KRT17 Facilitates Proliferation, Migration and Invasion in Esophageal Squamous Cell Carcinoma by Regulating mTOR/S6K1 pathway

Lei Liu

Sichuan Provincial People's Hospital: Sichuan Academy of Medical Sciences and Sichuan People's Hospital

Mengqi Shao

Sichuan Provincial People's Hospital: Sichuan Academy of Medical Sciences and Sichuan People's Hospital

Youyu Wang

Sichuan Provincial People's Hospital: Sichuan Academy of Medical Sciences and Sichuan People's Hospital

Gang Li

Sichuan Provincial People's Hospital: Sichuan Academy of Medical Sciences and Sichuan People's Hospital

Shenglong Xie

Sichuan Provincial People's Hospital: Sichuan Academy of Medical Sciences and Sichuan People's Hospital

Kegang Jia

Sichuan Provincial People's Hospital: Sichuan Academy of Medical Sciences and Sichuan People's Hospital

Gang Feng (✉ scsrmmyxwk@126.com)

Sichuan Provincial People's Hospital: Sichuan Academy of Medical Sciences and Sichuan People's Hospital

---

Research

Keywords: KRT17, proliferation, migration, invasion, esophageal squamous cell carcinoma, mTOR/S6K1

Posted Date: November 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1038602/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive malignancies worldwide which originates from the malignant transformation of esophageal epithelial cells. Dysregulated expression of Keratin17 (KRT17) has been claimed in a variety of malignancies, while its role in ESCC remains unclear. Therefore, our study aimed to explore the potential function and underlying molecular mechanism of KRT17 in ESCC.

Methods

Data-independent acquisition-mass spectrometry (DIA-MS) workflow was used to analysis KRT17 expression between ESCC and adjacent non-cancerous esophageal tissues. The online database gene expression profiling interactive analysis (GEPIA) was used to further determine the differential expression of KRT17 in tissues. The function of KRT17 in ESCC was tested on two human esophageal cancer cell lines (EC9706 and ECA109). Small interfering RNA (siRNA) was used to inhibit KRT17 expression. Cell proliferation was examined by cell counting kit 8 (CCK8) reagent, colony formation assay, cell cycle distribution analysis and apoptosis. Cell migration was examined by transwell and wound healing assay. Cell invasion was also examined by transwell assay. Western blot and quantitative real-time PCR (qRT-PCR) was used to evaluate protein and mRNA levels, respectively.

Results

KRT17 expression was higher in cancer tissues compared with normal tissues. Transfected with siKRT17 attenuated protein and mRNA levels of KRT17, inhibited proliferation, migration and invasion, and decreased mTOR/S6K1 phosphorylation levels in EC9706 and ECA109.

Conclusion

KRT17 facilitates proliferation, migration and invasion in ESCC cells, and these cell viability functions were mediated by mTOR/S6K1 pathway.

Background

Esophageal cancer is a leading cause of cancer-related deaths in the world with a low 5-year survival rate, about 19% [1]. Esophageal cancer can histologically classify into two predominate subtypes, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) [2], while ESCC is more prevalent than EAC, account for approximately 90% of cases with esophageal cancer worldwide [3]. Despite rapid progress in medicine, many ESCC patients are still diagnosed at an advanced stage. Patients who receive prompt treatment also suffer from local or systemic recurrence and have a poor prognosis [4]. Hence, it is meaningful to investigate the pathogenesis and underlying molecular

mechanism of ESCC, which may aim of developing better strategies to improve ESCC prevention, management and treatment.

Keratin17 (KRT17) belongs to type Ⅱ intermediate filament, which molecular weight is 48 KDa [5]. KRT17 is involved in multiple biological processes, represented by skin inflammation and cell proliferation [6]. The dysregulated expression of KRT17 has reported in several types of cancer, including gastric, bladder, pancreatic and colorectal cancer [7]. However, the role of KRT17 in the ESCC has not been elucidated.

