Heliyon 10 (2024) e36897

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The role of ESM1 in the lipids metabolic reprogramming and angiogenesis of lung adenocarcinoma cells

Wenchang Feng [a], Yi Ting [b], Xing Tang [c], Dan Liu [c], Wen-chao Zhou [c], Yukun Li [c,*], Zhenyu Shen [d,**]

[a] Cardiology Department, The Third Xiangya Hospital, Central South University, Changsha, China
[b] Department of Trauma Center, Zhuzhou Central Hospital, Xiangya Hospital Zhuzhou Central South University, Central South University, Zhuzhou, Hunan, China
[c] Department of Assisted Reproductive Centre, Zhuzhou Central Hospital, Xiangya Hospital Zhuzhou Central South University, Central South University, Zhuzhou, Hunan, China
[d] Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Hunan University of Medicine, Huaihua, 418000, China

ARTICLE INFO

Keywords:
Lung adenocarcinoma
ESM1
AKT pathway
Tumor metabolic reprogramming
Angiogenesis

ABSTRACT

Background: Lung adenocarcinoma (LUAD) is one of the respiratory diseases with high mortality and incidence. As an important angiogenic factor, (Endothelial cell-specific molecule 1) ESM1 plays an important role in the occurrence and development of LUAD. However, the role and molecular mechanism of ESM1 on LUAD metabolic reprogramming and angiogenesis remain unclear.

Methods: We used multiple databases to analyze the prognostic significance and potential function of ESM1 in patients with LUAD. The expression of ESM1 in LUAD cells was down-regulated/overexpressed by RNA interference, and the effects of ESM1 on the proliferation, migration, lipid metabolism and angiogenesis of LUAD cells in vitro and in vivo were analyzed using MTT, EdU, wound healing, oil red O, tubule formation, xenograft tumor model and chicken embryo allantoic model.

Results: ESM1 is closely associated with poor prognosis in LUAD patients. ESM1 promotes LUAD proliferation, migration, fatty acid synthesis and angiogenesis. It also accelerates the proliferation, migration, lipid synthesis and tubule formation of endothelial cells in the tumor microenvironment in the form of secreted protein. Mechanically, ESM1 can promote the activation of AKT signaling pathway and up-regulate the expression of SCD1 and FASN.

Conclusion: Our results suggest that ESM1 promotes the proliferation, migration, lipid reprogramming, and angiogenesis of LUAD cells by activating the AKT signaling pathway, suggesting that ESM1 may be a potential therapeutic target and prognostic marker in LUAD patients.

1. Introduction

As one of the most common tissue types of lung cancer, the incidence of lung adenocarcinoma (LUAD) is increasing year by year, and the mortality rate is still very high despite abundant clinical treatment options [1]. At present, the clinical treatment of LUAD

* Corresponding author.
** Corresponding author.
E-mail addresses: yukun_li@foxmail.com, yukun_li@csu.edu.cn (Y. Li), ZhenYuShen0701@126.com (Z. Shen).

https://doi.org/10.1016/j.heliyon.2024.e36897
Received 4 June 2024; Received in revised form 22 August 2024; Accepted 23 August 2024
Available online 24 August 2024

