Received: 3 December 2018 | Revised: 25 June 2019 | Accepted: 7 July 2019

DOI: 10.1111/cas.14129

REPORT

Cancer Science WILEY

Mucin 21 is a key molecule involved in the incohesive growth pattern in lung adenocarcinoma

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Abstract

Decreased cell adhesion has been reported as a significant negative prognostic factor of lung cancer. However, the molecular mechanisms responsible for the cell incohesiveness in lung cancer have not yet been elucidated in detail. We herein describe a rare histological variant of lung adenocarcinoma consisting almost entirely of individual cancer cells spreading in alveolar spaces in an incohesive pattern. A whole exome analysis of this case showed no genomic abnormalities in *CDH1* or other genes encoding cell adhesion molecules. However, whole mRNA sequencing revealed that this case had an extremely high expression level of mucin 21 (MUC21), a mucin molecule that was previously shown to inhibit cell-cell and cell-matrix adhesion. The strong membranous expression of MUC21 was found on cancer cells using mAbs recognizing different *O*-glycosylated forms of MUC21. An immunohistochemical analysis of an unselected series of lung adenocarcinoma confirmed that the strong membranous expression of MUC21 correlated with incohesiveness. Thus, MUC21 could be a promising biomarker with potential diagnostic and therapeutic applications for lung adenocarcinoma showing cell incohesiveness.

KEYWORDS

adenocarcinoma, cell adhesion, lung cancer, MUC21, STAS

1 | INTRODUCTION

The importance of decreased cell adhesion in cancer progression and metastasis is being increasingly recognized.[1-5] Cancer cells showing decreased cell adhesion are associated with cancer spread through vessels or alveolar spaces.[2,5,6] However, the molecular mechanisms underlying cell incohesiveness have not yet been elucidated.

The mucin family is involved in various aspects of cancer progression, such as cell adhesion, epithelial-mesenchymal transition, cell signaling, and the tumor microenvironment.[7] Mucins either alone or through interactions with receptor tyrosine kinases have been shown to mediate cell signals for the growth and survival of lung cancer cells.[8]

We herein report a rare histological variant of lung adenocarcinoma, in which individual cancer cells spread in the alveolar

spaces in an incohesive pattern. This histological variant of adenocarcinoma has only rarely been reported in English published works.[9,10] In the present study, a whole genomic analysis and immunohistochemical validation allowed us to identify mucin 21 (MUC21) as a molecule associated with cell incohesiveness in lung adenocarcinoma.

2 | MATERIALS AND METHODS

2.1 | Case description

Computed tomography detected an irregular mass in the S6 segment of the right lower lobe of a 72-year-old Japanese woman with no history of smoking. She had no previous history of tuberculosis or other lung diseases. She underwent right lower lobectomy and the resected specimen showed that she had stage 3A (pT2AN2M0) disease. She received 4 courses of adjuvant chemotherapy (a combination of carboplatin and gemcitabine), followed by uracil/tegafur given orally at a dose of 600 mg/d as maintenance therapy for 3 years. She showed no signs of recurrence for 45 months after surgery. However, she died 50 months after surgery due to an unknown cause.

2.2 | Genome-wide analysis

DNA and RNA were extracted from the frozen tissue of the resected specimen. DNA was subjected to whole exome next-generation sequencing (NGS) analyses using the HiSeq 2500 platform (Illumina) as previously described.[11] RNA was subjected to whole transcriptome sequencing.

2.3 | Electron microscopic analysis

A small tissue sample was excised from a paraffin block of the tumor specimen, dewaxed, rehydrated, and processed for a transmission electron microscopic analysis.

2.4 | Immunohistochemical analysis

Sections were stained for thyroid transcription factor-1 (TTF-1), Napsin A, CD68, E-cadherin, and β-catenin, as described previously.[11] Mucin 21 was detected using 3 mouse monoclonal anti-MUC21 antibodies (heM21A, C, and D) with the ability to distinguish the different glycoforms of MUC21,[12,13] as shown in Table S1.

