RNF213 inhibits migration via mediating KRT16 ubiquitination in lung adenocarcinoma cell

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Research Article

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Abstract

Background

Non-small cell lung cancer (NSCLC) is one of the most common malignant tumors, and lung adenocarcinoma (LUAD) accounts for 40% of NSCLC. Ring finger protein 213 (RNF213) has been shown to inhibit the progression of many different cancers including glioblastoma and breast cancer. However, the role of RNF213 in LUAD has not been reported. The aim of this study was to investigate the effect of RNF213 on the progression of LUAD.

Methods

The expression of RNF213 in LUAD tissues was analyzed by western blot, TCGA (The Cancer Genome Atlas) and GTEx (Genotype_Tissue Expression Project) databases. Kaplan-Meier Plotter database was used to predict the clinical significance of RNF213 in LUAD. We determined the role of RNF213 in LUAD cell lines through CCK-8 assay, migration and invasion assay. The relationship of RNF213 and KRT16 were demonstrated via co-immunoprecipitation, ubiquitination, protein degradation assay and immunoblotting. We next confirmed the oncogenetic role of KRT16 using migration and rescue assay. The clinical roles of RNF213 and KRT16 were explored by immunohistochemical staining assay (IHC) and Kaplan-Meier survival analysis.

Results

Our data manifested that RNF213 expression was reduced in LUAD, thereby affecting the prognosis of LUAD. The molecular study revealed that RNF213 functioned by promoting KRT16 ubiquitination. IHC data analysis showed that KRT16 was negatively correlated with RNF213 protein expression, and downregulation of RNF213 was associated with poor overall survival.

Conclusion

RNF213 is a potential tumor suppressor that inhibits migration of LUAD cell by mediating KRT16 ubiquitination.

Introduction

Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancers and is the leading cause of cancer-related death worldwide. Moreover, lung adenocarcinoma (LUAD) is the most common histopathological type of NSCLC[1, 2]. The therapy of LUAD mainly includes surgical treatment[3], radiotherapy[4], chemotherapy[5], biological immunotherapy[6], molecular targeted therapy[7], and traditional Chinese medicine therapy[8]. However, a multitude of patients with LUAD have local deep invasion or extensive metastasis at the time of diagnosis, and lose the opportunity for surgery [9]. Over the past decade, molecular targeted drugs and immune checkpoint inhibitors have been introduced into the clinical treatment for treating patients with advanced LUAD, improving the patients
Unfortunately, these treatments are only beneficial for a limited subset of patients, and most patients with advanced die within 5 years of diagnosis. It has been shown that metastasis is a major cause of cancer-related death in LUAD patients. Hence, exploring the molecular mechanism of the progression of LUAD is of great significance to search for a new prognosis marker.

Ring finger protein 213 (RNF213) is a gene located in the 17q25.3 region of human chromosome and converts a zinc finger binding protein with a molecular weight of 591kDa. What is more, the ring finger domain is an E3 ligase. Recently studies have confirmed that RNF213 is a crucial ATPase, which consists of two ATPase modules. These two types of components include Walker A and Walker B structures, both of which are necessary for RNF213 proteins to exert ATPase activity. Therefore, the combination of Walker A motif and ATP lead to the formation of RNF213 hexamer, while the Walker B motif hydrolyzes and dissociates ATP to maintain its stability. To sum up, RNF213 protein has both ATPase and ubiquitin ligase activity.

Although prior researches have revealed that RNF213 is mainly involved in the pathogenesis of moyamoya disease. In recent years, a host of reports have discovered that RNF213 gene may be involved in the progression of many different tumors including liver cancer, stomach cancer, ovarian cancer, head and neck cancer, pancreatic ductal cancer, and lymphoma. Thus, RNF213 exercises a promising function in cancer. But what is the role of RNF213 in LUAD?

This paper explored the effect of RNF213 in the progression of LUAD and researched the molecular mechanism of RNF213 in LUAD. Further, we investigated the clinical significance of RNF213 in LUAD.

**Materials and Methods**

**Data sources**

RNA-sequencing expression (level 3) profiles and corresponding clinical information for RNF213 were downloaded from the TCGA dataset (https://portal.gdc.com). The current-release (V8) GTEx datasets were obtained from the GTEx data portal website (https://www.gtexportal.org/home/datasets). Kaplan–Meier Plotter (https://kmplot.com/analysis/) was used to analyze the association of related genes with overall survival (OS) of LUAD patients. R software version 4.0.3 was used for data processing (R Foundation for Statistical Computing, Vienna, Austria).

