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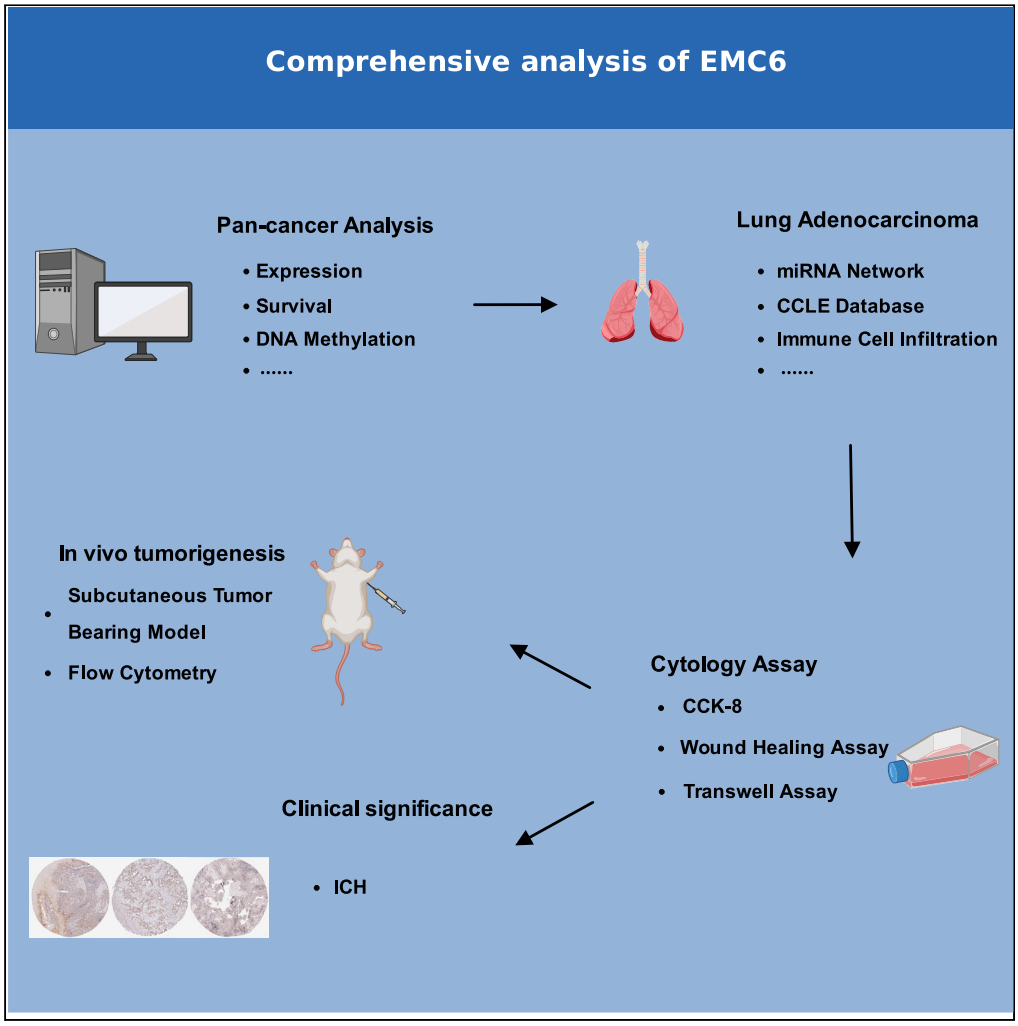

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Highlights

Our study comprehensively analyzed the functional mechanisms of EMC6 in all cancers

Our study validated the role of EMC6 in lung cancer by *in vivo* and *in vitro* assays

EMC6 regulates the infiltration of immune cells in lung adenocarcinoma

EMC6 regulates the occurrence of ferroptosis and cuproptosis in lung adenocarcinoma

Zhou et al., iScience *27*, 108648
January 19, 2024
https://doi.org/10.1016/j.isci.2023.108648

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Pan-cancer analysis identifies EMC6 as a potential target for lung adenocarcinoma

Xin Zhou,[1,5,6,*] Bowen Xiao,[2,5,*] Manman Jiang,[3,5] and Jun Rui[4,*]

SUMMARY

Endoplasmic reticulum membrane protein complex subunit 6 (EMC6) plays an important function in both physiological and pathological states of cells. Nevertheless, there are few studies focused on the role of EMC6 in tumors. At first, we performed a series of bioinformatics analyses on 33 kinds of cancers, including differential expression analysis, tumor mutational burden analysis, prognostic analysis, and clinicopathological staging analysis. Then, we corroborated the important role of EMC6 in lung cancer by cytological and *in vivo* experiments. We found that the reduction of EMC6 expression did effectively inhibit the proliferation, invasion, and metastasis of A549. Finally, EMC6 is indeed involved in the regulation of ferroptosis, cuproptosis, and immune response in LUAD. In a word, our study not only comprehensively analyzed the functional mechanisms of EMC6 in all cancers but also validated the regulatory role of EMC6 in lung cancer for the first time.

INTRODUCTION

As the saying goes, every coin has two sides. Although advances in technology have improved the quality of our life and provided highly accurate medical treatments, we are also obsessed by the various health problems that come with it. Among these health problems, cancer is currently the most frightening disease to us. The latest statistics show that approximately 20 million people are diagnosed with tumors each year in the world,[1] and this number is increasing every year. Cancer has become the sixth leading cause of death worldwide.[2] The most horrible thing about cancer is the rapid growth of cancer cells and their ability to invade and metastasize. For patients with early-stage cancer, we can completely limit the progression of cancer by surgical resection. But for those advanced cancer patients whose tumors have metastasized to other organs, it is difficult to maintain their lives with the existing treatments.[3] Nevertheless, we know very little about how cancer cells metastasize and how to control them. In fact, cancer cells are derived from normal tissue cells, which may be subjected to chemical or physical stimuli that force them to change their normal physiological functions, or it may also be the consequence of changes in some genes caused by genetic factors.[4] In addition, as our knowledge of cancer becomes more advanced, the importance of genetic alterations is becoming more and more prominent.[5] Therefore, it is particularly important to explore the treatment of cancer at the genetic level.

The endoplasmic reticulum (ER), a vital organelle within the cell, is essential for cell signaling and maintaining cellular homeostasis.[6] The role of ER is even more prominent for cancer cells with complex functions and high metabolism.[7] ER membrane protein complex subunit 6 (EMC6), also known as transmembrane protein 93 (TMEM93), is a structurally conserved enzyme that is widely expressed in a variety of cells.[8] ER membrane protein complex (EMC) in mammals contains 10 subunits, of which EMC6 is an important member.[9] According to the results of Li.'s study, EMC6 was identified as a regulator protein involved in cellular autophagy.[10] Then, Shen et al.[11] reconfirmed this finding in glioblastoma cells. EMC6, an emerging disease marker, has been studied mainly in pancreatic and gastric diseases. Initially, EMC6 was identified as a novel gastric cancer suppressor.[12] Subsequently, it was shown that EMC6 regulates the chemosensitivity of gastric cancer cells by participating in the mitochondrial-mediated apoptosis pathway.[13] The two diseases that occur in the pancreas are pancreatitis and pancreatic cancer. On the one hand, EMC6 mediates the apoptosis of acinar cells through APAF1 and thus regulates the progression of pancreatitis.[14] On the other hand, EMC6 was identified as a potential biomarker for regulating the prognostic of pancreatic cancer patients.[15] The critical role of EMC6 in tumors is gradually being explored.

