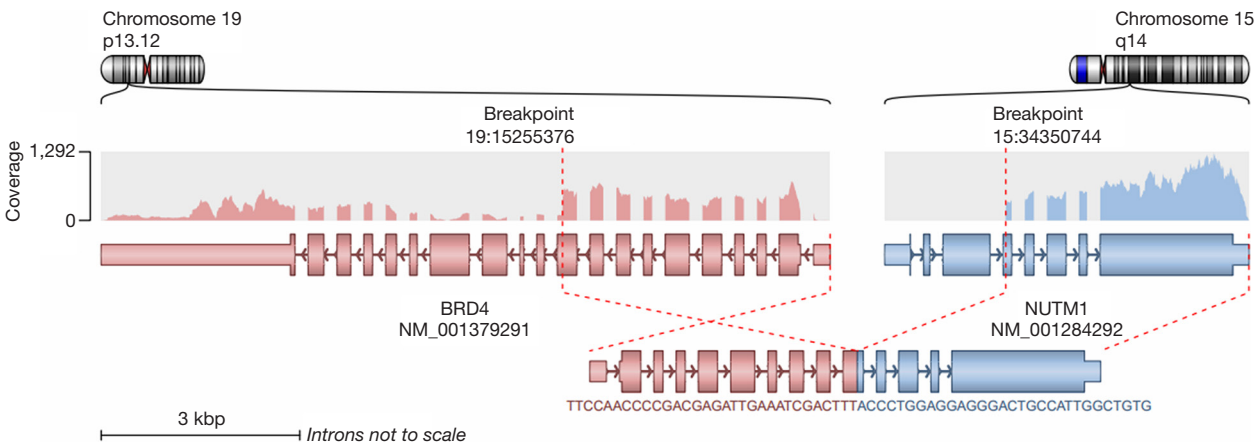

Figure 3 Genomic structure of *BRD4-NUT* translocation in this case.

cavity cancer diagnosed 14 years ago also formed solid alveoli of poorly differentiated cells (*Figure 2A*). In the lung tumor, poorly differentiated proliferative cells with heteromorphic nuclei infiltrated and formed large and small solid alveoli with keratinization (*Figure 2B*). No pericardial involvement or lymph node metastases were observed. Immunohistochemical analysis revealed tumor cells in both the nasal cavity and lung with diffuse strong NUT expression (*Figure 2C*,*2D*). Although the nasal cavity cancer may have metastasized to the lung, the nasal cavity cancer was histologically more malignant in comparison with the lung cancer.

RNA-seq (Macrogen, Seoul Korea) of the lung cancer tissue was performed, and Arriba analysis of whole transcriptomic sequence data showed the fusion of *BRD4* exon 10 to *NUTM1* exon 4 (*Figure 3*). Breakpoints are usually located at exon 11 or 15 in *BRD4* and exon 1b or 2

Table 1 Top 30 genes specifically expressed in NUT carcinoma compared to normal lung tissue

Gene symbol	NUT tissue (TPM)	Normal lung (TPM)	Fold change
PTHLH	1,598.0	0.6	1,000.5
SOX2	1,594.0	0.6	974.8
XIST	466.9	0.1	428.8
MIF	1,669.8	3.2	399.1
IGF2	3,969.3	9.2	389.1
TFAP2B	319.4	0.0	316.1
KRT5	1,389.7	3.6	303.9
CXCL11	547.3	1.5	219.0
LGALS7B	351.2	0.7	211.5
SOX2-OT	206.0	0.1	196.3
CA9	243.6	0.3	192.5
MYB	476.2	1.8	169.8
TP63	272.2	0.6	169.6
IRX4	158.4	0.0	156.4
SYT8	1,165.3	7.3	139.7
TFAP2A	171.2	0.2	138.3
ART3	161.8	0.3	128.1
RPL41P1	196.0	0.6	126.9
CALB1	114.1	0.1	100.6
GPR87	111.6	0.1	99.1
RN7SL4P	123.7	0.3	98.9
FOXD1	126.3	0.3	97.3
SNORA67	131.9	0.4	93.4
PNCK	129.2	0.4	93.0
PLEKHG4B	159.0	0.7	92.8
P2RY12	247.5	1.7	91.1
COL7A1	587.0	5.7	87.7
ZDHHC8P1	152.7	0.8	83.2
SERPINB5	92.8	0.1	82.8
TRIM74	124.7	0.5	82.1