**Clinical samples**

The study complied with ethical regulations regarding human participants and had the informed consent of all donors. In this study, 49 cases of LUAD were collected from the Fifth Affiliated Hospital of Sun Yat-sen University. And complete clinical follow-up data were available. All the above LUAD tissues were confirmed by histopathology. The study was approved by the Medical Ethics Committee of The Fifth Affiliated Hospital of Sun Yat-sen University (2019-L063-1).
Cell culture
The human cell lines A-549, NCI-H1975 and HEK293T were obtained from American Type Culture Collection (ATCC, USA). All experiments were performed with mycoplasma-free cells. Cells were cultured with F-12K medium, RPMI-1640 medium and DMEM (Thermo Scientific, USA) supplemented with 10% fetal bovine serum (FBS) (Thermo Scientific, USA).

Cell transfection
All transient transfections of plasmids and siRNA into cell lines followed the standard protocol for Lipofectamine 3000 Transfection Reagent (Thermo Fisher, USA). HEK-293T cells were cultured in six-well plates with complete culture medium the day before and transfected when cell fusion was about 90%. We used a plasmid lentivirus packaging system, and two packaging plasmids psPAX2 and PMD2.G (Thermo Scientific, USA) and the target gene were mixed in a ratio of 3 μg to 2 μg to 1 μg and then polyvinylimine (PEI) was added to a quarter of the plasmid. Finally, 200 μL Opti-MEM medium was added to the mixture and the combination was uniformly appended to the cells. The solution was replaced by a full culture medium after 6 hours. 48h later, the cell culture supernatant was collected and filtered with 0.4μm filter to obtain the virus solution.

Western blot
In brief, cells were lightly washed 3 times with cooled PBS and dealt with RIPA Lysis Buffer (Beyotime, China) including 1% protease inhibitor (Beyotime, China) and phosphatase inhibitor Cocktail (MedChemExpress, USA). The proteins were run on SDS-PAGE microgel at 80V and 120V, shifted to a PVDF membranes (Millipore, USA) for 1.5 hours at 350 mA. The membranes were blocked with 5% skim milk at room temperature for 1h. Then the PVDF membrane was incubated with primary antibody at 4°C overnight, and with secondary antibody at room temperature for 1 h. The specific bands were detected by an enhanced chemiluminescence (Vazyme, China).

Plasmids, cloning, and lentivirus production
To construct RNF213 and KRT16 knockout human LUAD cells, we designed short hairpin RNA (shRNA) or short interfering RNA (siRNA) sequences targeting the RNF213 and KRT16 coding sequence. The shRNAs and siRNAs used are listed in Table 1.
Table 1
The information of shRNAs and siRNAs used in this report.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Targeting sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>shRNF213#1</td>
<td>GCTGAAATGGAATCGAGAAATCTC</td>
</tr>
<tr>
<td>shRNF213#2</td>
<td>GCCTCAGCTAAGTATTCTGTTCTC</td>
</tr>
<tr>
<td>siKRT16#1</td>
<td>AGACCTGGTTCTGAGCAA</td>
</tr>
<tr>
<td>siKRT16#2</td>
<td>CCAACAGCGAACTGGTACA</td>
</tr>
</tbody>
</table>

Cell counting kit-8 (CCK-8) assay

CCK-8 assay was used to explore the cell proliferation. Cells in each group were seeded in 96-well plates at 2000 cells per well, and left to adhere for 4 h. Then cells were incubated for 1d, 2d, 3 d, 4 or 5d. At 1d, 2d, 3 d, 4 or 5d, the culture medium was discarded, and CCK-8 detection reagent was added. After incubated at 37°C for 1 h, the absorbance of cells was measured at 450 nm.

Migration and invasion assays

The LUAD cells migration and invasion ability were detected by transwell assay using 24-well Boyden chambers (Corning, USA) with 8µm pores coated with (invasion) or without (migration) Matrigel. We seeded 5×10^4 A549 cells and NCI-H1975 cells per well on the transwell inserts and cultured them in 300 µL of serum-free media at 37°C in the top chambers for 12 hours and 24 hours, whereas to the lower chamber was added a DMEM containing 10% FBS. After 12 hours of incubation, the cells were fixed, stained, and examined under microscope.