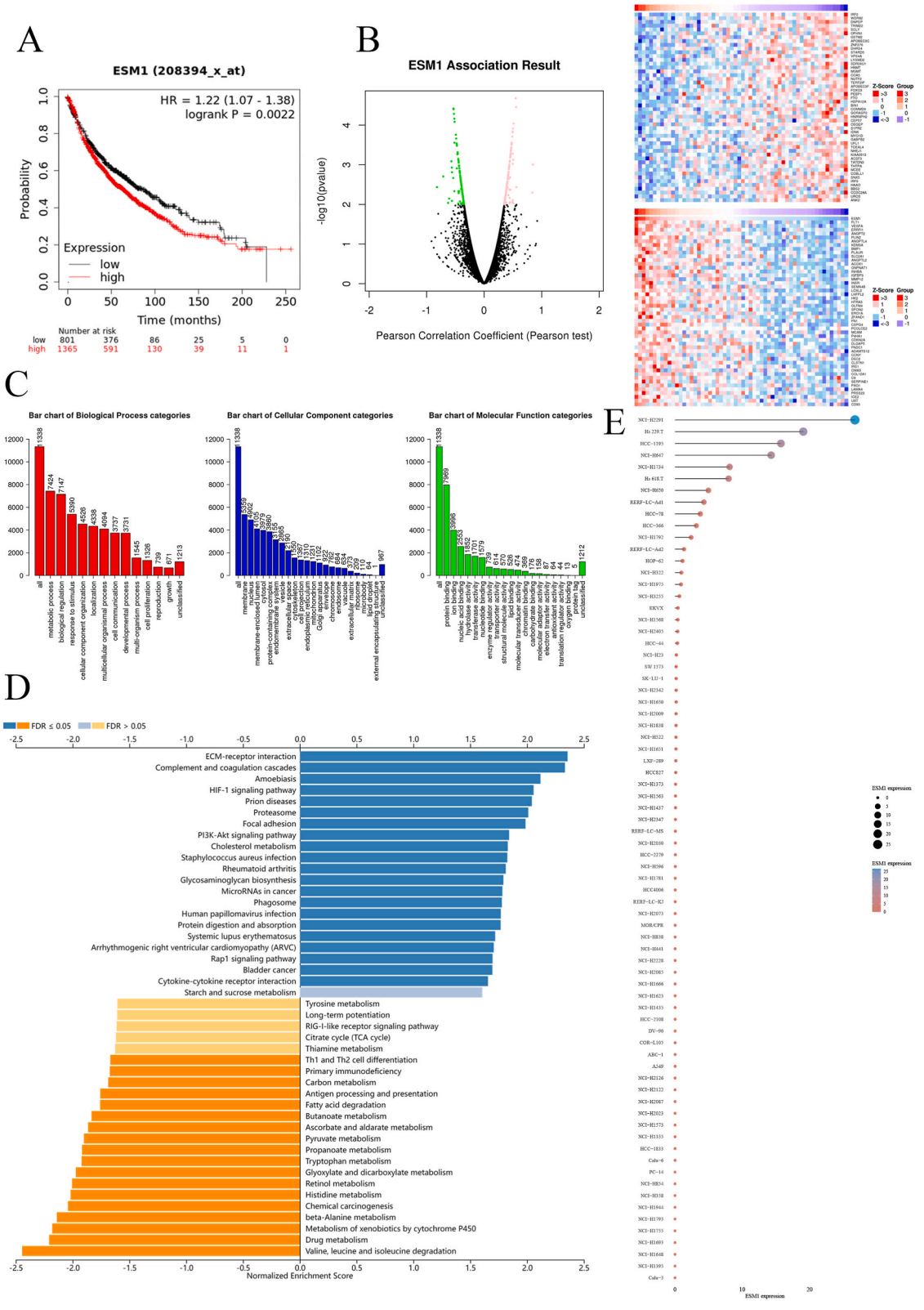

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Fig. 1. The prognostic significance and potential functions of ESM1 in LUAD patients based on multiple databases. A. Survival analysis of ESM1 mRNA expression in LUAD patients was performed using KM-plot database. B. Visualization of ESM1-related genes using volcano maps and heat maps based on LinkedOmics database. C. GO enrichment analysis for ESM1-related genes. D. GSEA analysis for ESM1-related genes. E. The expression of ESM1 in multiple LUAD cancer cell lines based on CCLE database.

includes surgical resection, traditional chemoradiotherapy and targeted therapy. Among them, anti-vascular therapy in targeted therapy plays an important role in the clinical treatment of LUAD[2]. It is important to note that anti-angiogenesis drugs have become an essential part of the treatment of LUAD. There is currently antiangiogenic therapy available in clinics for the treatment of late-stage lung cancer patients, but resistance to antiangiogenic therapy often arises [3]. Therefore, in the field of basic medical research, it is extremely important to find molecular targets and mechanisms to solve the problem of bevacizumab resistance.

Endothelial cell-specific molecule 1 (ESM1), as an important angiogenic factor, is primarily expressed in endothelial cells of the lungs and kidneys [4]. Previous studies have indicated that ESM1 has significantly high expression changes in a variety of tumors, including cervical cancer [5], gastric cancer [6], colorectal cancer [7], prostate cancer [8], and melanoma [9]. Moreover, our previous studies have shown that ESM1 also plays an important role in ovarian cancer by promoting the activation of Akt signaling pathway and interacting with ANGPTL4 to accelerate ovarian cancer cell proliferation, migration, invasion, angiogenesis and lipid metabolism reprogramming [10,11]. However, the function and molecular mechanisms of ESM1 in lung cancer remain unknown.

Reprogramming lipid metabolism is one of the malignant characteristics of cancer cells [12]. This reprogramming allows cancer cells to better adapt to the tumor microenvironment and promotes rapid growth by altering lipid uptake, storage, and expulsion [13]. In lung cancer, two crucial enzymes, FASN and SCD1, play significant roles in lipid metabolism [14,15]. Prior research has indicated a strong correlation between bevacizumab resistance in tumor patients and lipid metabolic reprogramming. Additionally, it has been observed that in cases where microvessel count is minimal, fatty acids can expedite tumor growth [16]. Consequently, investigating the molecular regulatory mechanism underlying tumor angiogenesis and lipid reprogramming holds significant potential in advancing clinical tumor therapy.

In this study, we identified the prognostic significance of ESM1 in LUAD patients through molecular biology, and further analyzed its possible downstream molecular biological functions and mechanisms. In molecular biology experiments, we found that ESM1 knockdown can inhibit the proliferation, migration, lipid metabolism reprogramming and angiogenesis of LUAD cells, and its molecular mechanism is related to the activation of Akt signaling pathway by ESM1 to promote FASN and SCD1.

2. Methods

2.1. Bioinformatic analysis

We analyzed the prognostic value of ESM1 mRNA in LUAD patients using the KM-plot database (https://kmplot.com/) [17]. We used LinkedOmics database (https://www.linkedomics.org/) [18] to extract ESM1-related genes in LUAD patients, and conducted subsequent GO and GSEA enrichment analysis. The Cancer Cell Line Encyclopedia (CCLE) database (https://sites.broadinstitute.org/) [19] was used to identify differences in ESM1 expression in LUAD cell lines.

2.2. Cell culture and transfection

Human normal lung bronchial epithelial cells (BEAS-2B), human umbilical vein endothelial cell (HUVECs) and LUAD cancer cell lines (H1975, H1299, H1734, A549 and H2291) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA), which were cultured with RPMI-1640 medium (Hyclone, USA) with 10 % fetal bovine serum (Gibco, USA) at 37 °C and 5 % CO_2. ESM1 shRNAs and overexpressed plasmids were purchased from HornorGene (Changsha, China). Lipofectamine® 3000 (Thermo Fisher Scientific, Inc.) was used in the cell transfection experiment, and the specific method was referred to the instructions of the kit. ESM1 shRNA target sequences were as follows: shESM1: GCAATAATTATGCGGTGGACT.

2.3. Proliferation assay

For MTT assay, 5000 cells were seeded in 96-well plates and cultured in incubators for 1,2,3 days. Then, 20 μl MTT solution (5 mg/ml, Sigma–Aldrich; Merck KGaA) was added to each well and after further culture for 4 h, 150 μl of DMSO was added to each well to fully dissolve precipitates. Finally, OD values were confirmed by microplate reader (Molecular Devices, LLC). For EdU staining assay, operations were following the EDU kit's operating guidelines (RiboBio, Guangzhou, China).

2.4. Wound healing

When $5X10^5$ LUAD cells were planted in six-well plates and cultured to a cell density of 70 % in an incubator, scratches were performed in the six-well plates using a 100 μL pipette gun head, and then the dewalled cells were cleaned with PBS, and complete media was added to each hole. The migration of cells in the scratches was observed at 0 h and 24 h, respectively.

