ABCA3, a tumor suppressor gene, inhibits the proliferation, migration and invasion of lung adenocarcinoma by regulating the epithelial-mesenchymal transition process

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Abstract. Lung adenocarcinoma (LUAD) is one of the most common types of lung cancer, which affects the life and health of patients. The role of ATP-binding cassette subfamily A member 3 (ABCA3) in the occurrence and development of LUAD is unclear; therefore, ABCA3 expression in LUAD and other tumors was analyzed in the present study. In addition, ABCA3 expression in patients with LUAD and their survival were analyzed using a public database. ABCA3 co-expressed genes were identified and their enriched pathways were analyzed. Furthermore, ABCA3 expression was knocked down in LUAD cell lines. The proliferation, invasion and migration of cells, and the process of epithelial-mesenchymal transition (EMT), were studied through cytological and molecular biology experiments. Compared with that in normal lung tissues, ABCA3 expression was significantly reduced in tumor tissues. Patients with low ABCA3 expression had a markedly worse overall survival compared with those with high ABCA3 expression. Notably, abnormal ABCA3 expression has been observed in a variety of tumors. Subsequently, multiple pathophysiological pathways enriched by ABCA3 and its co-expressed genes were explored. Furthermore, the malignant behavior of tumor cells was enhanced when ABCA3 expression was knocked down, and the EMT process was activated after ABCA3 expression was knocked down. In conclusion, as a tumor suppressor gene, ABCA3 serves a protective role in the development of tumors, and may have a potential role in clinical applications, and thus, is worthy of further study.

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Introduction

Lung cancer is not only a life-threatening disease that causes thousands of deaths (1), but it is also a severe social problem that causes billions in economic burden every year (2). The 5-year survival rate of lung cancer is <20%. Non-small cell lung cancer (NSCLC) is the most common form of lung cancer with lung adenocarcinoma (LUAD) being the most common subtype of NSCLC. In recent years, programmed death-1/programmed death-ligand 1 immune checkpoint therapy and driver gene mutation-oriented targeted therapy have resulted in marked survival benefits for some patients with LUAD (3). However, the problem of drug resistance gradually hinders the effect of treatment, and more potential novel targets are needed for the prediction and treatment of LUAD (4).

ATP-binding cassette (ABC) subfamily A member 3 (ABCA3) is a gene located on chromosome 16p13.3*-. Studies on ABCA3 have revealed that this gene serves an important role in the development of malignant disease; Schimanski *et al* (5) revealed that decreased ABCA3 expression in breast cancer was associated with poor prognosis. Steinbach *et al* (6) showed that ABCA3 was upregulated in childhood acute myeloid leukemia (AML) compared with in healthy bone marrow, and ABCA3 was identified as the most likely transporter that causes drug resistance. Although there are a few studies on the effects of ABCA3 in lung cancer, the exact role of ABCA3 in LUAD is not clear (7,8). In the present study, a series of public databases such as The Cancer Genome Atlas (TCGA) and the Clinical Proteomic Tumor Analysis Consortium (CPTAC) were analyzed, and cytological and molecular experiments were performed to explore the role of ABCA3 in the occurrence and development of LUAD.

Materials and methods

Data analysis from public databases. mRNA expression data from TCGA were obtained from the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN; http://ualcan.path.uab.edu/analysis.html) (9) and UCSC XENA browser (https://xenabrowser.net/) (10), and protein expression data from the CPTAC were also

obtained from the University of Alabama at Birmingham Cancer Data Analysis Portal (https://ualcan.path.uab.edu/analysis.html). Survival data from TCGA were obtained from UCSC XENA browser and Kaplan-Meier plotter online analysis database (https://kmplot.com/analysis/index.php?p=service&cancer=lung). Co-expressed genes were obtained from UALCAN and cBioportal (http://www.cbioportal.org/index.do) (11,12). Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analyses were performed with ClueGo V2.5.2 (13) in Cytoscape software V3.7.0 (14). The protein-protein interaction network was analyzed using the Search Tool for the Retrieval of Interacting Genes/Proteins V11.5 (https://cn.string-db.org/) (15).

Cell culture and transfection. The A549 LUAD cell line was obtained from Hebei Medical University (Shijiazhuang, China), and the cells were cultured in a 5% CO_2 incubator at 37˚C. DMEM (Corning, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and penicillin-streptomycin (100 U/ml) (Gibco; Thermo Fisher Scientific, Inc.) was used for cell culture. During the culture process, PCR detection was performed once a week to prevent mycoplasma contamination, and cell passage was performed when the cell confluence reached 70%. To knock down the expression of ABCA3, cell transfection was performed when the cell confluence reached 70%. ABCA3 and negative control (NC) small interfering RNA (siRNA) sequences were synthesized by Sangon Biotech Co., Ltd. Lipofectamine® RNAiMAX Transfection Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used to transfect siRNA into the cells. According to the instructions of the transfection reagent, the siRNA concentration was 50 nM and the transfection process was performed in a 37˚C incubator; the initial incubation duration was 5 min and the final incubation time was 72 h. The effect of protein knockdown (KD) was detected by western blotting 72 h after transfection. The siRNA sequences were as follows: NC forward, 5'-UUCUCCGAACGUGUCACGUTT-3' and reverse: 5'-ACGUGACACGUUCGGAGAATT-3'; and ABCA3 forward, 5'-CGCUGUUCCUCAAGCAGAAAU-3' and reverse, 5'-UUCUGCUUGAGGAACAGCGAG-3'.