NUT, nuclear protein in testis; TPM, transcripts per million.

in *NUTM1* (4). Differential gene expression analysis comparing RNA-seq data from this case and the GTEx (https://www.gtexportal.org/) normal lung dataset (*Table 1*) identified *SOX2* and *TP63*, which were previously reported as NC-related genes (5). Immunohistochemical analysis of parathyroid hormone-like hormone (*PTHLH*), which was highly expressed in lung NC (*Table 1*), revealed negative staining in the previous nasal cavity cancer (*Figure 4A*) but positive in the lung cancer (*Figure 4B*). This finding suggests that the lung and nasal cavity NCs were metachronous multiple primary cancers. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee for Human Research of the Faculty of Medicine of Nagoya University (#2017-0034). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

Discussion

NC is characterized by *NUT* rearrangements. The t(15:19) translocation, which brings *NUT* in frame with *BRD4*, is the most common rearrangement. *BRD4* is a widely expressed transcriptional activator that promotes the expression of the *BRD4-NUT* fusion gene (6). Standard chemotherapeutic drugs and radiotherapy are ineffective in treating NC, and the prognosis and outcomes for patients with NC are unfavorable, with a median survival time of 6.5 months (7).

Currently, no standard treatment options are available for patients with NC. The only treatment for this case was surgery. However, new treatments that specifically address unique tumorigenic mechanisms are under investigation, including bromodomain and extraterminal (BET) inhibitors, histone deacetylase inhibitors (HDACi), and immunotherapies (8-10). BET inhibitors bind to the bromodomain and acetylated histones of BET proteins to reduce the expression of *MYC*, *SOX2*, and *TP63*. Unfortunately, the efficacy of BET inhibitors is limited, response rates are moderate, and response durations are only 2–3 months (11). Therefore, BET inhibitors are combined with other chemotherapies. HDACi reduce hyperacetylated chromatin in the BRD4 mega-domain and return cellular transcription to normal levels (12). To date, evidence supporting the efficacy of HDACi is lacking, and HDACi treatment cannot be recommended as a standard treatment for patients with NC.

In this case, we differentiated between metastatic and metachronous cancers. *PTHLH* was highly expressed in the lung cancer but not in the nasal cavity cancer. Although

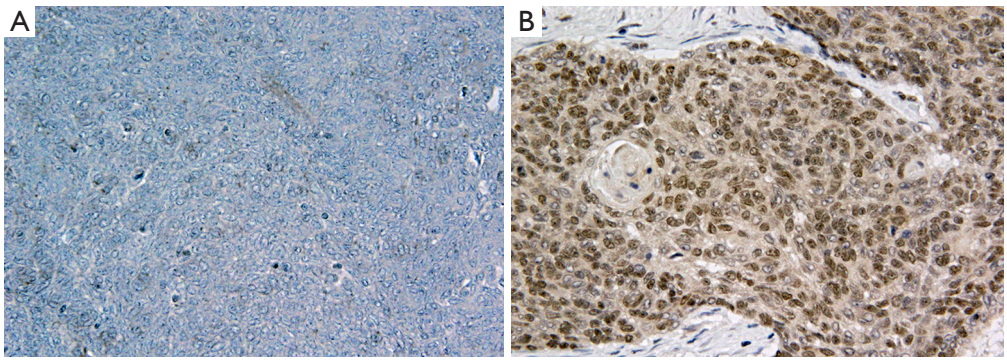

Figure 4 Immunohistochemical staining for *PTHLH* was negative in the nasal cavity cancer (A, ×200) and positive in the lung cancer (B, ×100). *PTHLH*, parathyroid hormone-like hormone.

tumor heterogeneity and clonal evolution may influence the differences in protein expression between these two cancers (13), the higher pathological malignancy of the nasal cavity cancer and the long disease-free duration indicated that the cancers were metachronous primary cancers (14). Furthermore, *PTHLH* is a marker of poor prognosis. *PTHLH* stimulates *in vitro* cell proliferation in squamous cell carcinoma through the promotion of cell-cycle progression (15). Cyclin genes, including *CCNA2*, *CCNE2*, and *CDC25A*, are stimulated by *PTHLH* and contribute to cell-cycle checkpoint evasion and phase transition. Therefore, therapeutic agents that inhibit *PTHLH* expression, such as roxithromycin, may prevent the proliferation of NCs expressing *PTHLH* and complications associated with *PTHLH*-induced cancer (14).