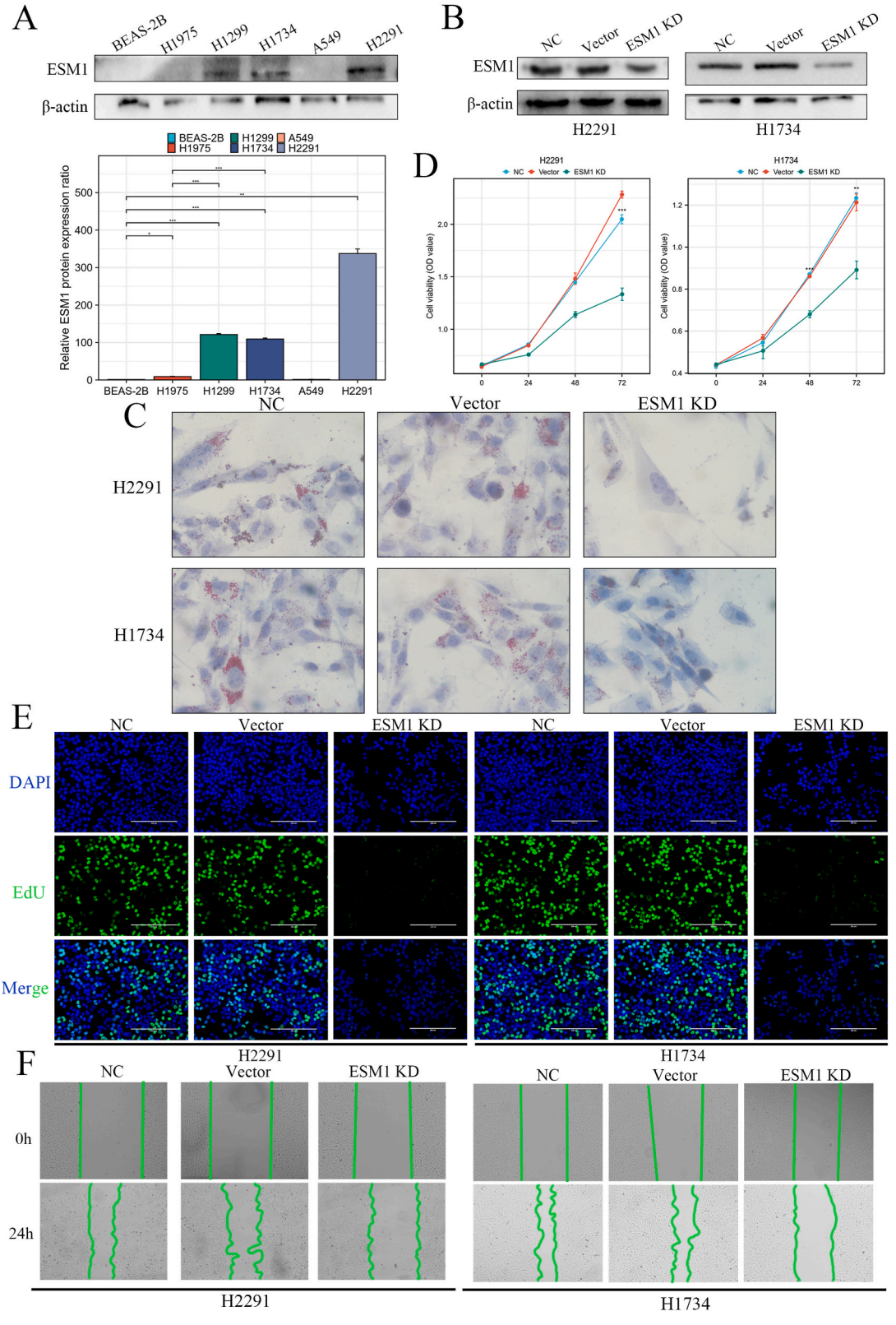

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Fig. 2. The effects of ESM1 knockdown on LUAD cell proliferation, migration and lipid metabolism reprogramming. A. The expression of ESM1 protein in multiple LUAD cancer cell lines by Western blot (The Original data could be seen in Supplementary Material-Original data of WB-Fig. 2A). B. The expression of ESM1 in H2291 and H1734 with ESM1 knockdown or not by Western blot (The Original data could be seen in Supplementary Material-Original data of WB-Fig. 2B). C. Oil red O staining showed the effect of ESM1 knockdown on fatty acids in LUAD cells. D. MTT analysis showed the effect of ESM1 knockdown on the proliferation of LUAD cells. E. EdU staining was used to analyze the effect of ESM1 KD on the proliferation of LUAD cells. F. The effect of ESM1 knockdown on the migration ability of H2291 and H1734 cells was analyzed by wound healing assay. *P < 0.05, **P < 0.01, ***P < 0.001 represents significant differences compared with the vector group.

2.5. Tube formation assay

Matrigel (200 μl) was introduced into each well of a 48-well plate, followed by the seeding of 1 × 104 primary HUVECs in 50 μl of conditioned medium obtained from H2291, H1734 or A549 cells. Subsequently, the plate was incubated at 37 °C for 6 h, and images were captured using a fluorescence microscope.

2.6. Western blot and IHC staining

Please refer to our previous studies for details [10,11]. In WB analysis, the cell samples required for the experiment were lysed by adding an appropriate amount of a mixture of RIPA (P0013B, Biyuntian) and PMSF (ST505, Biyuntian). The protein concentration of the resulting supernatant was quantified using a BCA kit (E112-01, Vazyme). Subsequently, the protein supernatant was combined in proportion with 5 × protein loading buffer (SL1170-1 ml, Solebo) and incubated at 100 °C for 5 min. The prepared protein samples were then sequentially loaded onto a 10 % SDS-PAGE gel (SK6010-250T, Coolaber), followed by electrophoresis and membrane transfer. After gently picking up the PVDF membrane with tweezers, immerse it in TBST containing 5 % skim milk and incubate for 2 h at room temperature. Subsequently, wash the PVDF membrane in TBST and add the appropriate primary antibody working solution. Incubate the membrane for 12 h on a shaker at 4 °C. Following the primary antibody incubation, wash the PVDF membrane in TBST and incubate it with the corresponding HRP-labeled goat anti-rabbit IgG secondary antibody (L3012, Solebo) or HRP-labeled goat anti-mouse IgG secondary antibody working solution for 1 h. The PVDF membrane was washed with TBST, subsequently incubated with the standard ECL luminescence solution (K-12045-D10, Advansta), and then subjected to development and imaging using the chemiluminescence imaging analysis system (MiniChemi610, System).