Cell behavior experiments. All cell experiments were performed 72 h after transfection. Cell proliferation was measured using an MTT assay. DMSO (MilliporeSigma) was used to dissolve the purple formazan and the absorbance was measured at 490 nm by spectrophotometry. Cell migration was measured using a wound healing assay, which was performed in cells cultured in DMEM without FBS. A wound was generated with a sterile pipette tip when cell confluence reached 80%, the scratched cells were washed with PBS and the width of the wound was measured every 12 h using an IX71 light microscope (Olympus Corporation). The relative width of the wound was calculated as follows: The width of the initial wound at the start of the assay was considered the baseline value, and the ratio of the width measured at each time point to the baseline value was considered the relative width. Cell invasion was measured using a Transwell assay. The 24-well Transwell chamber (diameter, 6.5 mm; pore size, 8.0 μm; cat. no. 3422, Corning Inc.) was coated with Matrigel (Corning Inc.) for 24 h at 37˚C, and the cells were seeded in DMEM containing 5% FBS in the upper chamber ($2x10^5$ per chamber per well). DMEM with 20% FBS was placed in the bottom chamber. Crystal violet (2%) was used to stain the cells under the bottom of the chamber at room temperature for 10 min, and cells were observed via an IX71 light microscope (Olympus Corporation).

Protein extraction and western blotting. Cell proteins were extracted using RIPA lysis reagent (Thermo Fisher Scientific, Inc.) with protease and phosphatase inhibitors. The total protein concentration was determined using a Pierce™ BCA protein assay kit (Thermo Fisher Scientific, Inc.). Proteins were stored at -80˚C and were then separated by SDS-PAGE on a 12% gel at a voltage of 120 V for 2 h. Subsequently, proteins were transferred to PVDF membranes, which were blocked with 5% BSA (Merck & Co., Inc.) for 2 h, incubated with primary antibodies for 4 h at room temperature and incubated with a secondary antibody for 2 h at room temperature. Finally, electrochemiluminescence was captured by a GE LAS-600 Imaging system with SuperSignal™ West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, Inc.). The gray value of bands was semi-quantified using ImageJ software V1.5.0 (National Institutes of Health). Details of the antibodies involved in this study are shown in Table I.

Statistical analysis. All assays have been verified with experimental repeats >3 times. SPSS (v22.0; IBM Corp.) software was used for statistical analysis. The differences in data between groups were detected by a t-test, and Z-values that represent standard deviations from the median across samples for the given cancer type were presented for the CPTAC database. Gene mRNA levels were divided into high and low expression groups based on the receiver operating characteristic (ROC) curve by MedCalc V18 software (MedCalc Software Ltd.). The survival difference between different groups was analyzed by Kaplan-Meier survival analysis and log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Abnormal ABCA3 expression is observed in TCGA-LUAD dataset. Through analysis of TCGA-LUAD dataset, it was observed that ABCA3 expression in primary tumor tissues was significantly reduced compared with that in normal tissues in TCGA normal lung tissue (Fig. 1A). Subgroup analysis revealed that similar results could be observed in all age groups, sexes and Tumor-Node-Metastasis stages (16) across TCGA samples (Fig. 1B-D). In the CPTAC database, a similar result was observed regarding the protein expression levels of ABCA3 in LUAD cancer tissues compared with in normal tissues (Fig. 1E).

Abnormal ABCA3 expression is observed in a variety of tumors. Pan-cancer analysis was performed to investigate ABCA3 expression in different types of cancer. The results indicated that for glioblastoma, head and neck squamous cell carcinoma, LUAD, lung squamous cell carcinoma (LUSC), thyroid carcinoma and uterine corpus endometrial carcinoma, ABCA3 expression was significantly lower in tumor tissues than in normal tissues (Fig. 1F). For some other types of tumors,

Table I. Antibody information.

Antibody	Brand	Catalog number	Dilution
Primary antibodies			
ABCA3	Abcam	ab99856	1:2,000
E-cadherin	Cell Signaling Technology, Inc.	14472	1:1,000
N-cadherin	Cell Signaling Technology, Inc.	13116	1:1,500
Vimentin	Proteintech Group, Inc.	60330-1	1:5,000
β-actin	Proteintech Group, Inc.	20536-1	1:3,000
Secondary antibodies			
Anti-mouse	Proteintech Group, Inc.	SA00001-1	1:5,000
Anti-rabbit	Proteintech Group, Inc.	SA00001-2	1:5,000

ABCA3, ATP-binding cassette sub-family A member 3.