Co-immunoprecipitation (Co-IP)

To investigate the interaction between endogenous RNF213 and KRT16, cell lysates (50 µg total protein) were incubated with anti-RNF213 and anti-KRT16 antibodies (Cell Signaling Technology) or control IgG (Sigma Aldrich) pass the night at 4°C. Protein A/G agarose beads (50 µl, Santa Cruz) were appended to each specimen. The beads were cleaned with RIPA buffer 5 times for 3 hours, subsequently subjected to western blot. For the Co-IP assay, cell lysates (50 µg total protein) were hatched with anti-HA-agarose (Sigma Aldrich), anti-V5-agarose, or anti-FLAG-agarose (Sigma Aldrich) gels pass the night at 4°C. Whereafter, the sediments were cleaned with RIPA buffer 5 times and measured by western blot.

Protein degradation assay
The stable cell A549 of RNF213 was knocked down with a certain density. Cycloheximide (CHX) solution was added at a ratio of 1:1000 to the medium and treated for 0h, 2h, 4h and 8h, respectively. Protein levels were detected by western blot.

**Antibodies**

Antibodies specific for RNF213 (Thermo Fisher, Cat#PA5-51902), KRT16(Santa, Cat#sc-377224), control IgG (Sigma Aldrich), Flag-Tag (Cell Signaling, Cat#14793S), HA-Tag (Cell Signaling, Cat#3724S), V5-Tag (Cell Signaling, Cat#13202S), β-actin (Proteintech, Cat#81115-1-RR) and GAPDH (Genetex, Cat#GTX100118) were purchased from the indicated companies.

**Rescue assay**

SiRNA targeting RNF213 and KRT16 were applied to knock down RNF213 and KRT16 in A549 and NCI-H1975 cells. After 48 hours, the cells were seeded in 5×10^4 cells into transwell chambers and cultured in 300µL serum-free medium for 12 hours at 37 °C. However, DMEM containing 10% FBS was added to the lower chamber. After 12 hours of incubation, the cells were fixed, stained, and observed under a microscope.

**Immunohistochemistry staining**

LUAD tissues were fastened in 4% paraformaldehyde for 16 h at room temperature and then dehydrated in decreasing ethanol concentration. The tumor sections were hatched in 3% H_2O_2 solution at room temperature for 20 min and cleaned with PBS 3 times. Then, the cells were probed with monoclonal anti-RNF213 (1:200) and monoclonal anti-KRT16(1:50) at 4°C overnight. After 3 times of cleaning with PBS, histological sections were dealt with goat anti-rabbit at room temperature for 2 hours. Streptavidin/peroxidase complex and diaminobenzidine were used for immunostaining, and hematoxylin was used for counterstaining.

**Statistical analysis**

All data were attained from three independent experiments and are displayed as the mean ± SD. Graphs were drawn using GraphPad Prism 8.0 software. Survival analyses were performed with the Kaplan–Meier method and the differences were tested by log-rank test. Analysis was performed with SPSS using unpaired, Student’s t test. Statistical significance was defined as P < 0.05.

**Results**

RNF213 is downregulated and predicts poor survival in LUAD patients
As reported, the gene RNF213 is mutated in multiple tumor tissues. However, many aspects of RNF213 activity in LUAD remain undetermined. In order to explore the expression of RNF213 in LUAD, we analyzed the TCGA database and GTEx database analysis and found that the expression of RNF213 in LUAD tissues (n = 516) was significantly lower than that in normal lung tissues (n = 578) (P = 0.00012, Fig. 1A). To verify the results of the database, western blot was employed to identify the expression of RNF213 in 15 cases of LUAD and adjacent tissues. As shown in Fig. 1B-E, RNF213 protein level was lower in LUAD tissues (P < 0.0001). Additionally, Kaplan-Meier Plotter database was used to predict the clinical significance of RNF213 in LUAD. And the results proved that the patients with high RNF213 expression had longer overall survival (OS) (P = 0.033, Fig. 1F). These results confirmed that RNF213 was downregulated in LUAD, and prognostic analysis suggested that the lower the expression level of RNF213, the worse the prognosis of patients.

