STRIP2, a member of the striatin-interacting phosphatase and kinase complex, is implicated in lung adenocarcinoma cell growth and migration

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Lung adenocarcinoma (LUAD) accounts for ~40% of lung cancer cases, and the 5-year relative survival rate is no more than 1%. Dysregulation of components of striatin-interacting phosphatase and kinase (STRIPAK) complexes is associated with various diseases, including cancer. Striatin-interacting protein 2 (STRIP2), also called Fam40b, has been reported to regulate tumor cell growth and migration. Here, we investigated the role of STRIP2 in LUAD growth, migration and the underlying mechanisms. Analysis of data from The Cancer Genome Atlas database revealed that STRIP2 is highly expressed and predicted poor outcomes in patients with LUAD. Moreover, quantitative RT-PCR (qRT-PCR) analysis revealed that the mRNA expression of STRIP2 is greater in all tested LUAD cells than in a normal lung cell line. To investigate the function of STRIP2, we overexpressed STRIP2 in SPC-A1 cells and depleted STRIP2 in Calu-3 cells. Cell proliferation was evaluated by Cell Counting Kit-8 and colony-forming assays, and Transwell assay was employed to test cell invasion and migration. Our results indicate that STRIP2 depletion suppressed cell proliferation, invasion and migration in Calu-3 cells, and overexpression of STRIP2 had the opposite effects in SPC-A1 cells. Moreover, we discovered that STRIP2 depletion reduced the protein levels of p-Akt and phosphorylated-mammalian target of rapamycin (p-mTOR) in Calu-3 cells, whereas STRIP2 overexpression increased levels of these proteins in SPC-A1 cells. Furthermore, we found that silencing of STRIP2 clearly enhanced protein levels of E-cadherin and reduced levels of N-cadherin, Vimentin and matrix metalloproteinase-9 in Calu-3 cells, whereas overexpression of STRIP2 had the opposite effect in SPC-A1 cells. Our data indicate that STRIP2 promotes the proliferation and motility of LUAD cells, and this may be mediated through the regulation of the Akt/mTOR pathway and epithelial–mesenchymal transition. These results may facilitate the development of therapeutic strategies to treat LUAD.

Abbreviations
CCK-8, Cell Counting Kit-8; EMT, epithelial–mesenchymal transition; LUAD, lung adenocarcinoma; MMP-9, matrix metalloproteinase-9; p-mTOR, phosphorylated-mammalian target of rapamycin; qRT-PCR, quantitative RT-PCR; STRIP2, striatin-interacting protein 2; STRIPAK, striatin-interacting phosphatase and kinase; TCGA, The Cancer Genome Atlas.
Lung cancer, with 2.1 million new cases and 1.8 million new deaths forecasted in 2018, is the prime cause of tumor occurrence and fatalities around the world [1,2]. Lung adenocarcinoma (LUAD), accounting for ~40% of lung cancer cases, is the most widespread form of lung cancer [3]. Although bronchoscopy and computed tomography are often used to diagnose lung cancer and LUAD, these methods have certain limitations [4]. The low detection rate of LUAD in early stage is the main barrier to the recovery of patients [5]. Although cancer treatment has made some progress, especially in immunotherapy and molecular-targeted therapy, the prognosis of patients with LUAD is still poor, and its 5-year relative survival rate is no more than 1% [6]. Therefore, to perform latent diagnosis in the early stage of LUAD, further studies are warranted to develop the deep mechanism of LUAD, including identifying molecular characteristics associated with patient survival, which may contribute to gene-targeted therapy.

Striatin-interacting phosphatase and kinase (STRIPAK) complexes show many physiological functions as a whole or as an individual component, which are highly conserved both in fungal and in mammalian eukaryotes [7,8]. Increasingly, dysregulation of STRIPAK components is involved in various diseases including cancer [9]. Striatin-interacting protein 2 (STRIP2), as one member of the STRIPAK complex and also called Fam40b, took part in regulating tumor cell growth and migration [10,11]. According to the research by Madsen et al. [11], STRIP2 ablation lessened cell migration of breast cancer cells. Moreover, evidence of one study indicated that PC3 prostate cancer cell migration was also decreased by silencing STRIP2 [12]. Up to now, there has been no report on the role of STRIP2 in LUAD growth, migration and the underlying mechanisms.