The primary antibodies used were as follows: ESM1 (Abcam, ab103590), Akt (Abcam, ab8805), p-AKT (Abcam, ab38449), mTOR (Abcam, ab245370), p-mTOR (Abcam, ab109268), PCNA (Abcam, ab265609), FASN (Abcam, ab99359), SCD1 (Abcam, ab236868) and β-actin (Abcam, ab8226).

2.7. Oil red O stain

Cells that were cultured in 6-well plates underwent three washes with PBS and were subsequently fixed in a 3.7 % aldehyde solution for a duration of 30 min prior to staining. Following fixation, the cells were rinsed with distilled water and exposed to 60 % isopropyl alcohol for a period of 30 s. Subsequently, the cells were stained with oil red O solution under room temperature and in the absence of light for a duration of 12 min. To eliminate the dye solution, a 7-s wash with 60 % isopropyl alcohol was performed. The cells were then rinsed twice with distilled water and subsequently photographed for the purpose of quantification.

2.8. Xenograft tumor model

A cohort of $4*10^6$ H1299 cells transfected with vector and H1299 cells transfected with ESM1 knockdown were subcutaneously injected into the left and right abdomen of 4-week-old female mice, respectively. Tumor growth was monitored every ten days using vernier calipers, and upon euthanization of the nude mice on the 30th day, the tumors were excised and weighed. The transplanted tumors were fixed with formalin and processed into paraffin sections for subsequent IHC staining.

2.9. Chorioallantoic membrane assay

Please refer to our previous studies for details [10,11]. In brief, the entire incubation process was conducted at a temperature of 38 ± 0.5 °C and a relative humidity ranging from 65 % to 70 %. After a 7-day incubation period, chicken embryos were extracted. The position of the egg chamber was marked using a lamp pen, and the shell surface was disinfected with iodine solution. A small hole was then created in the surface of the egg chamber using a sharp needle, and the air chamber was opened with surgical forceps to create a window approximately 1.5 cm by 1.5 cm in size. Drop a small amount of saline in dent place, gently shake off the inner membrane, exposed the CAM. A silicone ring was placed on the chick embryo allantoic membrane, and 100 μl of different groups of conditioned medium was added to the ring. Then use sterile waterproof breathable stickers will air chamber mouth closed. After 24 h open air chamber, in asana microscope chicken embryo allantois membrane angiogenesis and take photos.

2.10. Statistical analysis

R language was used for T test and one-way analysis of variance in all experiments. When the *p*-value was less than 0.05, it was

