High expression of CXCL14 is a biomarker of lung adenocarcinoma with micropapillary pattern

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Funding information
Japan Agency for Medical Research and Development, Grant/Award Number: 19ck0106263h0003; Chiba University; Japan Society for the Promotion of Science, Grant/Award Number: 17K16605

Abstract
Lung adenocarcinoma with micropapillary pattern (MPP) has an aggressive malignant behavior. Limited resection should be avoided because of its high recurrence rate. If adenocarcinoma with MPP is diagnosed preoperatively, the selection of proper treatment is possible. To explore a preoperative biomarker for diagnosing MPP, we undertook RNA sequencing analysis of 25 clinical samples as the training set, including 6 MPP, 16 other adenocarcinoma subtypes, and 3 normal lung tissues. Unsupervised hierarchical clustering analysis suggested a presence of subgroup with MPP showing different gene expression phenotype. We extracted differentially expressed genes with high expression levels in MPP samples, and chose VSIG1, CXCL14, and BAMBI as candidate biomarkers for MPP. Reverse transcription-quantitative PCR analysis confirmed a significantly higher expression of VSIG1 (\( P = .03 \)) and CXCL14 (\( P = .02 \)) in MPP than others. In a validation set of 4 MPP and 4 non-MPP samples, CXCL14 expression was validated to be significantly higher in MPP than in non-MPP (\( P = .04 \)). Comparing a total of 10 MPP and 20 non-MPP samples, the area under the curve of CXCL14 to distinguish MPP from others was 0.89. The threshold value was 0.0116, corresponding to sensitivity 80% and specificity 90%. In immunostaining of CXCL14, the staining score was significantly higher in MPP cases than others, where not only the MPP component but also other components showed heterogeneous staining in adenocarcinoma tissues with MPP. Moreover, a higher staining score of CXCL14 was significantly associated with poorer prognosis in all patients (\( P = .01 \)) or within cases in stage I-III (\( P = .01 \)). In summary, we identified CXCL14 as a possible diagnostic biomarker of MPP.

Key words
biomarker, CXCL14, lung adenocarcinoma, micropapillary pattern, RNA-seq
1 | INTRODUCTION

In 2011, an international multidisciplinary panel of lung cancer experts presented a novel classification system of lung adenocarcinoma by the predominant histologic subtype presenting more than 5% of tumor. The basis for the classification of lung adenocarcinomas was accepted in the recently published 2015 WHO classification of lung tumors, 4th edition, where the predominant histologic subtype of adenocarcinoma and its correlation with oncological prognosis were also mentioned.

Histologic subtypes of adenocarcinoma differ in prognosis, and in particular, adenocarcinoma subtypes with MPP and solid pattern have been reported to show a poorer prognosis than other subtypes. In our recent publication, MPP not only showed worse overall survival, but also the coexistence with free tumor clusters resulted in a further negative impact on the postoperative prognosis. Adenocarcinoma with MPP with free tumor clusters was observed in approximately half of the cases in our previous cohort, and showed higher incidence of nodal metastasis, so that the survival curve was nearly equivalent to that in T3 cases of MPP-negative adenocarcinoma.

Biomarkers to predict the existence of MPP are therefore expected to be developed, in order to diagnose lung adenocarcinoma with MPP prior to surgery and select appropriate treatment, e.g., lobectomy or supplemental treatment in addition to surgery. However, there is difficulty in the diagnosis using biopsied samples or frozen tissue samples obtained during surgery.

We undertook a transcriptome analysis in each subtype of lung adenocarcinoma by RNA-seq of a training set of lung adenocarcinoma samples and explored the useful biomarker to diagnose the existence of MPP within adenocarcinoma. Through validation of the expression by RT-qPCR and immunohistochemistry in the training set as well as the validation set of lung adenocarcinoma samples, we identified CXCL14 as a preoperative diagnostic biomarker of adenocarcinoma with MPP.