Herein, we discovered that STRIP2 was highly expressed in patients with LUAD, and high expression of STRIP2 was involved in worse overall survival in LUAD. Intriguingly, we disclosed that ablation of STRIP2 restrained the proliferation, invasion and migration of Calu-3 cells, and overexpression of STRIP2 accelerated the proliferation, invasion and migration of SPC-A1 cells, which all might be modulated by the Akt/mTOR pathway and epithelial–mesenchymal transition (EMT). These findings will provide a probable blueprint for the detection and treatment of LUAD.

Materials and methods

LUAD patient samples

In this study, the lung cancer RNA sequencing expression data were acquired from The Cancer Genome Atlas (TCGA) database (https://cancergenome.nih.gov; TCGA-LUAD Project). There were 535 LUAD patient samples and 59 normal samples, a total of 594 samples for analysis, which are listed in Table S1. The chi-square test statistical analysis method was used to evaluate the correlation between gene expression and clinical features. The clinical features were grouped to analyze the relationship between the expression of STRIP2 gene and clinical features. The survival analysis was analyzed by the Kaplan–Meier method, and the difference between the two groups was analyzed by log rank test. To investigate whether STRIP2 can be used as an independent predictor of LUAD prognosis, we performed Cox regression analysis on the samples. The data source for clinical correlation, survival and Cox regression analysis is presented in Table S2.

Cell preparation

Human LUAD cell lines (NCI-H1395, SPC-A1, NCI-H2009, A549 and Calu-3) and normal lung cell line (BEAS2B) were acquired from American Type Culture Collection (ATCC, Rockefeller, MD, USA). The cells were maintained in RPMI 1640 medium with 10% FBS, 0.1 mg·mL⁻¹ streptomycin and 100 U·mL⁻¹ penicillin at 37 °C and 5% CO₂ incubator.

Cell transfection

When the cell confluence in six-well plates reached 80%, the antibiotic-free medium was replaced 2 h before transfection, then transfection sequences—silencing(si)-con, 5′-AATTCTCCGAACGTGTCACGT-3′; si-STRIP2#1, 5′-GT GTACAGCCTTCCGC-3′; and si-STRIP2#2: 5′-CCCCC TAAAGCAGCACA-3′—were transfected into Calu-3 cells using the Lipofectamine 2000 transfection kit, respectively. The empty vector pcDNA3.1 and overexpression vector pcDNA3.1-STRIP2 were obtained from Shanghai GenePharma Co., Ltd. (Shanghai, China); then they were transfected into SPC-A1 cells according to the Lipofectamine 2000 transfection kit. After the cells were incubated in the incubator for 6 h, the complete medium was replaced. The expression of the transferred gene can be observed after 48 h.

qRT-PCR assay

The TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA of SPC-A1 and Calu-3 cells. A total of 1 µg extracted RNA was then reverse transcribed into cDNA using a reverse transcriptase kit (Takara, Dalian, China). Using Power SYBR Green PCR Kit (Applied Biosystems, Foster City, CA, USA), we executed qRT-PCR to quantify the expressions of STRIP2, along with an internal control of GAPDH. Finally, melting curves were analyzed. Primers used were as follows: STRIP2, F 5′-
AAAGAAGGTCCTGCTCCTGC-3', R 5'-GGCCTGGGT AATCCAACCAA-3'; GAPDH, F 5'-ACACCCACTCCTC-CACCTTT-3', R 5'-TTACTCCTTGGAGGCCATGT-3'.

Western blot

Cells were directly lysed in radioimmunoprecipitation assay lysis buffer. A total of 20 µg protein was separated using 10% SDS/PAGE. Then the proteins were transferred onto a poly(vinylidene difluoride) membrane. After being enclosed with 5% defatted milk at room temperature for 1 h, the membrane was then hatched with the primary antibodies at 4 °C overnight and the secondary antibodies at 37 °C for 2 h. They were visualized by using enhanced chemiluminescence reagents. Western blot data were carried out in three independent experiments.

Cell Counting Kit-8

Cell proliferation was assessed by Cell Counting Kit-8 (CCK-8; CWBio, Beijing, China) according to the manufacturer's guidelines. Survival curves were generated, and OD_{450} values were derived using GRAPHPAD PRISM v7 (GraphPad Software, San Diego, CA, USA).

Transwell assays

To explore the influence of STRIP2 on SPC-A1 and Calu-3 cell migration and invasion, we conducted Transwell assays. Cell suspension was prepared after 24-h transfection, and 1 × 10^5 cells were added to the upper chamber covered with (Transwell invasion assay) or without (Transwell migration assay) Matrigel. Six hundred microliters of complete medium was filled into the lower chamber. After being cultivated at 37 °C overnight, the cells were stained by 0.1% crystal violet dye. Finally, the invaded or migrated cells were photographed using a microscope. The cell numbers were counted in five random fields.

