Overexpression of Family with Sequence Similarity 83, Member A (FAM83A) Predicts Poor Clinical Outcomes in Lung Adenocarcinoma

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Background: The aim of this study was to explore the expression levels of family with sequence similarity 83, member A (FAM83A) in lung adenocarcinoma (LUAD) and investigate its clinical prognostic value.

Material/Methods: Bioinformatics mining methods were used to predict the differential expression levels of FAM83A mRNA in LUAD and normal lung tissues based on the TCGA and Oncomine databases. Immunohistochemical staining was performed to demonstrate the FAM83A protein expression levels in 83 cases of LUAD combined with paired normal lung tissues. The correlation between clinico-pathologic factors and FAM83A differential expression levels in LUAD was explored by the chi-square test. Kaplan-Meier univariate and Cox multivariate survival analyses were performed to investigate the clinical prognostic value of FAM83A expression in LUAD patients.

Results: Results from TCGA and Oncomine databases revealed that FAM83A mRNA expression level was significantly higher in LUAD than that in normal lung tissues (both \( P < 0.05 \)). Immunohistochemical findings demonstrated that the high positive rate of FAM83A in LUAD was 73.49% (61/83), while that of matched normal lung tissues was only 22.89% (19/83). Moreover, LUAD patients with FAM83A mRNA or high protein levels had dramatically lower OS times than those with FAM83A mRNA or low protein levels (All \( P < 0.05 \)). Lastly, Cox multivariate survival analysis showed that FAM83A differential expression level (low vs. high) was the only independent factor predicting the prognosis of LUAD patients (\( P = 0.001 \)).

Conclusions: FAM83A was overexpressed in LUAD, and FAM83A overexpression could be used as an independent factor of poor prognosis in LUAD patients.

MeSH Keywords: Lung Neoplasms • Oncogene Protein v-cbl • Prognosis

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Background

Lung cancer is the leading cause of cancer-related mortality [1]. According to the histology, lung cancer is divided into 2 main subtypes: small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC), accounting for 15% and 85% of all cases, respectively [2]. Among NSCLC, lung adenocarcinoma (LUAD) is one of the most common types of lung cancer, and is characterized by distinct cellular and molecular features, including gland and/or duct formation and/or production of significant amounts of mucus. Surgery is the standard treatment for early NSCLC [3]. Besides chemotherapy and radiotherapy, the presence of targeted drugs significantly increases the overall survival of patients with advanced NSCLC, especially for LUAD [4], but there is still a risk of recurrence in early postoperative lung cancer patients [5]. Meanwhile, chemotherapy and targeted therapies are faced with extensive drug resistance [6,7]. Thus, to understand the causes of drug resistance, establish methods of overcoming drug resistance and exploring new therapeutic targets have become important topics in the field of lung cancer.

Family with sequence similarity 83, member A (FAM83A), also known as BJ-TSA-9 [8], is located on chromosome 8, locus q24.13, and spans 27 566 base pairs. There is a promoter approximately 4000 base pairs upstream as predicted by the Eldorado tool by Genomatix. Deletions in this part of the chromosome, including the FAM83A gene, often result in Langer-Giedion syndrome [9]. Recently, some studies have found that dysregulated FAM83A is a potential biomarker in lung, prostate, and bladder cancers [8,10,11]. However, the exact expression levels of FAM83A in LUAD and its clinical prognostic value have been unknown.

Therefore, in the present study, bioinformatics methods were performed initially to predict the differential expression levels of FAM83A mRNA in LUAD and normal lung tissues through mining cancer-related databases (TCGA and Oncomine). Then, immunohistochemical staining was carried out to examine the FAM83A protein expression levels in 83 cases of LUAD combined with paired normal tissues. Moreover, the chi-square test was used to investigate the relationship between clinicopathologic parameters and differential expression levels of FAM83A protein in LUAD. Additionally, Kaplan-Meier univariate and Cox multivariate survival analyses were used to identify the prognostic value of FAM83A expression in LUAD patients.