2 | MATERIALS AND METHODS

2.1 | Clinical samples

From 52 patients of lung adenocarcinoma who underwent lung surgery at Chiba University Hospital between 2009 and 2018, we collected 30 frozen primary lung adenocarcinoma samples, 3 frozen normal lung tissues, and 52 FFPE tissues of lung adenocarcinoma, with written informed consent. Twenty-two frozen primary lung adenocarcinoma samples and 3 normal lung samples were used as training samples for RNA-seq as well as RT-qPCR analysis. The other 8 frozen samples were used for the RT-qPCR analysis to validate the results of the training set. For immunohistochemistry, 52 FFPE samples were analyzed, including the 30 samples undergoing RT-qPCR analysis. Clinicopathological data of these patients, including age, gender, pathological stage of tumors, mutation of EGFR, smoking history, presence/absence of COPD, recurrence of lung cancer, and overall survival, were extracted from our clinical record and used for correlation analyses between these factors and marker expression. EGFR mutation was analyzed by the peptide nucleic acid-locked nucleic acid PCR clamp method. In addition to adenocarcinoma, FFPE samples of 15 SCLC and 15 SqCC tissues were also collected, and used for immunohistochemistry. The study was approved by the institutional review board at Chiba University (No. 1027).

2.2 | RNA preparation and RNA-seq analysis

All tissue samples were microscopically confirmed by 2 independent pathologists to confirm tumor cell content higher than 50%, and dissection was carried out if it was necessary to enrich tumor cells. RNA was extracted using an RNeasy mini kit (Qiagen). Libraries for RNA-seq were prepared using the TruSeq Stranded mRNA Sample Prep Kit (Illumina), following the manufacturer’s protocol. Deep sequencing was carried out on the Illumina HiSeq 1500 platform using the TruSeq Rapid SBS Kit (Illumina) in 50-base single-end mode according to the manufacturer’s protocol. Gene expression levels were shown by FPKM. The RNA-seq analysis was undertaken for 3 normal lung tissues and 22 adenocarcinoma samples including 3 MPP predominant, 8 papillary predominant (including 3 cases with 20%, 30%, and 30% of MPP fraction), 5 lepidic predominant, 3 acinar predominant, and 3 solid predominant cases. Among the 22 adenocarcinoma cases, 3 MPP dominant cases and 3 papillary dominant cases with MPP fraction (20% or higher) were regarded as MPP cases, and the remaining 16 adenocarcinoma subtypes were regarded as non-MPP cases.

2.3 | Clustering analysis

Unsupervised 2-way hierarchical clustering was carried out to assess the presence of gene expression phenotypes in lung adenocarcinoma. Genes (n = 2153) showing significantly different gene expression levels between 22 adenocarcinoma and 3 normal lung samples (P < .05, t-test) were extracted, and used for clustering analysis.

2.4 | Extraction of highly expressed genes

The following criteria were used to extract candidate marker genes using RNA-seq data of lung adenocarcinoma with and without MPP. We found 161 genes that met the following criteria: (i) average value of FPKM in adenocarcinoma with MPP is greater than 10; (ii) average FPKM value in adenocarcinoma with MPP is greater than 2-fold compared with the average value of FPKM in non-MPP adenocarcinoma; (iii) average FPKM value in normal lung tissue is greater than 10; and (iv) top 2 z-score values in adenocarcinoma with MPP are greater than 0. We similarly found 58 genes that met the following criteria: (i) average FPKM value in non-MPP adenocarcinoma is
greater than 10; (ii) average FPKM value in non-MPP adenocarcinoma is greater than 2-fold compared with the average FPKM value in adenocarcinoma with MPP; (iii) average FPKM value in normal lung tissue is less than 10; and (iv) top 6 z-score values in non-MPP adenocarcinoma are greater than 0.

2.5 | Gene ontology analysis

Gene annotation enrichment analysis was undertaken based on GO (biologic process, cellular component, and molecular function) using the Functional Annotation tool at DAVID Bioinformatics Resources (https://david.ncifcrf.gov/).

2.6 | Reverse transcription-qPCR analysis

Total RNA was treated with DNase I (Ambion), and cDNA was synthesized from 10 μg total RNA using a Superscript II kit (Life Technologies). Quantitative PCR was carried out using SYBR Green PCR Core Reagents (PE Biosystems) and an iCycler Thermal Cycler (Bio-Rad Laboratories). The number of molecules of a specific gene in a sample was measured by comparing its amplification with the amplification of standard samples that contained 10^2-10^10 copies of the gene.12 The quantity of mRNA of each gene was normalized with that of ACTB. The RT-qPCR primers are shown in Table S1.

2.7 | Immunohistochemistry

To evaluate the protein expression in cancer tissue, FFPE tissue samples were analyzed by immunohistochemistry, as previously described.13 Rabbit anti-CXCL14 Ab (ab46010; Abcam) was used to evaluate expression of CXCL14, which was scored by assigning a percentage of positive cells and an intensity score. Immunohistochemical staining intensity of 0, 1, 2, or 3, indicated absent, weak, moderate, or strong expression, respectively. The percentages of positive cells were scored as follows: 0, no staining; 1, 1%-5%; 2, 5%-25%; 3, 25%-50%; 4, 50%-75%; and 5, 75%-100%. The intensity score was multiplied by the percentage score to obtain the staining score.