Colony-forming assay

The log-phase cells were trypsinized and pipetted into individual cells to make cell suspensions and then counted. The 60-mm dish containing 5 mL of 37 °C prewarmed medium was inoculated at a gradient density of 4000 cells per dish and gently rotated to evenly disperse the cells. After that, the cells were incubated in a culture incubator at 37 °C with 5% CO2 and saturated humidity for 1–2 weeks. When the macroscopic clones appeared in the culture dish, the culture was terminated. Discarding the supernatant, the precipitate was washed twice with PBS. The cells were fixed by 4% paraformaldehyde for 30 min. Then removing the fixing solution, the cells were stained by 0.1% crystal violet for half an hour. Finally, the clones were counted.

Statistical analysis

The experimental data were analyzed by SPSS 22.0 statistical (IBM Corporation, Chicago, IL, USA) and GRAPHPAD PRISM v7 (GraphPad Software, San Diego, CA, USA).

Table 1. The correlation between STRIP2 expression and the clinicopathological factors of patients with LUAD. The lung cancer RNA sequencing expression data were acquired from TCGA database (TCGA-LUAD Project).

| Characteristics          | Expression of STRIP2 (n) |
|--------------------------|--------------------------|
|                          | Low | High   | P-value |
| Age (years)              |     |        |         |
| < 60                     | 57  | 79     | 0.026*  |
| ≥ 60                     | 188 | 166    |         |
| Gender                   |     |        |         |
| Female                   | 153 | 116    | 0.001*  |
| Male                     | 97  | 133    |         |
| Pathological stage       |     |        |         |
| I + II                   | 202 | 183    | 0.046*  |
| III + IV                 | 44  | 62     |         |
| Pathological-T           |     |        |         |
| T1 + T2                  | 220 | 213    | 0.345   |
| T3 + T4                  | 28  | 35     |         |
| Pathological-N           |     |        |         |
| N0                       | 169 | 154    | 0.091   |
| N1                       | 73  | 92     |         |
| Pathological-M           |     |        |         |
| M0                       | 164 | 167    | 0.456   |
| M1                       | 10  | 14     |         |

* P < 0.05.
PRISM 7.0 analysis software. The comparison of the two groups was performed by Student’s t-test. The mean comparison among multiple samples was analyzed by one-way ANOVA and post hoc Bonferroni test. A P-value < 0.05 was considered statistically significant.

**Results**

**STRIP2 is highly expressed in patients with LUAD, and high expression of STRIP2 involves worse overall survival in LUAD**

In this paper, the differential expression of STRIP2 between patients with LUAD and normal samples was investigated by using data acquired from TCGA database (TCGA-LUAD Project). CI, confidence interval; HR, hazard ratio.

**Table 2.** Cox proportional hazards model analysis of clinicopathological features for overall survival in patients with LUAD. The lung cancer RNA sequencing expression data were acquired from TCGA database (TCGA-LUAD Project). CI, confidence interval; HR, hazard ratio.

| Variables | Univariate analysis | Multivariate analysis |
|-----------|---------------------|----------------------|
|           | P-value | HR | 95% CI | P-value | HR | 95% CI |
| STRIP2 expression (low/high) | 0.000** | 1.811 | 1.345–2.437 | 0.001* | 1.835 | 1.286–2.619 |
| Clinical stage (I + II/III + IV) | 0.000** | 2.554 | 1.874–3.480 | 0.218 | 1.367 | 0.832–2.247 |
| Pathological-T (T1 + T2/T3 + T4) | 0.000** | 2.293 | 1.565–3.359 | 0.028* | 1.703 | 1.060–2.735 |
| Pathological-M (M0/M1) | 0.006** | 2.119 | 1.237–3.630 | 0.407 | 1.314 | 0.689–2.505 |
| Pathological-N (N0/N1 + N2 + N3) | 0.000** | 2.559 | 1.903–3.442 | 0.001* | 2.004 | 1.343–2.990 |
| Age (<60/≥60 years) | 0.746 | 1.056 | 0.760–1.468 |               |        |        |
| Gender (female/male) | 0.667 | 1.066 | 0.796–1.427 |               |        |        |

*P < 0.05; **P < 0.01.