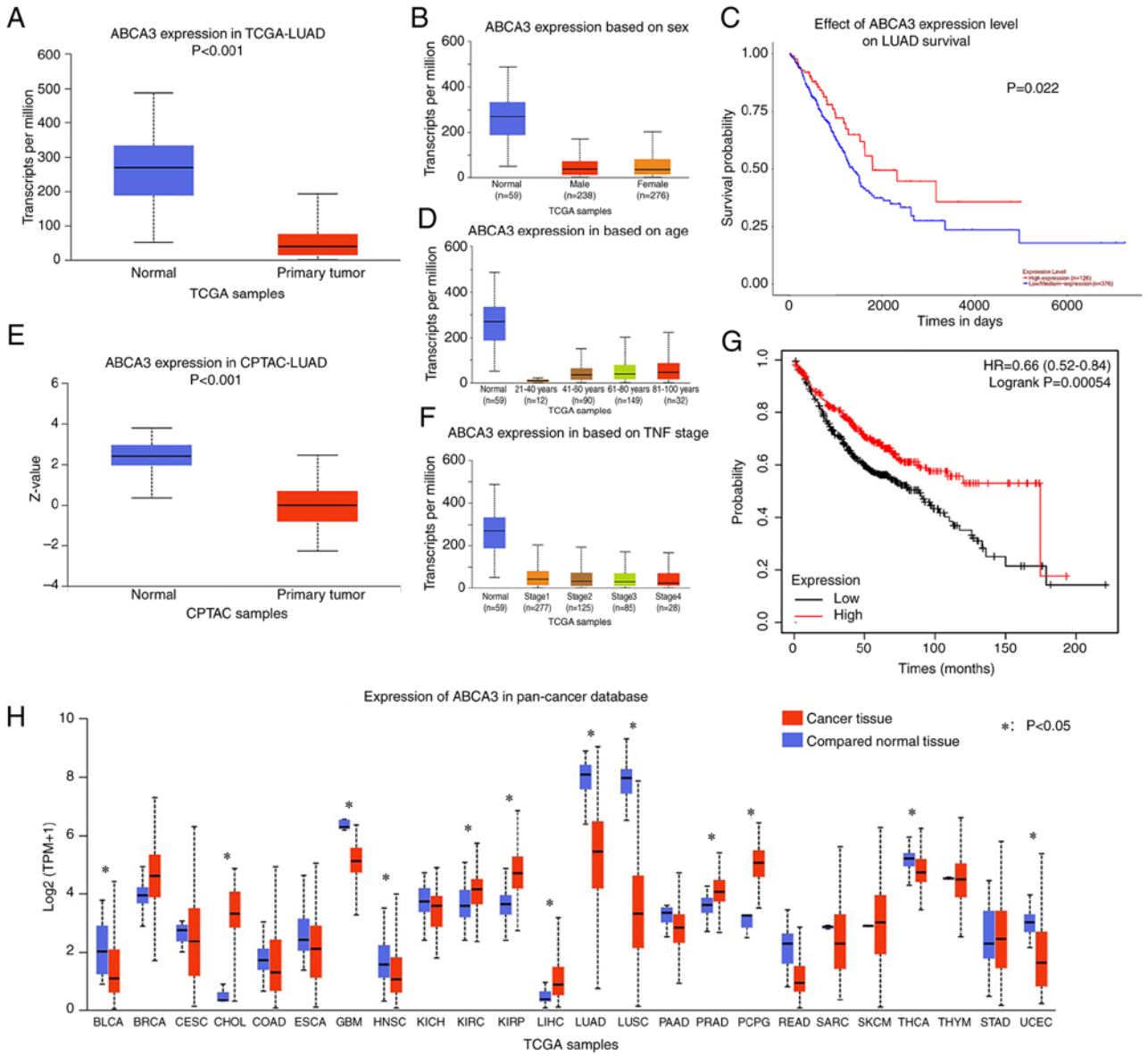

Figure 1. ABCA3 in public databases. (A) ABCA3 expression in TCGA-LUAD dataset based on different sample types. ABCA3 expression in TCGA-LUAD dataset based on (B) sex, (D) age and (F) TNM stage. (E) ABCA3 expression in the CPTAC-LUAD dataset based on different sample types. (H) Pan-cancer analysis of ABCA3 expression in TCGA. (C and G) Kaplan-Meier survival analysis of OS in different ABCA3 expression groups in (C) TCGA-LUAD dataset and (G) Kaplan-Meier plotter database. *P<0.05. LUAD, lung adenocarcinoma; ABCA3, ATP-binding cassette sub-family A member 3; TCGA, The Cancer Genome Atlas; CPTAC, Clinical Proteomic Tumor Analysis Consortium; TNM, Tumor-Node-Metastasis; TPM, transcripts per million; HR, hazard ratio.