Expression of MUC21 was also analyzed in a consecutive series of 120 surgically resected lung adenocarcinomas. The relationships between MUC21 expression and morphological features, such as histological patterns and cell incohesiveness in air spaces, were examined. The results obtained were independently evaluated by two pathologists (DM and TY). Evaluation criteria for each finding are shown in Table S2. If there was any discrepancy in the evaluation, 2 pathologists discussed and reached an agreement.

3 | RESULTS

Representative images of the present case are shown in Figure 1. The tumor was 4.0 × 2.5 × 2.5 cm in size, and its cut surface showed a 2.0 × 1.2 cm fibrotic focus, which was surrounded by a medullary zone that merged with a poorly marginated, pneumonia-like area (Figure 1A). A microscopic examination revealed that tumor cells mostly grew in an incohesive pattern in alveolar spaces or bronchioles, mimicking alveolar macrophages (Figure 1B,C). This incohesive pattern was consistently found in tumor cells that invaded vessels, bronchi, and pleura, and also in those that metastasized to the lymph nodes (Figure S1). A lepidic or papillary pattern was found in only a small area. Tumor cells had mucin on their surfaces under periodic acid-Schiff (not shown) and Alcian blue staining (Figure 1D).

Immunohistochemically, tumor cells were positive for TTF-1 (Figure 1E) and Napsin A (not shown), and negative for CD68 (Figure 1F). E-Cadherin and β-catenin were positive; however, their location was mainly cytoplasmic (Figure 1G,H).

Ultrastructurally, tumor cells had numerous long villi on their surfaces (Figure 1I,J).

Whole exome sequencing with NGS detected the E746-A750 frame deletion in exon 19 of the *EGFR* gene. No other mutations in other major driver genes or single nucleotide variants of major cell adhesion molecules (such as CDH1 and CTNNB1) were detected (Table S3). No significant CNAs were found in these genes, including CDH1 (Figure S2).

Next, we examined transcriptome data to identify the key molecules involved in the tumor incohesiveness of this case. We selected genes that were more strongly expressed in this case than in a cohort of 40 lung adenocarcinoma cases. Among the 4 genes that were expressed at levels more than 20-fold higher in this case than the average of the cohort, we focused on *MUC21* because its absolute value was the highest among these 4 genes (Figure 2A and Table S4). Furthermore, MUC21 is a large glycoprotein that inhibits cell adhesion through steric hindrance on the cell surface.[12,13]

We validated these results by immunohistochemistry using 3 mouse mAbs (heM21A, heM21C, and heM21D) that recognize different glycoforms of MUC21.[12,13] As shown in Figure 2B-D, the majority of cancer cells in the present case showed strong membranous staining with all 3 MUC21 Abs.

We then stained a series of 121 lung adenocarcinoma cases with these MUC21 Abs. When evaluated as a whole, the frequencies of MUC21 positivity for each mAb were 31.4% (38/121) for heM21A, 21.5% (26/121) for heM21C, and 4.1% (5/121) for heM21D. The relationships between their expression and clinicopathological variables are shown in Table S5. As MUC21 was heterogeneously expressed within the same tumor, we evaluated MUC21 expression for each histological pattern (Figure 3A). Each anti-MUC21 antibody was consistently positive in the incohesive pattern. The frequency of MUC21 positivity was lower in other histological patterns, but was slightly higher in the micropapillary, papillary, and lepidic patterns than in the