Nevertheless, we found that the tumor-related studies on EMC6 were limited to two cancers, namely, gastric cancer and pancreatic cancer. Consequently, a comprehensive analysis of EMC6 in multiple cancers is extremely necessary. In our study, we used multiple databases and analytical tools to perform a synthetical analysis of The Cancer Genome Atlas (TCGA) data for expression level and prognosis about EMC6. Then, we performed correlation analysis of EMC6 with genes related to tumor mutational burden (TMB), chemokines, chemokine

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https://doi.org/10.1016/j.isci.2023.108648

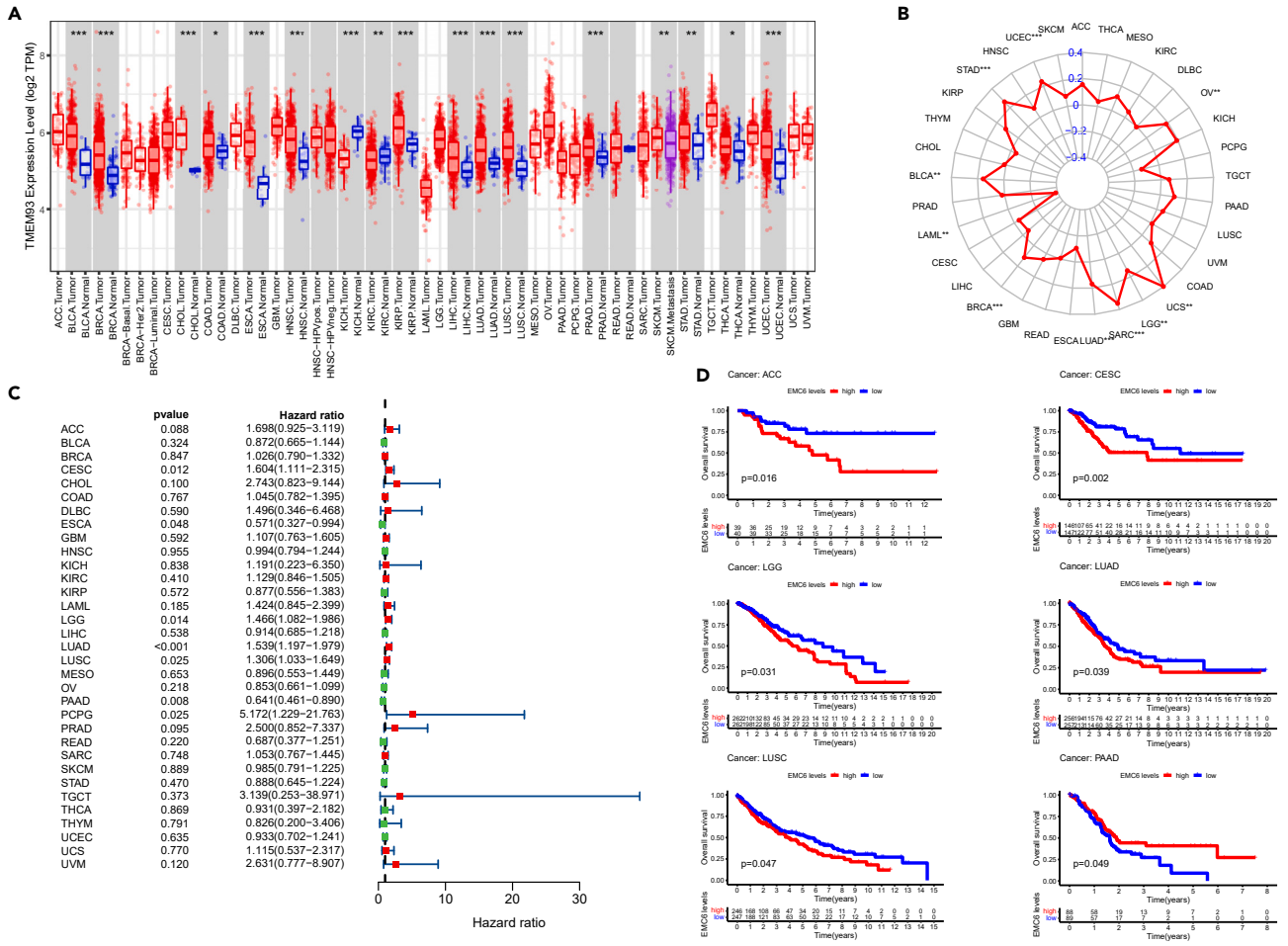

Figure 1. High expression of EMC6 in multiple cancers is closely related to the patient prognosis

(A) Comparison of EMC6 expression between tumor and normal tissues; (B) correlation between TMB and EMC6 expression; (C) forest plot exhibits the relationship of EMC6 expression with patient overall survival (OS); (D) Kaplan-Meier analyses show the association between EMC6 expression and OS. (*p < 0.05, **p < 0.01, ***p < 0.001).

receptors, etc. Finally, based on the results of the previous pan-cancer analysis, we found that EMC6 showed the best functional performance in lung adenocarcinoma (LUAD). Therefore, we selected the LUAD cell line (A549) for the subsequent cell assays. We designed and constructed an EMC6-knockdown A549 cell line using small interfering RNA (siRNA) and used the cell line for Cell Counting Kit-8 (CCK-8) proliferation assays, transwell invasion and migration assays, wound healing test, and subcutaneous tumor bearing model. What is more, we made a reasonable speculation on the functional mechanism of EMC6 in LUAD by the results of correlation analysis and functional enrichment analysis. The results of the aforementioned analysis can indicate that EMC6 is a potential biomarker for a variety of cancers and may be closely related to tumor immune response. More importantly, the reduced expression level of EMC6 could largely reduce the proliferation, invasion, and migration of LUAD cells and it is likely that EMC6 is involved in the process of ferroptosis and cuproptosis in LUAD cells. Our study not only comprehensively analyzed the functional mechanisms of EMC6 in all cancers but also validated the regulatory role of EMC6 in lung cancer for the first time. These results would provide more directions and theoretical support for the subsequent research of EMC6 on cancer therapy.

RESULTS

High expression of EMC6 in multiple cancers is closely related to the patient prognosis

Based on the expression of EMC6 in 33 kinds of cancers presented in the TIMER database, we found that the expression of EMC6 was extremely higher in the majority of cancers than in the corresponding normal tissues, except for significantly decreased expression levels in kidney chromophobe (KICH) and kidney renal clear cell carcinoma (KIRC) (Figure 1A). Furthermore, the elevated expression levels of EMC6 were particularly evident in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), esophageal carcinoma (ESCA),

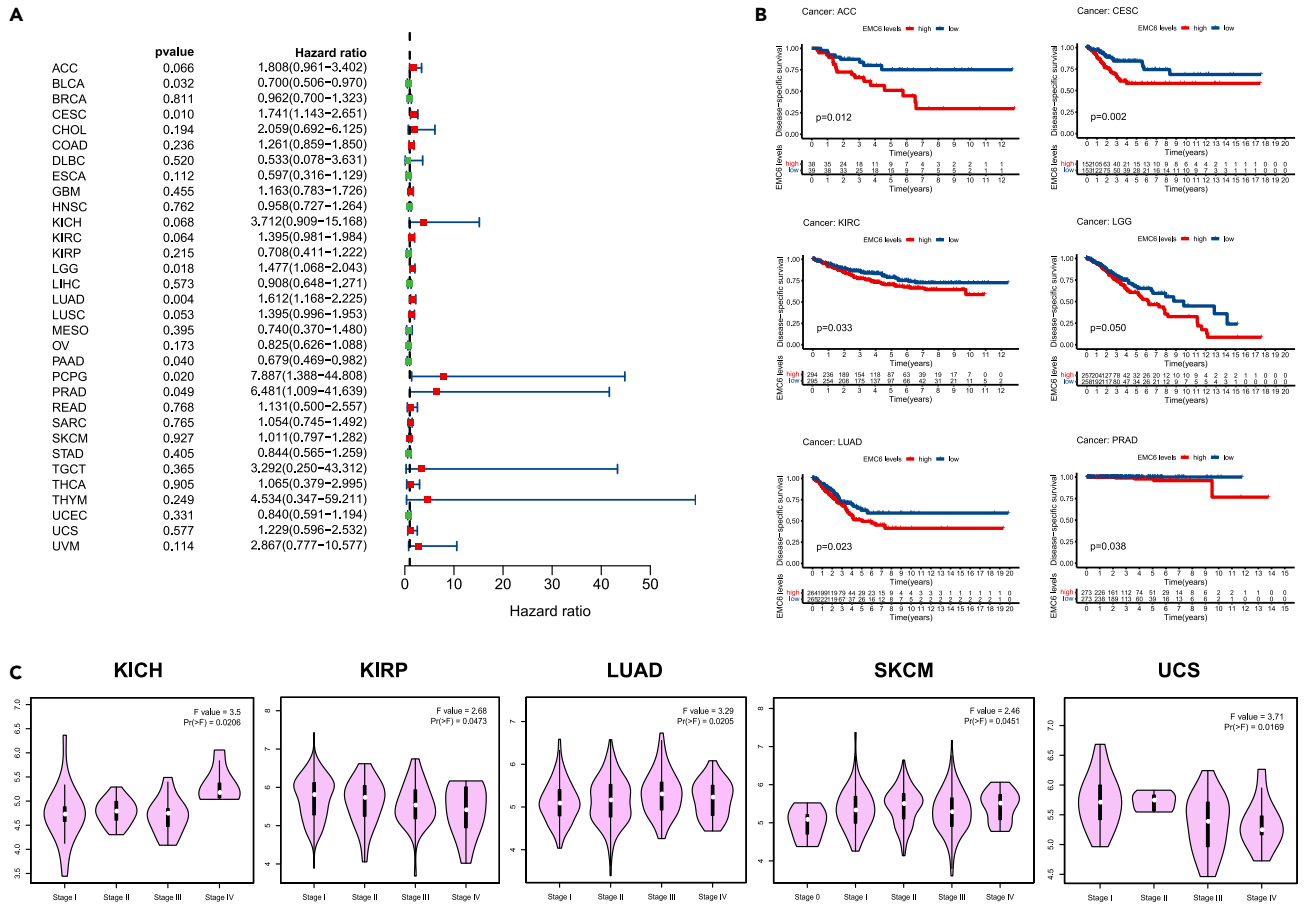

Figure 2. EMC6 is involved in the malignant progression of many cancers

(A) Forest plot exhibits the relationship of EMC6 expression with patient disease-specific survival (DSS); (B) Kaplan-Meier analyses show the association between EMC6 expression and DSS; (C) the association of EMC6 expression with pathological stages.