**RNF213 inhibits the invasion and migration of LUAD cell**

To investigate the biological function of RNF213 in LUAD, A549 and NCI-H1975 cells were transfected with the shRNAs targeting RNF213. Stable cell lines were selected and confirmed by western blot (Fig. 2A). Firstly, CCK-8 assay was used to explore the effect of RNF213 on the proliferation of LUAD cells. The results showed that there was no significant change in the proliferation of LUAD cells after knocking down RNF213 (Fig. 2B-C). We then applied transwell assay to explore the role of RNF213. As shown in Fig. 2D-G, RNF213 knockout promoted the migration and invasion of A549 and NCI-H1975 cells. In addition, in order to further evaluate the phenomenon, we transfected A549 and NCI-H1975 cells with RNF213 overexpressing plasmid (Fig. 2H). Stable cell lines were selected and conducted transwell experiments. The results showed that overexpression of RNF213 inhibited the migration and invasion of A549 and NCI-H1975 cells (Fig. 2I-L). In summary, these results indicated that RNF213 could suppress the migration and invasion of LUAD cell.

**RNF213 mediates KRT16 ubiquitination**

KRT16, a gene associated with promoting LUAD metastasis, was found in the previous study[24]. Our previous research on KRT16 conducted protein mass spectrometry experiments (MS) and found potential correlation between KRT16 and RNF213. To confirm the relationship of RNF213 and KRT16, the results of co-immunoprecipitation revealed that endogenous RNF213 interacted with KRT16 (Fig. 3A-B).

Additionally, RNF213 was knocked down in A549 and NCI-H1975 cell lines, and the expression of KRT16 was increased (Fig. 3C). Conversely, KRT16 expression decreased after overexpression of RNF213 (Fig. 3D). In order to explore how RNF213 regulated KRT16, prior researches have proven that RNF213 protein has ubiquitin ligase activity. Co-transfection of RNF213 and KRT16 in HEK293FT cells led to substantial poly-ubiquitination of KRT16, which was not observed when transfecting KRT16 alone (Fig. 3E). Consistently, ectopic expression of RNF213 led to substantially increased KRT16 poly-ubiquitination in both A549 and NCI-H1975 cell lines (Fig. 3F). To detect RNF213-mediated degradation of KRT16 in A549 cells, cells were treated with 40µM CHX for specific time period prior to detection. The
results showed that knockout of RNF213 delayed the degradation of KRT16 (Fig. 3G-H). These results revealed that RNF213 could increase the polyubiquitination of KRT16, leading to degrade KRT16.

**RNF213 represses LUAD cells migration via regulating KRT16**

Prior work substantiated that KRT16 was as an oncogene in LUAD via epithelial–mesenchymal transition[24]. To verify the function of KRT16 in LUAD, we overexpressed KRT16 in A549 and NCI-H1975. The results of migration assay indicated that overexpression of KRT16 promoted the migration of A549 and NCI-H1975 cells (Fig. 4A-C). Furthermore, KRT16 was knockdown in A549 and NCI-H1975 cells, and migration assay confirmed that siKRT16 inhibited their migration (Fig. 4D-F). To confirm that Whether RNF213 inhibited LUAD migration by regulating KRT16, we applied siRNAs targeting RNF213 and KRT16 in A549 and NCI-H1975 cells. And migration assay manifested that knockdown of RNF213 and KRT16 inhibited cell migration (Fig. 4G-J). These results manifested that RNF213 inhibited LUAD cells migration by regulating KRT16.

**RNF213 and KRT16 are negatively correlated in LUAD samples**

The above results have indicated that RNF213 increased ubiquitination degradation of KRT16, and that RNF213 expression is associated with prognosis in LUAD patients. To further investigate the clinical significance of RNF213 in LUAD, RNF213 and KRT16 immunohistochemical staining were performed on these sections from 34 LUAD patients. Simple linear regression analysis manifested a negative correlation between KRT16 and RNF213 protein expression (r=-1.026, Fig. 5A-B). According to RFN213 H-score cut-off value, 34 LUAD patients were divided into RNF213 high expression group and RNF213 low expression group. The results revealed that patients with high RNF213 expression (n = 11) had longer OS than patients with low RNF213 expression (n = 23) (P < 0.0001, Fig. 5C). In addition, it was found that patients with low KRT16 expression had longer OS (P = 0.0127, Fig. 5D). Finally, the results verified that LUAD patients in the high expression of RNF213 + low expression of KRT16 group had longer OS (P = 0.0003, Fig. 5E). In conclusion, KRT16 was negatively correlated with RNF213 protein expression in tissue samples of LUAD patients, and high expression of RNF213 could prolong the overall survival of LUAD patients.

