Katanin P80 correlates with larger tumor size, lymph node metastasis, and advanced TNM stage and predicts poor prognosis in non–small-cell lung cancer patients

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Abstract

Objective: The present study aimed to investigate the correlation of katanin P80 expression with clinicopathological features and survival profile in non–small-cell lung cancer (NSCLC) patients.

Methods: Totally, 398 NSCLC patients treated by pulmonary resection were enrolled and their tumor specimens were collected to determine katanin P80 expression by immunohistochemistry (IHC) assay. Clinical data were collected at diagnosis, and survival data including disease-free survival (DFS) and overall survival (OS) were assessed after treatment.

Results: There were 195 (49.0%) patients with katanin P80 high expression and 203 (51.0%) patients with katanin P80 low expression, respectively. Meanwhile, katanin P80 high expression was associated with larger tumor size (P = .001), lymph node (LYN) metastasis (P = .005), and advanced TNM stage (P = .001). As for survival data, katanin P80 high expression was correlated with reduced DFS (P < .001) and OS (P < .001). And forward stepwise multivariate Cox’s regression revealed that katanin P80 high expression was an independent predictor for decreased DFS (P < .001) and OS (P < .001). Besides, further analysis indicated that DFS (P < .001) and OS (P < .001) were the shortest in patients with katanin P80 high+++ expression, followed by patients with katanin P80 high++ expression and then those with katanin P80 high + expression and katanin P80 low expression.

Conclusion: Katanin P80 correlates with larger tumor size, LYN metastasis, and advanced TNM stage, and serves as a potential biomarker for predicting poor survival in NSCLC patients.

KEYWORDS
disease-free survival, Katanin P80, non–small-cell lung cancer, overall survival, tumor features
INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality leading to approximately 1.59 million deaths globally according to World Health Organization, and non–small-cell lung cancer (NSCLC) accounts for estimated 80%-85% of all lung cancer cases. Common treatments for NSCLC include surgical removal of the tumor, chemotherapy, radiotherapy, and targeted therapy, and NSCLC patients need long-term surveillances for treatment-related complications, tumor relapse, and occurrence of second primary lung cancer. However, the prognosis of NSCLC is still unfavorable due to its highly aggressive nature, multidrug resistance, and chemotherapy/radiotherapy-caused serious toxicity. Therefore, it is crucial to explore novel biomarkers which can monitor tumor progression and predict prognosis in NSCLC patients.

Katanin, an ATPase family member protein, severs the microtubule and is essential for fundamental cellular processes including cell division and differentiation. Katanin has heterodimeric structure, which consists of katanin P60 and katanin P80 encoded by KATNA1 and KATNB1. Previous studies indicate that katanin P80 is essential for the regulation of microtubule dynamics through combining with cytoplasmic dynein and nuclear mitotic apparatus protein, and participates in the spindle formation, neurogenesis, as well as neuronal migration via microtubule organization. Furthermore, recent studies demonstrate that katanin P80 might participate in the pathology of some cancers, such as breast cancer and prostate cancer. In addition, one study indicates that katanin is targeted by an effective microtubule-targeting agent, which inhibits the severing activity of katanin and activates JNK signaling in NSCLC treatment. Meanwhile, we conducted a pilot study with a small sample size (20 cases) before our present study, which observed that there was trend between katanin P80 levels with poor prognosis in NSCLC patients. Based on the previous studies and the results of our pilot study, we hypothesized that katanin P80 might be involved in the development and progression of NSCLC. Thus, we conducted this present study with a larger sample size (398 cases) to investigate the correlation of katanin P80 expression with clinicopathological features and survival profile in NSCLC patients.

MATERIALS AND METHODS

Patients

A total of 398 NSCLC patients treated by pulmonary resection in our hospital from January 2010 to December 2014 were analyzed in this study. The inclusion criteria were as follows: (a) newly diagnosed as primary NSCLC confirmed by clinical and histological, and pathological examinations, (b) received surgical resection without neoadjuvant therapy, (c) formalin-fixed paraffin-embedded (FFEP) tumor specimens were well-preserved, and (d) had complete medical data. The exclusion criteria were as follows: (a) secondary NSCLC, (b) patients complicated with other malignant tumors, and (c) tumor specimens or clinical data were missing. The approval for this study was obtained from the Institutional Review Board of our hospital, and the written informed consent or verbal agreement with tape recording was acquired from each patient or guardians (refer to people with the authority and duty to care for the interests of another one). This retrospective study was approved by the Ethics Committee of Renmin Hospital with the approval number "2018-008."