The mammalian target of rapamycin (mTOR) is an atypical serine/threonine kinase which plays vital roles in cell growth, metabolism, autophagy and immunity [8]. mTOR is frequently dysregulated in the pathogenesis of cancer, thus it has the potential to be a therapeutic target [9]. S6 kinase 1 (S6K1) is encoded by RPS6KB1 on chromosome 17 and can phosphorylated and activated by mTOR [10, 11]. The overexpression of S6K1 is closely related with worse prognosis and metastasis in multifarious human cancers, represented by breast, colorectal, lung and ovarian cancer [12]. The mTOR-S6K1 pathway has been considered to be an irreplaceable regulator in many pathological processes.

In the present study, we aimed to determine the expression of KRT17 in tissues and explore the potential function and underlying molecular mechanism of KRT17 in the pathogenesis of ESCC. We showed that KRT17 was overexpression in ESCC and knockdown of KRT17 attenuated the proliferation, migration and invasion of ESCC cell lines by inhibiting mTOR/S6K1 pathway.

Materials And Methods

Patients and Specimens

27 pairs of human ESCC tumor tissues and adjacent non-cancerous esophageal tissues were collected from patients with ESCC at Sichuan Provincial People's Hospital from May to November in 2019. None of the patients received radiotherapy, chemotherapy, or other anticancer treatment before surgery. The specimens were collected immediately following radical resection of esophageal carcinoma and flash frozen in liquid nitrogen. The staging of esophageal cancer should be performed according to the tumor-node-metastasis (TNM) classification published by the current American Joint Council on Cancer (AJCC). The histological features of all specimens were evaluated by pathologists according to the WHO criteria. Informed consent was collected from each participant. All the methods in this study were in accordance with approved guidelines, and this study was approved by the Medical Ethics Committee of Sichuan Provincial People's Hospital.

Tissue sample preparation

We extracted total protein with a standard protocol. Frozen tissue was scraped and added with SDT lysate to transfer to Lysing Matrix A tube. The BCA protein assay (Beyotime, China) was used for protein quantification. The samples were stored at -80°C. For each sample, 20µg of protein was added into 6X sample loading buffer, and the samples were bathed in boiling water for 5min. 12% SDS-PAGE

electrophoresis (constant pressure 250V, 40min) was performed, and the samples were stained with Coomase blue. Protein samples were digested according to the manufacturer's protocol for filter-aided sample preparation (FASP) [13].

Agilent 1260 Infinity HPLC System were used to fractionate the peptides of all samples. The sample was loaded onto the chromatographic column (Waters, XBridge Peptide BEH C18 Column, 130Å, 5 µm, 4.6 mm X 100 mm) equilibrated with the buffer A (5% ACN, 10 mM, pH 10.0) and separated at the flow rate of 1 mL/min. Liquid chromatography separation was performed using linear gradient from the buffer B (85% ACN, 10mM HCOONH₄, pH 10.0) concentration of 5% to 45% over 40 minutes at a constant column temperature of 30°C. Each fraction was dried in a vacuum concentrator. After lyophilization, 0.1% formic acid aqueous solution was used to re-dissolved the samples.

Spectral Library Generation

Taken from each fraction, 6µL samples were added into 1ul 10×iRT peptide and then injected 6µL after mixing, which were separated by nano-LC and analyzed by on-line electrospray tandem mass spectrometry. The complete set of the liquid chromatography-tandem mass spectrometry system is as follows: (1) Liquid phase system: EASY-nLC system (Thermo Fisher Scientific), (2) Mass spectrometry system: Q-Exactive HF-X (Thermo Fisher Scientific). Buffer A was 0.1% formic acid aqueous solution and buffer B was 0.1% formic acid in acetonitrile (80% acetonitrile). Sample was loaded on an analytical column (Thermo Fisher Scientific, Acclaim PepMap RSLC 50um X 15cm, nano viper, P/N164943) at the flow rate of 300nL/min and separated with a nonlinear gradient: 0-5min, 1% B; 5-95min, from 1% to 28% B; 95-110min, from 28% to 38% B; 110-115min, from 38% to 100% B; 115-120min, 100% B. The electrospray voltage applied was 2.0 kV.