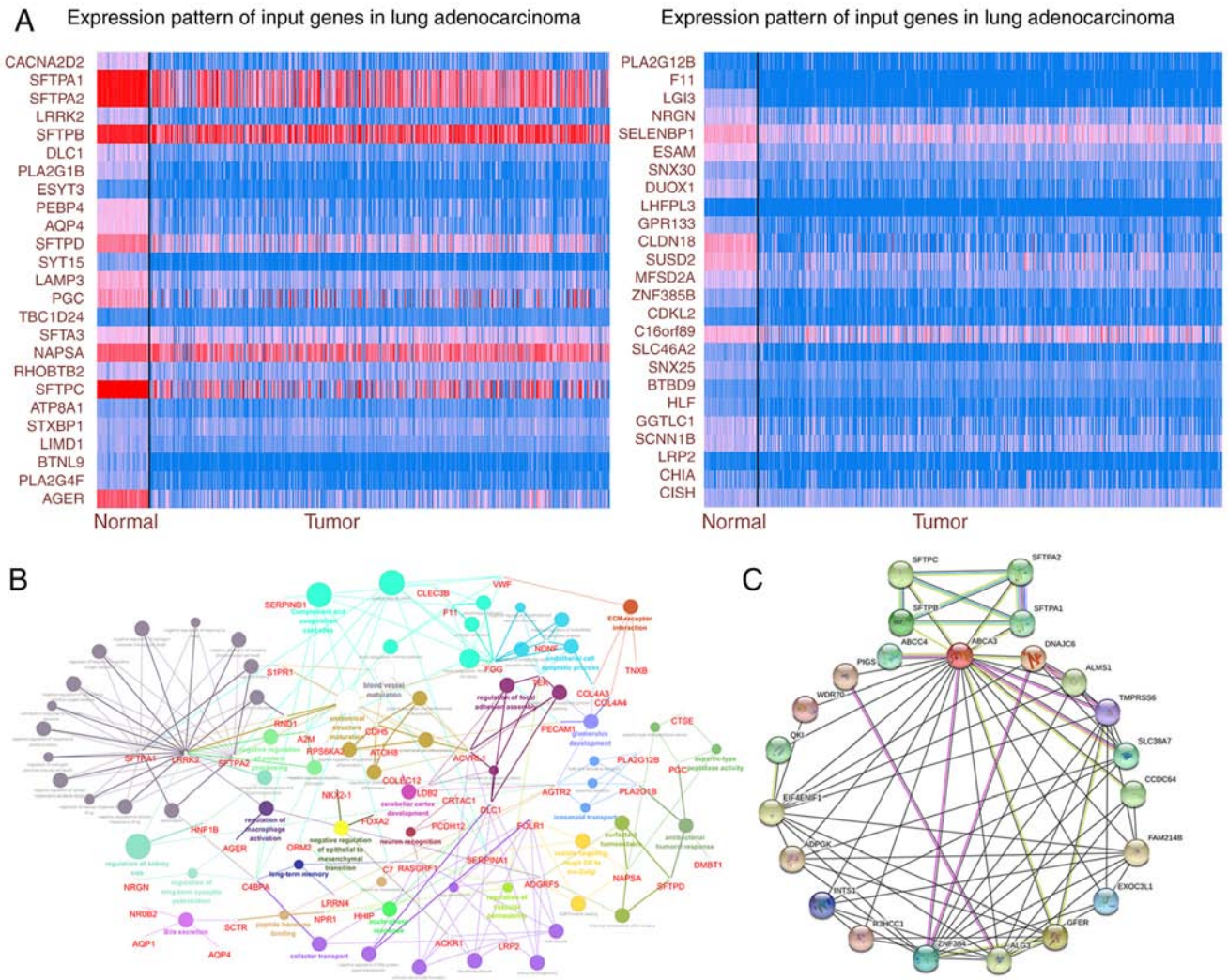

Figure 2. ABCA3 and its co-expressed genes in public databases. (A) Top 50 co-expressed genes related to ABCA3 expression. (B) Kyoto Encyclopedia of Genes and Genomes and Gene Ontology analyses of ABCA3 and related genes, different colors represent different gene clusters; all displayed pathway analysis results showed are statistically different (P<0.05). (C) Protein-protein interaction network of ABCA3, different line colors represent different associations. Blue or purple lines represent interactions from curated databases or experiments recorded in previous literature, respectively; green, red and yellow lines represent genes that are adjacent to ABCA3 on the chromosome, genes that fuse with ABCA3 (chromosomal translocation, interstitial deletion and chromosomal inversion) or genes that appear together with ABCA3 in literature studies respectively. The P-values of interactions were P<0.05. ABCA3, ATP-binding cassette sub-family A member 3.

such as breast invasive carcinoma, cholangiocarcinoma, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma, prostate adenocarcinoma, and pheochromocytoma and paraganglioma, ABCA3 expression in cancer tissues was higher than that in normal tissues (Fig. 1F).