and LUAD. The value of TMB shows the situation of gene mutation in tumor cells. These tumor cells with more genetic mutations are more able to stimulate the immune response. Therefore, the value of TMB could also show the prognosis of patients in some way. The result of TMB analysis of 33 kinds of cancers showed that the expression of EMC6 was strongly associated with gene mutations in stomach adenocarcinoma (STAD), sarcoma (SARC), uterine corpus endometrial carcinoma (UCEC), and LUAD (Figure 1B). The impact of the variation in gene expression on patient prognosis is a critical standard to measure whether the gene is an important biomarker for this kind of cancer. The forest plots of single-factor Cox regression analysis showed that altered expression of EMC6 had a significant impact on patient prognosis in seven kinds of cancers, including cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), ESCA, brain lower grade glioma (LGG), and so on (Figure 1C). It was worth noting that the expression of EMC6 had the most significant impact on the prognosis of LUAD patients ($p < 0.001$). Next, in the overall survival (OS) analysis, we found that these patients with higher expression of EMC6 had a terrible prognosis compared with patients with lower expression of EMC6 in adrenocortical carcinoma (ACC, $p = 0.016$), CESC ($p = 0.002$), LGG ($p = 0.031$), LUAD ($p = 0.039$), lung squamous cell carcinoma (LUSC, $p = 0.047$), and pancreatic adenocarcinoma (PAAD, $p = 0.049$) (Figure 1D). All those no significant results could be found in supplementary files.

EMC6 is involved in the malignant progression of many cancers

Disease-specific survival (DSS) is an index that can more accurately evaluate the clinical prognosis of a disease. The single-factor Cox regression analysis of DSS in 33 kinds of cancers clearly demonstrated that the expression of EMC6 was strongly associated with DSS in a variety of cancers, such as BLCA ($p = 0.032$), CESC ($p = 0.01$), LGG ($p = 0.018$), PAAD ($p = 0.04$), and so on (Figure 2A). It was as expected that the expression of EMC6 still had the strongest effect on DSS of LUAD ($p = 0.004$). The result of Kaplan-Meier (KM) analysis showed that there were six kinds of cancers whose DSS was closely related to the expression of EMC6 (Figure 2B). LUAD ($p = 0.023$) was also included in these six types of cancer. The clinical stage of the patient showed the clinicopathological characteristics of the patient, which means the malignant progression of the tumor tissue and the prognosis of patients. Thus, we searched the GEPIA2 database for the expression of EMC6 at different

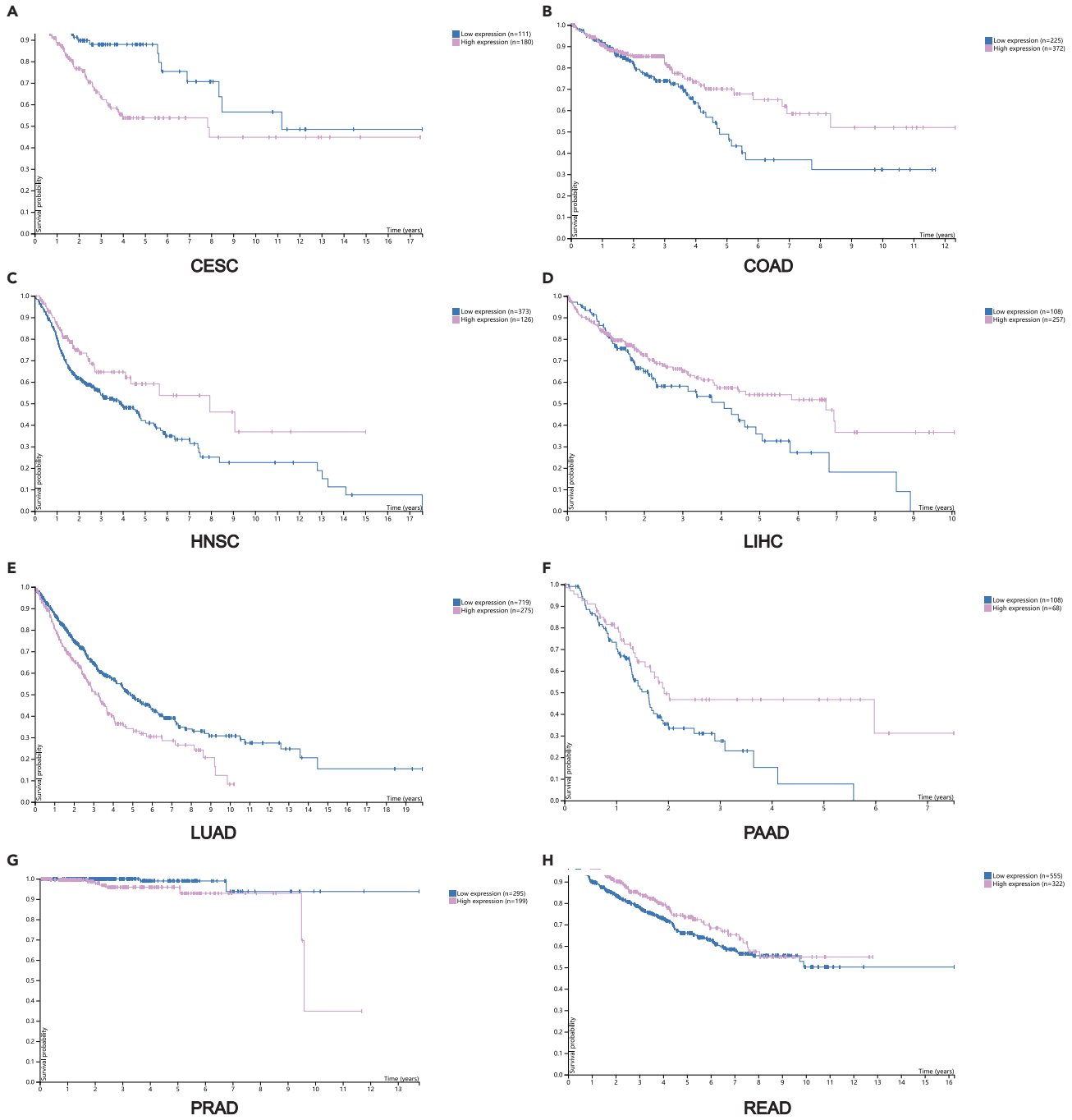

Figure 3. The correlation between EMC6 and the prognosis of various tumors has been demonstrated by clinical data

(A) Survival curve of EMC6 in CESC, p = 0.00077; (B) survival curve of EMC6 in COAD, p = 0.022; (C) survival curve of EMC6 in HNSC, p = 0.0062; (D) survival curve of EMC6 in LIHC, p = 0.04; (E) survival curve of EMC6 in LUAD, p = 0.000094; (F) survival curve of EMC6 in PAAD, p = 0.013; (G) survival curve of EMC6 in PRAD, p = 0.0076; (H) survival curve of EMC6 in READ, p = 0.042.

pathological stages in 33 kinds of cancers. Further, we found that the expression of EMC6 gradually increased with the progression of tumor in KICH (p = 0.0206), LUAD (p = 0.0205) and skin cutaneous melanoma (SKCM, p = 0.0451) (Figure 2C). In contrast, the expression of EMC6 gradually decreased with the progression of tumor in kidney renal papillary cell carcinoma (KIRP, p = 0.0473) and uterine carcinosarcoma (UCS, p = 0.0169) (Figure 2C). In order to better validate our results, we used survival analysis data from the HPA database (Figure 3) and DNA methylation status (Figure 4) to confirm our findings.[16–18]