**Fig. 2.** The expression of STRIP2 in LUAD cell lines. (A) The expression of STRIP2 in BEAS2B, NCI-H1395, SPC-A1, NCI-H2009, A549 and Calu-3 cell lines. *P < 0.05 versus BEAS2B group; **P < 0.01 versus BEAS2B group, one-way ANOVA. (B) The mRNA expression of STRIP2 in Calu-3 cells. **P < 0.01 versus si-control group, one-way ANOVA. (C, D) The protein levels of STRIP2 in Calu-3 cells. **P < 0.01 versus si-control group, one-way ANOVA. (E) The mRNA expression of STRIP2 in Calu-3 cells. **P < 0.01 versus vector group, Student’s t-test. (F, G) The protein levels of STRIP2 in Calu-3 cells. **P < 0.01 versus vector group, Student’s t-test. Error bars represent ± standard deviation of three independent experiments.
interestingly, in patients with higher-grade tumors, the STRIP2 expression level was higher. Univariate analysis demonstrated that STRIP2 expression, clinical stage, Pathological-T, Pathological-M and Pathological-N were obviously related to overall survival of patients with LUAD. Multivariate analysis hinted that STRIP2 can be used as an independent predictor of LUAD prognosis (*\(P < 0.05\); Table 2).

Overexpression and knockdown of STRIP2 in LUAD cell lines

We further studied the expression of STRIP2 in LUAD cell lines. BEAS2B is a normal lung cell line that we used to compare with five different LUAD cell lines: NCI-H1395, SPC-A1, NCI-H2009, A549 and Calu-3. A significant overexpression of STRIP2 was found in these five LUAD cell lines compared with the BEAS2B cell line. Furthermore, STRIP2 mRNA expression was relatively higher expressed in Calu-3 cells and lower expressed in SPC-A1 cells compared with other LUAD cells tested (Fig. 2A). Therefore, in the following assays, detection of STRIP2 knock-out effect was performed in the Calu-3 cell line.

Conversely, detection of the influences of STRIP2 overexpression was performed in the SPC-A1 cell line. The si-STRIP2 was transfected into the Calu-3 cell line, and the nonspecific sequence si-control was transfected as a control. After 48-h transfection, the total RNA and protein were extracted and detected by qRT-PCR and western blot. As illustrated in Fig. 2B–D, si-STRIP2#1 and si-STRIP2#2 could significantly lessen the expression of STRIP2 both in mRNA and in protein levels in Calu-3 cells. Also, compared with si-STRIP2#2, si-STRIP2#1 is beneficial to the follow-up experiment. Furthermore, as shown in Fig. 2E–G, pcDNA3.1-STRIP2 could significantly elevate the expression of STRIP2 both in mRNA and in protein levels in SPC-A1 cells.

Down-regulation of STRIP2 restrains the proliferation of Calu-3 cells, whereas overexpression of STRIP2 accelerates the proliferation of SPC-A1 cells

To investigate the effect of STRIP2 on the proliferation of LUAD cells, we knocked down the expression of STRIP2 in Calu-3 cells by transfection with
si-STRIP2, and the expression of STRIP2 was overexpressed in SPC-A1 cells by transfection with pcDNA3.1-STRIP2. STRIP2 knockdown could sharply reduce the proliferation in Calu-3 cells after being cultured for 48 and 72 h, but there was no significant effect at 24 h, as revealed by CCK-8 assay ($P < 0.01$; Fig. 3A). The clone formation abilities of Calu-3 cells were obviously restrained by silencing of STRIP2 when compared with si-con ($P < 0.01$; Fig. 3B,C). As displayed in Fig. 3D, the results indicated that the OD$_{450}$ value of SPC-A1 cells increased in the pcDNA3.1-STRIP2 group compared with the vector group, proving that overexpression of STRIP2 accelerated SPC-A1 cell proliferation. Moreover, the clone formation abilities of SPC-A1 cells were boosted after overexpression of STRIP2 when compared with the vector group ($P < 0.01$; Fig. 3E,F). All of these earlier findings insinuated that STRIP2 depletion had a repressive effect and STRIP2 overexpression had a stimulative effect on the growth of LUAD cells.