Patients with low ABCA3 expression have a poor OS. Patients in TCGA-LUAD dataset were divided into two groups based on ABCA3 expression, and the cut-off value was determined by ROC curve analysis. The results demonstrated that compared with patients in the high-expression group, patients in the low-expression group had a significantly shorter overall survival (OS) time (Fig. 1G and H).

ABCA3 may participate in multiple pathophysiological activities. To explore the possible pathological role of ABCA3 in LUAD, genes co-expressed with ABCA3 were identified using TCGA database. The expression of some significantly correlated co-expressed genes with a Pearson's correlation score of >0.4 in TCGA is shown in Fig. 2A. KEGG analysis was performed for ABCA3 and its co-expressed genes. The results showed that these genes were enriched in certain pathological pathways, such as 'extracellular matrix-receptor interaction', 'regulation of cellular response to drug' (GO:2001038; GO:2001024), 'regulation of endothelial cell apoptotic process' (GO:2000351; GO:2000352), 'regulation of cell-substrate junction assembly' (GO:0090109), 'endothelial cell apoptotic process' (GO:0072577), 'endothelial cell differentiation' (GO:0045446; GO:0045601), 'positive regulation of epithelial cell differentiation' (GO:0045603) and 'negative regulation of epithelial-mesenchymal transition' (GO:0010719) (EMT; Fig. 2B). Furthermore, the protein-protein interaction network of ABCA3 was explored (Fig. 2C), which indicated that there were multiple interaction relationships between ABCA3 and various proteins.

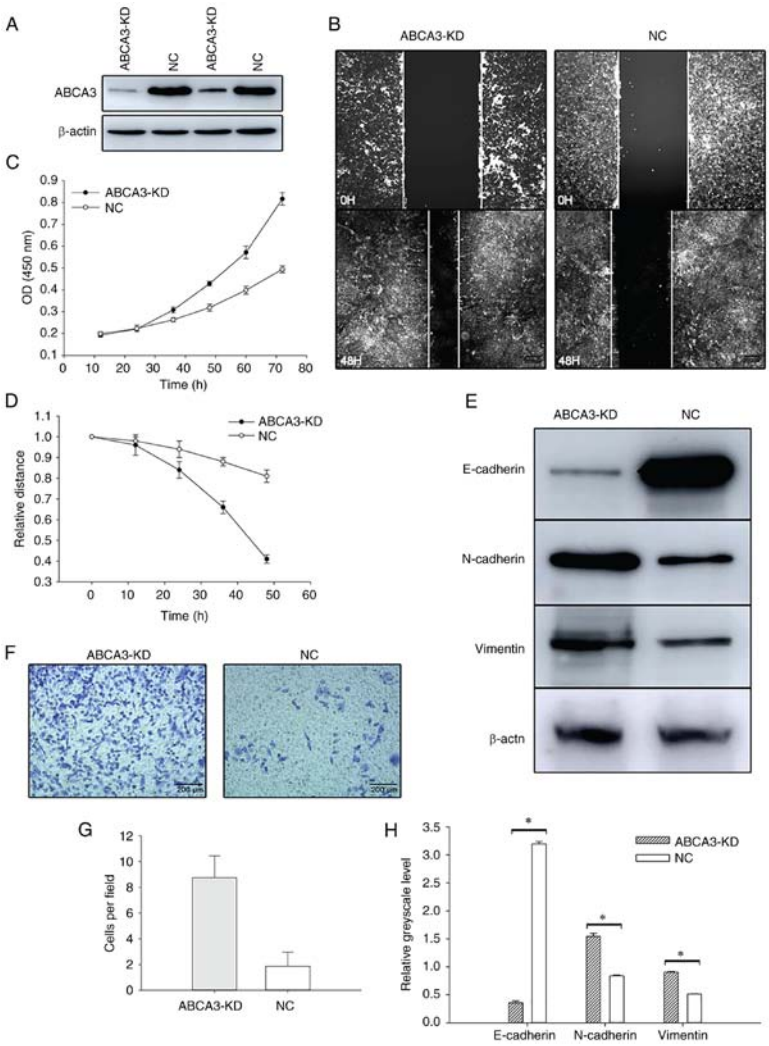

Figure 3. Biological behavior of A549 lung adenocarcinoma cells after knockdown of ABCA3 expression. (A) ABCA3 expression after small interfering RNA transfection. (C) MTT assay indicated the proliferation of ABCA3-KD and NC A549 cells. (B) Wound healing assay showing the migration of ABCA3-KD and NC A549 cells. (D) Curve of the wound healing assay of ABCA3-KD and NC A549 cells. (F) Transwell assay showing the invasion of ABCA3-KD and NC A549 cells. (G) Cell counts from the Transwell assay of ABCA3-KD and NC A549 cells. (E and H) Epithelial-mesenchymal transition-related protein expression was examined by western blotting in ABCA3-KD and NC A549 cells. *P<0.05. ABCA3, ATP-binding cassette sub-family A member 3; KD, knockdown; NC, negative control; OD, optical density.

