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Sameer Chhetri Aryal
Henry Ford Health, schhetr1@hfhs.org

Shereen Zia
Henry Ford Health, szia2@hfhs.org

Shannon Rodgers
Henry Ford Health, SRodger5@hfhs.org

Yulei Shen
Henry Ford Health, yshen2@hfhs.org

Kyle Perry
Henry Ford Health, KPERRY4@hfhs.org

See next page for additional authors

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Authors
Sameer Chhetri Aryal, Shereen Zia, Shannon Rodgers, Yulei Shen, Kyle Perry, and Lisi Yuan

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BRIEF REPORT

BRD3-NUTM1-expressing NUT carcinoma of lung on endobronchial ultrasound-guided transbronchial needle aspiration cytology, a diagnostic pitfall

Sameer Chhetri Aryal MD | Shereen Zia MD | Shannon Rodgers DO | Yulei Shen MD | Kyle Perry MD | Lisi Yuan MD, PhD

Department of Pathology and Laboratory Medicine, Henry Ford Health System, Detroit, Michigan, USA

Correspondence
Lisi Yuan, Department of pathology and Laboratory Medicine, 2799 W Gland Blvd, Detroit, MI 48202, USA.
Email: lyuan2@hfhs.org

Abstract

Background: Nuclear protein in testis (NUT) carcinoma (NC) is an aggressive type of poorly differentiated carcinoma with a variable degree of squamous differentiation. NC is defined by the presence of BRD-NUT fusion oncogenes, the most common fusion form being the BRD4-NUTM1 gene. Variant rearrangements involving the BRD3 and NSD3 genes. Variant rearrangements involving the BRD3 and NSD3 genes occur in approximately one-third of the cases.

Aims: This is the first case regarding the study of cytological features of NC of the lung with BRD3-NUTM1 fusion.

Materials and Methods: A 36-year-old female with chest heaviness and shortness of breath was found to have a right-sided pleural effusion; she was non-smoker and denied any significant past medical illness. CT-chest revealed an 8.5 cm heterogeneous mass in the right and mid-upper lung. She underwent endobronchial ultrasound-guided (EBUS) transbronchial fine-needle aspiration (FNA) of the lung mass. Thoracocentesis was performed, and pleural fluid was sent to the laboratory for cytological evaluation

Results: The cytopathological findings showed atypical squamoid cells with variably prominent single or multiple nucleoli. Monotonous-looking cells with high nuclear to cytoplasmic ratio and hyperchromasia were also present. The atypical squamoid cells showed abundant clear to eosinophilic cytoplasm with rare individual cell keratinization and focal keratin pearl formation. The atypical cells were positive for CK7, p40, p63, mCEA and equivocal for NUT-specific antibody. The cytopathological findings were consistent with squamous cell carcinoma with focal keratinization. The Fusion Panel-Solid Tumor (50 genes) revealed BRD3-NUTM1 fusion gene. Diagnosis was amended to pulmonary NC.

Discussion: NC is a diagnostic challenge for pathologists as it can morphologically mimic undifferentiated carcinoma, squamous cell carcinoma, or neuroendocrine carcinoma. The challenge is not how to diagnose NC but rather determining when to include it in the differential diagnosis and perform the diagnostic molecular tests (FISH or NGS) or IHC study for NUT-specific antibody.

Conclusion: When a specimen demonstrates a dual cell population of squamoid cells and primitive-looking tumor cells in the wrong clinical context (i.e., young patient with no smoking history), further molecular profiling is warranted to include the differential of a primary NC of the lung. The cytological features of NC itself have rarely

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been documented and moreover, that of a primary NC of the lung with BRD3-NUTM1 fusion has never been reported. We herein report cytological findings of a primary NC of the lung with BRD3-NUTM1 fusion gene.

KEYWORDS
BRD3-NUTM1 fusion gene, carcinoma, cytology, EBUS, NUT carcinoma

1 | INTRODUCTION

Nuclear protein in testis (NUT) carcinomas (NC) are aggressive, poorly differentiated carcinomas with varying degrees of squamous differentiation. NC is defined by the presence of BRD-NUT fusion oncogenes. NUT gene on chromosome 15q14 is involved in a balanced translocation with the BRD4 gene on chromosome 19p13.1.[1] Variant rearrangements involving the BRD3, and NSD3 gene occur in approximately one-third of the cases. These fusion genes promote cell growth and inhibit differentiation through aberrant histone acetylation and activation of MYC, contributing to the aggressive phenotype.[2] There is no known association with exposures to environmental toxins or infectious agents, smoking, or oncogenic viruses such as the Human papillomavirus or Epstein–Barr virus.[3]