Knockdown of STRIP2 suppresses cell invasion and migration in Calu-3 cells, and overexpression of STRIP2 augments cell invasion and migration in SPC-A1 cells

The influence of STRIP2 on the invasion and migration of the LUAD cell lines Calu-3 and SPC-A1 was investigated using Transwell assays. Silencing of STRIP2 significantly reduced the number of crystal violet-stained Calu-3 cells in the invasion and migration assays (Fig. 4A,B). On the contrary, overexpression of STRIP2 significantly fortified the number of crystal violet-stained SPC-A1 cells in Transwell assay (Fig. 4C,D). These consequences suggested that silencing of STRIP2 inhibited cell invasion and migration in
Calu-3 cells, and overexpression of STRIP2 augmented cell invasion and migration in SPC-A1 cells.

**STRIP2 can modulate the activation of the Akt/mTOR pathway in Calu-3 and SPC-A1 cells**

To gain a deeper understanding of the mechanism by which STRIP2 affects cell proliferation of Calu-3 and SPC-A1 cells, we tested the protein expression levels of Akt, p-Akt, mTOR and phosphorylated-mammalian target of rapamycin (p-mTOR) by western blot. As shown in Fig. 5A,B, the protein level of p-Akt was significantly heightened to 2.01-fold of the si-con group after silencing of STRIP2. Knockdown of STRIP2 significantly lessened the protein expression levels of N-cadherin, Vimentin and MMP-9 to 0.25-, 0.20- and 0.28-fold of the si-con group. Moreover, overexpression of STRIP2 obviously receded the protein expression levels of E-cadherin to 0.16-fold of the si-con group. Nevertheless, the protein levels of N-cadherin, Vimentin and MMP-9 were significantly enhanced to 2.01-, 1.97- and 1.96-fold of the vector group after overexpression of STRIP2 (Fig. 6C,D).

**Discussion**

Based on reported studies, we are the first to report the role of STRIP2 on LUAD. To be specific, we disclosed that high expression of STRIP2 was related to (MMP-9) are the markers of EMT. To gain a deeper understanding of the mechanism by which STRIP2 affects cell motility of Calu-3 and SPC-A1 cells, we tested the protein expression levels of E-cadherin, N-cadherin, Vimentin and MMP-9 by western blot. As shown in Fig. 6A,B, the protein level of E-cadherin was significantly heightened to 2.01-fold of the si-con group after silencing of STRIP2. Knockdown of STRIP2 significantly lessened the protein expression levels of N-cadherin, Vimentin and MMP-9 to 0.25-, 0.20- and 0.28-fold of the si-con group. Moreover, overexpression of STRIP2 obviously receded the protein expression levels of E-cadherin to 0.16-fold of the si-con group. Nevertheless, the protein levels of N-cadherin, Vimentin and MMP-9 were significantly enhanced to 2.01-, 1.97- and 1.96-fold of the vector group after overexpression of STRIP2 (Fig. 6C,D).
poor prognosis of patients with LUAD. Furthermore, knockdown of STRIP2 significantly restrained cell proliferation, invasion and migration possibly by inactivating the Akt/mTOR pathway and EMT in Calu-3 cells, whereas STRIP2 overexpression significantly promoted cell proliferation, invasion and migration, which might activate the Akt/mTOR pathway and EMT in SPC-A1 cells. Therefore, STRIP2 may be a novel promising target for LUAD treatment.

Our outcomes manifested that high expression of STRIP2 was related to poor prognosis of patients with LUAD. Growing evidence demonstrated that STRIP2 regulated STRIPAK complex activity by interacting with its key core striatin [13]. STRIP2 is a member of STRIPAK complex that may be concerned with modulating cell proliferation, migration, adhesion and so on [14]. Hence, STRIP2 may have significant effects in several biological courses, including cell growth, differentiation, and development of vascular and neural function of cardiac disease and cancer. Madsen et al. [11] hold the view that STRIP2 had a crucial role in tumor development and metastasis. It has been reported that MDA-MB231 breast cancer cell migration was decreased by STRIP2 deficiency [11]. Besides, according to a recent study, silencing of STRIP2 also decreased cell migration in prostate cancer [12]. To date, whether STRIP2 has a role in LUAD proliferation, migration and the underlying mechanisms has not been reported. In our paper, we constructed Calu-3 cells with down-regulated STRIP2 and SPC-A1 cells with overexpressed STRIP2. We revealed for the first time that knockdown of STRIP2 significantly repressed cell proliferation, invasion and migration, whereas STRIP2 overexpression significantly facilitated cell proliferation, invasion and migration in LUAD cells.