Malignant behavior of tumor cells is enhanced when ABCA3 expression is knocked down. To investigate the role of ABCA3 in LUAD, the A549 cell line was cultured for further research. siRNA was transfected into the A549 cell line to knock down ABCA3 expression, and the MTT assay showed that after siRNA transfection, the proliferation of A549 cells was increased (Fig. 3A and B). Wound healing assays showed that the wound healing speed in ABCA3-KD cells was faster than that in normal A549 cells (Fig. 3C and D). In the Transwell assay, compared with A549 cells transfected with a negative control, more ABCA3-KD cells passed through the Transwell membrane, which indicated that the invasion was significantly enhanced after transfection (Fig. 3E and F).

EMT is activated after KD of ABCA3. In the present study, the expression levels of three EMT-related proteins, E-cadherin, N-cadherin and vimentin, were explored by western blotting after siRNA transfection. The results indicated that E-cadherin expression was significantly reduced after transfection, whereas the expression levels of N-cadherin and vimentin in ABCA3-KD cells were significantly higher than those in NC A549 cells, indicating that the EMT process was activated after the KD of ABCA3 (Fig. 3G and H).

Discussion

Lung cancer is the most common malignant tumor of the respiratory system (1). It has a number of pathological subtypes due to different cell origins, such as small cell lung cancer, squamous cell carcinoma and adenocarcinoma. In recent years, smoking cessation has markedly reduced the incidence of squamous cell carcinoma, while with the popularization of CT scanning for medical examination, an increasing number of early lung cancer cases have been found through health examinations, with most of them being peripheral lung cancer represented by adenocarcinoma. At the same time, with the popularization of smoking cessation campaigns, the incidence of lung squamous cell carcinoma

has significantly decreased, and LUAD has gradually become the most common type of lung cancer (17). On the basis of traditional surgery, radiotherapy and chemotherapy, gene mutation-oriented targeted therapy and treatment with immune checkpoint inhibitors provide individualized treatment strategies for the comprehensive treatment of patients with LUAD, and notably improve prognosis compared with past anticancer treatments (18). At the same time, the issue of acquired drug resistance caused by these treatments has gradually emerged, and it has become the main factor affecting the treatment effect and long-term survival of patients (19). An important way to solve the issue of drug resistance is to explore the genes and proteins that could potentially be used as novel therapeutic targets in the occurrence and development of LUAD. In the present study, in-depth research was carried out on the survival-related gene ABCA3 in LUAD to develop novel treatment approaches.

ABCA3 is a gene located on chromosome 16p13.3, and the protein encoded by this gene belongs to the ABC transporter superfamily. Schimanski *et al* (5) demonstrated that ABCA3 expression was diminished in human breast cancer tissue, and Steinbach *et al* (6) hypothesized that ABCA3 may be a possible cause of drug resistance in childhood AML. Furthermore, de Lima *et al* (20) reported that ABCA3 4548-91 CC/CA genotypes were related to a poor complete molecular response in patients with chronic myeloid leukemia treated with standard-dose imatinib. However, there have been few studies on ABCA3 in LUAD (4), and the regulatory effect of ABCA3 on LUAD cells is unclear.

In the present study, ABCA3 expression was analyzed using TCGA and CPTAC databases, and a significant decrease in the mRNA and protein expression levels of ABCA3 was observed in LUAD cancer tissues compared with in normal tissues. This abnormal expression may indicate that ABCA3 serves a role in the occurrence and development of LUAD. In previous studies, the role of ABCA3 in tumorigenesis and development was not clear. Most studies have been limited to the difference in ABCA3 expression with regard to prognosis in tumors (5,8), Previous scholars have studied the expression of ABCA3 in lung cancer, both in SCLC and in NSCLC, but have not analyzed lung adenocarcinoma as a separate disease (8). Previous studies have demonstrated that LUAD is notably different from other types of lung cancer in terms of pathogenesis, treatment targets and drug resistance, all of which should be studied further (18,21). The follow-up pan-cancer analysis in the present study revealed that the ABCA3 expression range was different in multiple types of malignant tumors, with abnormal increases in some tumors and significant decreases in others. These results indicated that ABCA3 may serve different roles in different tumors. In some tumors, such as glioblastoma multiforme and LUAD, ABCA3 expression was revealed to be inhibited. We hypothesized that, in these tumors, ABCA3 has a negative regulatory effect on tumor cells, with ABCA3 expression being inhibited during tumor development.