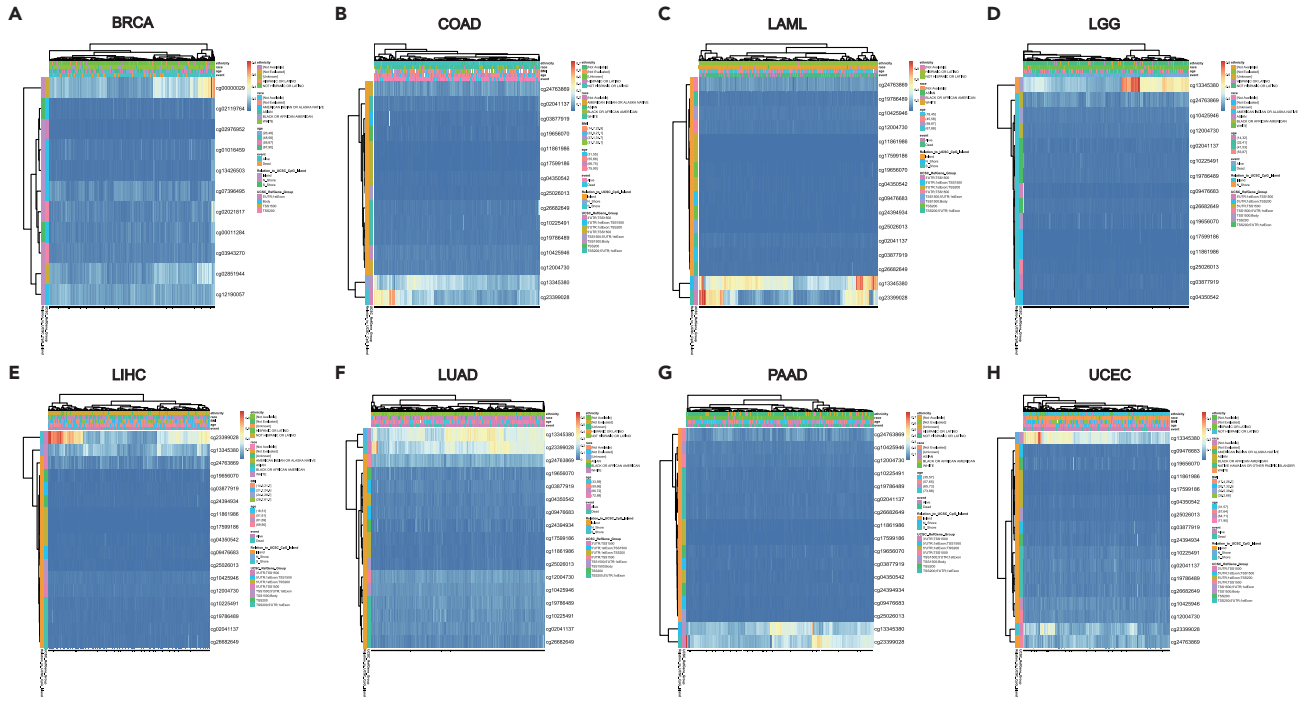

Figure 4. The correlation between EMC6 and DNA methylation

(A) DNA methylation of EMC6 in BRCA; (B) DNA methylation of EMC6 in COAD; (C) DNA methylation of EMC6 in LAML; (D) DNA methylation of EMC6 in LGG; (E) DNA methylation of EMC6 in LIHC; (F) DNA methylation of EMC6 in LUAD; (G) DNA methylation of EMC6 in PAAD; (H) DNA methylation of EMC6 in UCEC.

Relationship between the expression of EMC6 and tumor immune

As we all know, the immune response is the most important mechanism for our body to resist the development of tumor cells.[19,20] In order to explore whether EMC6 is involved in tumor immunity in 33 kinds of cancers, we performed co-expression analysis of EMC6 with a series of immune-related genes on TCGA data. The heatmap of co-expression analysis demonstrated that the expression of EMC6 was closely associated with immune activation genes (Figure 5A), chemokines (Figure 5B), chemokine receptors (Figure 5C), and major histocompatibility complex (MHC) (Figure 5D) in almost all types of cancer, except for in ACC, lymphoid neoplasm diffuse large B cell lymphoma (DLBC), mesothelioma (MESO), and UCS. Interestingly, we found that the expression of EMC6 was significantly positively correlated with immune responses in cancers such as KICH and uveal melanoma (UVM) and significantly negatively correlated with immune responses in cancers such as cholangiocarcinoma (CHOL) and LUAD (Figure 5).

The critical role of EMC6 in LUAD

Through the earlier analysis we found that EMC6 is involved in tumor immune response in various cancers; especially in LUAD the expression of EMC6 showed a significant negative correlation with various immune-related genes, which indicated that EMC6 is likely to be involved in the immune escape process of LUAD. Thus, we first used differential expression analysis (Figures 6A and 6B) and survival analysis (Figures 6C and 6D) from multiple GEO datasets (GSE19188, GSE44077, GSE31201, and jacob-00182-HLM) to confirm the key role of EMC6 in LUAD. Then, we constructed a microRNA (miRNA) regulatory network of EMC6 in LUAD (Figure 6E) and observed the expression of EMC6 in various LUAD cell lines in the CCLE database (Figure 6F). Next, we re-evaluated the relationship of EMC6 with tumor immune in LUAD by CIBERSORT algorithm. The result of this analysis showed that EMC6 had significant negative correlations with mast cells ($p = 3.9e\text{-}08$), $CD4^+$ T cells ($P = 1e\text{-}07$), immune scores ($p = 4.8e\text{-}05$), and stromal scores ($p = 1.9e\text{-}07$) in LUAD (Figure 7A). To validate our analysis, we constructed the EMC6-knockdown LUAD cell line A549 using siRNA and verified the efficiency of EMC6 knockdown using qPCR (Figure 7B). Then, we performed a series of cell function experiments using the constructed EMC6-knockdown A549 cell line. At first, the outcomes of CCK-8 assay showed that the reduction of EMC6 protein level greatly diminished the proliferation ability of A549 (Figure 7C). Secondly, the results of transwell assay showed that the number of migrated and invaded EMC6-knockdown A549 cells were significantly fewer compared to normal A549 cells in the same time (Figure 7D). At last, the results of the wound healing assay again demonstrated that the existence of EMC6 played a vital role in the migration ability of A549 (Figure 7E). Now that we have explored the effect of EMC6 on A549 cells, we wanted to explore the mechanism of EMC6 in LUAD. As we all know, EMC6 has been proved to be a key gene of autophagy,[10] so we thought whether EMC6 is also involved in the process of ferroptosis and cuproptosis in LUAD. Interestingly, co-expression analysis exhibited that EMC6 was indeed closely linked with these genes related to ferroptosis and cuproptosis (Figure 7F). To make this finding more clinically applicable, we used connectivity map