Immunohistochemistry (IHC) assay

FFEP tumor specimens of patients were collected from the pathology sample storage room of our hospital after approval. The specimens were sectioned at a 4-µm thickness and baked overnight for IHC assay. Prior to immunostaining, deparaffinization and hydration were performed in xylene and graded ethanol to distilled water. During hydration, blocking for endogenous peroxidase was performed in H2O2. Then, heat-induced epitope retrieval (HIER) in retrieval buffer was carried out with microwave heating. After blocked with normal goat serum, the sections were incubated with primary antibody rabbit polyclonal to katanin P80 (Sigma-Aldrich) overnight at 4°C. Next day, the sections were incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G antibody (Abcam). Subsequently, the sections were stained with diaminobenzidine (DAB) (Dako), counterstained with hematoxylin (Sigma-Aldrich), dehydrated in a graded series of ethanol and xylene, and sealed with neutral resin (Sango Biotech). Finally, the sections were mounted for examination and photographed on a microscope (Nikon Instruments).

Semi-quantitative scoring for IHC staining

The sections were examined and scored by one pathologist. Briefly, 5 high-power fields were randomly selected, and 100 cells in each selected field were counted to assess the density and intensity of positive stained cells. The staining density was assessed by the percentage of positively stained tumor cells and was scored as follows: 0, 0%; 1, <25%; 2, 26% ~ 50%; 3, 51% ~ 75%; and 4, >75%. And the staining density was scored as follows: 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). Finally, multiplying the density score by the intensity score, a final IHC score was obtained, which ranged from 0 to 12. The katanin P80 expression was evaluated according to the IHC score: The IHC score ≤3 was defined as katanin P80 low expression; correspondingly, the IHC score >3 was defined as katanin P80 high expression. Further, the katanin P80 high expression was classified as high+ (IHC score 4 ~ 6), high++ (IHC score 7 ~ 9), and high+++ (IHC score 10 ~ 12).

Clinical and survival data collection

Clinical data at diagnosis were collected by reviewing patients’ medical records, which were mainly comprised of age, gender, history of smoke
and drink, complications (hypertension, hyperlipidemia, and diabetes), differentiation of tumor, tumor size, lymph node (LYN) status, TNM stage, and carcinoembryonic antigen (CEA) level. Adjuvant treatment after surgery was administered to patients based on clinical status and TNM stage. Observation, chemotherapy, or radiation therapy (RT) ± chemotherapy was given to stage I patients; chemotherapy or chemoradiotherapy ± chemotherapy was performed for stage II patients; and chemotherapy ± RT or chemoradiotherapy ± chemotherapy was conducted for stage III patients. Patients were regularly followed up to December 31, 2018, and the median follow-up duration was 48.0 months. Survival data were collected from the follow-up records; then, the disease-free survival (DFS) and overall survival (OS) were assessed. The DFS was defined as the duration from initial therapy to disease recurrence, disease progression, or death. The OS was defined as the time interval from initial therapy to death.

2.5 | Statistical analysis

SPSS 20.0 software (IBM) was used for data analyses, and GraphPad Prism 6.01 software (GraphPad Software) was applied to construct graphs. Quantitative data were described as mean ± standard deviation (SD) or median and interquartile range (IQR), and qualitative data were displayed as number (percentage). Clinical characteristic comparison between katanin P80 high expression patients and low expression patients was determined by the Student t test, chi-square test, or Wilcoxon rank-sum test. DFS and OS curves were constructed using Kaplan-Meier method and determined by the log-rank test among groups. Factors affecting DFS and OS were determined by univariate and forward stepwise multivariate Cox’s proportional hazard regression model analyses. P value < .05 was considered as statistically significant.

3 | RESULTS

3.1 | Study flow

Initially, 627 NSCLC patients treated by pulmonary resection were retrospectively reviewed, and 206 of them were excluded, including 122 patients who received neoadjuvant therapy, 41 patients without tumor specimens, 34 patients with incomplete clinical and follow-up data, and 9 patients complicated with other malignant tumors at initial diagnosis (Figure 1). The remained 421 NSCLC patients met screening criteria, while 23 of them were excluded due to being unable to acquire informed consents. Finally, 398 NSCLC patients were included for analysis in this study.