NC presents most commonly in midline structures of the body, such as in the head, neck, or mediastinum, and also in non-midline structures including the lung, pancreas, kidney, bladder, endometrium, and salivary gland. The WHO classification of tumors removed the word "midline" from the name of this type of tumor and redefined it as NC in 2015.[4] Dutta et al. reported 10 cases of primary pulmonary NC.[5] NC of the lung was reported occurring near the hilum of the lung; however, the majority of cases involving lung also demonstrate mediastinum involvement at the time of diagnosis.[5,6] Thus, these cases were designated as thoracic NUT carcinoma as it is difficult to decide the exact site of origin.

The treatment guidelines for pulmonary NC are similar to the guidelines for non-small cell lung cancer. Recently bromodomain and extraterminal inhibitors and histone deacetylase inhibitors have emerged as two promising classes of targeted therapy.[7,8]

This is the first case regarding the study of cytological features of NC of the lung with BRD3-NUTM1 fusion. The histocytomorphology of NCs ranges from monomorphic, primitive appearing tumor cells that do not often stain for lineage-specific markers to carcinoma with squamous differentiation and abrupt keratinization. National comprehensive cancer network (NCCN) guidelines version 3.2020 for non-small cell lung cancer recommends testing for NUT-expression in poorly differentiated carcinoma, and particularly in non-smokers or in patients presenting at a young age, for consideration of pulmonary NC. Definite diagnosis of NC is made by demonstration of NUT rearrangement by FISH or immunostaining by NUT-specific antibodies. We herein report cytological findings of a primary NC of the lung with the BRD3-NUTM1 fusion gene.

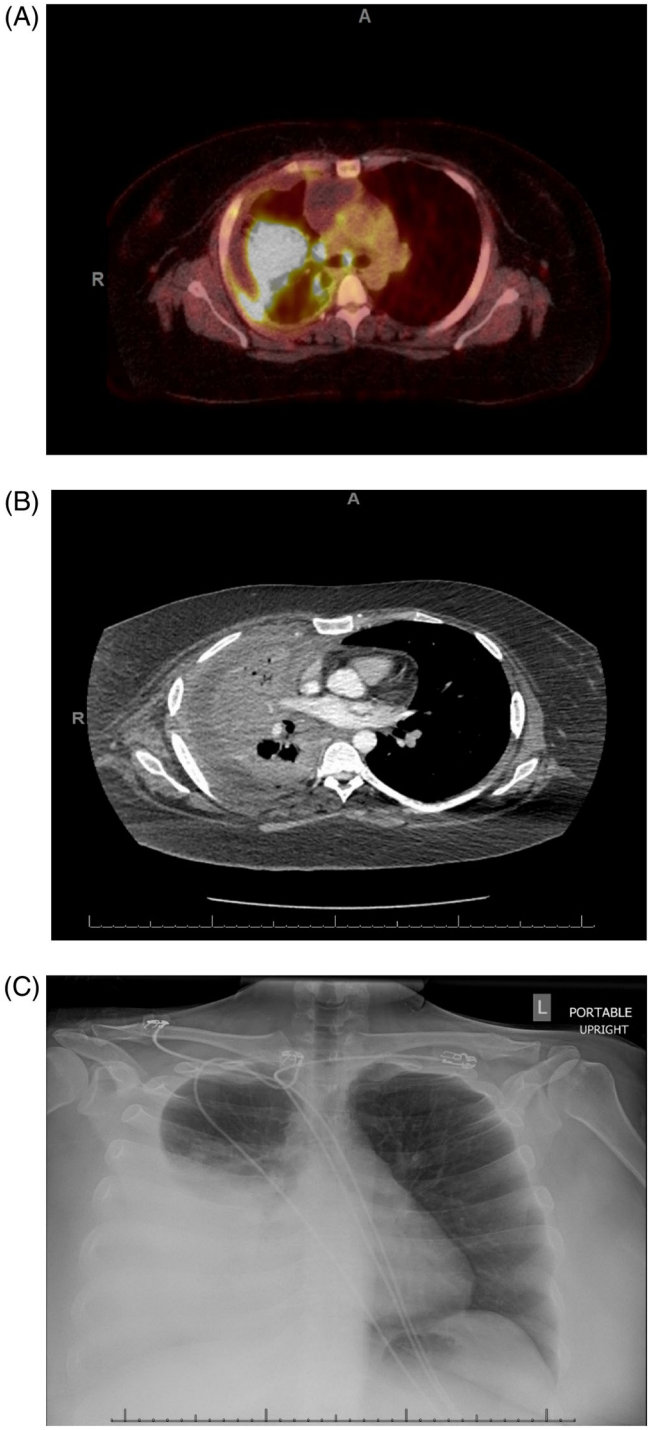

FIGURE 1 (A) PET-CT scan shows a right lung mass involving pleura. (B) CT scan of the chest revealed a mass in right middle and upper lung. (C) Chest X-ray with right sided pleural effusion