2.8 | Statistical analysis

Z-score was calculated by R and the heatmap was drawn in Java TreeView (http://jtreeview.sourceforge.net/). Unsupervised 2-way hierarchical clustering was undertaken using Cluster 3.0 software (http://bonsai.hgc.jp/~mdehoon/software/cluster/). Fisher’s exact test, the χ^2 test, and Wilcoxon’s signed rank test were used for analysis of clinical characteristics. The relative expression by RT-qPCR and the staining score in immunohistochemistry were analyzed by Wilcoxon’s signed rank test with P < .05 considered statistically significant. Analyses of the AUC of the ROC curve were carried out using JMP Pro 13.0.0 (SAS Institute). Overall survival was undertaken using Kaplan-Meier survival curves and the log-rank statistic and Cox proportional hazard regression analyses were applied to calculate risk ratios and 95% confidence intervals for each risk factor using JMP Pro 13.0.0 software.

3 | RESULTS

3.1 | Clinical characteristics

The composition of all adenocarcinoma subtypes and present/absent EGFR mutation are shown in Table S2, and clinicopathologic features are summarized in Table 1. There was no significant difference in age, gender, pathological stage, smoking index, or COPD between MPP and non-MPP adenocarcinoma, while death and recurrence after surgery were more frequently observed in adenocarcinoma with MPP than in non-MPP adenocarcinoma. EGFR mutations were found in 40.3% of adenocarcinomas; there was no significant difference in frequencies of EGFR mutations between MPP and non-MPP adenocarcinoma, or among any predominant subtypes of adenocarcinoma, when analyzing a total of 52 adenocarcinoma samples (Table 1).

3.2 | Transcriptome analysis by RNA-seq

Using RNA-seq data of 22 lung adenocarcinoma and 3 normal lung tissue samples, we undertook unsupervised 2-way hierarchical clustering to assess the presence of any gene expression phenotypes corresponding to predominant patterns (Figure S1). Three normal samples formed a distinct cluster, and 22 cancer samples were classified into 3 clusters. All the 3 micropapillary predominant cases were included in a cluster of 4 samples (P < .05), and the remaining sample was a papillary predominant case also containing MPP fraction. As 4 of 6 MPP cases showed similar gene expression phenotype, we hypothesized that there might be marker genes with differential expression patterns to predict MPP cases.

We then extracted 161 genes showing high expression and 58 genes showing low expression in 6 adenocarcinoma samples with MPP, which contained an MPP component of at least 20% (Figure 1A). Gene ontology analysis of the 2 groups showed significant enrichment of GO terms such as immune response, inflammatory response, and chemokine activity in the low expression group (Figure 1B), and GO terms such as alpha-amylose activity, extracellular exosome, and extracellular space in the high expression group (Figure 1C). Genes related with extracellular space included CXCL14, and 2 other genes, BAMBI and VSIG1, were selected as the candidate marker genes for adenocarcinoma with MPP.

3.3 | Validation of RNA-seq results by RT-qPCR

To quantitatively validate the results of the RNA-seq analysis, RT-qPCR was initially carried out using the training set, including 6 MPP
and 16 non-MPP samples. Although there was no marked difference in expression level of \textit{BAMBI}, we found a significantly higher expression level of \textit{CXCL14} (\(P = .02\)) and \textit{VSIG1} (\(P = .03\)) in adenocarcinoma with MPP than non-MPP (Figure 2A). Then, we additionally validated expression levels of the 2 genes using the validation set of 4 MPP and 4 non-MPP samples and confirmed significantly higher expression of \textit{CXCL14} in adenocarcinoma with MPP than non-MPP (\(P = .04\)) (Figure 2B). Using a total of 30 samples, including 10 MPP and 20 non-MPP samples, we further compared \textit{CXCL14} expression level between adenocarcinoma with MPP and without MPP (Figure 2C), resulting in significant difference (\(P = .0006\)). When the AUC and ROC curves were evaluated using the relative expression level of \textit{CXCL14}, the AUC was calculated to be 0.89. The \textit{CXCL14} threshold value, which offered the best sensitivity-specificity relationship, was calculated to be 0.0116, with sensitivity 80\% and specificity 90\% (Figure 2D).