3.2 | Katanin P80 expression by IHC assay in NSCLC patients

Katanin P80 expression in NSCLC tumor tissues was evaluated by IHC assay, and according to the score assessed by a semiquantitative scoring method, there were 195 (49.0%) patients with katanin P80 high expression (IHC score >3) and 203 (51.0%) patients with low expression (IHC score 0-3), respectively (Figure 2). Katanin P80 high expression was further classified as high+ (IHC score 4-6), high++ (IHC score 7-9), and high+++ (IHC score 10-12), and there were 83 (42.6%), 91 (46.7%), and 21 (10.8%) patients with katanin P80 high+, high++, and high+++ expression, respectively.

3.3 | Correlation of katanin P80 with characteristics in NSCLC patients

Katanin P80 high expression was associated with larger tumor size \((P = .001)\), LYN metastasis \((P = .005)\), and advanced TNM stage \((P = .001)\), while there was no correlation of katanin P80 expression with age \((P = .668)\), gender \((P = .222)\), history of smoke \((P = .641)\), history of drink \((P = .750)\), hypertension \((P = .300)\), hyperlipidemia \((P = .554)\), diabetes \((P = .395)\), tumor differentiation \((P = .301)\), or CEA \((P = .298)\) (Table 1).

3.4 | Correlation of katanin P80 with disease-free survival in NSCLC patients

Disease-free survival was reduced in patients with katanin P80 high expression compared with patients with katanin P80 low expression \((P < .001)\) (Figure 3A). And DFS was the shortest in patients with katanin P80 high+++ expression, followed by patients with katanin P80 high++ expression and then those with katanin P80 high+ expression and katanin P80 low expression \((P < .001)\) (Figure 3B).
3.5 | Factor affecting disease-free survival in NSCLC patients

Univariate Cox’s regression presented that katanin P80 high expression (P < .001), age (≥60 years) (P = .014), differentiation (poor) (P = .005), tumor size (>5 cm) (P = .002), LYN metastasis (P < .001), higher TNM stage (P < .001), and CEA (>5 ng/mL) (P = .041) were associated with decreased DFS in NSCLC patients (Table 2). And forward stepwise multivariate Cox’s regression indicated that katanin P80 high expression (P < .001), LYN metastasis (P < .001), and CEA (>5 ng/mL) (P = .031) were independent predictive factors for decreased DFS in NSCLC patients.

**TABLE 1** Correlation of Katanin P80 expression with NSCLC patients’ characteristics

| Items                          | Total NSCLC patients (N = 398) | Katanin P80 expression | P value |
|-------------------------------|---------------------------------|------------------------|---------|
| Age (years), mean ± SD        | 61.8 ± 10.5                     | 62.0 ± 10.4            | 61.6 ± 10.5 | .668 |
| Gender, No. (%)               |                                 |                        |         |
| Female                        | 90 (22.6)                       | 51 (25.1)              | 39 (20.0) | .222 |
| Male                          | 308 (77.4)                      | 152 (74.9)             | 156 (80.0) | .641 |
| History of smoke, No. (%)     | 217 (54.5)                      | 113 (55.7)             | 104 (53.3) | .750 |
| History of drink, No. (%)     | 154 (38.7)                      | 77 (37.9)              | 77 (39.5) | .395 |
| Hypertension, No. (%)         | 149 (37.4)                      | 71 (35.0)              | 78 (40.0) | .554 |
| Hyperlipidemia, No. (%)       | 121 (30.4)                      | 59 (29.1)              | 62 (31.8) | .300 |
| Diabetes, No. (%)             | 67 (16.8)                       | 31 (15.3)              | 36 (18.5) | .395 |
| Differentiation, No. (%)      |                                 |                        |         |
| Well                          | 56 (14.1)                       | 30 (14.8)              | 26 (13.4) | .301 |
| Moderate                      | 255 (64.1)                      | 135 (66.5)             | 120 (61.5) | .554 |
| Poor                          | 87 (21.8)                       | 38 (18.7)              | 49 (25.1) | .300 |
| Tumor size (cm), mean ± SD    | 5.2 ± 2.1                       | 4.9 ± 2.0              | 5.6 ± 2.2 | .298 |
| LYN metastasis, No. (%)       | 138 (34.7)                      | 57 (28.1)              | 81 (41.5) | .031 |
| TNM stage, No. (%)            |                                 |                        |         |
| I                             | 138 (34.7)                      | 86 (42.4)              | 52 (26.7) | .001 |
| II                            | 122 (30.6)                      | 61 (30.0)              | 61 (31.3) | .031 |
| III                           | 138 (34.7)                      | 56 (27.6)              | 82 (42.0) | .001 |
| CEA (ng/mL), median (IQR)     | 6.7 (3.1-27.6)                  | 6.2 (2.8-25.5)         | 7.0 (3.3-30.0) | .298 |