2 | CASE PRESENTATION

A 36-year-old female presented with chest heaviness and shortness of breath. She was a non-smoker and had good health until her current illness. Chest X-ray (Figure 1C) showed massive right-sided pleural effusion. CT-chest revealed an 8.5 x 6.8 cm heterogeneous mass in the right middle, and upper lung (Figure 1B). PET-CT showed a hypermetabolic mass (7.9 x 6.7 cm) spanning the right upper, middle and lower lung fields (Figure 1A). There was also mediastinal and right hilar lymphadenopathy without distant metastasis. The mass was clinically Stage IVA (cT4, cN2, pM1a).

Endobronchial ultrasound-guided (EBUS) transbronchial fine-needle aspiration (FNA) of the lung mass was done. Thoracocentesis was done, and pleural fluid was sent to the laboratory for cytological evaluation. ThinPrep smears were air-dried and stained with Diff-Quick or wet fixed in 95% ethanol for Papanicolaou staining. Cell blocks were prepared, and 4um sections were stained with hematoxylin and eosin staining.

2.1 | Cytological findings

ThinPrep smear and cell block preparation of FNA material from right lung mass showed cohesive groups of atypical squamoid cells with

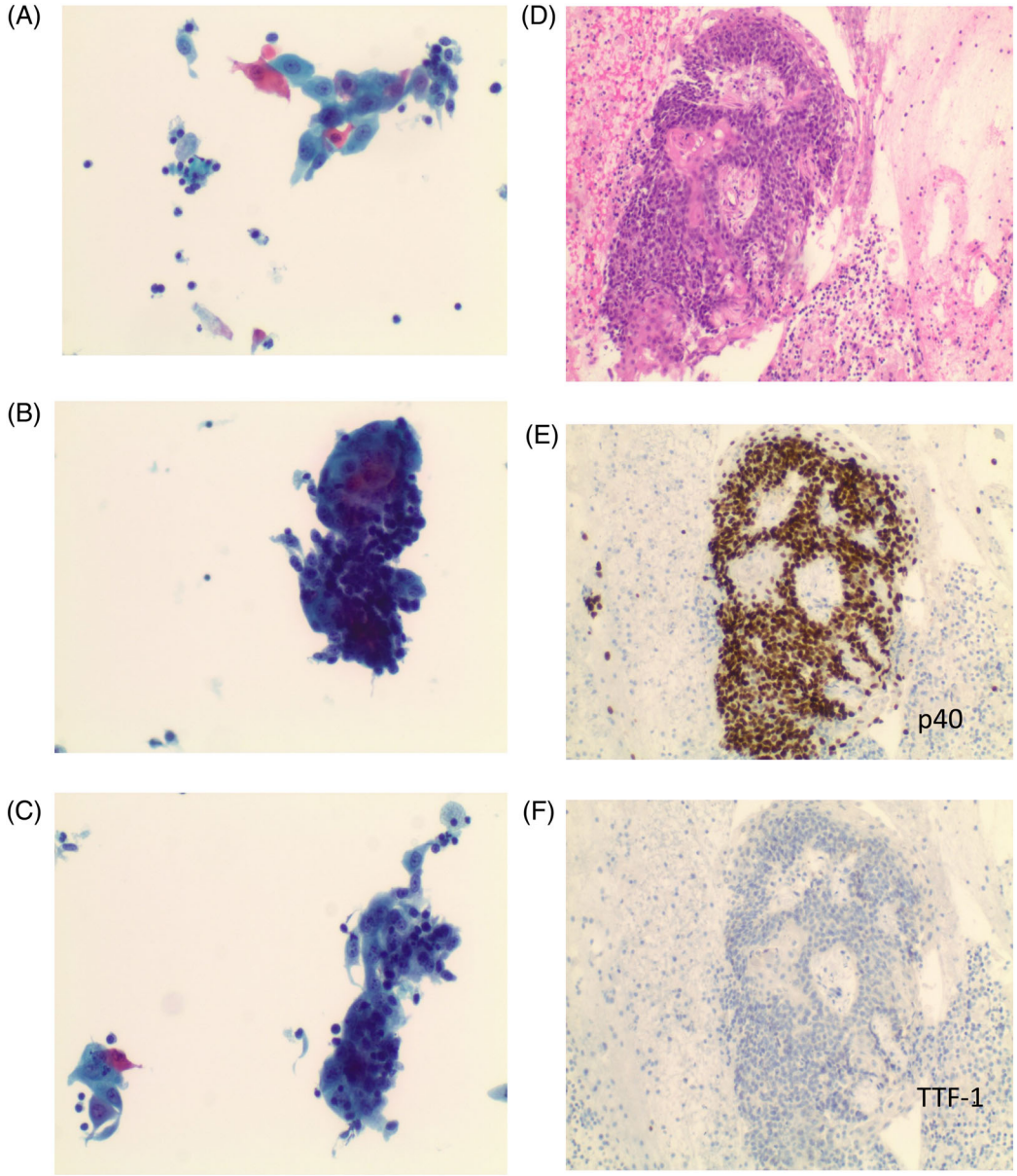

FIGURE 2 (A) Cohesive fragment of atypical squamous cells with keratinized squamous cells (ThinPrep smear of FNA, Papanicolaou, x200). (B, C) Cohesive cluster of atypical cells with high n/c ratio and focus of squamous differentiation (ThinPrep smears of FNA, Papanicolaou, x200). (D) Cohesive sheet of atypical cells with a subset having high n/c ratio, indistinct cell borders, and enlarged nuclei. Within this group, a second population of tumor cells have smaller nuclei, distinct cell borders and abundant clear to eosinophilic cytoplasm (H & E, x200). (E and F) Immunohistochemistry shows atypical cells positive for p40 and negative for TTF-1, respectively (IHC, x200)

abundant dense cytoplasm and occasional orangeophilic keratinized cells (Figure 2A). A focus of squamous differentiation was identified within a cell group (Figure 2B). Cohesive fragments of atypical cells with oval to spindle-shaped nuclei with hyperchromasia and high nuclear-to-cytoplasmic (n/c) ratio were also seen (Figure 2C). Cell block preparation showed a cohesive sheet of atypical cells with a high n/c ratio, indistinct cell borders, and enlarged nuclei. Within this group, there was a second population of tumor cells that had smaller nuclei, distinct cell borders, and abundant clear to eosinophilic cytoplasm (Figure 2D). The tumor cells were diffusely positive for p40 (Figure 2E) and negative for TTF-1 by immunohistochemistry (IHC; Figure 2F).