The parameters of mass spectrometry system were set as follows. (1) MS: scan range (m/z) =350-1500; resolution=60,000; AGC target=3e6; maximum injection time=30 ms; include charge states=2-7; Filter Dynamic Exclusion: exclusion duration=30s; (2) dd-MS2: Isolation window=1.6 m/z, resolution=15,000; AGC target=1e5; maximum injection time=45 ms; NCE=28%.

Via Spectronaut Pulsar X (version 12, Biognosys AG), mass spectrometry RAW data were combined, analyzed, searched and established a spectrogram database named Uniprot_HomoSapiens_20386_20180905 with download link <http://www.uniprot.org> in June, 2018. Trypsin was set as the enzyme allowing two missed cleavage sites. The search parameters were as follows: fixed modification of Carbamidomethyl (C) and variable modifications of Oxidation(M) and acetyl (Protein N-term). The standards were 1% Precursor FDR, 1% Protein FDR and 1% Peptide FDR.

DIA mass spectrometry analysis

6ul of each sample was added to 1µL 10×iRT peptide segment. After mixing, 6µL of each sample was injected and separated by Nano-LC. Then the sample was analyzed by online electrospray tandem mass spectrometry. The whole liquid-mass tandem system was as follows: 1) Liquid phase system: Easy nLC

system (Thermo Fisher Scientific); 2) mass spectrometry system: Q-Exactive HF-X (Thermo Fisher Scientific). The sample was separated by nonlinear growth gradient at a flow rate of 300nL/min on analytical columns (Thermo Fisher Scientific, Acclaim PepMap RSLC 50um X 15cm, Nano Viper, P/N164943).

Online database

KRT17 expression datasets were obtained from gene expression profiling interactive analysis (GEPIA, <http://gepia.cancer-pku.cn/index.html>).

Cell culture and transfection

ESCC cell lines (EC9706 and ECA109) were purchased from Shanghai Cell Bank of the Institute of the Chinese Academy of Sciences (Shanghai, China), and were cultured in DMEM (Hyclone, USA) supplemented with 10% FBS, 100U/ml penicillin and 100 µg/ml streptomycin (Gibco, USA) at 37°C with 5% CO₂. siKRT17 was synthesized by GenePharma (Shanghai, China), the sequences were listed as follow, sense 5'-CCUGCUACAGAUUGACAAUTT-3' and antisense 5'-AUUGUCAAUCUGUAGCA

GGTT-3'. EC9706 and ECA109 were transfected by incubating with Lipofectamine™ 2000 (Invitrogen, USA) for 24h according to the manufacturer's protocol.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from cells by using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. Reverse transcription reaction was conducted with 37°C for 15min, subsequent 85°C for 5s. qRT-PCR reaction were performed by using the SYBR® PrimeScript™ RT-PCR Kit (Takara, Japan) and GAPDH served as the internal control. The fold changes were calculated by using the $2^{-\Delta\Delta C_t}$ method. Primer sequences were listed as follow, KRT17 forward 5'-GGTGGGTGGTGAGATCAATGT-3' and reverse 5'-CGCGGTTCA GTTCCTCTGTC-3'; GAPDH forward 5'-GGACCTGACCTGCCGTCTAG-3' and reverse 5'-GTAGCCCAGGATGCCCTTGA-3'.

Western blot

Total protein was extracted from cells by using a mixture composed of radioimmunoprecipitation lysis buffer, phenylmethylsulfonyl fluoride (Beyotime, China) and phosphatase inhibitor (Solarbio, China), then separated by SDS-PAGE and transferred onto a PVDF membrane (Millipore, USA). Transferred membranes were blocked with 5% non-fat milk for 1h at room temperature and then incubated overnight at 4°C with following primary antibodies, KRT17, mTOR, p-mTOR, S6K1, p-S6K1 (1:1000, Proteintech, USA) and β-actin (Bioss, China), followed by incubated with the HRP labeled goat antibody against rabbit IgG (Beijing Zhongshan Biotechnology Co. Ltd., China) for 1h at room temperature. After that, the density of protein bands was captured by chemiluminescence and analyzed by ImageJ (NIH, USA)

Cell counting kit 8 (CCK8)

The 96-well plates were precultured in the incubator for 24 hours with 100 μ l cell suspension per well, then added 10 μ l substance with different concentrations. Incubated plates in the incubator for an appropriate period of time, add 10 μ l CCK8 solution (Solarbio, China) to each well and put plates in the incubator for 1-4 hours. Plates were measured by a microplate reader (Biotek, USA) at a corresponding wavelength.