Note: Comparison was determined by Student’s t test, chi-square test, or Wilcoxon rank-sum test. Abbreviations: CEA, carinoembryonic antigen; IQR, interquartile range; LYN, lymph node; NSCLC, non-small-cell lung cancer; SD, standard deviation.
3.6 Correlation of katanin P80 with OS in NSCLC patients

OS was decreased in patients with katanin P80 high expression compared with patients with katanin P80 low expression ($P < .001$) (Figure 4A). OS was the shortest in patients with katanin P80 high+++ expression, followed by patients with katanin P80 high++ expression, and then patients with katanin P80 high+ expression and patients with katanin P80 low expression ($P < .001$) (Figure 4B).

3.7 Factors affecting OS in NSCLC patients

Univariate Cox's regression indicated that katanin P80 high expression ($P < .001$), differentiation (poor) ($P < .001$), tumor size ($>5$ cm) ($P < .001$), LYN metastasis ($P < .001$), higher TNM stage ($P < .001$), and CEA ($>5$ ng/mL) ($P = .002$) were correlated with reduced OS in NSCLC patients (Table 3). Further forward stepwise multivariate Cox's regression revealed that katanin P80 high expression ($P < .001$), differentiation (poor) ($P = .004$), LYN metastasis ($P < .001$), and CEA ($>5$ ng/mL) ($P = .003$) were independent predictive factors for decreased OS in NSCLC patients.

### Table 2 Factors affecting DFS

| Items                          | Cox's regression model | $P$ value | HR (95% CI)          |
|-------------------------------|------------------------|-----------|----------------------|
| Katanin P80 high expression   | <.001                  | 1.701 (1.360-2.129) |
| Age ($\geq 60$ y)             | .014                   | 1.329 (1.060-1.666) |
| Gender (male)                 | .904                   | 1.017 (0.776-1.332) |
| History of smoke              | .266                   | 0.882 (0.706-1.101) |
| History of drink              | .462                   | 0.918 (0.730-1.153) |
| Hypertension                  | .886                   | 1.017 (0.809-1.279) |
| Hyperlipidemia                | .830                   | 1.027 (0.807-1.307) |
| Diabetes                      | .404                   | 0.879 (0.650-1.190) |
| Differentiation (poor)        | .005                   | 1.294 (1.082-1.547) |
| Tumor size ($>5$ cm)          | .002                   | 1.415 (1.130-1.771) |
| LYN metastasis                | <.001                  | 2.607 (2.068-3.288) |
| Higher TNM stage              | <.001                  | 1.467 (1.281-1.679) |
| CEA ($>5$ ng/mL)              | .041                   | 1.264 (1.009-1.584) |
| Katanin P80 high expression   | <.001                  | 1.655 (1.320-2.073) |
| LYN metastasis                | <.001                  | 2.545 (2.017-3.213) |
| CEA ($>5$ ng/mL)              | .031                   | 1.282 (1.024-1.607) |

Note: Factors affecting DFS were determined by univariate and forward stepwise multivariate Cox's proportional hazard regression model analyses.

Abbreviations: CEA, carcinoembryonic antigen; CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; LYN, lymph node.

4 DISCUSSION

In our study, we found that (a) Katanin P80 high expression was associated with advanced tumor features, including larger tumor size, LYN metastasis, and advanced TNM stage in NSCLC patients. (b) Katanin P80 high expression was correlated with decreased DFS and OS in NSCLC patients, and it was an independent predictive factor for worse DFS and OS.