### 3.4 Immunostaining for \textit{CXCL14}

Protein expression of \textit{CXCL14} was analyzed by immunohistochemistry. We first analyzed FFPE tissue samples of the training set of 22 samples, including 6 MPP and 16 non-MPP; frozen samples underwent RNA-seq analysis. The staining scores of \textit{CXCL14} and the ratio of \textit{CXCL14} positive cells are summarized in Table S3. Expression of \textit{CXCL14} was detected in the cytoplasm of the tumor cells, confirming its expression in the tumor cells themselves (Figure 3A-E), and \textit{CXCL14} staining was also clearly detected in the free tumor clusters in adenocarcinoma with MPP (Figure 3F). The staining score was significantly higher in MPP than non-MPP samples (\(P = .01\), Figure 3G). An additional 30 FFPE tissue samples, including 15 MPP and 15 non-MPP, were analyzed as a validation set. The staining score was also significantly higher in MPP than non-MPP samples (\(P = .02\), Figure 3H), while the score was not significantly different amongst subtypes of non-MPP adenocarcinoma (data not shown).

Interestingly, the expression of \textit{CXCL14} was observed not only in the MPP component; homogeneous or scattered expression of \textit{CXCL14} was also observed in other components in adenocarcinoma with MPP (Figure 3I,J). In 21 MPP cases, \textit{CXCL14}-positive cells were observed at 78.3\% in the MPP component and also observed at 61.9\% in components of other subtypes (Table S3).

\[\text{TABLE 1} \quad \text{Clinicopathologic features of adenocarcinoma with and without micropapillary pattern (MPP)}\]

|                      | RNA sequencing (n = 22) | RT-qPCR (n = 30) | Immunohistochemistry (n = 52) |
|----------------------|------------------------|------------------|-------------------------------|
|                      | MPP        | Non-MPP      | \(P\) value | MPP        | Non-MPP      | \(P\) value | MPP        | Non-MPP      | \(P\) value |
| No. of samples       | 6          | 16          | —           | 10         | 20          | .6           | 21         | 31          | .5           |
| Age (y)              | 68.2       | 67.5        | .6          | 70.7       | 66.1        | .1           | 69.0       | 67.8        | .5           |
| Gender               |            |              |             |            |              |             |            |              |             |
| Male                 | 3          | 8           | .6          | 6          | 11          | .3           | 11         | 18          | .3           |
| Female               | 3          | 8           | .6          | 4          | 9           | .3           | 10         | 13          | .3           |
| Stage, \(n(\%)}     |            |              |             |            |              |             |            |              |             |
| I                    | 2 (33)     | 9 (56)      | .2          | 4 (40)     | 12 (60)     | .5           | 9 (43)     | 18 (58)     | .3           |
| II                   | 0 (0)      | 3 (19)      |            | 0 (0)      | 3 (15)      |             | 2 (10)     | 6 (19)      |             |
| III                  | 4 (67)     | 4 (25)      |            | 5 (50)     | 5 (25)      |             | 8 (38)     | 6 (19)      |             |
| IV                   | 0 (0)      | 0 (0)       |            | 1 (10)     | 0 (0)       |             | 2 (10)     | 1 (3)       |             |
| EGFR mutation (+), \(n(\%)} | 0 (0) | 11 (69) | .01 | 1 (10) | 11 (55) | .006 | 6 (29) | 15 (48) | .2 |
| Smoking (pack-y)     | 39.2       | 24.9        | .2          | 31.0       | 24.8        | .5           | 20.7       | 28.5        | .1           |
| COPD, \(n(\%)}      | 3 (50)     | 2 (13)      | .1          | 4 (40)     | 4 (20)      | .2           | 5 (24)     | 11 (36)     | .2           |
| Recurrence after surgery, \(n(\%)} | 5 (83) | 3 (19) | .01 | 7 (70) | 5 (25) | .02 | 15 (71) | 8 (26) | .001 |
| Mortality during follow-up, \(n(\%)} | 4 (67) | 3 (19) | .02 | 5 (50) | 3 (15) | .02 | 6 (29) | 4 (13) | .08 |

Clinicopathologic factors were compared between MPP and non-MPP cases, in the cohort assessed by RNA sequencing (n = 22), RT-quantitative (q)-PCR (n = 30), and immunohistochemistry (n = 52).