Colony formation assay

Seeded with 1000 cells/well in 6-well plates and continued to culture for 14 days, then fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Cell number in each well were counted by an optical microscope (Nikon, Japan).

Flow cytometry

Transfected cells were treated by the Cell Cycle Testing Kit and Cell Apoptosis Detection Kit (BestBio, China) according to the manufacturer's protocol, then cells were immediately measured by a flow cytometer (Beckman, USA).

Wound healing assay

Transfected cells were seeded into 12-well plates until cell confluence >90%, a 10 μ l sterile pipette tip was scratched along the longitudinal direction of the cavity, replaced plates with new medium (0.5% FBS). Images of cell migration were captured at 0h, 24h and 48h by an inverted microscope.

Transwell migration and invasion assay

Both migration and invasion capacity can be assessed by using transwell inserts. Transfected cells were seeded into the upper chambers of inserts and were cultured at 37°C. Then inserts were inverted on absorbent paper to wipe the medium and remove non-migratory cells with a cotton swab. After that, cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet, counted in 3 random fields per sample.

Statistical analysis

All statistical analyses were performed with SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Data were presented as mean $\pm$ SD. One-way ANOVA or Student's t-test was used for the analyses. $P < 0.05$ were considered statistically significant.

Results

KRT17 was overexpression in ESCC

We collected human ESCC tumor tissues and adjacent non-cancerous esophageal tissues to screen out the differentially-expressed proteins (DEPs). In Fig. 1A, significantly up-regulated and down-regulated proteins were shown in the volcano plot, and the number of DEPs were listed in Fig. 1B, the volcano plot of DEPs of ESCC tumor (T) and non-cancerous esophageal tissues (N) groups is based on differences in protein expression. The heatmap of the 1200 significantly differentially expressed proteins was shown in Fig. 1C, blue represents the downregulation and red indicates upregulation. The Gene Ontology contains biological process, molecular function and cellular components, which can detailedly and accurately demonstrate gene products function. The top 20 of biological process, molecular function and cellular components were shown in Fig. 1D, Fig. 1E and Fig. 1F, respectively. A total of 7344 terms were enriched for the DEPs, and the amount of DEP enriched in each term were listed in Fig. 1G. The above results revealed the crucial DEP, KRT17.

To further demonstrate the role of KRT17 in ESCC, we explored KRT17 expression according to online database. Our study showed that compared to normal tissues, KRT17 was overexpression in tumor tissues (Fig. 2A), and the overall survival was also closely related with KRT17 levels (Fig. 2B). The results implied us that KRT17 may act as a tumor promoter, its inhibition may improve the status of ESCC.

siKRT17 inhibited the overexpression of KRT17 in ESCC cell lines

To explore the effect of siKRT17 in KRT17 expression, its mRNA and protein levels were measured in EC9706 and ECA109. Fig. 2C showed that both EC9706 and ECA109 KRT17 mRNA level in siKRT17 group was significantly lower than that in NC group. Western blot results were corresponded with qRT-PCR assay, as shown in Fig. 2D, siKRT17 down-regulated KRT17 expression in both cell lines. These results suggested that siKRT17 effectively down-regulated KRT17 expression in EC9706 and ECA109.

KRT17 inhibition attenuated proliferation, migration and invasion in ESCC cell lines

To determine the role of KRT17 in the pathogenesis of ESCC, we evaluated KRT17 function on proliferation, migration and invasion in EC9706 and ECA109. The effect of KRT17 on cell proliferation was verified by cell viability, CCK8 assay showed that compared with siCtrl group, cell lines transfected with siKRT17 had slower cell proliferation (Fig. 3A). Cell proliferation was also indicated by colony formation assay, as shown in Fig. 3B, the number of cell clones in siKRT17 group was reduced obviously ($P < 0.01$), and the analysis of EC9706 and ECA109 are consistent. The change of DNA content can reflect cell cycle, compared with siCtrl group, EC9706 and ECA109 siKRT17 group had lower cell count in S and G2/M phase, while had higher cell count in G1 phase (Fig. 3C). Moreover, we measured the percentage of apoptotic cells to reflect cell proliferation laterally, EC9706 and ECA109 transfected with siKRT17 had a higher apoptosis rate. These results strongly suggested that KRT17 facilitated ESCC cell proliferation.