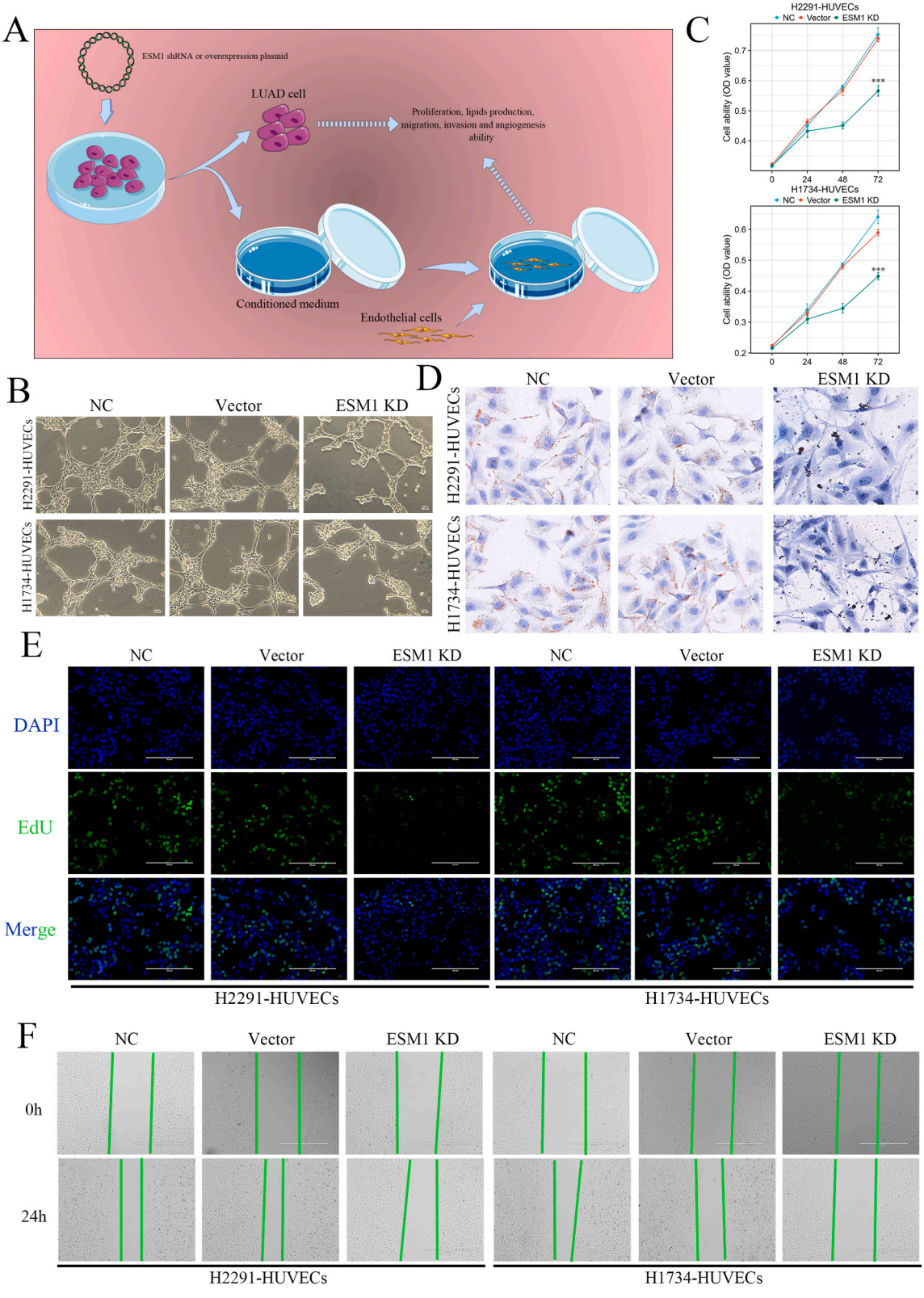

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Fig. 3. ESM1 knockdown promote LUAD angiogenesis by regulating HUVEC proliferation, migration and lipid metabolism reprogramming. A. Illustration of the workflow for collecting LUAD conditional media for processing HUVECs. B. The tube formation ability of HUVECs in LUAD conditional media with ESM1 knockdown or not by tube formation assay. C. MTT analysis showed the effect of ESM1 knockdown on HUVECs in LUAD conditional media. D. Oil red O staining showed the effect of ESM1 knockdown on fatty acids in HUVECs with LUAD conditional media. E. EdU staining was used to analyze the effect of ESM1 KD on the proliferation of HUVECs with LUAD conditional media. F. The effect of ESM1 knockdown on the migration ability of HUVECs in the conditional media of H2291 and H1734 cells was analyzed by wound healing assay. *P < 0.05, **P < 0.01, ***P < 0.001 represents significant differences compared with the vector group.

considered statistically significant.

3. Results

3.1. The prognostic significance and molecular functions in LUAD

In previous studies, ESM1 expression was found to be elevated in non-small cell lung cancer, and its circulating serum level can indicate the level of angiogenic stimulation and prognosis in patients [20]. We used KM-plot database to confirm the prognostic significance of ESM1 in LUAD patients, which showed that high level ESM1 mRNA was significantly correlated with poor prognosis (Fig. 1A). Moreover, we extracted genes associated with ESM1 in LUAD patients based on TCGA database (Fig. 1B). GO enrichment showed metabolic process, biological regulation, response to stimulus, protein-containing complex, mitochondrion, protein binding, lipid binding, and molecular adaptor activity (Fig. 1C). GSEA enrichment showed that ESM1 related genes were enriched in HIF-1 signaling pathway, PI3K-Akt signaling pathway, cholesterol metabolism, cytokine-cytokine receptor interaction, fatty acid degradation, pyruvate metabolism, and drug metabolism (Fig. 1D). The expression of ESM1 mRNA in multiple LUAD cell lines were further confirmed in CCLE database (Fig. 1E).

3.2. The effects of ESM1 on LUAD cell lipid metabolism reprogramming, proliferation and migration

Next, we confirmed the ESM1 expression in human normal lung bronchial epithelial cells (BEAS-2B) and multiple LUAD cancer cell lines (H1975, H1299, H1734, A549 and H2291) by Western blot (Fig. 2A). We further knockdown ESM1 expression in H2291 and H1734 cell lines (Fig. 2B). Oil red O staining showed that ESM1 knockdown significantly inhibited lipid synthesis in LUAD cells (Fig. 2C). MTT analysis showed that ESM1 knockdown inhibited the proliferation of LUAD cells (Fig. 2D). EdU staining also showed that the proliferation ability was repressed by ESM1 knockdown in H2291 and H1734 cell (Fig. 2E). The migration ability of H2291 and H1734 was obviously inhibited by ESM1 knockdown by wound healing assay (Fig. 2F).

3.3. ESM1 knockdown inhibits LUAD angiogenesis in vitro

Then, we cultured endothelial cells with conditioned media of LUAD cells in different groups, and further examined the proliferation, migration, lipid reprogramming, and tubule formation of these endothelial cells (Fig. 3A). Tube formation analysis showed that ESM1 knockdown could significantly inhibit the angiogenesis level of HUVECs co-cultured with conditioned media (Fig. 3B). MTT analysis showed that the proliferation ability of HUVECs was inhibited in the conditioned media derived from LUAD cells after ESM1 knockdown (Fig. 3C). Oil red O staining showed that ESM1 knockdown significantly inhibited lipid synthesis in HUVECs co-cultured with conditioned media of LUAD cells (Fig. 3D). EdU staining also showed that the proliferation ability was repressed by ESM1 knockdown in endothelial cells co-cultured with conditioned media of H2291 and H1734 cell (Fig. 2E). Moreover, the migration ability of HUVECs co-cultured with conditioned media of H2291 and H1734 cell with ESM1 knockdown was significantly decreased compared to NC and vector group (Fig. 3F).

3.4. ESM1 knockdown inhibits LUAD growth, lipids production and angiogenesis by inactivating AKT signaling pathway in vivo

Furthermore, we constructed xenograft tumor model for further confirming the effects of ESM1 knockdown on LUAD growth, lipids production and angiogenesis in vivo (Fig. 4A). The tumor weight and volume were both significantly decreased in ESM1 KD group of H2291 (Fig. 4B). IHC staining showed the expression of ESM1, p-AKT, p-mTOR, SCD1, FASN and PCNA were both inhibited in xenografts with ESM1 knockdown compared to vector group (Fig. 4C).

CAM is an in vitro model commonly used to study angiogenesis and tumor growth. In this model, the chicken embryo allantois membrane angiogenesis is used as a research platform, because it is a highly vascularized outer membrane and the innervation of the embryo, is suitable for simulating the angiogenesis induced by tumor [21]. In tumor research, various cancer cell types can be transplanted into CAM for tumor cultivation, which provides a relatively simple model for studying tumor formation in different cancers. CAM model has a high degree of repeatability and cost effective, and does not require ethics committee approval, suitable for cell transplantation and drug tests [22]. In our study, CAM assay was utilized to detect the role of ESM1 in LUAD angiogenesis in vivo, which showed that ESM1 knockdown could obviously repress the density of blood vessels (Fig. 4D).