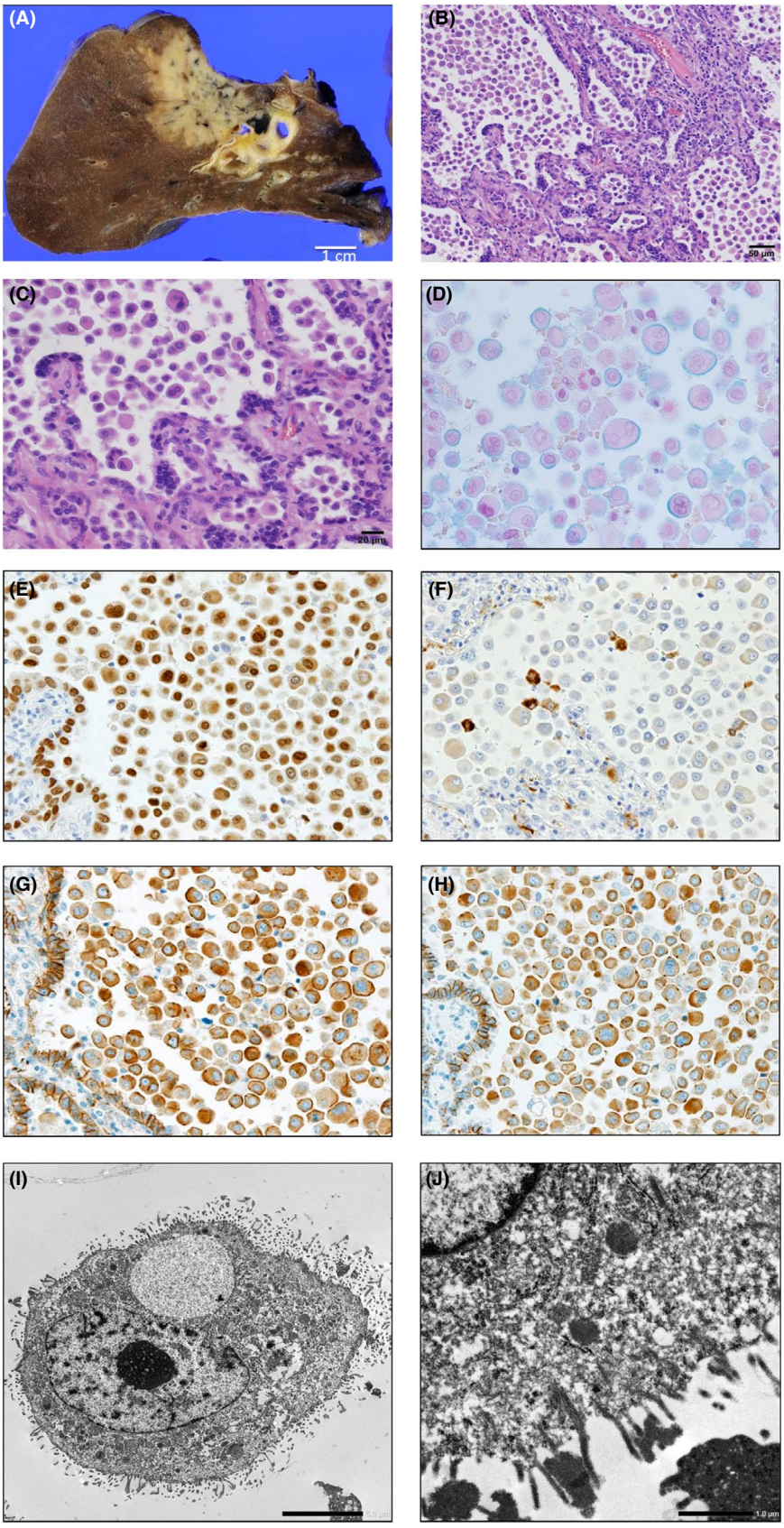

FIGURE 1 Lung adenocarcinoma of a 72-year-old Japanese woman. A, Gross view of the tumor. B,C, Microscopically, tumor cells predominantly grew in an incohesive pattern mimicking alveolar macrophages. The lepidic or papillary pattern was minimal (H&E stain). D, Tumor cells showed mucin on their surface (Alcian blue stain). E,F, Tumor cells were positive for TTF-1 (E) and negative for CD68 (F). G,H, E-Cadherin and β-catenin were positive, but mainly cytoplasmic. I,J, Ultrastructurally, tumor cells showed numerous long villi. Original magnification: (B) 200×, (C) 400×, (D) 600×, (E-H) 400×, (I) 3000×, (J) 15 000×