COPD, chronic obstructive pulmonary disease; EGFR, epidermal growth factor receptor.
Survival analysis

Correlation with overall survival was analyzed (Figure 4C-F). Although MPP generally showed poorer prognosis than non-MPP cases (Table 1), significant difference was not observed in the comparison of overall survival between MPP and non-MPP using a total of 52 samples ($P = .08$, log-rank test) (Figure 4C). Cases with higher staining score of CXCL14 (5 or higher) showed significantly worse overall survival than those with lower staining (less than 5) (Figure 4D). While prognosis was significantly different among pathological stages ($P = .01$), patients at stage IV showed the worst prognosis (Figure 4E). When analyzing patients at stage I-III only, higher staining score of CXCL14 (5 or higher) significantly correlated with worse overall survival (Figure 4F).

Finally, correlation between overall survival and clinicopathologic factors was analyzed by the Cox proportional hazard model (Table 2). Only CXCL14 staining score was found to be significant in univariate analysis ($P = .02$) and in multivariate analysis ($P = .02$).

DISCUSSION

The presence of MPP is known to be a factor associated with poor prognosis in lung adenocarcinoma. While the preoperative detection of MPP is difficult, limited resection including wide wedge resection and segmentectomy to adenocarcinoma with MPP reportedly resulted in a worse prognosis than lobectomy.\textsuperscript{15} Approximately 50% of adenocarcinoma with MPP reportedly showed small tumor clusters, spreading in air space up to 2.5 cm from the main tumor.\textsuperscript{7,16} Residual free tumor clusters in the resection margin could lead to tumor recurrence, thus it has been considered necessary to search for biomarkers enabling preoperative diagnosis of adenocarcinoma with MPP. In the present study, we undertook RNA-seq screening in lung adenocarcinoma and identified that high expression of CXCL14 could be a biomarker for adenocarcinoma with MPP and poorer prognosis.

There have been abundant studies to analyze gene expression in lung cancer, and some articles pointed out their correlation with subgroups of adenocarcinoma. Bhattacharjee et al\textsuperscript{17} classified 186
lungs tumor samples into 4 distinct subgroups by hierarchical clustering analysis, and identified a poorer prognosis in the subgroup with neuroendocrine gene expression, in contrast to a relatively good prognosis in the subgroup containing bronchioloalveolar carcinoma. Garber et al. classified lung adenocarcinoma into 3 subgroups and reported that less differentiated adenocarcinoma showed a poorer prognosis. In terms of the correlation between molecular features and adenocarcinoma subtypes, The Cancer Genome Atlas reported that adenocarcinoma subtypes and prognostic diversity did not necessarily match the molecular features defined by transcriptional profiles, CpG island methylation, or oncogene mutations. Conversely, Choi et al. undertook hierarchical clustering analysis using the mutation profile, and classified the acinar and the papillary subtypes of adenocarcinoma in 1 cluster, and the micropapillary and the solid subtypes in a different cluster. Another report showed that the solid and the lepidic subtypes showed a different gene expression profile from other subtypes. Our hierarchical clustering analysis in this study showed that all the 3 MPP dominant cases and 1 papillary dominant case with MPP component formed a clearly distinct cluster (Figure S1), suggesting a presence of molecular marker genes to distinguish adenocarcinoma subtype with MPP component. We extracted genes differentially expressed at higher levels in adenocarcinoma with MPP, which showed significant enrichment of GO terms, eg alpha-amylase activity, extracellular exosome, and
extracellular space. A gene related with extracellular space, CXCL14, was validated to show significantly higher expression in adenocarcinoma with MPP and was thus identified as a possible biomarker of this subtype.

CXCL14, also called BRAK, was first identified to be expressed in the breast and kidney, and shown to be ubiquitously expressed in almost all cell types, especially epithelial cells, but not in many tumor cell lines in vitro.\textsuperscript{22,23} In colorectal, breast, papillary thyroid cancer, and osteosarcoma, high expression of CXCL14 reportedly associated with metastasis and poor prognosis.\textsuperscript{24-27} Shaykhiev et al\textsuperscript{28} reported that clinical lung adenocarcinoma with high expression of CXCL14 had a poor prognosis. In this study, high staining score of CXCL14 significantly correlated with worse overall survival in lung adenocarcinoma (\(P = .01\)) (Figure 4D). Pathological stages also significantly correlated with worse prognosis (\(P = .01\)) (Figure 4E), and significant association was confirmed between high staining score of CXCL14 and advanced
pathological stage (P < .05) (Table S4). We therefore excluded pathological stage from clinicopathologic factors in the univariate and multivariate analyses (Table 2), where only CXCL14 staining score was considered as a predictor of prognosis. In the analysis of stage I-III patients who were eligible for surgery, high expression of CXCL14 (staining score ≥ 5) significantly correlated with worse prognosis (P = .01) (D). Overall survival was significantly different among pathological stages (I, II, III, and IV), and cases at stage IV showed worst prognosis (E). Even in analysis of stage I-III cases only, high expression of CXCL14 (staining score ≥ 5) significantly correlated with worse prognosis (P = .01) (F).