Based on our previous GSEA results (Fig. 1D), we decided to further examine the effect of ESM1 on the AKT signaling pathway. The Western blot showed that ESM1 knockdown could repress the activity of AKT signaling pathway and the expression of FASN and SCD1, and the AKT agonist (SC79) could reverse the molecular effects of ESM1 knockdown (Fig. 4E). Therefore, ESM1 could upregulate FASN

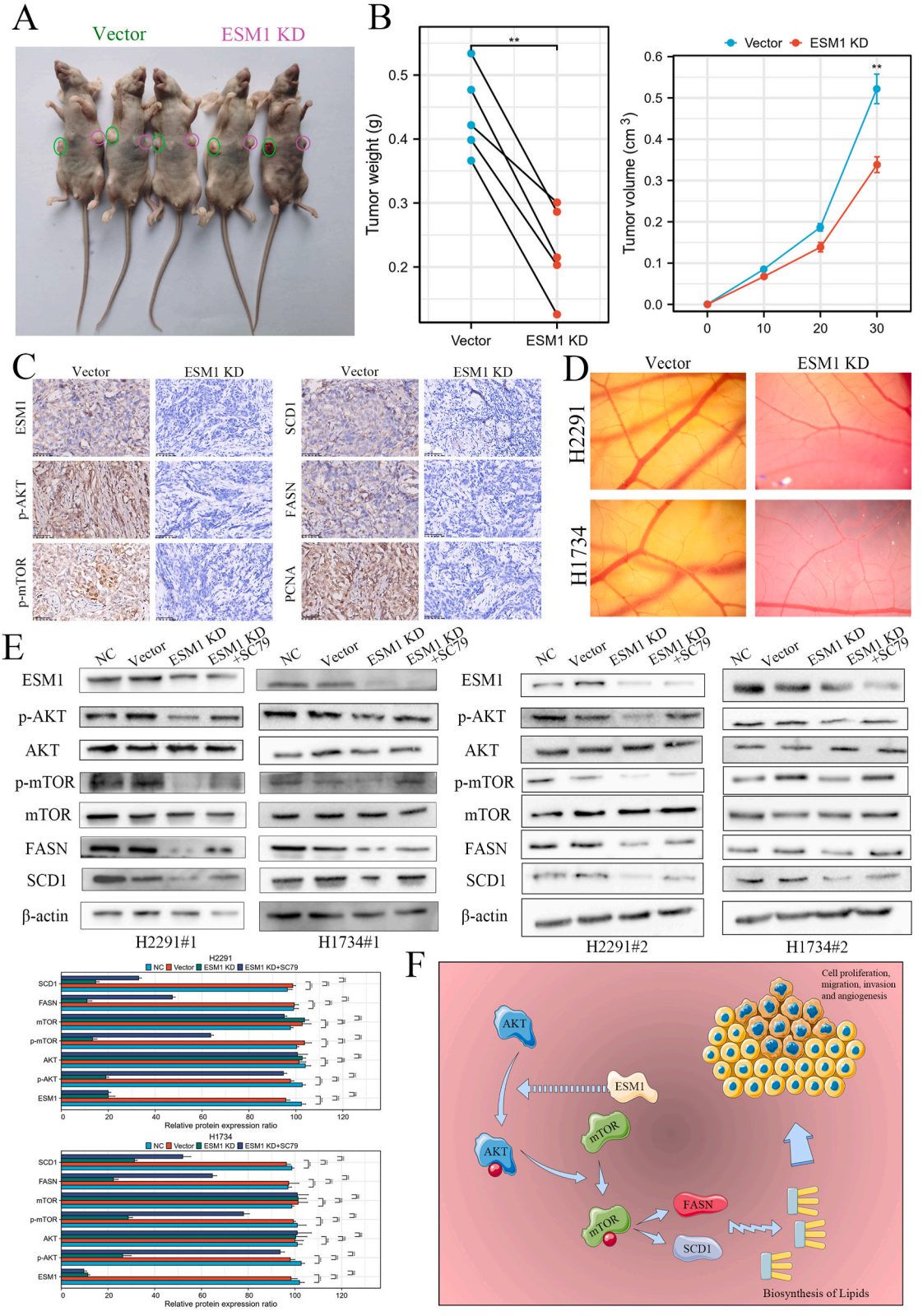

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Fig. 4. ESM1 knockdown impeded LUAD growth and angiogenesis by activating AKT pathway. A. The effects of ESM1 shRNA lentivirus plasmid transfection or empty vector lentivirus plasmid transfection on H2291 growth in vivo by xenograft model. The green circle marks the Vector group and the purple circle marks the ESM1 knockdown group. B. Differences in xenografts volume and weight. C. The effects of ESM1 knockdown on p-AKT, p-mTOR, SCD1, FASN and PCNA were determined by IHC staining. D. The effect of ESM1 knockdown on the angiogenesis of LUAD in vivo was examined in chick embryo allantoic model. E. The effect of ESM1 knockdown on AKT signaling pathway was detected by Western blot (The Original data could be seen in Supplementary Material-Original data of WB-Fig. 4E#1 and Fig. 4E#2). F. Mechanism diagram of ESM1 promoting LUAD cell proliferation, migration, lipid reprogramming, and angiogenesis via AKT signaling pathway. *P < 0.05, **P < 0.01, ***P < 0.001 represents significant differences compared with the vector group.

and SCD1 to promote biosynthesis of lipids, proliferation, migration and angiogenesis by activating AKT pathway (Fig. 4F).

3.5. ESM1 overexpression promote LUAD cell proliferation, migration and angiogenesis by AKT pathway

Last but not least, we constructed ESM1 overexpressed A549 cell and detected the effects of AKT inhibitor (LY294002) on ESM1 in A549 cell (Fig. 5A). Oil red O staining showed that ESM1 could promote lipid synthesis compared to NC and vector group, which could be reversed by LY294002 (Fig. 5B). MTT assay showed that ESM1 promoted A549 proliferation and reversed by LY294002 (Fig. 5C). EdU assay also showed that ESM1 promoted A549 proliferation, and LY294002 could counter the effects of ESM1 (Fig. 5D). wound healing assay showed that ESM1 could significantly enhance the migration ability in A549 cell, which could be reversed by LY294002 (Fig. 5E).