Tumor cells can acquire migration and invasion phenotype to accelerate cancer progression, and KRT17 was overexpression in ESCC based on previous results. Hence, we explored KRT17 function in cell migration and invasion. As shown in Fig. 4A, compared with siCtrl group, wound healing assay showed that the migration rate in siKRT17 group was reduced at 48h in EC9706 and ECA109. Transwell assay

revealed that KRT17 inhibition in both cell lines effectively reduced the invasion capacity (Fig. 4B). We further used transwell assay to assess cell migration, the result showed that EC9706 and ECA109 transfected with siKRT17 had a lessened migration capacity (Fig. 4C).

KRT17 regulated proliferation, migration and invasion via mTOR/S6K1 pathway

The mTOR pathway was reported to play a role in proliferation, migration and invasion [14]. The activation of mTOR brought about S6K1 phosphorylation, and mTOR/S6K1 pathway is also associated with angiogenesis [15]. To investigate the role of mTOR/S6K1 pathway in KRT17 related proliferation, migration and invasion, we used western blot to detect the protein levels of mTOR and S6K1. The results showed that EC9706 and ECA109 transfected with siKRT17 significantly decreased mTOR (Fig. 5A) and S6K1 (Fig. 5B) phosphorylation levels.

Discussion

ESCC is the main subtype of esophageal cancer with high mortality in the world [16]. Despite the remarkable advances in diagnostic methods and treatments including surgical excision, chemotherapy and radiotherapy in recent years, the situation of ESCC patients remains unsatisfied with most cases diagnosed at an advanced stage and existing treatments have not brought about long-term survival benefits [17]. The relatively low survival rate has led to extensive research on ESCC aimed at changing the poor status, while the molecular mechanisms in the pathogenesis of ESCC still not clear. KRT17 belongs to type $\square$ intermediate filament with multiple functions, deregulated expression of KRT17 has been shown to be related with the pathogenesis of diversified cancers [18]. Recent study showed that KRT17 was overexpression in osteosarcoma, and KRT17 inhibition reduced osteosarcoma cell proliferation via regulating the AKT/mTOR/HIF1 α pathway [19]. KRT17 was also up-regulation in cervical cancer, knockdown of KRT17 attenuated proliferation and migration, and stimulate cervical cancer cell apoptosis [20]. In gastric cancer, KRT17 function as a promoter to facilitate tumor growth, motility and invasion [21]. However, the role of KRT17 in ESCC has not been clarified.

The present study aimed to identified the potential function and underlying molecular mechanism of KRT17 in ESCC. First of all, we needed to determine KRT17 expression. DIA-MS is a proteomic methodology for deep and proteome-wide profiling with high accuracy and reproducibility in proteomic quantification [22, 23], we used DIA-MS to investigate the DEPs, and the result showed that the expression of KRT17 was up-regulated in ESCC tumor tissues, the differential expression was further determined by the data obtained from GEPIA. Also, the overall survival of ESSC patients was also closely related with KRT17 levels. These results implied that KRT17 potentially act as an oncogene in the pathogenesis of ESCC, knockdown of KRT17 can better explore its function, EC9706 and ECA109 transfected with siKRT17 inhibit the expression of KRT17 by the evidence of significantly decreased protein and mRNA levels.