ThinPrep smears of the pleural fluid showed cohesive groups of cells with enlarged, hyperchromatic nuclei and prominent nucleoli (Figure 3A). Cell block preparation showed cohesive sheets of atypical squamoid cells with distinct cell borders and abundant clear-to-eosinophilic cytoplasm along with a monotonous population of tumor cells with a high n/c ratio and indistinct cell borders (Figure 3B). Tumor cells with pavement-like stratification were also appreciated (Figure 3C). Mitotic figures were identifiable; however, there was no nuclear molding, apoptosis, or necrosis.

IHC studies of the atypical cells in pleural fluid showed diffuse positivity for p40, p63, CK7, and mCEA (Figure 4A–D), while negative for TTF-1, Napsin A, D2-40, Calretinin, MOC31, BerEP4, and PDL-1. The initial diagnosis of this case was squamous cell carcinoma of the lung with pleural metastasis, although the clinical scenario was less typical for it. The more primitive-looking groups of cells were thought to represent the non-keratinized or less well-differentiated areas. Of note, there was no history of HPV-related oropharyngeal lesion or HPV-related cervical dysplasia. Molecular studies were negative for coding variants or copy number alterations detected in the EGFR, KRAS, NRAS, BRAF, MET, and ERBB2 targeted regions tested. Given this uncharacteristic diagnosis in such a young and non-smoker patient, we performed Fusion Panel-Solid Tumor (50 genes) study searching for translocations and fusions with known and novel fusion partners of 50 genes including NUT, considering the NCCN guidelines. The Fusion-panel was positive for BRD3-NUTM1 gene fusion (Figure 4F). These findings confirmed the diagnosis of primary pulmonary NC with the BRD3-NUTM1 fusion gene with pleural metastasis. Retrospectively, the IHC for NUT-specific antibody (C52B clone, rabbit monoclonal antibody, 1:200 dilution, cell signaling technology) was performed, the results were equivocal as approximately 50% of the tumor cells showed a weakly positive nuclear stain for it (Figure 4E).