In our study, we discovered that the influence of STRIP2 on LUAD cell proliferation might be activated by the Akt/mTOR pathway. In controlling cell survival, differentiation and malignant transformation, the Akt/mTOR pathway is one of the main pathways [15]. Suppression of this pathway is considered a latent antitumor therapy [16]. The Akt/mTOR pathway is a critical pathway for LUAD cell proliferation [15,17]. Through the Akt/mTOR pathway, xeroderma pigmentosum complementation group C repression rescued cisplatin resistance in LUAD cells [17]. However, a few articles reported that STRIP2 was involved in the modulation of the Akt/mTOR pathway. The deficiency of STRIP2 has been illustrated to restrain the proliferation and migration of vascular smooth muscle cells through affecting the Akt pathway [10]. In line with the earlier reports, our article manifested that overexpression of STRIP2 promoted cell proliferation, which
might be achieved by the activation of the Akt/mTOR pathway in LUAD cells.

In our article, we also found that the impact of STRIP2 on LUAD cell invasion and migration was possibly regulated by EMT. EMT is a vital process in embryonic upgrowth and tumor migration [18,19]. The invasion and migration ability of tumor cells are obviously heightened after the incidence of EMT [20]. Through targeting the PI3K/Akt pathway, CNTN-1 boosted chemoresistance in LUAD via abdution of EMT [21]. Studies have indicated that the expression levels of some vital factors (such as E-cadherin, N-cadherin, Vimentin and MMP-9) have been detected during the process of EMT [22,23]. As reported, E-cadherin is a suppressor, whereas N-cadherin, Vimentin and MMP-9 are promoters [24,25]. E-cadherin can serve as a major conditioning agent in epithelial cell adhesion [26]. Using a mouse model, ablation of E-cadherin facilitated cancer progression and metastasis in LUAD [27]. N-cadherin, which is an adhesion molecule of EMT, was first identified in the nervous system [28]. As reported, depletion of N-cadherin suppressed cell invasion and migration in LUAD [29]. As a biomarker of EMT, Vimentin is an intermediate filament protein that plays a role in cell migration to keep structure and motility [30]. Through maintaining heterotypic cancer cell–cancer-associated fibroblast interactions during collective invasion, Vimentin was required for LUAD metastasis [30]. MMPs, which can proteolyze all elements of matrix in extracellular, are a big class of endopeptidases [31]. MMP-9, as one member of the MMP family, can be used as a controller for tumor neovascularization [32]. The effect of MMP-9 on cancer growth is achieved by type IV collagen degradation (the main constituent part of the basement membrane) [33]. Wen and Li [34] indicated that MMP-9 was highly expressed in LUAD. In our findings, we identified that overexpression of STRIP2 obviously reeded the protein expression levels of E-cadherin and enhanced the protein levels of N-cadherin, Vimentin and MMP-9 in LUAD, suggesting that STRIP2 exhibited a promoting effect on the EMT of LUAD cells.

Conclusions

Taken together, we argue for the first time that STRIP2 was highly expressed in patients with LUAD, and high expression of STRIP2 was involved in worse overall survival in LUAD. In addition, knockdown of STRIP2 significantly suppressed cell proliferation, invasion and migration in Calu-3 cells, whereas STRIP2 overexpression significantly promoted cell proliferation, invasion and migration in SPC-A1 cells, which might be modulated by the Akt/mTOR pathway and EMT. Our observations will provide novel insight into the regulation of STRIP2 during the pathogenesis of LUAD and have important clinical implications for patients with LUAD. However, more in vivo studies are needed to further verify our results.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

L-MQ and H-TM designed this work. L-MQ, Y-HS, T-TC and J-JC conducted the experiments. L-MQ wrote the paper. H-TM revised the paper. All the authors approved the final version of the paper.