CCK8 assay can directly reflect the role of KRT17 on cell proliferation, the result showed that knockdown of KRT17 induced by siRNA manifested a lower proliferation capacity in EC9706 and ECA109. Consistent

with CCK8 assay, siKRT17 group had a fewer number of cell clones. In EC9706 and ECA109 transfected with siKRT17, the percentage of cells in S and G2/M phase decreased while the percentage of cells in G1 phase increased when compared to that transfected with siCtrl. Also, EC9706 and ECA109 transfected with siKRT17 enhance the proportion of apoptotic cells. Transwell and wound healing assay still revealed negative effects of siKRT17 on migration and invasion. All in all, knockdown of KRT17 in EC9706 and ECA109 led to the alleviation of proliferation, migration and invasion capacity, these results adequately demonstrated the close relationship between KRT17 and these cell viability functions.

mTOR is associated with diverse molecular and biological aspects of cancer, and overexpression of mTOR can promote tumor growth [24]. As a mTOR downstream effector, S6K1 overexpression is associated with poor cancer prognosis [25]. Growing studies have focused on the relationship between mTOR/S6K1 pathway and cancer. Our present study revealed that KRT17 inhibition decreased the phosphorylation levels of mTOR and S6K1 in EC9706 and ECA109. These results showed that knockdown of KRT17 attenuated proliferation, migration and invasion of ESCC cell lines by inhibiting mTOR/S6K1 pathway.

Conclusion

Our study demonstrated that KRT17 was overexpression in ESCC tissues. Knockdown of KRT17 led to the alleviation of proliferation, migration and invasion capacity in ESCC cell lines, and these processes was mediated by inhibiting mTOR/S6K1 pathway. KRT17 may be a novel and effective target for ESSC treatment.

Declarations

Acknowledgements

None

Authors' contributions

LL performed the experiments and wrote the manuscript. KJ and GF designed the study and reviewed the manuscript. MS and YW collected the data and conducted analysis. LG and SX collected the data and reviewed the manuscript. All the authors read and approved the final manuscript.

Availability of data and materials

All data can be obtained from the manuscript or from request to the author.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

Author details

¹ Department of Thoracic surgery, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, China/Chinese Academy of Sciences Sichuan Translational Medicine Research Hospital, Chengdu 610072, China. ² Department of Thoracic surgery, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, China/Chinese Academy of Sciences Sichuan Translational Medicine Research Hospital, Chengdu 610072, China/School of Medicine, University of Electronic Science and Technology of China, Chengdu, Sichuan, China

References

- [1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2019, *CA Cancer J Clin* 69(1) (2019) 7-34.
- [2] Y. Mao, Z. Fu, Y. Zhang, L. Dong, Y. Zhang, Q. Zhang, X. Li, J. Liu, A seven-lncRNA signature predicts overall survival in esophageal squamous cell carcinoma, *Sci Rep* 8(1) (2018) 8823.
- [3] R.J. Zeng, C.W. Zheng, J.E. Gu, H.X. Zhang, L. Xie, L.Y. Xu, E.M. Li, RAC1 inhibition reverses cisplatin resistance in esophageal squamous cell carcinoma and induces downregulation of glycolytic enzymes, *Mol Oncol* 13(9) (2019) 2010-2030.
- [4] L.Y. Chu, Y.H. Peng, X.F. Weng, J.J. Xie, Y.W. Xu, Blood-based biomarkers for early detection of esophageal squamous cell carcinoma, *World J Gastroenterol* 26(15) (2020) 1708-1725.
- [5] Z. Wang, M.Q. Yang, L. Lei, L.R. Fei, Y.W. Zheng, W.J. Huang, Z.H. Li, C.C. Liu, H.T. Xu, Overexpression of KRT17 promotes proliferation and invasion of non-small cell lung cancer and indicates poor prognosis, *Cancer Manag Res* 11 (2019) 7485-7497.
- [6] L. Yang, S. Zhang, G. Wang, Keratin 17 in disease pathogenesis: from cancer to dermatoses, *J Pathol* 247(2) (2019) 158-165.
- [7] J. Wu, H. Xu, H. Ji, B. Zhai, J. Zhu, M. Gao, H. Zhu, X. Wang, Low Expression of Keratin17 is Related to Poor Prognosis in Bladder Cancer, *Onco Targets Ther* 14 (2021) 577-587.
- [8] H. Hua, Q. Kong, H. Zhang, J. Wang, T. Luo, Y. Jiang, Targeting mTOR for cancer therapy, *J Hematol Oncol* 12(1) (2019) 71.