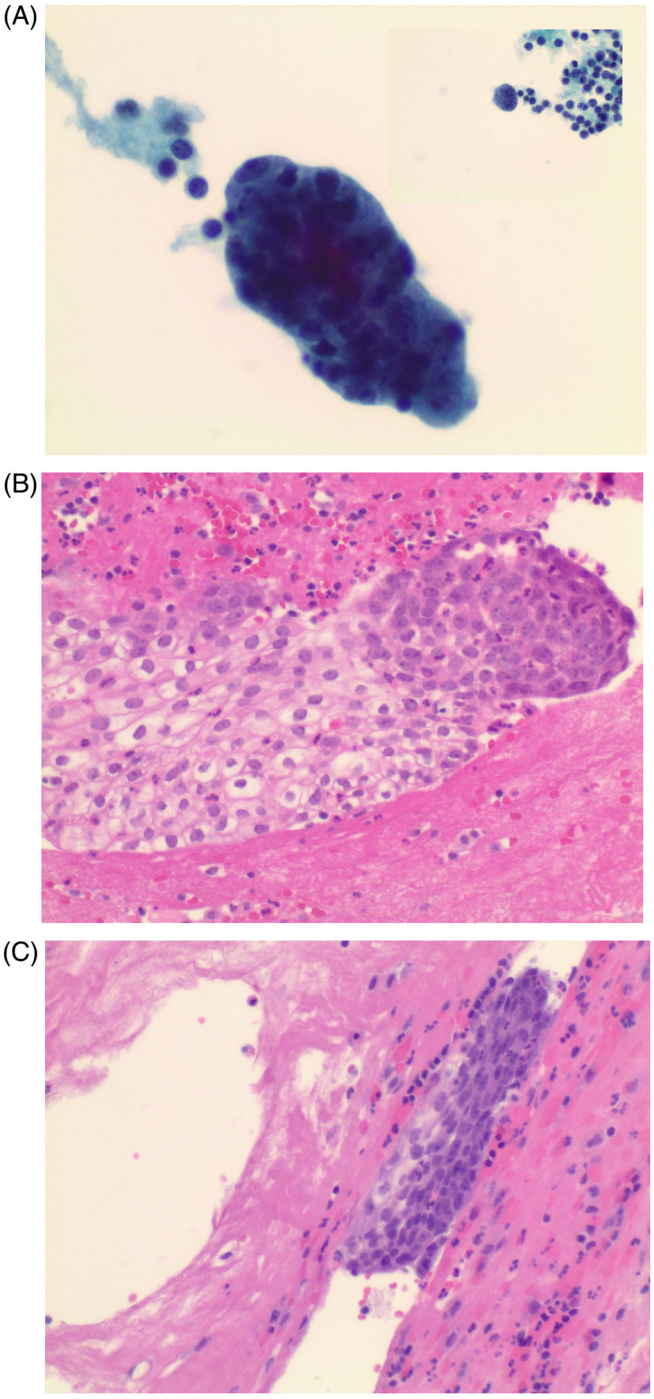

FIGURE 3 (A) ThinPrep smear shows a cohesive group with enlarged, hyperchromatic cells with prominent nucleoli (ThinPrep smear of pleural fluid, Papanicolaou, x200). (B, C) Fragments of atypical tumor cells with focal squamous differentiation (H&E, x200)

2.2 | Treatment and follow up

The patient received chemotherapy consisting of carboplatin+Nab-Paclitaxel+Pembrolizumab. She received Denosumab for symptomatic hypercalcemia. She passed away eight months after the initial presentation.

3 | DISCUSSION

NC is a rare and aggressive type of carcinoma arising most often in a midline structure. Although poorly differentiated, they display at least focal squamous differentiation in 33–40% of cases.[9] Its true incidence remains unknown due to the low detection rate. NC is a diagnostic challenge for pathologists as it can morphologically mimic undifferentiated carcinoma, squamous cell carcinoma, or neuroendocrine carcinoma. The challenge is not how to diagnose NC but rather determining when to include it in the differential diagnosis and perform the diagnostic molecular tests (FISH or NGS) or IHC study for NUT-specific antibody.