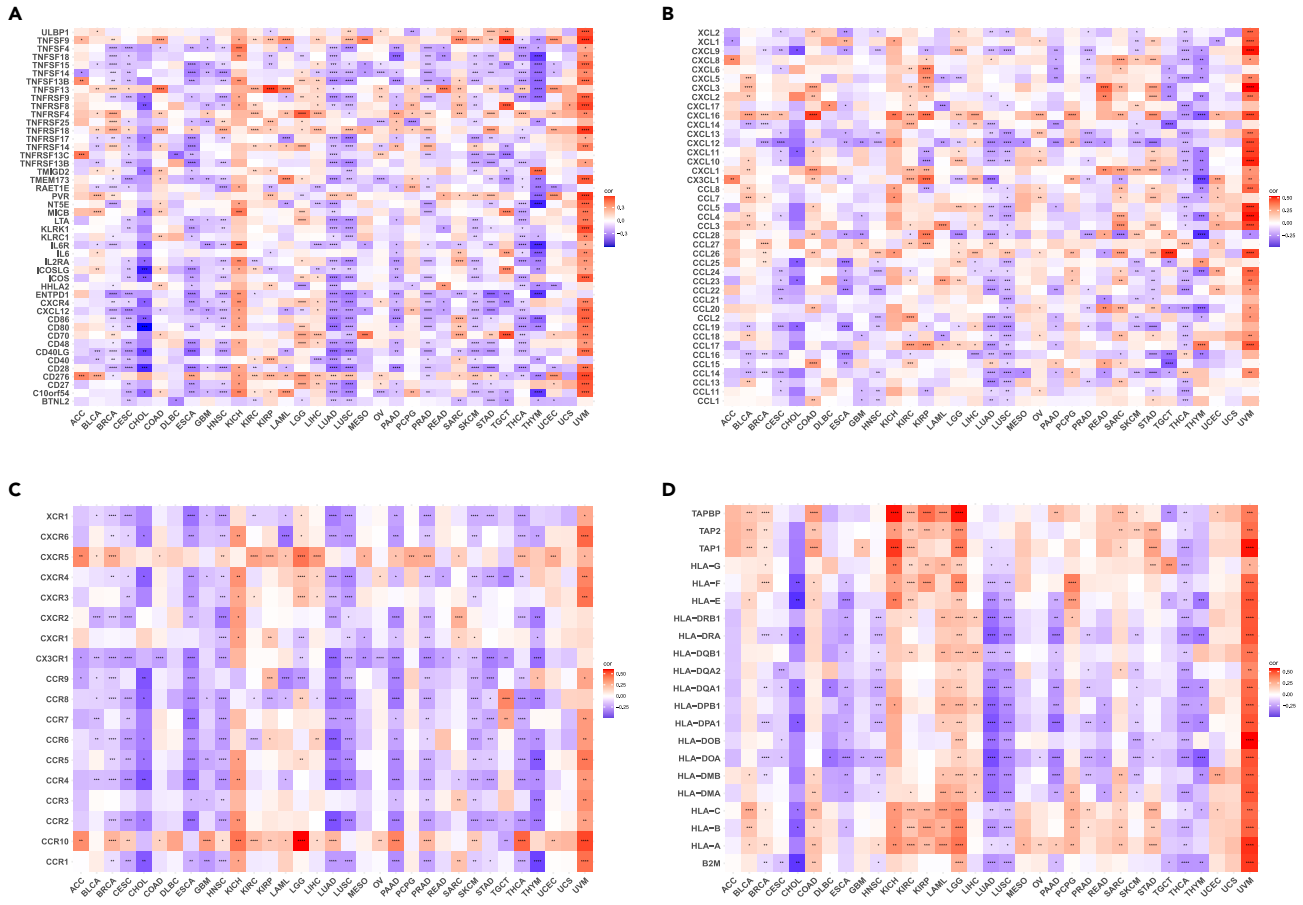

Figure 5. Relationship between the expression of EMC6 and tumor immune

(A) Correlation analyses of the EMC6 expression with immune activation-related genes in pan-cancers; (B) correlation analyses of the EMC6 expression with chemokines-related genes in pan-cancers; (C) correlation analyses of the EMC6 expression with chemokines receptors-related genes in pan-cancers; (D) correlation analyses of the EMC6 expression with MHC-related genes in pan-cancers. (*p < 0.05, **p < 0.01, ***p < 0.001).

(CMap) to predict EMC6-related small-molecule compounds and their corresponding target genes (Table S1).[21] Based on these results, we found that "mitotane" is likely to be able to regulate the association between lung cancer and diabetes. Then, we performed functional enrichment analysis of EMC6-related genes in LUAD. The major enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were JAK-STAT signaling pathway, cell adhesion molecules (CAMs), mitogen-activated protein kinase (MAPK) signaling pathway, and ribosome (Figure 7G). The enriched molecular functions (MFs) were structural constituent of ribosome and cytokine receptor activity (Figure 7G). In addition, the biological processes (BPs) of EMC6 were generation of precursor metabolites and energy and regulation of GTPase activity (Figure 7G). The cellular components (CCs) enriched were mitochondrial inner membrane and mitochondrial matrix and receptor complex (Figure 7G). What is more, the results of the TIMER database indicate that the expression level of EMC6 has a significant effect on the infiltration of a wide range of immune cells in LUAD (Figure 7H). However, cytology experiments merely mimic the biological processes of cells *in vitro*, but it is *in vivo* experiments that provide a better view of the real processes. Therefore, we constructed a subcutaneous hormonal tumor model using C57 mice (Figure 8A). The results showed that reducing the expression of EMC6 in LUAD cells could significantly reduce the growth rate of the tumor (Figure 8B) and could significantly increase the infiltration of immune cells ($CD4^+$ T cells, $CD8^+$ T cells, and macrophages) in the tumor tissue (Figures 8C and 8D). Finally, to improve the clinical significance of this study, we observed the immunohistochemical results of EMC6 in LUAD tissues and normal lung tissues from the HPA database. The results showed that the expression of EMC6 in LUAD tissues was significantly higher than that in normal lung tissues (Figure 9).

DISCUSSION

As the largest organelle in the cell, the ER is involved in several basic physiological functions of the cell, including synthesis and transport of protein, metabolism and storage of lipid and sugar, and various cell signaling pathways.[22] It is its complex and crucial physiological functions that determine the rich variety of proteins that the ER has. Therefore, it goes without saying that ER proteins certainly play a critical role in physiological and pathological states.[23,24] The abnormal state of the cell caused by significant changes in the structure and function of ER

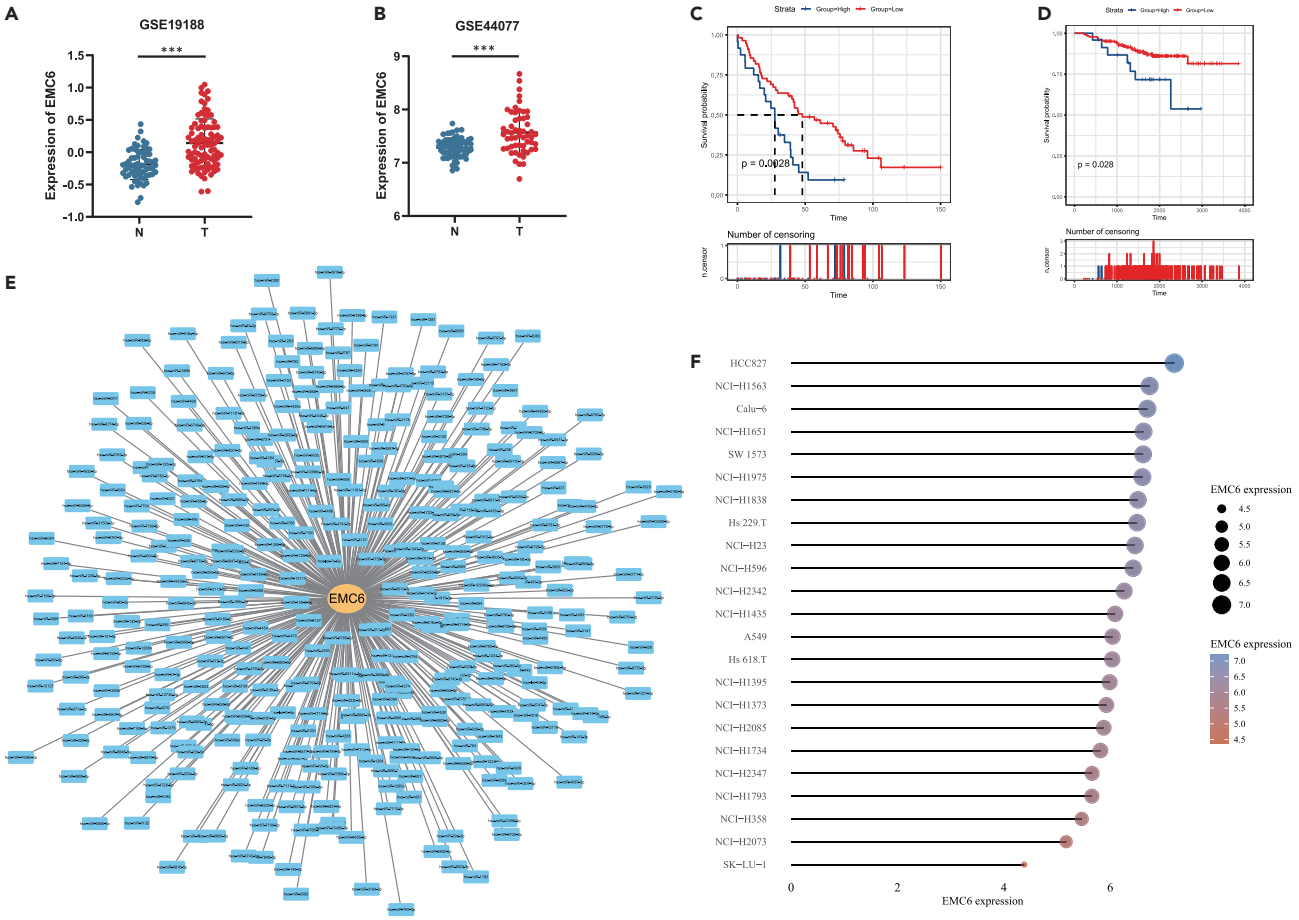

Figure 6. The correlation between EMC6 and LUAD

(A) In GSE19188, the expression of EMC6 in LUAD is higher than in normal lung tissues; (B) in GSE44077, the expression of EMC6 in LUAD is higher than in normal lung tissues; (C) survival curve of EMC6 in GSE31201; (D) survival curve of EMC6 in jacob-00182-HLM; (E) the miRNA regulatory network of EMC6 in LUAD; (F) in CCLE, the expression of EMC6 in various LUAD cells.