The katanin, as a microtubule-severing enzyme, is important for remodeling microtubule structures, which affects cell division, motility, morphogenesis, and signaling.\(^{15}\) Katanin consists of p60 and p80 subunits, and katanin P60 is responsible for microtubule-severing activity directly via phosphorylation, while this activity is enhanced by the katanin P80.\(^{16}\) Previous studies have revealed the role of katanin P60 in the initiation and development of several cancers.\(^{17-19}\) For example, one study illustrates that katanin P60 is highly expressed in breast cancer bone metastatic tissues compared with primary tumor tissues, and its overexpression promotes cell migration but inhibits cell proliferation in breast cancer, suggesting the important role of katanin P60 in breast cancer metastasis.\(^{17}\) Another study investigates...
the association of katanin P60 with clinicopathological features and survival profile in breast cancer patients, which demonstrates that katanin P60 positive expression is correlated with higher lymph node metastasis, and its high expression independently predicted worse prognosis in breast cancer patients.\(^{18}\) In addition, katanin P60 is aberrantly expressed during the prostate cancer progression, and its overexpression is responsible for cancer cell metastasis via promoting cell motility in prostate cancer.\(^{19}\) Regarding katanin P80, as another subunit of katanin, it is also involved in the microtubule remodeling through microtubule-severing activities and is of important value in regulation of various cell processes, including cellular homeostasis, proliferation, and invasion during meiosis and mitosis.\(^{12,20}\) With regard to its role in tumorigenesis, there are only two related studies available. One study reveals that katanin P80 high expression is associated with higher N stage and advanced TNM stage in patients with breast cancer.\(^{21}\) Another study indicates that katanin P80 interacts with LAPSER1, which participates in cytokinesis and potentially causes the genetic instability in prostate cancer.\(^{11}\) However, the role of katanin P80 in NSCLC and the association of katanin P80 with clinicopathological features remain undetermined. Therefore, we performed this study and observed that katanin P80 high expression was associated with larger tumor size, LYN metastasis, and advanced TNM stage in NSCLC patients. The possible reasons might include that: (a) According to the previous reports, katanin P80 regulated the severing of cytoplasmic microtubules and intercellular bridge microtubules during cytokinesis to promote cell morphogenesis and migration. Therefore, katanin P80 high expression enhanced the abnormal cell proliferation, invasion, and migration in NSCLC, and clinically, its high expression was associated with worse tumor features in NSCLC patients. (b) Katanin P80 high expression might enhance cell division via regulating spindle length,\(^{17}\) further promoting tumor cell proliferation in NSCLC; therefore, katanin P80 high expression was correlated with advanced tumor features in NSCLC patients.

With regard to the association of katanin P80 with prognosis in cancer, few related studies have been reported. Only one previous study shows that katanin P80 expression is of value in predicting worse OS in patients with breast cancer.\(^{21}\) In order to explore the value of katanin P80 in predicting prognosis in NSCLC patients, we performed the present study to compare the survival profile among NSCLC patients with different katanin P80 expressions and found that katanin P80 expression was negatively associated with DFS and OS; meanwhile, katanin P80 high expression was an independent predictor for decreased DFS and OS in NSCLC patients. The possible reasons might include that: (a) Based on the findings in our study,
katanin P80 was associated with advanced tumor features in NSCLC patients. Thus, katanin P80 high expression might influence poor prognosis via interacting with these tumor features. (b) Katanin P80 high expression might promote tumor cell morphogenesis and migration via regulating severing of cytoplasmic microtubules, which increase the risk of tumor relapse and occurrence of tumor metastasis; thus, NSCLC patients with katanin P80 high expression have worse survival profile. (c) Katanin P80 high expression might decrease the sensitivity of microtubule-targeting agents and inactivate related antitumor signaling pathway (such as JNK signaling); therefore, patients with katanin P80 high expression might have increased drug resistance compared with those with katanin P80 low expression, which led to poor prognosis. 

However, there still exist some limitations in our study. (a) In order to avoid confounding factors, we excluded the NSCLC patients who did not receive pulmonary resection or who underwent neoadjuvant therapy as well as pulmonary resection. Therefore, the role of katanin P80 in those patients was not investigated, and the wide application of Katanin P80 in NSCLC patients needed further investigation. (b) Since the present study was a single-centered study, larger sample size from multiple centers was needed for validation. (c) The underlying mechanism of katanin P80 in NSCLC is needed to be supported by cellular experiments. (d) Considering the correlation of katanin P80 with clinicopathological features and survival profile in NSCLC patients, katanin P80 might be a potential therapeutic target in NSCLC, which needed further molecular experiments for validation.

In summary, katanin P80 correlates with larger tumor size, LYN metastasis, and advanced TNM stage, and serves as a potential biomarker for predicting poor survival in NSCLC patients.

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