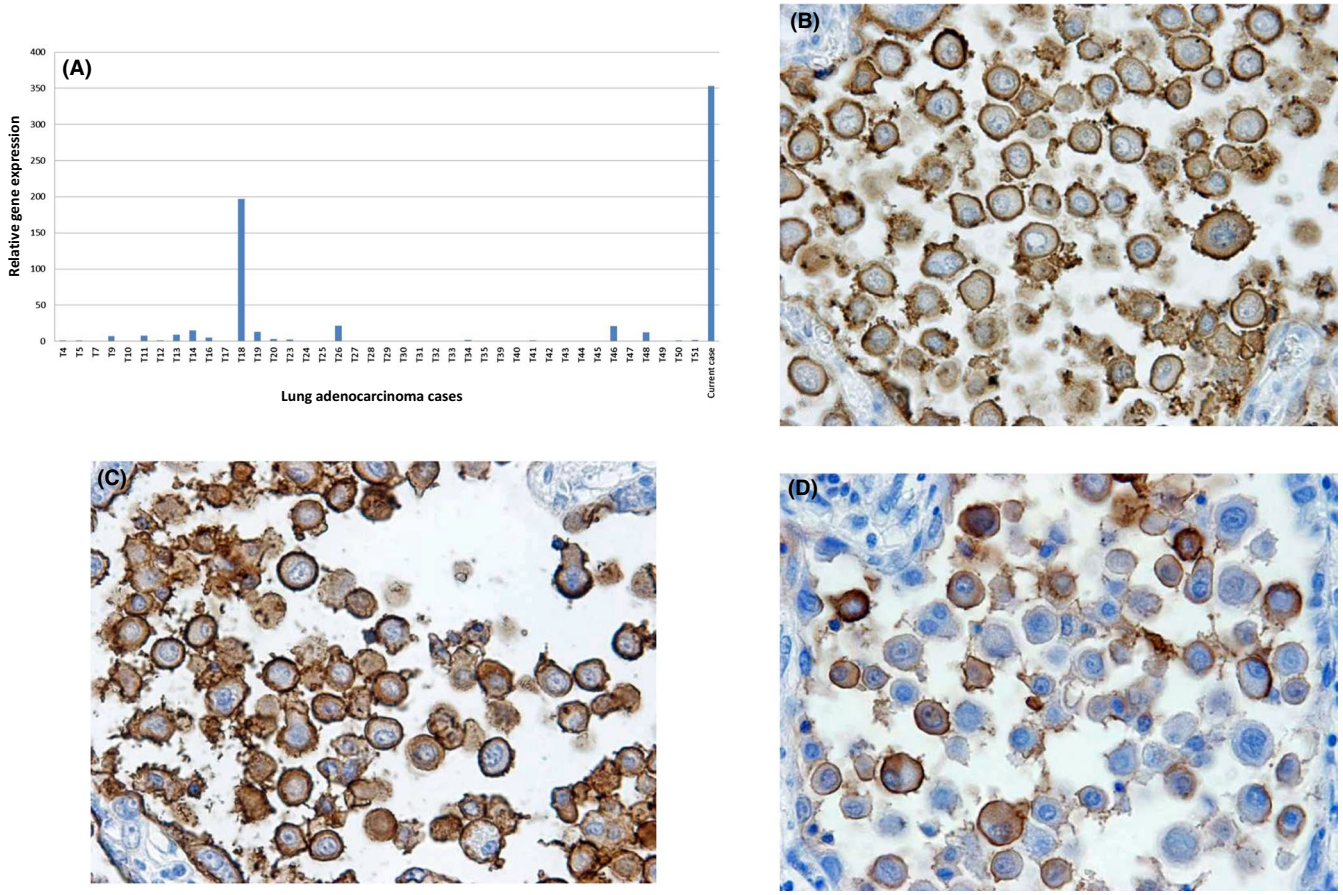

FIGURE 2 A, Expression levels of mucin 21 (MUC21) in this case and a cohort of 40 cases. The vertical line shows the reads per kilobase of exon per million mapped reads. B-D, The majority of cancer cells showed strong membranous staining with all 3 MUC21 Abs: heM21A (B), heM21C (C), and heM21D (D). Original magnification: 400×

acinar and solid patterns (Figure 3B-D). Representative figures are shown in Figure S3.