proteins in response to certain stimuli is called ER stress state.[25] Currently, a large number of studies have demonstrated the widespread presence of ER stress states in tumor cells due to the complexity of the tumor microenvironment (TME).[26] More importantly, in a variety of malignancies, ER stress is able to remodel its function and metabolism by inducing autophagy, which in turn is required to maintain tumor cells' rapid growth.[27] EMC6 is an important ER protein that has been identified as being involved in autophagy in recent years. However, we found that there were only few studies focusing on the regulatory role of EMC6 in cancers. To the best of our knowledge, this study was the first to perform a pan-cancer analysis of EMC6 in tumors, which will provide new ideas and directions for further research on ER proteins.

Altered expression levels in tumor tissues are a prerequisite for genes to perform important regulatory functions. Satisfactorily, by analyzing TCGA data we discovered that the expression of EMC6 in a variety of tumors was significantly different from the corresponding paracancerous tissues. Subsequently, TMB analysis, OS analysis, and DSS analysis also presented that EMC6 expression was closely correlated with the clinical prognosis of many kinds of cancers, especially LGG and LUAD. Clinicopathological staging is a more accurate complement to differential expression analysis of cancer and paracancerous tissues and better demonstrates the impact of gene expression on cancer progression. The clinical data from the HPA database and DNA methylation data from the METHSURV database further support the important role of EMC6 in various cancers. The results of clinicopathological staging analysis fully illustrated the effect of EMC6 expression on the malignancy of a variety of tumors.

The results of all the aforementioned analyses could demonstrate that EMC6 is a critical diagnostic and therapeutic target for a variety of cancers. We believe that the development of specific inhibitors or activators targeting EMC6 can effectively improve the disease progression and prognosis of cancer patients. However, the research of targeted drugs is not limited to biomarkers that modulate tumor cell function. In addition, tumor immunotherapy also has been a promising treatment against tumors. We have been searching for biomarkers that activate tumor immune response and induce immune escape. To our delight, the results of pan-cancer analysis revealed that EMC6 may play a critical role in the immune response to a variety of cancers. Chemokines are a group of relatively small-molecular-weight secreted proteins that

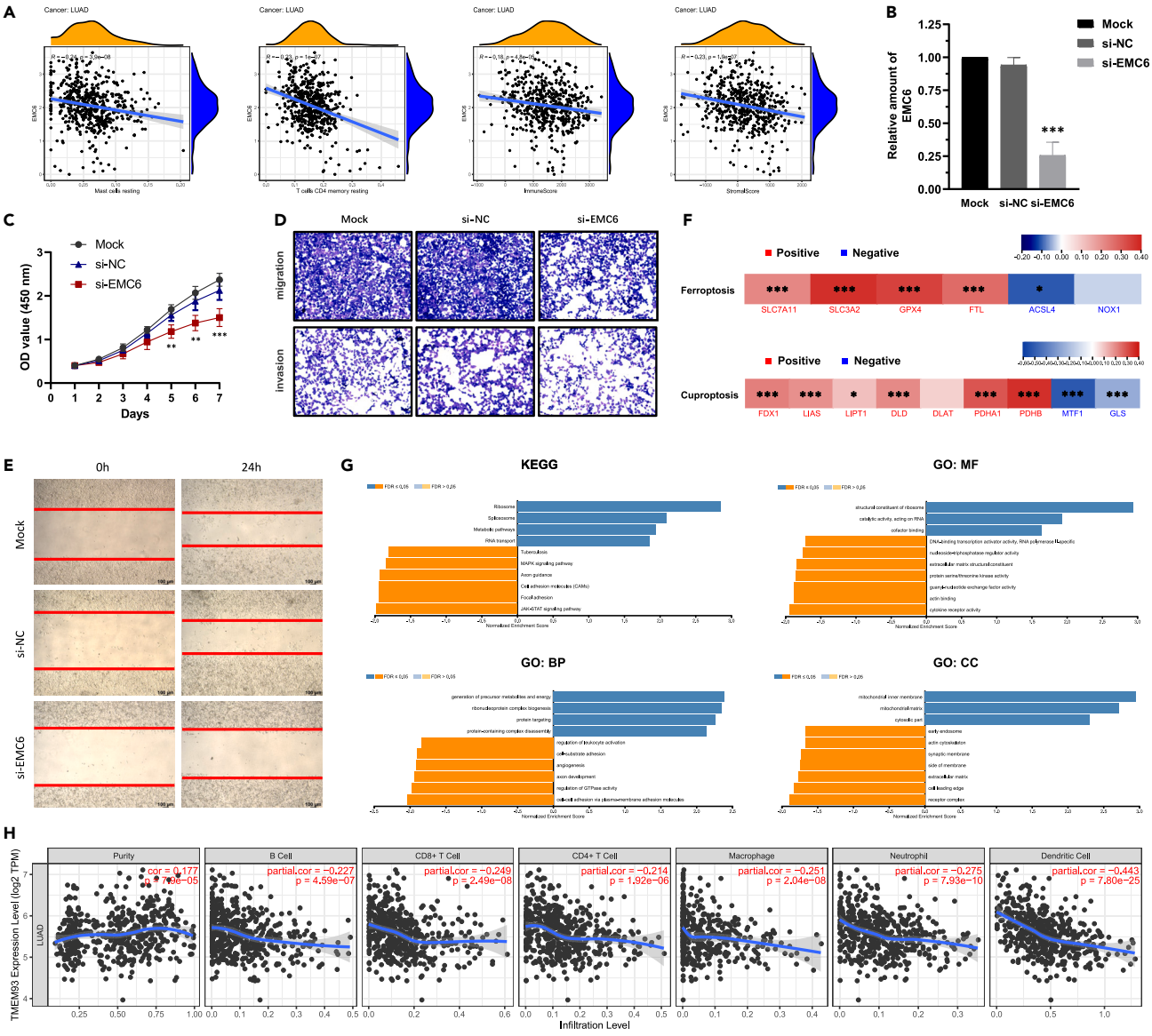

Figure 7. The critical role of EMC6 in LUAD

(A) Correlation between EMC6 and mast cells resting, T cells CD4 memory resting, immune score, stromal score in LUAD; (B) the relative expression of EMC6 was determined by qPCR; (C) detection of A549 viability by CCK-8 assay; (D) comparison of the migration and invasion ability of A549, A549 transfected with si-NC, and A549 transfected with si-EMC6 by transwell assay; (E) wound healing assays in A549, A549 transfected with si-NC, and A549 transfected with si-EMC6; (F) correlation analyses of the EMC6 expression with ferroptosis- and cuproptosis-related genes in LUAD; (G) functional enrichment analysis of EMC6 in LUAD. (H) The relationship between EMC6 and immune cells that infiltratethe tumor tissue in LUAD.

induce immune cell movement and function by interacting with chemokine receptors.[28] The MHC, which is widely considered to be involved in some processes such as antigen presentation and processing, is essential for the immune response to various human diseases.[29] Our co-expression analysis presented that EMC6 is closely correlated with the expression of these related genes of chemokines, chemokine receptors, and MHC in various cancers, which strongly suggests that EMC6 is likely to be essential for immunotherapy in a variety of tumors. This finding fully corroborated the previous study that tumor cells whose ER stress were induced are able to release certain factors that promote their own growth and inhibit the function of tumor-killing immune cells.[30,31] Abnormal expression of EMC6 probably also mediates the release of certain small molecules from tumor cells to regulate the process of tumor immune escape, which will be the molecular mechanism to be explored in our subsequent research.