References

1 Shan L, Zhao M, Lu Y, Ning H, Yang S, Song Y, Chai W and Shi X (2019) CENPE promotes lung adenocarcinoma proliferation and is directly regulated by FOXM1. Int J Oncol 55, 257–266.
2 Zhang L, Li M, Deng B, Dai N, Feng Y, Shan J, Yang Y, Mao C, Huang P, Xu C et al. (2019) HLA-DQB1 expression on tumor cells is a novel favorable prognostic factor for relapse in early-stage lung adenocarcinoma. Cancer Manag Res 11, 2605–2616.
3 Justilien V, Ali SA, Jamieson L, Yin N, Cox AD, Der CJ, Murray NR and Fields AP (2017) Ect2-dependent rRNA synthesis is required for KRAS-TRP53-driven lung adenocarcinoma. Cancer Cell 31, 256–269.
4 Buttner R, Wolf J, Kron A and Nationales Netzwerk Genomische Medizin (2019) [The national Network Genomic Medicine (nNGM): model for innovative diagnostics and therapy of lung cancer within a public healthcare system]. Pathologe 40, 276–280.
5 KleczkoEK, Kwak JW, Schenk EL and Nemenoff RA (2019) Targeting the complement pathway as a therapeutic strategy in lung cancer. Front Immunol 10, 954.
6 Yerukala Sathipati S and Ho SY (2017) Identifying the miRNA signature associated with survival time in
patients with lung adenocarcinoma using miRNA expression profiles. *Sci Rep* 7, 7507.
7 Tang Y, Chen M, Zhou L, Ma J, Li Y, Zhang H, Shi Z, Xu Q, Zhang X, Gao Z et al. (2019) Architecture, substructures, and dynamic assembly of STRIPAK complexes in Hippo signaling. *Cell Discov* 5, 3.
8 Hwang J and Pallas DC (2014) STRIPAK complexes: structure, biological function, and involvement in human diseases. *Int J Biochem Cell Biol* 47, 118–148.
9 Shi Z, Jiao S and Zhou Z (2016) STRIPAK complexes in cell signaling and cancer. *Oncogene* 35, 4549–4557.
10 Dai L, Zhou J, Li T, Qian Y, Jin L, Zhu C and Li S (2019) STRIP2 silencing inhibits vascular smooth muscle cell proliferation and migration via P38-AKT-MMP-2 signaling pathway. *J Cell Physiol* 234, 22463–22476.
11 Madsen CD, Hooper S, Tozluoglu M, Bruckbauer A, Fletcher G, Erler JT, Bates PA, Thompson B and Sahai E (2015) STRIPAK components determine mode of cancer cell migration and metastasis. *Nat Cell Biol* 17, 68–80.
12 Bai SW, Herrera-Abreu MT, Rohn JL, Racine V, Tajadura V, Suryavanshi N, Bechtel S, Wiemann S, Baum B and Ridley AJ (2011) Identification and characterization of a set of conserved and new regulators of cytoskeletal organization, cell morphology and migration. *BMC Biol* 9, 54.
13 Suryavanshi N, Furmston J and Ridley AJ (2018) The STRIPAK complex components FAM40A and FAM40B regulate endothelial cell contractility via ROCKs. *BMC Cell Biol* 19, 26.
14 Sabour D, Srinivasan SP, Rohani S, Wagh V, Gaspar JA, Panek D, Ardestani MA, Doss MX, Riet N, Abken H et al. (2017) STRIP2 is indispensable for the onset of embryonic stem cell differentiation. *Mol Ther Methods Clin Dev* 5, 116–129.
15 Cao P, Liu B, Du F, Li D, Wang Y, Yan X, Li X and Li Y (2019) Scutellarin suppresses proliferation and promotes apoptosis in A549 lung adenocarcinoma cells via AKT/mTOR/4EBP1 and STAT3 pathways. *Thorac Cancer* 10, 492–500.
16 Li L, Wang L, Prise KM, Yu KN, Chen G, Chen L, Mei Y and Han W (2017) Akt/mTOR mediated induction of bystander effect signaling in a nucleus independent manner in irradiated human lung adenocarcinoma epithelial cells. *Oncotarget* 8, 18010–18020.
17 Teng X, Fan XF, Li Q, Liu S, Wu DY, Wang SY, Shi Y and Dong M (2019) XPC inhibition rescues cisplatin resistance via the Akt/mTOR signaling pathway in A549/DDP lung adenocarcinoma cells. *Oncol Rep* 41, 1875–1882.
18 Zhao M, Ang L, Huang J and Wang J (2017) MicroRNAs regulate the epithelial-mesenchymal transition and influence breast cancer invasion and metastasis. *Tumour Biol* 39, https://doi.org/10.1177/1010428317691682.
19 Zhou J, Cheng H, Wang Z, Chen H, Suo C, Zhang H, Zhang J, Yang Y, Geng L, Gu M et al. (2019) Bortezomib attenuates renal interstitial fibrosis in kidney transplantation via regulating the EMT induced by TNF-alpha-Smurf1-Akt-mTOR-P70S6K pathway. *J Cell Mol Med* 23, 5390–5402.
20 Oral D, Erkekoglu P, Kocer-Gumusel B and Chao MW (2016) Epithelial-mesenchymal transition: a special focus on phthalates and bisphenol A. *J Environ Pathol Toxicol Oncol* 35, 43–58.
21 Zhang R, Sun S, Ji F, Liu C, Lin H, Xie L, Yang H, Tang W, Zhou Y, Xu J et al. (2017) CNTN1 enhances chemoresistance in human lung adenocarcinoma through induction of epithelial-mesenchymal transition by targeting the PI3K/Akt pathway. *Cell Physiol Biochem* 43, 465–480.
22 Setyaningsih WAW, Arfian N, Suryadi E, Romi MM, Tranggono U and Sari DCR (2018) Hyperuricemia induces Wnt5a/Ror2 gene expression, epithelial-mesenchymal transition, and kidney tubular injury in mice. *Iran J Med Sci* 43, 164–173.
23 Luo Y, Ren Z, Du B, Xing S, Huang S, Li Y, Lei Z, Li D, Chen H, Huang Y et al. (2019) Structure identification of ViceninII extracted from Dendrobium officinale and the reversal of TGF-beta1-induced epithelial(-)mesenchymal transition in lung adenocarcinoma cells through TGF-beta/Smad and PI3K/Akt/mTOR signaling pathways. *Molecules* 24, 144–161.
24 Tang Y, Xuan Y, Qiao G, Ou Z, He Z, Zhu Q, Liao M and Yin G (2019) MDM2 promotes epithelial-mesenchymal transition through activation of Smad2/3 signaling pathway in lung adenocarcinoma. *Onco Targets Ther* 12, 2247–2258.
25 Mao W, Sun Y, Zhang H, Cao L, Wang J and He P (2016) A combined modality of carboplatin and photodynamic therapy suppresses epithelial-mesenchymal transition and matrix metalloproteinase-2 (MMP-2)/MMP-9 expression in HEP-2 human laryngeal cancer cells via ROS-mediated inhibition of MEK/ERK signalling pathway. *Lasers Med Sci* 31, 1697–1705.
26 Hong L, Han K, Wu K, Liu R, Huang J, Lunney JK, Zhao S and Yu M (2017) E-cadherin and ZEB2 modulate trophoblast cell differentiation during placental development in pigs. *Reproduction* 154, 765–775.
27 Sinkevicius KW, Bellaria KJ, Barrios J, Pessina P, Gupta M, Brainson CF, Bronson RT and Kim CF (2018) E-cadherin loss accelerates tumor progression and metastasis in a mouse model of lung adenocarcinoma. *Am J Respir Cell Mol Biol* 59, 237–245.
28 Niu M, Ma F, Qian J, Li J, Wang T, Gao Y and Jin J (2018) N-cadherin attenuates nucleus pulposus cell senescence under high magnitude compression. Mol Med Rep 17, 2879–2884.