The subsequent survival analysis revealed that compared with the high ABCA3 expression group, patients with low ABCA3 expression had significantly worse OS. By contrast, Overbeck *et al* (8) reported the opposite results from an analysis of 89 patients with lung cancer; patients with high ABCA3 expression had a worse OS time. The present study examined LUAD cases, while ~70% of cases in the study by Overbeck *et al* (8) were LUSC cases. Through further analysis of the LUSC data in TCGA (TCGA-LUSC), it was demonstrated that the relationship between ABCA3 and survival in LUSC is opposite to that in LUAD; in TCGA-LUSC dataset, the high ABCA3 expression group had a worse OS than the low ABCA3 expression group.

To verify the hypothesis that ABCA3 may serve a protective role in LUAD as a tumor suppressor that is downregulated in tumor tissue, cell behavior experiments were performed. The A549 cell line, as the most commonly used LUAD cell line, was used in the current study. siRNA was transfected into A549 cells to knock down ABCA3 expression. The results demonstrated that, in the A549 cell line, ABCA3 expression was negatively associated with malignant behavior. When ABCA3 expression was knocked down, the proliferation, migration and invasion of A549 cells was enhanced. This result indicated that ABCA3 may inhibit the development of tumors, which confirmed that ABCA3 has an inhibitory effect on the occurrence and development of LUAD, and this may provide novel potential ideas for the treatment of LUAD.

In further experiments, the pathological pathways that ABCA3 and co-expressed genes were enriched in were identified through GO analysis; a number of these pathways were related to the regulation of EMT, epithelial cells and endothelial cells, which indicated that the EMT process may serve an important role in ABCA3 inhibition of tumor cells. EMT is an important process in the occurrence and development of malignant tumors (22). The EMT process can regulate the proliferation, migration and invasion of tumor cells, and affect the growth and metastasis of tumors in patients, which has an important impact on their prognosis (23). When the EMT process occurs, the polarity of cells begins to disappear, the epithelial characteristics are weakened and the mesenchymal characteristics are notably enhanced (24). Therefore, in subsequent experiments, the expression of the following EMT-related proteins was investigated: The epithelial marker E-cadherin (19), and the mesenchymal markers N-cadherin (25) and vimentin (26). The results demonstrated that when ABCA3 expression was knocked down, the expression levels of E-cadherin were markedly decreased, whereas those of N-cadherin and vimentin were significantly increased, indicating that the EMT process was activated.

In addition, GO pathway analysis showed that ABCA3 was related to the RNA regulation function of some substances in the cell and before protein synthesis. Kaminski *et al* (27) proposed that ABCA3 may function as a lung surfactant lipid transporter, and ABCA-subfamily transporters serve critical functions in human physiology, which, when they are defective, cause disease. Matsumura *et al* (7) reported that ABCA3 mediated ATP-dependent choline-phospholipid uptake into intracellular lysosomal associated membrane protein 3-positive vesicles in A549 cells. Fish *et al* (28) demonstrated that silencing of ABCA3 could markedly increase lung cancer growth in a study of the regulatory process mediated by transactivation-responsive RNA-binding protein 2 (TARBP2), and

a notable increase in the mature mRNA levels and a decrease in the relative pre-mRNA levels of ABCA3 upon TARBP2 KD were observed in H1299 LUAD cells. These results supported the previous conclusions of the bioinformatics analysis that ABCA3 may have a negative regulatory role in the development of tumor cells, and ABCA3 could act as a protective factor against tumor development.