Combined with the results of our earlier analysis, EMC6 had the most significant regulatory role in LUAD, in terms of both prognosis and tumor immune modulation. Consequently, we chose to explore the role of EMC6 in LUAD to validate our analytical results. Lung cancer, the

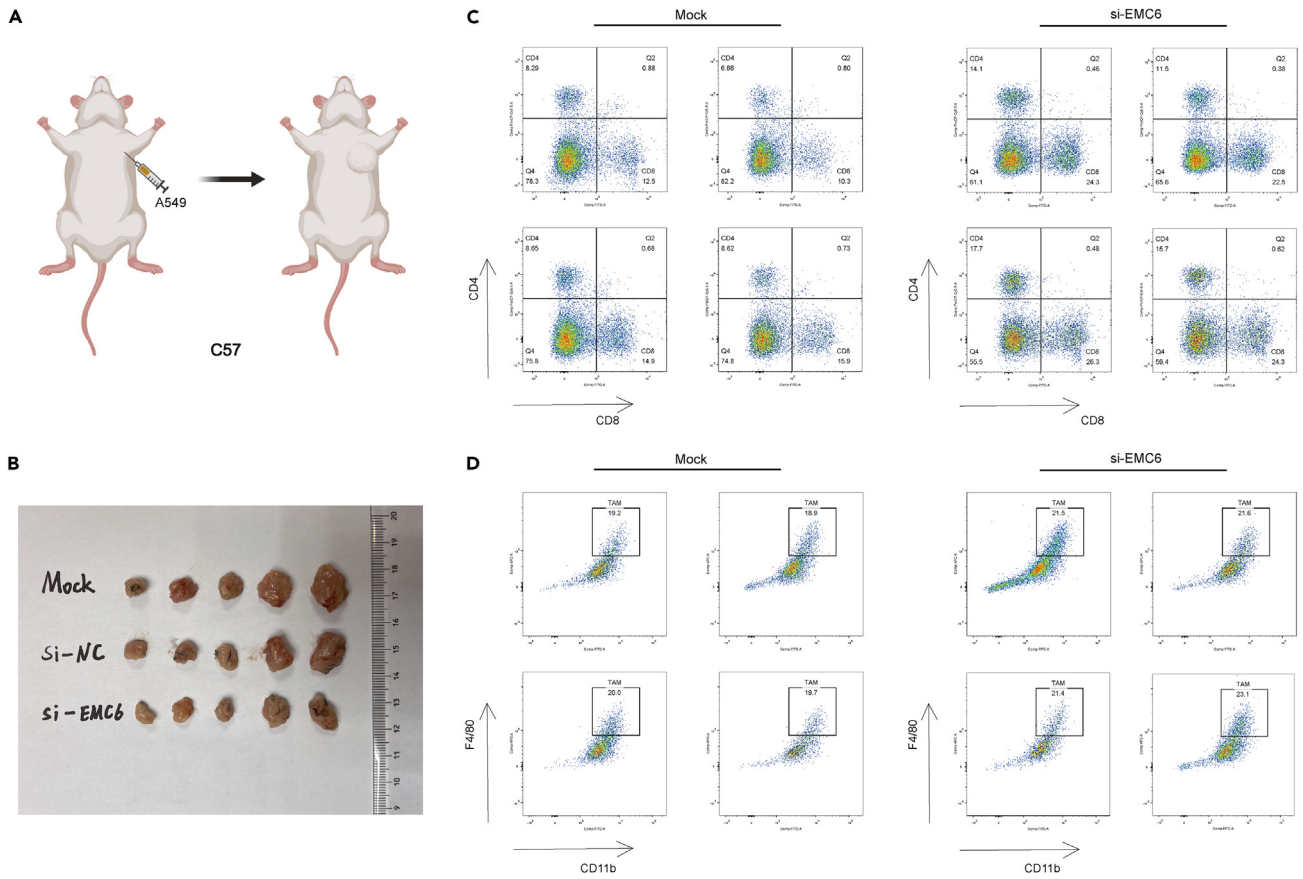

Figure 8. *In vitro* experiments, EMC6 has been shown to regulate tumor growth and immune infiltration

(A) Construction of a subcutaneous tumor bearing model in mice (C57 mice, A549 cells); (B) subcutaneous tumors removed after 15 days in each group; (C) T cells isolated from each subcutaneous tumor; (D) macrophages isolated from each subcutaneous tumor.

leading cause of cancer-related mortality around the world, is one of the most dreaded diseases.[32] As the most common sub-type of lung cancer, LUAD has a very poor clinical prognosis.[33] By searching the expression levels of EMC6 in various LUAD cell lines in the CCLE database, we found that the expression levels of EMC6 in the A549 cell line were stable and high.[34,35] Therefore, we chose the A549 cell line as the target cell line for this study. Afterward, we designed and transfected siRNA si-EMC6 to construct EMC6-knockdown LUAD cell line (A549). Through a series of *in vivo* and *in vitro* experiments, we found that the reduction of EMC6 expression did effectively inhibit the proliferation, invasion, and metastasis of A549. These results of functional enrichment analysis also indicated that EMC6 was involved in several immunomodulatory-related signaling pathways. These results sufficiently supported our speculation that EMC6 was an important biomarker for LUAD. It is well known that immunotherapy is one of the main treatments for LUAD. Our finding that EMC6 was closely associated with tumor immune response in LUAD and the impact is particularly significant especially for T cells may well explain the process of immune escape occurring in LUAD.

Finally, we had made other speculations and verifications on the mechanism of action of EMC6 in LUAD. Based on the recent findings that both ferroptosis[36] and cuproptosis[37] are inextricably linked to ER function, we then investigated the relationship between EMC6 and ferroptosis and cuproptosis in LUAD. Interestingly, EMC6 is indeed involved in the regulation of ferroptosis and cuproptosis in LUAD. This finding also enriches the theoretical study of these two modes of cell death.

In a word, our study demonstrates the important role of EMC6 in a variety of cancers and validates and speculates on its regulatory role in LUAD. All these findings would provide new ideas and support for the regulatory role of ER proteins in tumor therapy, while its clinical significance is of vital importance.

Limitations of the study

Nevertheless, there are some limitations in our study. Regulation of ferroptosis and cuproptosis by EMC6 only stays at the level of data analysis, and there are not enough experimental data to confirm our results. Therefore, we will arrange reasonable assays to verify our conjecture step by step.

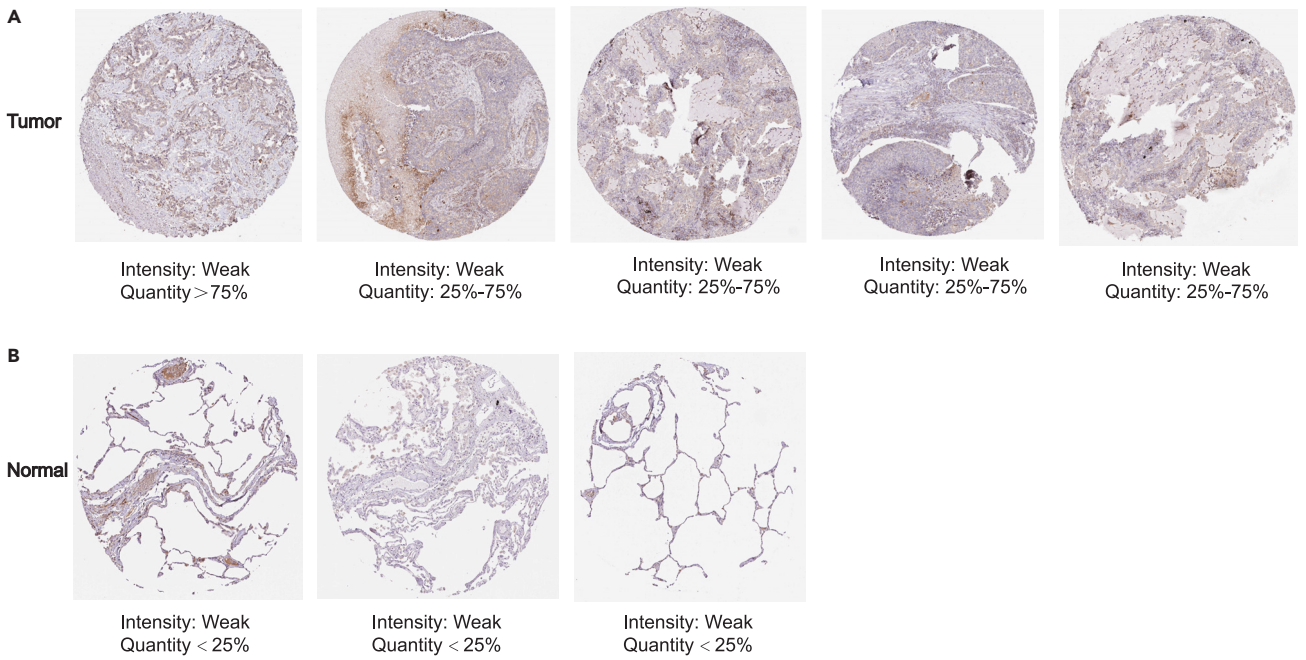

Figure 9. Immunohistochemistry of EMC6

(A) Immunohistochemistry of EMC6 in LUAD; (B) immunohistochemistry of EMC6 in normal lung tissues.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.108648.