Material and Methods

Bioinformatics mining

We downloaded 483 cases of LUAD and 347 cases of normal lung tissues containing FAM83A mRNA expression information from the TCGA database (https://cancergenome.nih.gov/). Then, RStudio software was used to analyze the differential expression levels of FAM83A mRNA between groups and draw the overall survival (OS) and disease-free survival (DFS) curves. The Oncomine database (https://www.oncomine.org) [12] was also screened to explore the differential expression levels of FAM83A between LUAD and normal groups. A total of 4 GEO-sourced datasets were included. Additionally, Kaplan-Meier Plotter (http://kmplot.com/analysis/index.php?p=service&cancer=lung) [13] was used to draw the OS and DFS curves based on the GEO data.

Collected LUAD tissues and patients’ clinicopathological data

We retrospectively collected 83 cases of LUAD and matched adjacent normal lung tissues for immunohistochemical staining. All the patients had received radical surgery in the Department of Cardiovascular Surgery in our hospital from January 2006 to December 2012. The last follow-up time was June 2017. The last pathologic diagnosis of all patients was LUAD after surgery. As listed in Table 1, besides FAM83A expression levels, the corresponding clinical information of patients, including sex, age, T stage, and TNM stage, was concluded in detail. The present study was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University and informed consent was obtained from each patient.

Immunohistochemistry and judging of results

According to the manufacturer’s protocol, immunohistochemistry analysis was performed to investigate the differential expression levels of FAM83A in 83 cases of LUAD and matched normal lung tissues. FAM83A monoclonal antibody (ab128245) was purchased from Abcam Corporation and its working concentration was 1: 200. The results of immunohistochemical staining were independently judged and interpreted by 2 pathologists who were double-blinded. Based on methods used in previous other studies [14–16], the immunoreaction score (IRS) was calculated from each slice, with the IRS equal to staining intensity (SI)×number of stained cells (PP). IRS scores ranged from 0 to 12. When the IRS was larger than 4, FAM83A was defined as high expression, and with IRS ≤4, FAM83A was defined as low expression.
Statistical analysis

SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA) was used to statistically analyze the experimental data. Mean and standard deviation were used to express the quantitative data. TCGA and Oncomine data were analyzed using the independent-samples t test to compare the differential expression levels of FAM83A mRNA between LUAD and normal lung tissue groups. The chi-square test was used to analyze the correlation between FAM83A differential levels and clinicopathological factors of LUAD patients. Kaplan-Meier univariate and Cox multivariate survival analyses were used to verify the prognostic value of FAM83A differential levels in LUAD patients. If the P value was less than 0.05, the difference was considered statistically significant.

Results

FAM83A was overexpressed in LUAD compared to normal lung tissues

Through mining the TCGA data, we found that FAM83A mRNA expression levels were significantly higher in LUAD than those in normal lung tissues (P<0.05, Figure 1A). By searching the Oncomine database, a total of 4 GEO-sourced datasets...
were found. The meta-analysis revealed that FAM83A mRNA levels were dramatically higher in LUAD than in normal lung tissues ($P=3.38\times10^{-13}$, Figure 1B). Moreover, as shown in Figure 2, results from each dataset were consistent with findings of the above meta-analysis.

Then, to confirm the above predictive results, immunohistochemistry was carried out. As shown in Figure 3A, FAM83A was positively stained in cytoplasm and cytomembrane of LUAD cells and the high positive rate of FAM83A staining in LUAD was 73.49% (61/83), while that of matched normal lung tissues was only 22.89% (19/83) (Figure 3B). Levels of FAM83A protein were remarkably higher in LUAD than those in matched normal lung tissues ($\chi^2=42.562$, $P=0.000$, Table 2).

### Association between FAM83A differential levels and clinicopathological factors of LUAD patients

Based on the immunohistochemical staining results, chi-square testing was done for FAM83A protein differential levels (low vs. high) and different subgroups of each clinicopathological factors. As shown in Table 1, there was no significant difference in FAM83A expression levels between the 2 subgroups of clinicopathological factor in LUAD patients (All $P>0.05$).

### Potential prognostic value of FAM83A gene in LUAD patients

As shown in Figure 4, through mining the TCGA database and use of Kaplan-Meier Plotter, we found that LUAD patients with high levels of FAM83A mRNA had dramatically decreased OS and DFS compared to those with low levels of FAM83A mRNA (All $P$ values <0.05, the cut-off value of FAM83A mRNA expression was set as 50%). Then, immunohistochemistry was performed to verify the predictive results. Kaplan-Meier survival analysis showed that LUAD patients with high levels of FAM83A protein had remarkably lower OS than those with low levels of FAM83A protein ($P=0.002$, Figure 5). Log-rank univariate survival analysis demonstrated that together with FAM83A differential levels, lymph node metastasis, T stage, TNM stage, and EGFR status were all the statistically significant parameters affecting the OS of LUAD patients (Table 3). Additionally, the above 5 factors were used in the subsequent Cox multivariate survival analysis, showing that FAM83A differential expression level (low vs. high) was the only independent factor predicting the prognosis of LUAD patients ($P=0.001$, Table 4).