Reportedly, CXCL14 bound to glycoproteins harboring heparan sulfate proteoglycans and sialic acids, leading to the proliferation and migration of cancer cells. In contrast, CXCL14 expression also reportedly suppressed tumor growth by CXCL14-mediated antitumor CD8$^+$ T cell response in human papillomavirus-related head and neck cancer, and CXCL14 expression was a good prognostic factor contributing to decreased metastasis. These reciprocal features of CXCL14 expression in cancer, tumorigenic or antitumorigenic, might depend on cancer cell types; the present study showed significant correlation between high CXCL14 expression and lung adenocarcinoma with MPP. Although MPP was reportedly recognized as a poor prognostic factor and generally tended to show poorer prognosis in our cohort (Table 1), significant difference was not observed in the Kaplan-Meier analysis of overall survival (P = .08). This might be influenced by the relatively small sample size in our study (n = 52), whereas more than 120 cases were analyzed in the reported studies.

Cigarette smoke extract reportedly induced CXCL14 expression in the differentiated airway epithelium, and epidermal growth factor also mediated the upregulation of CXCL14. Both SCLC and SqCC, which are known to be related to smoking in cancer development, showed high smoking index and high CXCL14 protein expression (Figure 4A,B). The MPP cases that showed lower smoking index, however, showed higher staining score of CXCL14, suggesting that high CXCL14 expression in adenocarcinoma with MPP could be independent of smoking.
Takiguchi et al. established clones of bone-seeking lung cancer cells, which showed high expression of CXCL14, and enhanced cancer cell tropism to the bone and anchorage-independent growth and/or recruitment of bone marrow cells. CXCL14 was also reported as a potent inhibitor of in vivo angiogenesis, stimulated by multiple angiogenic factors, e.g., interleukin-8, fibroblast growth factor-2, and vascular endothelial growth factor, to block endothelial cell chemotaxis in vitro.

Micropapillary pattern is characterized by a pattern showing micropapillary structures without a fibrovascular core; CXCL14 expression in lung adenocarcinoma cells and the surrounding environments might influence each other, which might contribute to formation of characteristic MPP features. Further studies should be investigated to explore the mechanism of developing MPP features and poor prognosis.

The limitation of this study might be the small number of frozen tissue samples examined by RT-qPCR, as small as 10 for adenocarcinoma with MPP and 20 for non-MPP. We evaluated the CXCL14 protein expression by immunohistochemistry using more FFPE tissue samples, including 21 MPP and 31 non-MPP samples; however, more frozen tissue samples should be additionally analyzed by RT-qPCR in order to evaluate the usefulness of CXCL14 as a preoperative diagnostic marker. Another limitation is that we have not evaluated preoperative biopsy specimens in this study. However, immunohistochemistry indicated that CXCL14 is highly expressed in adenocarcinoma with MPP, not only in the limited areas such as the free tumor clusters and MPP components, but also in other components homogenously or heterogeneously (Figure 3I,J). These scattered expression patterns of CXCL14 in other components could lead to advantage in analysis of biopsy specimens, because biopsy specimens of adenocarcinoma with MPP could show high expression of CXCL14 regardless of the content of the MPP lesions. Further studies to evaluate the usefulness of CXCL14 as a preoperative diagnostic marker should be carried out, by assessing CXCL14 expression and the content of MPP component in preoperative biopsy specimens.

In summary, we undertook a transcriptome analysis to search for biomarkers for lung adenocarcinoma with MPP and identified CXCL14 as a possible diagnostic biomarker of adenocarcinoma with MPP.

**ACKNOWLEDGEMENT**

We thank Eriko Ikeda and Haruka Maruyama for technical assistance. This work was supported by the Japan Agency for Medical Research and Development (AMED) (Practical Research for Innovative Cancer Control #19ck0106263h0003), Japan Society for the Promotion of Science (JSPS) (KAKENHI #17K16605), and a grant from Global and Prominent Research, Chiba University (2018-Y9).

**DISCLOSURE**

All authors have no conflict of interest to disclose.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.