In conclusion, ABCA3 may have an inhibitory role in the development of lung adenocarcinoma, as observed in the present cytological assays. The underlying pathway mechanisms require further exploration in subsequent research. The present findings indicated that ABCA3 may be a promising target for the treatment of lung adenocarcinoma, and inhibitors targeting ABCA3 may have the potential to provide survival benefits to patients with lung adenocarcinoma.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in The Cancer Genome Atlas (https://www.cancer.gov/tcga), NIH CPTAC database (https://proteomics.cancer.gov//), cBioportal (http://www.cbioportal.org/), University of Alabama at Birmingham Cancer Data Analysis Portal (http://ualcan.path.uab.edu/analysis.html), UCSC XENA (https://xenabrowser.net/) and Search Tool for the Retrieval of Interacting Genes/Proteins (https://cn.string-db.org/) repositories. The other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MS designed the study, conducted the experiments and drafted the manuscript. LG and JZ designed the study and analyzed the data. XX designed the study, revised the manuscript and given final approval of the version to be published. LG and JZ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethical approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD, Wagle NS and Jemal A: Cancer statistics, 2023. CA Cancer J Clin 73: 17-48, 2023.
2. Maomao C, He L, Dianqin S, Siyi H, Xinxin Y, Fan Y, Shaoli Z, Changfa X, Lin L, Ji P and Wanqing C: Current cancer burden in China: Epidemiology, etiology, and prevention. Cancer Biol Med 19: 1121-1138, 2022.
3. Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL and Paz-Ares L: Lung cancer: Current therapies and new targeted treatments. Lancet 389: 299-311, 2017.
4. Passaro A, Jänne PA, Mok T and Peters S: Overcoming therapy resistance in EGFR-mutant lung cancer. Nat Cancer 2: 377-391, 2021.
5. Schimanski S, Wild PJ, Treeck O, Horn F, Sigruener A, Rudolph C, Blaszyk H, Klinkhammer-Schalke M, Ortmann O, Hartmann A and Schmitz G: Expression of the lipid transporters ABCA3 and ABCA1 is diminished in human breast cancer tissue. Horm Metab Res 42: 102-109, 2010.
6. Steinbach D, Gillet JP, Sauerbrey A, Gruhn B, Dawczynski K, Bertholet V, de Longueville F, Zintl F, Remacle J and Efferth T: ABCA3 as a possible cause of drug resistance in childhood acute myeloid leukemia. Clin Cancer Res 12: 4357-4363, 2006.
7. Matsumura Y, Sakai H, Sasaki M, Ban N and Inagaki N: ABCA3-mediated choline-phospholipids uptake into intracellular vesicles in A549 cells. FEBS Let 581: 3139-3144, 2007.
8. Overbeck TR, Arnemann J, Waldmann-Beushausen R, Trümper L, Schöndube FA, Reuter-Jessen K and Danner BC: ABCA3 phenotype in non-small cell lung cancer indicates poor outcome. Oncology 93: 270-278, 2017.
9. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK and Varambally S: UALCAN: A portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 19: 649-658, 2017.
10. Goldman MJ, Craft B, Hastie M, Repečka K, McDade F, Kamath A, Banerjee A, Luo Y, Rogers D, Brooks AN, *et al*: Visualizing and interpreting cancer genomics data via the Xena platform. Nat Biotechnol 38: 675-678, 2020.
11. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, *et al*: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6: pl1, 2013.
12. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, *et al*: The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. Cancer Discov 2: 401-404, 2012.
13. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pagès F, Trajanoski Z and Galon J: ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics 25: 1091-1093, 2009.
14. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T: Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 13: 2498-2504, 2003.
15. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, *et al*: STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 47 (D1): D607-D613, 2019.
16. Detterbeck FC, Boffa DJ, Kim AW and Tanoue LT: The eighth edition lung cancer stage classification. Chest 151: 193-203, 2017.
17. Hutchinson BD, Shroff GS, Truong MT and Ko JP: Spectrum of lung adenocarcinoma. Semin Ultrasound CT MR 40: 255-264, 2019.
18. Ruiz-Cordero R and Devine WP: Targeted therapy and checkpoint immunotherapy in lung cancer. Surg Pathol Clin 13: 17-33, 2020.
19. Leonetti A, Sharma S, Minari R, Perego P, Giovannetti E and Tiseo M: Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. Br J Cancer 121: 725-737, 2019.
20. de Lima LT, Bueno CT, Vivona D, Hirata RD, Hirata MH, Hungria VT, Chiattone CS, Zanichelli MA, Chauffaille Mde L and Guerra-Shinohara EM: Relationship between SLCO1B3 and ABCA3 polymorphisms and imatinib response in chronic myeloid leukemia patients. Hematology 20: 137-142, 2015.
21. Liu J, Ren L, Li S, Li W, Zheng X, Yang Y, Fu W, Yi J, Wang J and Du G: The biology, function, and applications of exosomes in cancer. Acta Pharm Sin B 11: 2783-2797, 2021.

22. Lamouille S, Xu J and Derynck R: Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 15: 178-196, 2014.
23. De Craene B and Berx G: Regulatory networks defining EMT during cancer initiation and progression. Nat Rev Cancer 13: 97-110, 2013.
24. Singh M, Yelle N, Venugopal C and Singh SK: EMT: Mechanisms and therapeutic implications. Pharmacol Ther 182: 80-94, 2018.
25. Loh CY, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, Chong PP and Looi CY: The E-cadherin and N-cadherin switch in epithelial-to-mesenchymal transition: Signaling, therapeutic implications, and challenges. Cells 8: 1118, 2019.
26. Satelli A and Li S: Vimentin in cancer and its potential as a molecular target for cancer therapy. Cell Mol Life Sci 68: 3033-3046, 2011.
27. Kaminski WE, Piehler A and Wenzel JJ: ABC A-subfamily transporters: Structure, function and disease. Biochim Biophys Acta 1762: 510-524, 2006.
28. Fish L, Navickas A, Culbertson B, Xu Y, Nguyen HCB, Zhang S, Hochman M, Okimoto R, Dill BD, Molina H, *et al*: Nuclear TARBP2 drives oncogenic dysregulation of RNA splicing and decay. Mol Cell 75: 967-981.e9, 2019.