We also investigated the relationship between cell incohesiveness and MUC21 expression. MUC21 was strongly expressed in various patterns of incohesive cancer cells (Figure S4). As shown in Table 1, MUC21 expression scored as a whole tumor (as described in Table S2) correlated with the presence of incohesive cancer cells. We also evaluated MUC21 expression separately in the "Cohesive component" and "Incohesive component" in 13 cases that showed extensive cell incohesiveness. The total score for MUC21 expression was slightly higher in the incohesive component than in the cohesive component in these cases (Table S6). Although we did not observe a significant difference, cell adhesion of tumor cells with these cell incohesive patterns may have already decreased, even in the cohesive component.

We also analyzed the prognostic significance of MUC21 expression. No correlation was found between MUC21 expression (evaluated as in Table S2) and clinical outcomes (Figure S5A). As a proportion of papillary and acinar adenocarcinomas showed MUC21 expression only on the luminal side, but not on the basolateral side of the cell membrane (as shown in Figure S3C), we reasoned that these "only luminal" cases may be masking the prognostic impact of MUC21 expression. When we reassigned cases showing "only luminal" pattern expression as negative, heM21A and heM21C correlated with shorter disease-free survival (Figure S5B).

4 | DISCUSSION

We herein analyzed the molecular profile of a histological variant of lung adenocarcinoma that showed an incohesive growth pattern. This histology-driven, NGS-based analysis identified MUC21 as a key molecule associated with cell incohesive growth.

MUC21 is the newest member of the mucin family initially identified as a novel transmembrane mucin gene that is highly likely to correspond to murine epiglycanin.[12] MUC21 is expressed as a large glycoprotein at the cell surface and inhibits cell-cell and cell-matrix adhesion through steric hindrance caused by the large highly glycosylated TR domain.[14] These biological properties of MUC21 implicate it as a strong candidate molecule causally involved in cell incohesiveness in lung adenocarcinoma.

Decreased cell adhesion may play a role in tumor metastasis by facilitating cell invasion and spread through lymphatics,