### Discussion

Recently, accumulating studies have found that FAM83A was overexpressed in some malignancies [17,18], including...
Figure 2. Four datasets on FAM83A mRNA levels in LUAD vs. normal lung tissue by Oncomine database. The GSE number of each dataset was listed as follows: (A) Okayama Lung (GEO: GSE31210); (B) Hou Lung (GEO: GSE19188); (C) Selamat Lung (GEO: GSE32863); (D) Garber Lung (GEO: GSE3398).

Figure 3. FAM83A protein levels in 83 cases of LUAD and matched adjacent normal lung tissues by immunohistochemical staining. (A) High expression of FAM83A in LUAD; (B) Low expression of FAM83A in matched normal lung tissue. Bar=50 um.
lung [8,10,19], breast [20–23], pancreatic [24], and ovarian cancer [25]. Researchers observed that the FAM83A gene might be a good candidate for early detection of these cancers, especially lung cancer [8,10]. Lee et al. [20] revealed that FAM83A conferred EGFR-TKI drug resistance in breast cancer cells and in mice, and the underlying mechanisms were that FAM83A could interact with and cause phosphorylation of c-RAF and PI3K p85, upstream of MAPK and downstream of EGFR. Boyer et al. [21] performed the quantitative proteomics with siRNA screening, showing that FAM83A and MAPK1 were both involved in the trastuzumab resistance in HER2-positive breast cancers, and similar findings were observed by Bartel et al. [22].

| Tissues                  | FAM83A expression levels (Cases, %) | χ²   | P       |
|-------------------------|-------------------------------------|------|---------|
|                         | Low                                 | High |         |
| LUAD                    | 22 (26.51)                          | 61 (73.49) | 42.562 | 0.000  |
| Matched normal tissues  | 64 (77.11)                          | 19 (22.89) |        |        |

Table 2. Overexpression of FAM83A in 83 cases of LUAD tissues.

Figure 4. Overall survival (OS) and disease-free survival (DFS) curves of LUAD patients based on the differential expression levels FAM83A mRNA (low vs. high). (A) OS curve based on TCGA data; (B) DFS curve based on TCGA data; (C) OS curve based on GEO data (analyzed in Kaplan-Meier Plotter website); (D) DFS curve based on GEO data (analyzed in Kaplan-Meier Plotter website).
### Table 3. Kaplan-Meier univariate survival analysis of FAM83A and other clinicopathological parameters in LUAD patients.

| Clinicopathological parameters | Mean survival time (months) | 95% CI | P value |
|-------------------------------|-----------------------------|--------|---------|
| **FAM83A levels**             |                             |        |         |
| Low                           | 79.286                      | 61.241–97.331 | 0.002                           |
| High                          | 39.646                      | 32.438–46.854 |        |
| **Gender**                    |                             |        |         |
| Male                          | 49.569                      | 37.470–61.668 | 0.499                           |
| Female                        | 56.247                      | 43.320–69.173 |        |
| **Age (years)**               |                             |        |         |
| <60                           | 52.308                      | 40.891–65.400 | 0.971                           |
| >60                           | 53.146                      | 43.320–69.173 |        |
| **Histological differentiation** |                       |        |         |
| Poor                          | 46.440                      | 33.717–59.163 | 0.926                           |
| Well/moderate                 |                             |        |         |
| Tumor size (cm)               |                             |        |         |
| ≤3                            | 62.342                      | 42.342–82.220 | 0.110                           |
| >3                            | 42.342                      | 34.249–50.436 |        |
| **Tumor location**            |                             |        |         |
| Left lung                     | 48.324                      | 38.251–58.396 | 0.759                           |
| Right lung                    | 52.478                      | 40.361–64.594 |        |
| **Lymph node metastasis**     |                             |        |         |
| No                            | 72.237                      | 57.350–87.237 | 0.000                           |
| Yes                           | 35.560                      | 28.624–42.497 |        |
| **T stage**                   |                             |        |         |
| T1                            | 74.281                      | 52.032–96.530 | 0.030                           |
| T2–T4                         | 42.730                      | 35.721–49.739 |        |
| **TNM stage**                 |                             |        |         |
| I                             | 75.748                      | 59.631–91.866 | 0.001                           |
| II+III+IV                     | 37.119                      | 30.318–43.920 |        |
| **EGFR status**               |                             |        |         |
| Negative                      | 46.684                      | 46.684–67.268 | 0.046                           |
| Positive                      | 32.357                      | 21.979–42.735 |        |