- [9] A.K. Murugan, mTOR: Role in cancer, metastasis and drug resistance, *Semin Cancer Biol* 59 (2019) 92-111.
- [10] B. Magnuson, B. Ekim, D.C. Fingar, Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks, *Biochem J* 441(1) (2012) 1-21.
- [11] S. Sridharan, A. Basu, Distinct Roles of mTOR Targets S6K1 and S6K2 in Breast Cancer, *Int J Mol Sci* 21(4) (2020).
- [12] H. Shen, G.C. Wang, X. Li, X. Ge, M. Wang, Z.M. Shi, V. Bhardwaj, Z.X. Wang, R.G. Zinner, S.C. Peiper, A.E. Aplin, B.H. Jiang, J. He, S6K1 blockade overcomes acquired resistance to EGFR-TKIs in non-small cell lung cancer, *Oncogene* 39(49) (2020) 7181-7195.
- [13] J.R. Wisniewski, A. Zougman, N. Nagaraj, M. Mann, Universal sample preparation method for proteome analysis, *Nat Methods* 6(5) (2009) 359-62.
- [14] D. Li, X.F. Ni, H. Tang, J. Zhang, C. Zheng, J. Lin, C. Wang, L. Sun, B. Chen, KRT17 Functions as a Tumor Promoter and Regulates Proliferation, Migration and Invasion in Pancreatic Cancer via mTOR/S6k1 Pathway, *Cancer Manag Res* 12 (2020) 2087-2095.
- [15] H. Yang, L. Hu, Z. Liu, Y. Qin, R. Li, G. Zhang, B. Zhao, C. Bi, Y. Lei, Y. Bai, Inhibition of Gli1-mediated prostate cancer cell proliferation by inhibiting the mTOR/S6K1 signaling pathway, *Oncol Lett* 14(6) (2017) 7970-7976.
- [16] Z. Xie, T. Li, B. Huang, S. Liu, L. Zhang, Q. Zhang, Semaphorin 3F Serves as a Tumor Suppressor in Esophageal Squamous Cell Carcinoma and is Associated With Lymph Node Metastasis in Disease Progression, *Technol Cancer Res Treat* 19 (2020) 1533033820928117.
- [17] Y. Yang, H. Ge, Effective combinations of radiotherapy and immunotherapy in the treatment of esophageal squamous cell carcinoma, *Future Oncol* 16(31) (2020) 2537-2549.
- [18] C.H. Chiang, C.C. Wu, L.Y. Lee, Y.C. Li, H.P. Liu, C.W. Hsu, Y.C. Lu, J.T. Chang, A.J. Cheng, Proteomics Analysis Reveals Involvement of Krt17 in Areca Nut-Induced Oral Carcinogenesis, *J Proteome Res* 15(9) (2016) 2981-97.
- [19] X. Yan, C. Yang, W. Hu, T. Chen, Q. Wang, F. Pan, B. Qiu, B. Tang, Knockdown of KRT17 decreases osteosarcoma cell proliferation and the Warburg effect via the AKT/mTOR/HIF1alpha pathway, *Oncol Rep* 44(1) (2020) 103-114.
- [20] M. Dong, Z. Dong, X. Zhu, Y. Zhang, L. Song, Long non-coding RNA MIR205HG regulates KRT17 and tumor processes in cervical cancer via interaction with SRSF1, *Exp Mol Pathol* 111 (2019) 104322.
- [21] M. Chivu-Economescu, D.L. Dragu, L.G. Necula, L. Matei, A.M. Enciu, C. Bleotu, C.C. Diaconu, Knockdown of KRT17 by siRNA induces antitumoral effects on gastric cancer cells, *Gastric Cancer* 20(6)

(2017) 948-959.

[22] R. Lou, P. Tang, K. Ding, S. Li, C. Tian, Y. Li, S. Zhao, Y. Zhang, W. Shui, Hybrid Spectral Library Combining DIA-MS Data and a Targeted Virtual Library Substantially Deepens the Proteome Coverage, *iScience* 23(3) (2020) 100903.