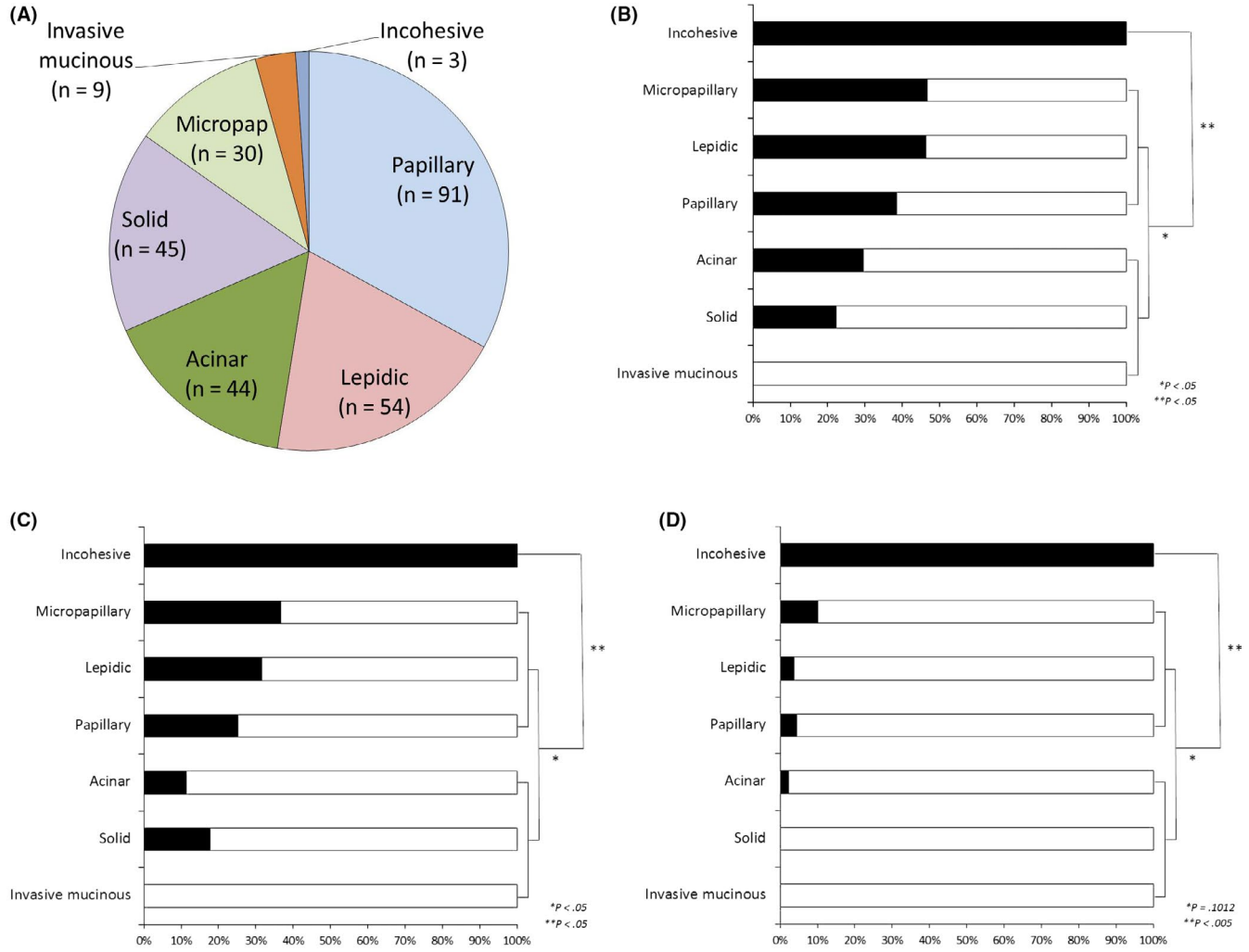

FIGURE 3 Mucin 21 (MUC21) expression in a series of lung adenocarcinomas. A, Frequencies of histological patterns in a series of 120 lung adenocarcinoma cases plus the present case. Micropap, micropapillary. B-D, The frequencies of heM21A (B), heM21C (C), and heM21D (D) positivities in each histological pattern. All 3 cases of incohesive patterns were positive for heM21A, C, and D. *P* values were calculated by using Fisher's exact test. *(Micropapillary + lepidic + papillary) vs (acinar + solid + invasive mucinous). **(Incohesive) vs (all the other patterns)

TABLE 1 Correlation between expression of mucin 21 and cell incohesiveness

	With incohesive component (n = 13)	Without incohesive component (n = 108)	*P* value
heM21A			
Positive	8	30	.0234
Negative	5	78	
heM21C			
Positive	6	20	.0328
Negative	7	88	
heM21D			
Positive	4	1	.0004
Negative	9	107	

P values calculated by Fisher's exact test.

vascular vessels, pleura, and air spaces.[2-6] The last may be represented by "spread through air spaces" (STAS), a recently proposed mode of tumor invasion.[6] Although there is a possibility that STAS could be a product of mechanical artifact,[15,16] we hypothesize that MUC21 plays a role in producing STAS by decreased cell adhesion.

MUC21 has clinical potential as a serum biomarker and therapeutic target.[7,8] The detection of MUC21 in serum may help to identify patients at high risk of recurrence preoperatively. MUC21 targeted therapy (including immunotherapy) may be envisioned for a fraction of refractory cancer cells floating in body fluids or tissues. Our results provide a rationale for further studies on this interesting molecule.

ACKNOWLEDGMENTS

We thank Tomoko Tamura for immunohistochemical work and Kiyomi Hidano for the electron microscopic analysis.

DISCLOSURE

The authors have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Yoshimoto T, Matsubara D, Soda M, et al. Mucin 21 is a key molecule involved in the incohesive growth pattern in lung adenocarcinoma. *Cancer Sci*. 2019;110:3006–3011. https://doi.org/10.1111/cas.14129