Chau *et al.* (7) recently established a predictive risk model of survival outcomes based on the largest study cohort of patients with NC to date. Patients were classified into the following three risk groups based on anatomic site and *NUTM1* fusion type: patients with nonthoracic primary NC and *BRD3*- or *NSD3-NUTM1* fusion, patients with nonthoracic primary NC and *BRD4-NUTM1* fusion, and patients with thoracic primary NC and any *NUTM1* fusion type. Patients with nonthoracic *BRD3*- and *NSD3-NUTM1* fusion tumors exhibited significantly longer overall survival rates than those with nonthoracic primary *BRD4-NUTM1* fusion tumors. Patients with thoracic primary tumors exhibited worse outcomes than the other patient groups, regardless of the *NUTM1* fusion type. In our case, the patient experienced no recurrence for 14 years after surgery for NC in the nasal cavity, but NC subsequently recurred in the left lung and metastasized to the chest wall 2 years after the second surgery. Although this case may be in line with previous reports, no metachronous NUT cancer cases separated by a long period have been reported due to the poor prognosis of NC.

Recurrence-free survival after surgery for thoracic NC was 2 years in this case. This prognosis was relatively favorable compared with that in previous reports (1,16). However, some thoracic NC cases with better prognoses have been reported. The cases with better outcomes were NUT variants involving *BRD3* or other uncharacterized partner genes (17). Based on RNA-seq results, the NC was characterized as a *BRD4-NUT* fusion tumor in our case. Although this is a typical fusion gene for NC, the breakpoint was *BRD4* exon 10 and *NUTM1* exon 4, which are not typical breakpoints (4). The most common oncogenic variation in NC is an in-frame fusion of *BRD4* exon 11 with the start of *NUTM1* exon 2 (18,19). In the case reported here, the predicted protein domain structure of *BRD4-NUT* fusion consisted of two NUT protein families and three well-characterized *BRD4* domains, including two bromodomains and the extraterminal (ET) domain (*Figure 5*) (20,21). The breakpoint of this case was in the ET domain of *BRD4*. Therefore, the ET domain of the fusion gene was incomplete. The ET domain regulates transcription factors (22,23). Thus, the low-grade malignancy and better prognosis of the NC may have been due to this new variant. Although complete resection of lung cancer improves survival and this is the only case with a breakpoint at *BRD4* exon 10 and *NUTM1* exon 4, this new variant may result in a favorable prognosis of NC.

Conclusions

We experienced a rare recurrence of lung NC 14 years after

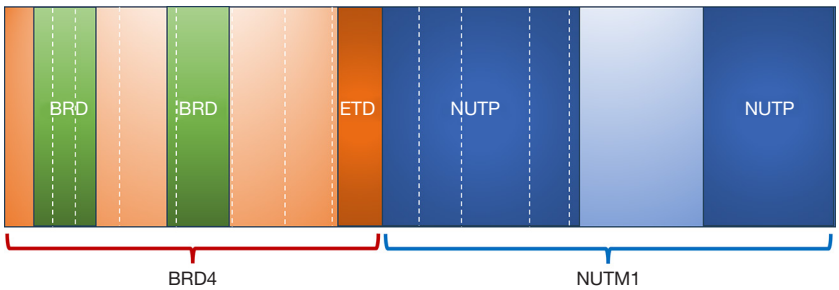

Figure 5 Predicted protein domain structure of *BRD4-NUT* fusion in this case. BRD, bromodomain; ETD, extraterminal domain; NUTP, NUT protein; NUT, nuclear protein in testis.

the initial surgery. The *BRD4-NUT* fusion consisted of a new breakpoint. Furthermore, the expression pattern of *PTHLH* suggested that the NCs in the nasal cavity and lung were metachronous multiple lung cancers. Although the prognosis of NC is usually very poor, this case highlights the possible identification of a more favorable prognosis of pulmonary NCs via gene fusion analysis. However, more effective treatment strategies for this malignancy are needed.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-259/rc

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-259/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-259/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee for Human Research of the Faculty of Medicine of Nagoya University (#2017-0034). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