29 Mo D, Yang D, Xiao X, Sun R, Huang L and Xu J (2017) MiRNA-145 suppresses lung adenocarcinoma cell invasion and migration by targeting N-cadherin. Biotechnol Lett 39, 701–710.

30 Richardson AM, Havel LS, Koyen AE, Konen JM, Shupe J, Wiles WGT, Martin WD, Grossniklaus HE, Sica G, Gilbert-Ross M, et al. (2018) Vimentin is required for lung adenocarcinoma metastasis via heterotypic tumor cell-cancer-associated fibroblast interactions during collective invasion. Clin Cancer Res 24, 420–432.

31 Garcia-Prieto CF, Gil-Ortega M, Vega-Martin E, Ramiro-Cortijo D, Martin-Ramos M, Bordiu E, Sanchez-Pernaute A, Torres A, Aranguez I, Fernandez-Alfonso M et al. (2019) Beneficial effect of bariatric surgery on abnormal MMP-9 and AMPK activities: potential markers of obesity-related CV risk. Front Physiol 10, 553.

32 Kessenbrock K, Plaks V and Werb Z (2010) Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 141, 52–67.

33 Matulka M, Konopka A, Mroczko B, Pryczynicz A, Kemono A, Groblewska M, Sieskiewicz A and Olszewska E (2019) Expression and concentration of matrix metalloproteinase 9 and tissue inhibitor of matrix metalloproteinases 1 in laryngeal squamous cell carcinoma. Dis Markers 2019, 3136792.

34 Wen YL and Li L (2015) Correlation between matrix metalloproteinase-9 and vascular endothelial growth factor expression in lung adenocarcinoma. Genet Mol Res 14, 19342–19348.

Supporting information

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

Table S1. Analyzed TCGA samples.

Table S2. Data source for clinical correlation, survival and Cox regression analysis.