[23] B.C. Searle, K.E. Swearingen, C.A. Barnes, T. Schmidt, S. Gessulat, B. Kuster, M. Wilhelm, Generating high quality libraries for DIA MS with empirically corrected peptide predictions, *Nat Commun* 11(1) (2020) 1548.

[24] M. Moschetta, A. Reale, C. Marasco, A. Vacca, M.R. Carratu, Therapeutic targeting of the mTOR-signalling pathway in cancer: benefits and limitations, *Br J Pharmacol* 171(16) (2014) 3801-13.

[25] H. Populo, J.M. Lopes, P. Soares, The mTOR signalling pathway in human cancer, *Int J Mol Sci* 13(2) (2012) 1886-918.

Figures

Figure 1

(A) Volcano plot of differentially expressed proteins between T and N groups. The Fold change and P-value of protein expression between the two groups of samples were used, the x-coordinate is the difference multiple (logarithmic transformation based on 2) and the y-coordinate is the P-value (logarithmic transformation based on 10). (B) The number of differentially expressed proteins in each group. (C) Heatmap of the 1200 significantly differentially expressed proteins between T and N groups. Blue represents the downregulation and red indicates upregulation. (D) Top 20 GO enrichment terms of biology process for the ESCC DEPs. (E) Top 20 GO enrichment terms of molecular function for the ESCC DEPs. (F) Top 20 GO enrichment terms of cellular component for the ESCC DEPs. (G) The number of

DEPs enriched in each term. Abbreviations: DEPs, differentially expressed proteins; ESCC, esophageal squamous cell carcinoma; N, Non-cancerous esophageal tissues; T, ESCC tumor tissues.

Figure 2

(A) The expression of KRT17 in ESCA group was significantly higher than that in normal group ($|\log_2FC|$ cutoff >1 , p-value cutoff <0.01). * $P<0.05$. Picture was obtained from GEPIA, Copyright © 2017 Zefang Tang, Chenwei Li, Boxi Kang. Zhang's Lab. GEPIA: <http://gepia.cancer-pku.cn/index.html>. (B) ESCA group with high KRT17 expression had a worse overall survival. Picture was obtained from GEPIA, Copyright © 2017 Zefang Tang, Chenwei Li, Boxi Kang. Zhang's Lab. GEPIA: <http://gepia.cancer-pku.cn/index.html>. (C) qRT-PCR analysis of KRT17 mRNA level among NC group and siKRT17 group in EC9706 and ECA109.

(D) Western blot analysis of KRT17 protein level among control group, NC group and siKRT17 group in EC9706 and ECA109. Data were shown as mean $\pm$ SD (n=3). **P<0.01 versus NC group. Abbreviations: ESCA, esophageal carcinoma; GEPIA, gene expression profiling interactive analysis; KRT17, keratin17; NC, negative control; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

Figure 3

(A) Cell proliferation in EC9706 and ECA109 transfected with siCtrl or siKRT17 were measured by CCK8. (B) Cloning counts in EC9706 and ECA109 transfected with siCtrl or siKRT17. Data were shown as mean $\pm$ SD (n=3). **P<0.01 versus siCtrl group. (C) Cell cycle distribution in EC9706 and ECA109 transfected with siCtrl or siKRT17 were detected by FCM. (D) Apoptosis in EC9706 and ECA109 transfected with siCtrl or siKRT17 were detected by FCM. Abbreviations: CCK8, cell counting kit 8; FCM, flow cytometry.

Figure 4

(A) Cell migration rate at 0h, 24h and 48h in EC9706 and ECA109 transfected with siCtrl or siKRT17 were measured by wound healing assay. (B) Cell invasion in EC9706 and ECA109 transfected with siCtrl or siKRT17 were measured by transwell assay. (C) Cell migration rate in EC9706 and ECA109 transfected with siCtrl or siKRT17 were measured by transwell assay.

Figure 5

(A) Western blot analysis of p-mTOR and p-S6K1 protein levels in EC9706 and ECA109 transfected with none, siCtrl or siKRT17.