**Discussion**

Metastasis plays a key role in the progression of LUAD, which is a major factor affecting the prognosis. In terms of molecular mechanism, it involves a variety of related signaling pathways and numerous molecular components, such as metastasis-associated genes, Wnt and TGF-β pathways[25–27]. It is well known that dominant genes in cancer can be divided into driver and suppressor genes[28]. Driver genes promote tumor growth and proliferation. In contrast, suppressor genes inhibit the occurrence and development of tumors[29, 30]. In this study, RNF213 was confirmed to be a tumor suppressor of LUAD. Our data discovered that the expression of RNF213 in tumor tissues was reduced, which was consistent
with the results of TCGA database and GTEx database analysis. This phenomenon may be due to the mutation of RNF213 gene in LUAD[31]. A host of studies have shown that RNF213 gene mutations occur in multiple tumor tissues. In patients with glioblastomas, RNF213 and SLC26A11 formed fusion gene resulting in abnormal copy number of RNF213 gene[32]. RNA-seq detection revealed that RNF213 and SLC26A11 gene fused to form a new fusion gene RNF213-SLC26A11 in patients with LML[33]. However, no corresponding studies for LUAD have been revealed. Therefore, subsequent experiments are needed to further explore the specific way in which RNF213 mutations occur in LUAD.

Little research has reported that RNF213 regulates relative signals associated with tumorigenesis and development. In breast cancer, PTP1B/RNF213/α-KGDD signaling is closely related to patient survival time[34]. Our previous research on KRT16 conducted protein mass spectrometry experiments (MS) and found potential correlation between KRT16 and RNF213. This study focused on the regulatory effect of RNF213 on KRT16, and confirmed that RNF213 could mediate the ubiquitination and degradation of KRT16. Keratin (KRTs) is a large family of proteins, and the KRT family has different subtypes of proteins. For non-small cell lung cancer, it has been reported that KRT-232 and navitoclax can enhance the anticancer activity of trametinib in xenotransplantation from KRAS-mutated non-small cell lung cancer patients [35]. Another research team detected the expression of keratin 19(KRT-19) in the blood samples of lung cancer patients, patients with benign lung disease and healthy people, and found that KRT-19 has a potential role as a tumor marker [36]. So far, it has not been known whether RNF213 can also regulate other proteins of the KRT family in lung cancer. If so, in what way? Does it also promote the degradation of keratin by inducing its ubiquitination? The above series of questions are the research contents to be explored in the follow-up experiments of this study.

As mentioned above, this study explored the effect and mechanism of RNF213 on the progression of LUAD. The results revealed that RNF213 could inhibit the migration of LUAD cells by increasing the ubiquitination degradation of KRT16, which may be conducive to our clearer understanding of the progression mechanism of LUAD. In this study, it was also unveiled that the expression of KRT16 decreased after overexpression of RNF213. Therefore, we hypothesized that RNF213 plaied a role in inhibiting tumor development by participating in the process of promoting KRT16 degradation. To verify this hypothesis, tissues from 34 patients with LUAD were obtained for immunohistochemical staining with RNF213 and KRT16. As shown in the results, there was a negative correlation between RNF213 and KRT16 protein levels in patient tissue sections. Importantly, we found that high expression of RNF213 in patients with LUAD was associated with longer overall survival. Accordingly, the detection of RNF213 in tissues of patients with LUAD may become a novel molecular marker to predict the prognosis of these patients, and has the clinical significance for the prognosis of patients with LUAD.

Conclusions

In conclusion, this study focused on elucidating the molecular mechanism of RNF213 gene in LUAD cell. The results revealed that RNF213 could inhibit the migration of LUAD cell by increasing the ubiquitination degradation of KRT16, suggesting that RNF213 played a cancer-suppressing effect in LUAD. Furthermore,
the expression of RNF213 is positively correlated with the overall survival of LUAD patients, which has clinical meaning for judging the prognosis of LUAD patients.