ACKNOWLEDGMENTS

This study was supported by the project of research and practice innovation plan for postgraduates in Jiangsu Province (No. KYCX20-2721). All authors have read and approved the version of the manuscript submitted for publication.

AUTHOR CONTRIBUTIONS

X.Z. and B.X. conceptualized the project, designed research, supervised experiments, and wrote the manuscript. J.R. and M.J. designed research, performed experiments, analyzed data, and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: June 8, 2023
Revised: August 14, 2023
Accepted: December 4, 2023
Published: December 8, 2023

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-mouse-CD45-APC	BioLegend	103111
anti-mouse-CD3-PE	BioLegend	100205
anti-mouse-CD4-PerCP-Cy 5-5	BioLegend	100433
anti-mouse-CD8-FITC	BioLegend	100705
anti-mouse-CD45-PE	BioLegend	103105
anti-mouse-CD11b-FITC	BioLegend	101205
anti-mouse-F4/80-APC	BioLegend	123115
Critical commercial assays		
Real-time Quantitative RCR Kit	Yeasen Biotechnology	11143ES50
Cell Counting Kit-8	NCM Biotech	C6005
Transwell Chambers	Corning	3470
Opti-MEM Medium	Gibco	31985
DMEM Medium	Biosharp	BL1124A
LipofecctamineTM 3000	Invitrogen™	L3000075
Software and algorithms		
R (version 4.0.4)	The R Foundation	https://www.r-project.org
ggplot2	R package	N/A
survival	R package	N/A
Graphpad Prism	N/A	https://www.graphpad-prism.cn/
Cytoscape	N/A	http://www.cytoscape.org/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Xin Zhou (denniszhouxin@163.com).

Materials availability

The study did not generate any new materials.

Data and code availability

- This paper analyzes existing, publicly available data. These accession numbers for the datasets are listed in method details.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The data analyzed in this study were obtained from The Cancer Genome Atlas (TCGA) database and the Gene Expression Omnibus (GEO) database. RNA-seq data and clinical survival information for LUAD samples were downloaded from the TCGA database. Gene expression profiles and clinical survival information for three independent LUAD cohorts were obtained from the GEO database.

A549 cells were maintained in high-glucose Dulbecco's-modified Eagle's medium (DMEM, Biosharp) supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin, 100 mg/ml streptomycin in a humidified incubator at 37°C with 5% CO_2. Cell lines were not authenticated internally. All cells were routinely tested and confirmed to be free of mycoplasma (MycoAlert PLUS Assay, Lonza).

METHOD DETAILS

Gene expression level analysis

TIMER database (https://cistrome.shinyapps.io/timer/) is a powerful online analysis platform containing a large amount of cancer data. We used the "Diff Exp" module of the TIMER database to visualize the expression of EMC6 in 33 cancers from TCGA. As for the data on the changes in the expression levels of EMC6 at different stages in various cancers, they were obtained from the GEPIA2 database. GEPIA2 (http://gepia2.cancer-pku.cn/) database contains the vast majority of data from the TCGA and GTEx projects and integrates them for more complex analysis.

Analysis of the relationship between EMC6, tumor mutational burden (TMB), and prognosis

Patient survival data and mutation data were all obtained from the TCGA database (downloaded from UCSC Xena). The correlation between the expression of EMC6 and TMB was analyzed by Spearman's rank correlation coefficient. After integrating EMC6 expression levels and survival time in cancer patients from TCGA, we used univariate Cox analysis model to explore the association between EMC6 and overall survival (OS) and disease-specific survival (DSS) in various cancers. The survival-associated forest plot and Kaplan-Meier (KM) analysis were performed by R language (version 4.0.4).

EMC6 and tumor immunity

EMC6 was analyzed in relation to tumor immunity in the following areas, including immune activation, chemokines, chemokine receptors, histocompatibility complex (MHC) and various immune cell infiltrations as calculated by CIBERSORT. All gene markers were obtained from previous studies.[38–40] In our study, we used flow cytometry to detect the infiltration of immune cells ($CD4^+$ T cell, $CD8^+$ T cell, and macrophages) in a mouse subcutaneous tumor bearing model. The antibodies used in this flow cytometry are as follows: anti-mouse-CD45-APC (BioLegend, cat:103111); anti-mouse-CD3-PE (BioLegend, cat:100205); anti-mouse-CD4-PerCP-Cy 5-5 (BioLegend, cat: 100433); anti-mouse-CD8-FITC (BioLegend, cat: 100705); anti-mouse-CD45-PE (BioLegend, cat: 103105); anti-mouse-CD11b-FITC (BioLegend, cat: 101205); anti-mouse-F4/80-APC (BioLegend, cat:123115).

Linkedomics database utilization

Linkedomics database (http://www.linkedomics.org/) is a user-friendly cancer data analysis platform that contains multi-omics data from all 32 TCGA Cancer types and 10 Clinical Proteomics Tumor Analysis Consortium (CPTAC) cancer cohorts. We analyzed the association between EMC6 and genes related to ferroptosis and cuproptosis using the "LinkFinder" module of Linkedomics database. EMC6 functional enrichment analysis in lung adenocarcinoma was from "LinkInterpreter" module of Linkedomics database.

Cell culture and transfection

A549 cells were cultured in six-well plates at a concentration of $1x10^6$ /ml. When the cell density reached about 80%, EMC6 small interfering RNA (si-EMC6) and siRNA negative control (si-NC) were respectively transfected with LipofecctamineTM 3000 (L3000075, Invitrogen™, USA) and Opti-MEM (31985, Gibco, USA) into A549 cells. Three groups of cells (siRNA interference group, siRNA control group and blank control) were collected after 48 hours.

Quantitative real time PCR (qRT-PCR)

The total RNA of the three groups of A549 cells was extracted and synthesized with reverse transcription kit to obtain c-DNA. EMC6 mRNA expression was detected by real-time quantitative RCR kit (11143ES50, Yeasen Biotechnology, China), in which GAPDH was used as an internal reference gene. EMC6 forward: 5′-GTCGCCAAGATTTGCTCCCT-3′, reverse:5′-AAACACACAATGCCGGTACAC-3′; GAPDH forward: 5′- ATCATCAGCAATGCCTCCTG-3′, reverse: 5′- ATGGACTGTGGTCATGAGTC-3′.

Cell proliferation experiment

Cell Counting Kit-8 (CCK-8, C6005, NCM Biotech, China) was used to detect the proliferation of A549 cells in siRNA interference group, siRNA control group and blank control group. Three groups of cells were putted in 96-well plates, and the assay was performed in a time course of 1–7 days. Each well was incubated at 37°C for 4 hours after the addition of CCK8 reagent, and the absorbance value at 450 nm was detected using a microplate reader.

Cell migration and invasion assay

Cell invasion assay require pre-coating the upper surface of Transwell chambers (3470, Corning, USA) with Matrigel glue. The three groups of A459 cells were inoculated in the upper chamber of fetal bovine serum-free medium, and the bottom chamber was the medium including 20% fetal bovine serum. Take out the upper chamber after 36 hours in the incubator, non-invading cells were removed. The upper chamber was fixed with methanol and stained in 0.1% crystal violet, and the number of invading cells was quantified by taking pictures of 5 visual fields in the chamber randomly selected by microscope. The cell migration assay was identical except that the step of applying Matrigel glue was ignored.

Wound healing assay

Three groups of A549 cells were cultured in 24 well plates. When the cell density reaches 100%, use the sterile gun head to scratch the cells in each group on the scale, and then scratch photography. The cells were cultured for 24 hours and photographed, and compared with the scratch area at 0 hour.

In vivo tumorigenesis

We injected $2x10^6$ three groups of A549 cells /0.1 ml subcutaneously into the armpit of each C57 mouse (5 mice in each group and 15 mice in total). After 15 days, due to the diameter of the largest tumor was close to 20 mm, we sacrificed all mice with cervical dislocation and removed all tumors for measurement and recording.

QUANTIFICATION AND STATISTICAL ANALYSIS

In our study, all plots were completed by R software (version.4.0.5). Unpaired t tests were used to compare two groups. The screening criteria for all data was P-value<0.05. All these assays were repeated at least three times.