**Figure 5.** Overall survival (OS) curve of LUAD patients based on the immunohistochemical data under differential expression levels FAM83A protein (low vs. high).
Table 4. Cox multivariate analysis of FAM83A and other clinicopathological parameters in LUAD patients.

| Covariates                          | HR    | 95% CI for HR | P value |
|-------------------------------------|-------|---------------|---------|
| FAM83A expression level (low vs. high) | 3.194 | 1.576–6.471   | 0.001   |
| Lymph node metastasis (no vs. yes)  | 1.294 | 0.585–2.864   | 0.524   |
| T stage (T1 vs. T2–T4)              | 1.299 | 0.614–2.748   | 0.494   |
| TNM stage (I vs. II+III+IV)         | 1.857 | 0.781–4.420   | 0.162   |
| EGFR status (negative vs. positive) | 1.904 | 0.942–3.846   | 0.073   |

Moreover, FAM83A overexpression was closely linked to the development, high disease stage, presence of distant metastasis, and poor prognosis in breast cancer [23]. Recently, Ning et al. [26] used the TCGA dataset to perform an integrated genomics analysis of lung squamous cell carcinoma, and found that 6 ceRNAs (PLAU, miR-31-5p, miR-455-3p, FAM83A-AS1, MIR31HG, and MIR99AHG) were significantly correlated with patient survival. However, the expression levels of FAM83A in LUAD and its prognostic value have not been investigated in detail.

Therefore, in the present study, the bioinformatics mining method was used to examine the differential expression of FAM83A mRNA between LUAD and normal lung groups based on TCGA and GEO (from Oncomine database) data. Results from TCGA and Oncomine databases revealed that FAM83A mRNA expression levels were significantly higher in the LUAD group than in the normal lung group. Then, to confirm the predictive results, immunohistochemistry was used to investigate the expression levels of FAM83A protein in 83 cases of LUAD and matched normal lung tissues. Identical with the above predictive findings, the high positive rate of FAM83A protein in LUAD was 73.49%, but was only 22.89% in the normal group. Compared with the normal group, the expression levels of FAM83A protein were dramatically higher in the LUAD group. All these results suggest that FAM83A is an oncogene involved in promoting the occurrence and development of LUAD.

Subsequently, the relationship between FAM83A and clinicopathological factors of LUAD patients and its potential clinical prognostic value were investigated. Through mining the TCGA database and use of Kaplan-Meier Plotter, we observed that LUAD patients with high FAM83A mRNA levels had dramatically lower OS and DFS time than those with low FAM83A mRNA levels. Based on the immunohistochemical data, Kaplan-Meier univariate survival analysis showed that LUAD patients with high levels of FAM83A protein had remarkably decreased OS times than those with low levels of FAM83A protein. Moreover, Cox multivariate survival analysis demonstrated that FAM83A differential expression levels (low vs. high) were the only independent factors predicting the prognosis of LUAD patients. In summary, these findings suggest that FAM83A overexpression could serve as an independent factor to predict the poor prognosis of LUAD patients, and FAM83A might be an important target gene involved in the growth and metastasis of LUAD.

The present study has certain deficiencies. First, the number of retrospectively collected samples was relatively small, and this might have influenced the statistical analysis to some extent. Second, some cell functional experiments, including cell proliferation, migration, and invasion, were not performed. Third, the underlying mechanisms of FAM83A overexpression in LUAD were not explored in this study. These limitations will be addressed in our future studies.

Conclusions

Our study demonstrated that FAM83A was overexpressed in LUAD, and FAM83A overexpression could serve as an independent factor to predict the poor prognosis of LUAD patients. FAM83A may become a new potential therapeutic target for the treatment of LUAD.

Conflicts of interest

None.

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