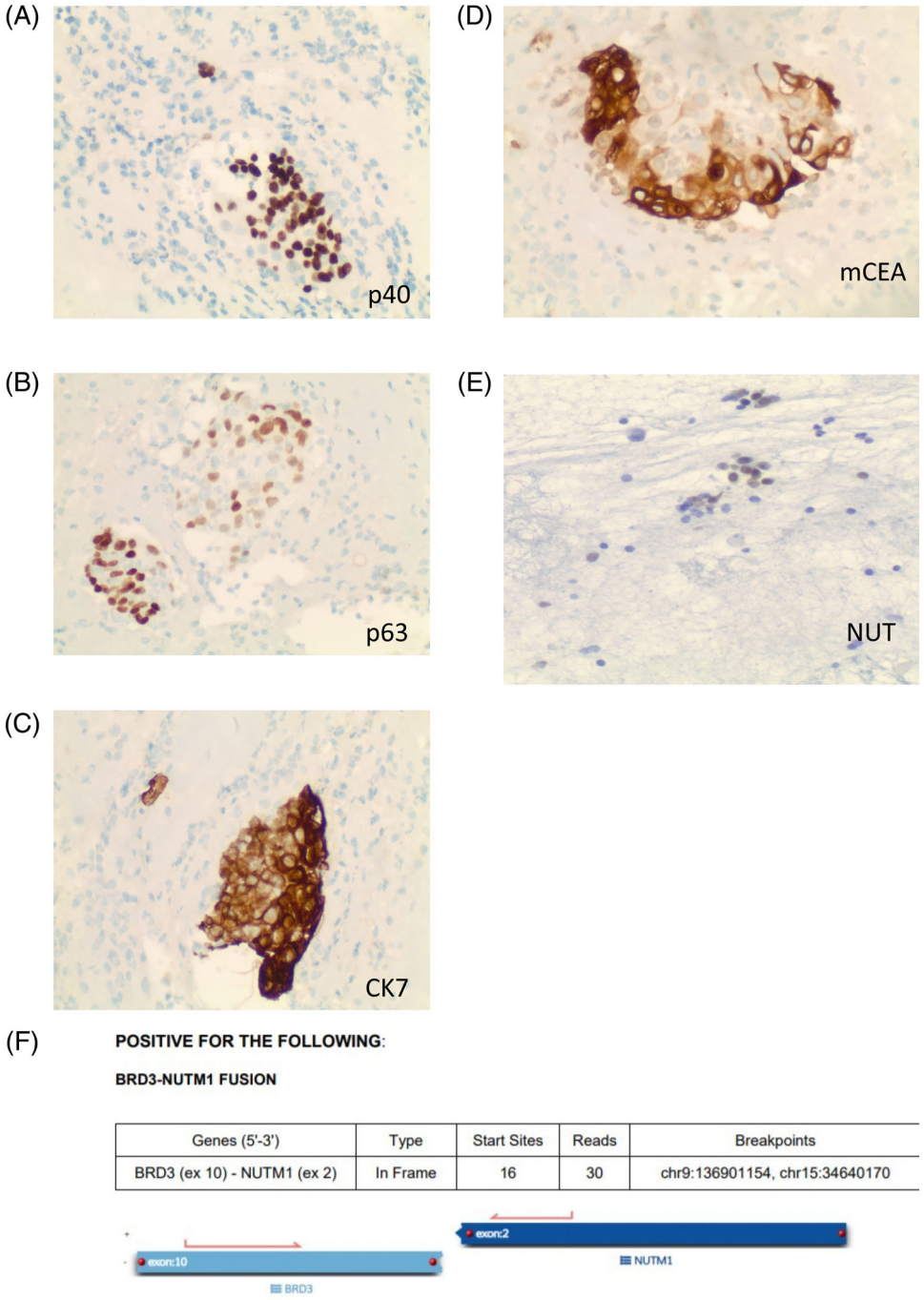

FIGURE 4 (A–D), Neoplastic cells positive for P40, P63, CK7 and mCEA (×200). (E), nuclear protein in testis specific antibody showed weak nuclear staining in up to 50% of the tumor cells (equivocal). (F), Fusion Panel- Solid Tumor (50 genes) revealed the presence of BRD3-NUTM1 fusion gene

Cytological findings of primary pulmonary NCs from previous studies show cohesive clusters and dispersed single monotonous, small to medium-sized cells with primitive-appearing features and scant cytoplasm. Squamous differentiation in the form of abrupt dyskeratotic cells or squamous cells with abundant dense cytoplasm is noted in rare cases.[10,11] A reported case of NC of the lung harboring an NSD3-NUT fusion showed cellular smear with a non-cohesive pattern of monomorphic cells with a round-to-oval nucleus, slightly irregular nuclear contours, variably prominent nucleoli, scant cytoplasm, foci of stratification, and overt keratin pearl formation.[12]