References

1. Harms A, Herpel E, Pfarr N, et al. NUT carcinoma of the thorax: Case report and review of the literature. Lung Cancer 2015;90:484-91.
2. French CA. The importance of diagnosing NUT midline carcinoma. Head Neck Pathol 2013;7:11-6.
3. Stevens TM, Morlote D, Xiu J, et al. NUTM1-rearranged neoplasia: a multi-institution experience yields novel fusion partners and expands the histologic spectrum. Mod Pathol 2019;32:764-73.
4. Moreno V, Saluja K, Pina-Oviedo S. NUT Carcinoma: Clinicopathologic Features, Molecular Genetics and Epigenetics. Front Oncol 2022;12:860830.
5. Kosno M, Currie SL, Kumar A, et al. Molecular features driving condensate formation and gene expression by the BRD4-NUT fusion oncoprotein are overlapping but distinct. Sci Rep 2023;13:11907.

6. Hellquist H, French CA, Bishop JA, et al. NUT midline carcinoma of the larynx: an international series and review of the literature. Histopathology 2017;70:861-8.
7. Chau NG, Ma C, Danga K, et al. An Anatomical Site and Genetic-Based Prognostic Model for Patients With Nuclear Protein in Testis (NUT) Midline Carcinoma: Analysis of 124 Patients. JNCI Cancer Spectr 2020;4:pkz094.
8. Kaplan HG, Subramaniam S, Vallières E, et al. Prolonged Survival of NUT Midline Carcinoma and Current Approaches to Treatment. Oncologist 2023;28:765-70.
9. Pan M, Chang JS. Durable Complete Remission of PD-L1 Positive NUT Carcinoma Treated With Concurrent Chemotherapy and Radiation. Perm J 2020;25:1-3.
10. Lauer UM, Hinterleitner M, Horger M, et al. NUT Carcinoma-An Underdiagnosed Malignancy. Front Oncol 2022;12:914031.
11. Eagen KP, French CA. Supercharging BRD4 with NUT in carcinoma. Oncogene 2021;40:1396-408.
12. Salati M, Baldessari C, Bonetti LR, et al. NUT midline carcinoma: Current concepts and future perspectives of a novel tumour entity. Crit Rev Oncol Hematol 2019;144:102826.
13. McGranahan N, Swanton C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. Cell 2017;168:613-28.
14. Chang WM, Lin YF, Su CY, et al. Parathyroid Hormone-Like Hormone is a Poor Prognosis Marker of Head and Neck Cancer and Promotes Cell Growth via RUNX2 Regulation. Sci Rep 2017;7:41131.
15. Lv Z, Wu X, Cao W, et al. Parathyroid hormone-related protein serves as a prognostic indicator in oral squamous cell carcinoma. J Exp Clin Cancer Res 2014;33:100.
16. Fekkar A, Emprou C, Lefebvre C, et al. Thoracic NUT carcinoma: Common pathological features despite diversity of clinical presentations. Lung Cancer 2021;158:55-9.
17. Fujioka N, French CA, Cameron MJ, et al. Long-term survival of a patient with squamous cell carcinoma harboring NUT gene rearrangement. J Thorac Oncol 2010;5:1704-5.
18. Haruki N, Kawaguchi KS, Eichenberger S, et al. Cloned fusion product from a rare t(15;19)(q13.2;p13.1) inhibit S phase in vitro. J Med Genet 2005;42:558-64.
19. Thompson-Wicking K, Francis RW, Stirnweiss A, et al. Novel BRD4-NUT fusion isoforms increase the pathogenic complexity in NUT midline carcinoma. Oncogene 2013;32:4664-74.
20. French CA. NUT Carcinoma: Clinicopathologic features, pathogenesis, and treatment. Pathol Int 2018;68:583-95.
21. French CA, Cheng ML, Hanna GJ, et al. Report of the First International Symposium on NUT Carcinoma. Clin Cancer Res 2022;28:2493-505.
22. Rahman S, Sowa ME, Ottinger M, et al. The Brd4 extraterminal domain confers transcription activation independent of pTEFb by recruiting multiple proteins, including NSD3. Mol Cell Biol 2011;31:2641-52.
23. Crowe BL, Larue RC, Yuan C, et al. Structure of the Brd4 ET domain bound to a C-terminal motif from γ-retroviral integrases reveals a conserved mechanism of interaction. Proc Natl Acad Sci U S A 2016;113:2086-91.

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