In terms of angiogenesis, we found that ESM1 significantly promoted the tube-forming ability of endothelial cells co-cultured in conditioned medium of A549 (Fig. 5F). Oil red O staining showed that ESM1 significantly enhanced lipid synthesis in HUVECs co-cultured with conditioned media of LUAD cells (Fig. 5G). MTT analysis showed that the proliferation ability of HUVECs was inhibited in the conditioned media derived from LUAD cells after ESM1 (Fig. 5H). EdU staining also showed that the proliferation ability was upregulated by ESM1 in endothelial cells co-cultured with conditioned media of A549, which could be rescued by LY294002 (Fig. 5I). Furthermore, the migration ability of HUVECs co-cultured with conditioned media of A549 cell with ESM1 overexpression was significantly increased compared to NC and vector group, and rescued by AKT inhibitor (Fig. 5J).

4. Discussion

In this study, we confirmed that ESM1 was a significant oncogene in LUAD fatty acid synthesis, proliferation, migration and angiogenesis. Multiple studies suggested that ESM1 was an important factor in promoting cancer progression, including gastric cancer, liver cancer, breast cancer, cervical cancer, and melanoma. Our previous study also found that ESM1 could promote ovarian cancer cell proliferation, migration, invasion, lipids accumulation and angiogenesis [10,11]. Lu et al. found that the level of ESM1 in pleural effusion had a valuable diagnostic and prognostic significance for lung cancer patients [23]. Grigoriu et al. indicated that ESM1 expression was significantly and positively correlated poor prognosis in human patients with lung cancer [20]. Gu et al. found that ESM1 could drive chronic intermittent hypoxia-mediated lung cancer stemness, invasion and proliferation via activating HIF-1α pathway [24]. However, whether ESM1 influences lipid metabolic reprogramming in lung cancer and the molecular mechanism of how it promotes LUAD development remain unclear.

In our previous study, we found ESM1 could promote ovarian cancer lipid metabolic reprogramming [11]. Therefore, we tested the effects of ESM1 on the lipids production in LUAD cell. ESM1 overexpression could significantly promote lipids accumulation in LUAD cell, and accelerate cell proliferation and migration. Lipid reprogramming plays an important role in maintaining the proliferation, migration and angiogenesis of LUAD cells. High lipid levels provide energy supply and necessary nutrient synthesis substrates for the molecular functions of LUAD cells [25,26]. Therefore, ESM1 may act as a key metabolic regulator in LUAD, promoting cell proliferation, migration, and angiogenesis by promoting lipid reprogramming in cancer cells and accelerating lipid synthesis.

SCD1 and FASN are key lipid metabolizing enzymes in cell metabolism, both of which promote the synthesis of fatty acids [27]. Wohlhieter et al. found that SCD1 is one of the key factors affecting ferroptosis in LUAD cells and is a potential therapeutic target for LUAD[28]. Shen et al. also found that Anlotinib could impede lipid metabolism to repress LUAD proliferation and growth by inhibiting FASN[29]. Zhu et al. found that FASN was significantly correlated with LUAD induced by $PM_{2.5}$ exposure [30]. In our study, we found ESM1 could upregulate the expression of SCD1 and FASN to promote proliferation, migration and angiogenesis in LUAD cell. These results both indicated that ESM1-SCD1/FASN axis played a key role in the development and progression of LUAD.

Based on the results of GSEA enrichment analysis in our study, we found that ESM1 may have a molecular biological role in activating the AKT signaling pathway (Fig. 1D). We further found by Western blot that ESM1 could activate the AKT signaling pathway and promote the expression of FASN and SCD1, the key enzymes for lipid acid synthesis. Yang et al. found that ESM1 could accelerate colorectal cancer angiogenesis by activating AKT signaling pathway [7]. Liu et al. confirmed that ESM1 promote breast cancer proliferation by activating AKT/NF-KB/Cyclin D1 pathway [31]. Lu et al. found that ESM1 could promote SYT13 to activating AKT pathway in cervical cancer [5]. Zhu eta al also suggested that AKT signaling pathway was downstream pathway of ESM1 in human gliomas to promote angiogenesis [32]. In ovarian cancer, we also found ESM1 could promote AKT pathway to accelerate cancer cell proliferation, migration, invasion and angiogenesis [10]. ESM1 could drive Warburg effect to induce vascular mimicry, lactic acid and fatty acid synthesis by interacting with PKM2 and ANGPTL4 in ovarian cancer [11,33]. Qi et al. found that ESM1 could promote cervical cancer EMT cascade by driving ZEB1/PI3K/AKT pathway [34]. Moreover, lactate could enhance ESM1 expression to inhibit immune infiltration and promote cisplatin resistance in ovarian cancer by activating SCD1/Wnt pathway [35]. Zhao et al. also found