**Abbreviations**

- NSCLC Non-small cell lung cancer
- LUAD Lung adenocarcinoma
- RFN213 Ring finger protein 213
- KRT16 Keratin 16
- IHC Immunohistochemistry
- TCGA The Cancer Genome Atlas
- GTEx Genotype Tissue Expression Project
- CHX Cycloheximide
- OS Overall survival

**Declarations**

**Funding**

This study was supported by the National Natural Science Foundation of China (grants 81972740).

**Ethics approval and consent to participate**

The authors confirm that all methods were conducted according to the principles of the Declaration of Helsinki. The study was approved by the Medical Ethics Committee of The Fifth Affiliated Hospital of Sun Yat-sen University (2019-L063–1). they confirm that informed consent was obtained from all subjects.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Acknowledgements**
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Data Availability

The data applied in the bioinformatics analysis were obtained from TCGA GTEx and Kaplan-Meier Plotter database open-access database. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contribution

X P, X L and H Z performed most of the experiments and analyzed data; F Z, C W and Z Z participated in the in vitro study. N C, H Z, X P and X L designed the overall study, supervised the experiments. X P and X L wrote the paper. N C and H Z revised the paper. H Z acquired the funding. All authors read and approved the final manuscript.

References


**Figures**

![Figure 1](image-url)
RNF213 is downregulated and predicted poor survival in LUAD patients

(A) The expression distribution of RNF213 gene in LUAD and normal tissues

(B-E) Expression of RNF213 in LUAD and adjacent normal samples. (The same gel)

(F) OS Kaplan-Meier curve was plotted based on RNF213 expression of LUAD tissues in the Kaplan–Meier plotter database. ***p <0.001 ****p <0.001.

Figure 2

RNF213 inhibits the invasion and migration of LUAD cells

(A) RNF213 expression levels in the transfected LUAD cell lines were detected using western blot. (The same gel)

(B-C) CCK-8 assay in cell transfection models.

(D-G) Migration and invasion assay in cell transfection models.

(H) RNF213 expression levels in the transfected LUAD cell lines were detected by western blot. (The same gel)
(I-L) Migration and invasion assay in cell transfection models. * p < 0.05; ** p < 0.01.

Figure 3

RNF213 mediates KRT16 ubiquitination

(A-B) Coimmunoprecipitation of RNF213 and KRT16 in NCI-H1975 cells. (The same gel)

(C) Western blot analysis of KRT16 expression in RNF213 knockdown cells. (Different gels)

(D) Western blot analysis of KRT16 expression in RNF213 overexpressing cells. (Different gels)

(E) Ubiquitination of KRT16 molecules in HEK293T cells with or without RNF213 overexpression. (Different gels)

(F) Ubiquitination of KRT16 molecules in RNF213-overexpressing cells. (Different gels)

(G-H) Protein degradation assay in cell transfection models. *p < 0.05. (Different gels)
Figure 4

RNF213 represses LUAD cells migration via regulating KRT16

(A) KRT16 expression levels in the transfected LUAD cell lines were detected using western blot. (Different gels)
(B-C) Migration assay in cell transfection models.

(D) KRT16 expression levels in the transfected LUAD cell lines were detected using western blot. (Different gels)

(E-F) Migration assay in cell transfection models.

(G-H) Rescue assay in A549 and NCI-H1975. (Different gels)

(I-J) Migration assay in A549 and NCI-H1975. ** p< 0.01; *** p< 0.001, **** p< 0.0001.

**Figure 5**

RNF213 and KRT16 are negatively correlated in LUAD samples

(A) Representative immunohistochemical (IHC) staining of RNF213 and KRT16 in 34 LUAD tissues.

(B) Correlation analysis of RNF213 and KRT16 protein levels in the LUAD tissues used in (A).

(C) Kaplan–Meier survival curves showed better overall survival in high RNF213 expression group.

(D) Kaplan–Meier survival curves showed better overall survival in low KRT16 expression group.

(E) Kaplan–Meier survival curves showed better overall survival in high expression of RNF213 + low expression of KRT16 group.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Rawdata.pdf
- SupplementaryFigure.6FulllengthgelsforFigures123and4.png