Similar to previously described cytological features of NC,[10,11] this case also showed monomorphic, small to midsize, primitive appearing tumor cells with scant cytoplasm. However, the majority of the tumor cells appear to be atypical squamoid cells with oval to elongated nuclei, abundant clear to eosinophilic cytoplasm and rare individual cell keratinization or keratin pearl formation. Our initial cytological diagnosis was squamous cell carcinoma; further molecular study identified the BRD3-NUTM1 fusion gene. The diagnosis was then amended to pulmonary NC with pleural metastasis. IHC for NUT specific antibody was performed retrospectively, which showed weak

TABLE 1 Cytological and immunophenotypic features of primary pulmonary NC

References	Age/sex	Cytological diagnosis	P63	P40	Keratin	NEM	mCEA	TTF-1	NUT	FISH
Dutta et al[6]	58/M	PDCA	ND	+	AE1/AE3+	+	ND	−	+	ND
Dutta et al[6]	35y/M	MRCT	ND	+	AE1/AE3+	−	ND	−	+	ND
Dutta et al[6]	65y/F	PDCA	ND	+	ND	ND	ND	ND	+	ND
Dutta et al[6]	23y/F	PDCA	ND	+	CK 5/6+	ND	ND	−	+	ND
Dutta et al[6]	32y/F	SQC	ND	+	CK 5/6+	ND	ND	−	+	ND
Dutta et al[6]	19/M	PDCA	ND	+	ND	ND	ND	−	+	ND
Dutta et al[6]	52/M	PDCA	+	ND	ND	ND	ND	ND	+	ND
Dutta et al[6]	37/M	PDCA	ND	+	ND	ND	ND	−	+	ND
Dutta et al[6]	42/F	PDCA	ND	ND	ND	ND	ND	ND	+	ND
Dutta et al[6]	17/F	MC	ND	+	ND	ND	ND	−	+	ND
Policarpio-Nicolas et al[7]	34y/M	NET	+	ND	−	−	ND	ND	+	ND
Bishop et al[11]	22y/M	NC	ND	ND	ND	ND	ND	ND	+	BRD4-NUT
Suzuki et al[15]	36y/F	UC	+	ND	AE1/AE3+	ND	ND	ND	+	NSD3-NUT
Lee at al[16]	45y/M	PDCA	ND	ND	AE1/AE3+	−	ND	−	+	ND
Lee at al[16]	32y/M	PDCA	+	ND	AE1/AE3+	−	ND	−	+	ND
Present study	36y/F	SQC	+	+	CK7+	ND	+	−	ND	BRD3-NUT

Abbreviations: − negative; + positive; MC, malignant cell present; MRCT, malignant round cell tumor; NC NUT, carcinoma; ND, not done; NEM, neuroendocrine markers; NET, neuroendocrine tumor; NUT, nuclear protein in testis; PDCA, poorly differentiated carcinoma with abrupt squamous differentiation; SCC, small cell carcinoma; SQC, squamous cell carcinoma; UC, undifferentiated carcinoma with cytoplasmic fine vacuoles.

nuclear staining in up to 50% of the tumor cells. The IHC result for NUT-specific antibody was equivocal, and interpretation was made according to the previous guideline.[13]

Sholl et al reviewed eight cases of pulmonary NCs with BRD4-NUT rearrangement. All tumors expressed keratin, p63, and NUT protein.[14] Suzuki et al. showed that the NSD3-NUT variant of NC expresses CK5/6, CK7, p40, p63, EMA, AE1/AE3, CAM 5.2, CD138, vimentin, and NUT protein.[15] A p63 negative primary pulmonary NUT midline carcinoma with the BRD4-NUT fusion has been reported.[9] These unusual patterns of immunostaining may reflect the stages of cellular differentiation of cancer cells. The cytological features and IHC findings of primary pulmonary NC are summarized in Table 1.[5,6,10,15,16]