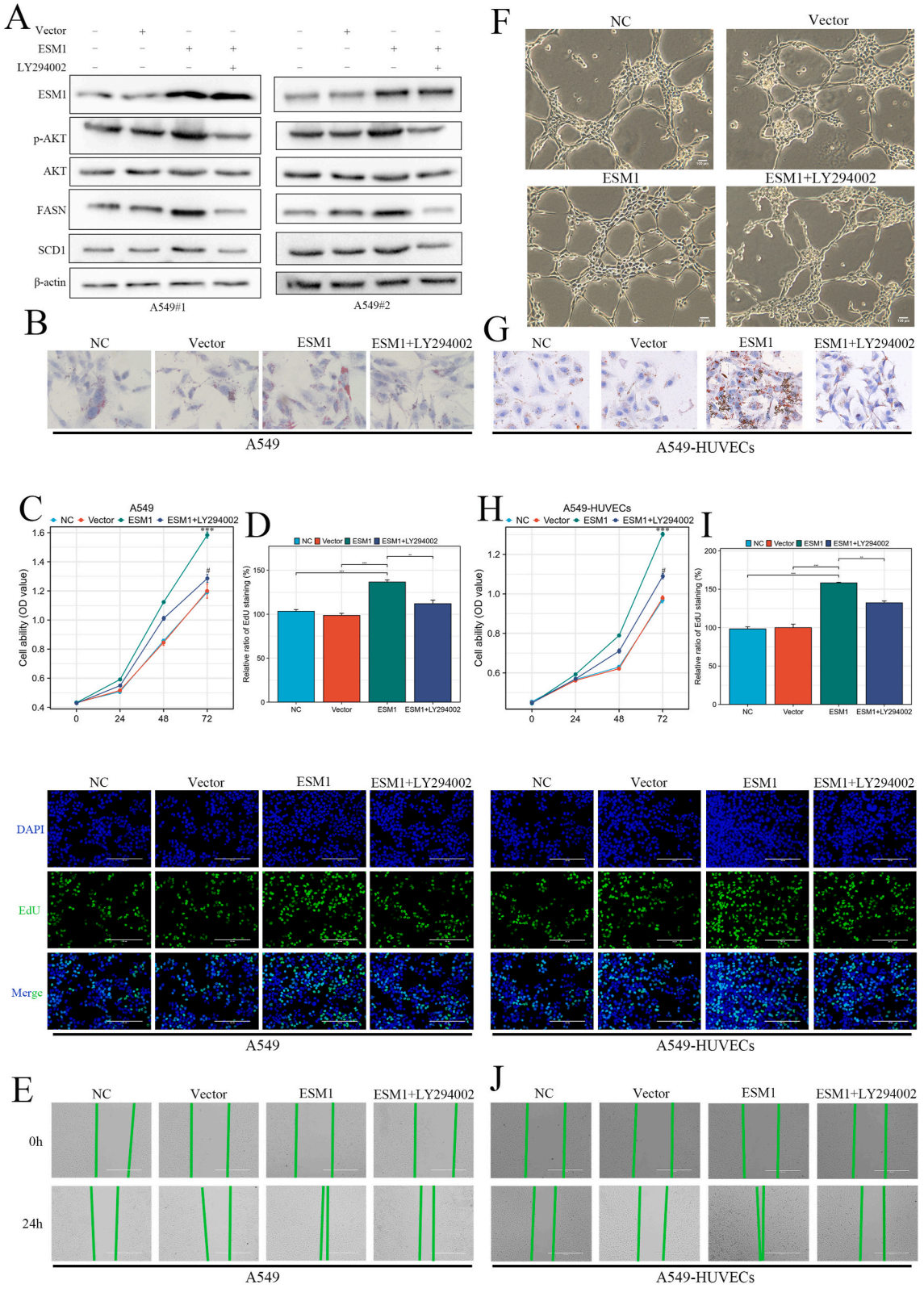

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Fig. 5. ESM1 overexpression promote A549 cell proliferation, migration, angiogenesis and lipid metabolism reprogramming by activating AKT pathway. A. The expression of ESM1 in A549 with vector plasmid transfection, ESM1 plasmid transfection, or ESM1 plasmid transfection plus LY294002 treatment by Western blot (The Original data could be seen in Supplementary Material-Original data of WB-Fig. 5A). B. The effects of ESM1 and ESM1 plus AKT inhibitor on lipid metabolism of LUAD cells were detected by oil red O staining. C. MTT analysis showed the effect of ESM1 and ESM1 plus AKT inhibitor on the proliferation of LUAD cells. D. EdU staining was used to analyze the effect of ESM1 and ESM1 plus AKT inhibitor on the proliferation of LUAD cells. E. The effect of ESM1 and ESM1 plus AKT inhibitor on the migration ability of A549 cells was analyzed by wound healing assay. F. The tube formation ability of HUVECs in LUAD conditional media with ESM1 and ESM1 plus AKT inhibitor by tube formation assay. G. Oil red O staining showed the effect of ESM1 and ESM1 plus AKT inhibitor on fatty acids in HUVECs with LUAD conditional media. H. MTT analysis showed the effect of ESM1 and ESM1 plus AKT inhibitor on HUVECs in LUAD conditional media. I. EdU staining was used to analyze the effect of ESM1 and ESM1 plus AKT inhibitor on the proliferation of HUVECs with LUAD conditional media. J. The effect of ESM1 and ESM1 plus AKT inhibitor on the migration ability of HUVECs in the conditional media of A549 cells was analyzed by wound healing assay. *P $<$ 0.05, **P $<$ 0.01, ***P $<$ 0.001 represents significant differences compared with the vector group, #P $<$ 0.05 represents significant differences compared with the ESM1 overexpression group.

that histone lactylation promote liver cancer development by upregulating ESM1 expression [36]. The cross-talk between metabolic reprogramming and the AKT signaling pathway is a key factor in promoting the development of various types of cancer, including colorectal cancer, ovarian cancer, prostate cancer, and lung cancer [35,37–39]. Thus, AKT signaling pathway is a key downstream signaling pathway for ESM1 and may be conserved and critical in multiple cancers, especially in LUAD.

In conclusion, we firstly elucidated the ESM1/AKT/mTOR/FASN/SCD1 axis in LUAD proliferation, migration, lipid metabolism reprogramming and angiogenesis. This work could further confirm that ESM1 was a potential target for LUAD patients.

Funding

This work was supported by the Natural Science Foundation of Hunan Province (Grant No.2024JJ9594).

Data availability statement

All the data generated or analyzed during this study are included in this published article (and its supplementary information files).

Compliance with ethical standards

Disclosure The authors declare that no competing interests exist.

Ethics approval

The questionnaire and methodology for this study were approved by the Human Research Ethics Committee of The Third Xiangya Hospital.

CRediT authorship contribution statement

Wenchang Feng: Writing – original draft, Methodology. **Yi Ting:** Methodology, Data curation. **Xing Tang:** Methodology, Investigation. **Dan Liu:** Methodology, Formal analysis. **Wen-chao Zhou:** Methodology. **Yukun Li:** Writing – review & editing, Conceptualization. **Zhenyu Shen:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36897.

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