In conclusion, although the distinction of NC from other poorly differentiated carcinomas based solely on morphology is difficult, cytology can be helpful, especially for identifying abrupt keratinization. The cytological and IHC findings can be compatible with keratinizing squamous cell carcinoma. However, when a specimen demonstrates a dual cell population of squamoid cells and primitive-looking tumor cells in the wrong clinical context (i.e., young patient with no smoking history), further molecular profiling is warranted to include the differential of a primary NC of the lung.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Lisi Yuan and Sameer Chhetri Aryal contributed to report conception, data acquisition, data analysis and interpretation, and manuscript writing and editing. Yulei Shen contributed to data acquisition. Shereen Zia contributed to manuscript writing. Kyle Perry and Shannon Rodgers contributed to manuscript editing.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ORCID

Sameer Chhetri Aryal https://orcid.org/0000-0001-7203-7951
Shannon Rodgers https://orcid.org/0000-0002-1530-5005
Lisi Yuan https://orcid.org/0000-0002-5935-2073

REFERENCES

1. French CA, Miyoshi I, Kubonishi I, Grier HE, Perez-Atayde AR, Fletcher JA. BRD4-NUT fusion oncogene: a novel mechanism in aggressive carcinoma. *Cancer Res*. 2003;63(2):304-307.
2. Grayson AR, Walsh EM, Cameron MJ, et al. MYC, a downstream target of BRD-NUT, is necessary and sufficient for the blockade of differentiation in NUT midline carcinoma. *Oncogene*. 2014;33:1736-1742.
3. Stelow EBBA, Bellizzi AM, Taneja K, et al. NUT rearrangement in undifferentiated carcinomas of the upper aerodigestive tract. *Am J Surg Pathol*. 2008;32:828-834.
4. Travis WD, Brambilla E, Nicholson AG, et al. The 2015 World Health Organization classification of lung tumors: Impact of genetic, clinical and radiologic advances since the 2004 classification. *J ThoracOncol*. 2015;10(9):1243-1260.
5. Dutta R, Nambirajan A, Mittal S, Roy-Chowdhuri S, Jain D. Cytomorphology of primary pulmonary NUT carcinoma in

different cytology preparations. *Cancer Cytopathol*. 2021 Jan; 129(1):53-61.
6. Policarpio-Nicolas MLC, de Leon EMB, Jagirdar J. Cytologic findings of NUT midline carcinoma in the hilum of the lung. *DiagnCytopathol*. 2015;43(9):739-742.
7. Filippakopoulos P, Qi J, Picaud S, et al. Selective inhibition of BET bromodomains. *Nature*. 2010;468(7327):1067-1073.
8. Maher OM, Christensen AM, Yedururi S, Bell D, Tarek N. Histone deacetylase inhibitor for NUT midline carcinoma. *Pediatr Blood Cancer*. 2015;62(4):715-717.
9. French CA. NUT Carcinoma: Clinicopathologic features, pathogenesis, and treatment. *Pathol Int*. 2018 Nov;68(11):583-595. doi:10.1111/pin.12727
10. Bishop JA, French CA, Ali SZ. Cytopathologic features of NUT midline carcinoma: A series of 26 specimens from 13 patients. *Cancer Cytopathol*. 2016;124(12):901-908.
11. Lee T, Choi S, Han J, Choi Y-L, Lee K. Abrupt dyskeratotic and squamoid cells in poorly differentiated carcinoma: case study of two thoracic NUT midline carcinomas with cytohistologic correlation. *J Pathol Transl Med*. 2018;52(5):349-353.
12. Kuroda S, Suzuki S, Kurita A, et al. Cytological features of a variant NUT midline carcinoma of the lung harboring the NSD3-NUT fusion gene: a case report and literature review. *Case Rep Pathol*. 2015;2015:572951.
13. Haack H, Johnson LA, Fry CJ, et al. Diagnosis of NUT midline carcinoma using a NUT-specific monoclonal antibody. *Am J Surg Pathol*. 2009 Jul; 33(7):984-991. doi:10.1097/PAS.0b013e318198d666
14. Sholl LM, Nishino M, Pokharel S, et al. Primary pulmonary NUT midline carcinoma: clinical, radiographic, and pathologic characterizations. *J Thorac Oncol*. 2015;10(6):951-959.
15. Suzuki S, Kurabe N, Ohnishi I, et al. NSD3-NUT-expressing midline carcinoma of the lung: first characterization of primary cancer tissue. *Pathol Res Pract*. 2015;211(5):404-408.
16. Lee T, Choi S, Han J, Choi YL, Lee K. Abrupt dyskeratotic and squamous cells in poorly differentiated carcinoma: case study of two thoracic NUT midline carcinoma with cytohistologic correlation. *J Pathol Transl Med*. 2018;52(5):349-353.

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