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ESM1 Restrains Taxol Resistance in Lung Cancer Cells via Interacting with KRT8

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KEYWORDS Endothelial Cell Specific Molecule 1. Lung Cancer. Keratin 8. Protein Kinase B. Taxol Resistance

ABSTRACT Molecules attract increasing attention in lung cancer treatment. This present study has investigated regulatory effects of ESM1 on lung cancer cell progression. ESM1 was detected to be upregulated in lung cancer tissue samples and cell lines using RT-qPCR. Thereafter, results of CCK-8, transwell and scratch test indicated that ESM1 upregulation facilitated A549 cell viabilities, invasiveness, and migration while ESM1 suppression hampered SK-MES-1 cell viabilities, migration, and invasiveness. Moreover, ESM1 upregulation facilitated A549 cell viabilities, suppressed apoptosis and accelerated EMT after Taxol treatment. However, ESM1 downregulation inhibited Taxol-treated lung cancer cell viabilities, elevated apoptosis and restrained EMT. Furthermore, overexpression of ESM1 upregulated KRT8 expressions. KRT8 upregulation also accelerated Taxol-treated lung cancer cell viabilities and inhibited apoptosis while KRT8 downregulation caused opposite results.

INTRODUCTION

Lung cancer is a critical cause of cancer-related death and it has been classified into different histologic subtypes including squamous carcinoma, large cell carcinoma (NSCLC), adenocarcinoma and small cell lung cancer (SCLC) (Ruiz-Cordero and Devine 2020). The reason of lung cancer on the top of the list is that this malignancy is usually diagnosed at an advanced stage (Nooreldeen and Bach 2021). SCLC takes about 15 percent of all kinds of lung cancer cases, but it is one of most intractable disorders in clinical stage due to the high recurrence rate and low survival rate (Wang et al. 2020). Meanwhile, NSCLC is the most common type with increasing morbidity worldwide (Friedlaender et al. 2020). According to previous evidence, smoking has been confirmed to be the most vital risk factor of lung cancer (MacRosty and Rivera 2020). Beyond that, air pollution and occupational exposure have been detected to be related to increasing lung cancer incidence (Cao and Chen 2019). Hence, smoking cessation and lung cancer screening are needed to decrease incidence rate and mortality of this malignancy (Duma et al. 2019; Peiffer et al. 2020). Options in lung cancer treatment include surgery, chemotherapy, radiotherapy, and targeted therapy (Lemjabbar-Alaoui et al. 2015). What is unsatisfactory is that despite advancement in these treatments, the prognosis of patients remains low (Miller and Hanna 2021). Hence, new drugs are needed to be discovered for lung cancer treatment. Taxol, a natural product segregated from the Pacific yew, has been observed to be one of the most effective chemotherapy drugs for lung cancer treatment (Alqahtani et al. 2019; Zhang et al. 2019). However, resistance of this drug limits its efficacy in lung cancer treatment. Hence, molecules that help increase Taxol effects might provide promising therapeutic approaches for lung cancer. Endothelial cell specific molecule 1 (ESM1), also named as endocan, is a 50kDa soluble endothelial cell-associated proteoglycan (Xu et al. 2019). ESM1 has been reported to act as an oncogene in many kinds of tumors and express preferentially in endotheli-

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um of tumors (Abid et al. 2006). ESM1 has also been verified to be upregulated in breast cancer cell tissues with accelerating migration, invasiveness and proliferation via activating AKT/NF-κB/CyclinD1 signaling pathway (Liu et al. 2021). ESM1 was discovered to be promoted by the overexpression of lncRNA small nucleolar RNA host gene 14, resulting in increased proliferative, migrative and invasive abilities and suppressed cell apoptosis of bladder cancer cells (Feng et al. 2021). ESM1 downregulation accelerated adrenocortical carcinoma cell apoptosis and suppressed cell invasiveness, migration and viabilities (Huang et al. 2021). In lung cancer tissues, ESM1 has been found to be upregulated and promote EGFR signaling through interacting with EGF-EGFR, resulting in facilitated tumorigenesis (Yang et al. 2020). However, deep molecular mechanisms about ESM1 in lung cancer remain unclear.

Keratin 8 (KRT8) is one of the most essential keratin proteins with increasing resistance to cell apoptosis (Miao et al. 2020). Overexpression of KRT8 were demonstrated to accelerate proliferation, EMT and migration of gastric cancer cells while the inhibition of KRT8 hampered gastric cancer cell migration and EMT but had no impacts on cell proliferation (Fang et al. 2017). Moreover, KRT8 has been verified to be an oncogenic biomarker in lung cancer. KRT8 expressions were increased in both LUSC and LUAD tissue samples, while it has correlation with LUAD development instead of LUSC (Xie et al. 2019). Additionally, the knockdown of KRT8 hindered LUAD cell proliferation, invasiveness, EMT and migration but induced cell apoptosis via inactivating NF-κB signaling pathway (Chen et al. 2022). Therefore, research about roles of KRT8 in lung cancer cells might bring novel approaches for lung cancer treatment. Herein, researchers hypothesized that ESM1 might interact with KRT8 to modulate Taxol sensitivity in lung cancer cells.

Objectives

The present study aimed at investigating impacts of ESM1 on modulating lung cancer cell viabilities, invasiveness, migration, apoptosis and EMT and effects of ESM1 on in modulating Taxol sensitivity in lung cancer cell progression with KRT8 and AKT signaling pathway.

Experimental

Main Reagents

Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA), fetal bovine serum (FBS, Gibco), penicillin/ streptomycin (Gibco), iScript cDNA Synthesis Kit (Bio-Rad, USA), SYBR Green Supermix (Bio-Rad), TRIzol reagent (Invitrogen), CCK-8 (Ameresco, USA), Taxol (HY-B0015, MedChemExpress, USA), Lipofectamine 2000 (Invitrogen, USA), trypsin (Gibco), PBS (Gibco), paraformaldehyde (Beyotime, Shanghai, China), Triton X-100 (Beyotime), DAPI buffer (Beyotime), matrigel (Corning, USA), formic acid (Thermo Scientific, USA), acetonitrile (Thermo Scientific), RIPA Lysis Buffer (Beyotime), anti-ESM1 (ab103590, Abcam, UK), anti-AKT (ab32505, Abcam), anti-p-AKT (ab81283, Abcam), anti-Bax (ab32503, Abcam), anti-Bcl2 (ab182858, Abcam), anti-caspase-3 (ab32351, Abcam), anti-E-cadherin (ab233611, Abcam), anti-N-cadherin (ab254512, Abcam), anti-Snail (ab216347, Abcam), anti-KRT8 (ab9023, Abcam), anti-GAPDH (ab9484, Abcam), goat anti-mouse IgG H&L (HRP) (ab97023, Abcam), BeyoECL Moon (Beyotime).

METHODOLOGY

Lung Cancer Tissue Samples Collection and Cell Culture

Lung cancer tissue samples (n=9) and adjacent normal tissue samples (n=9) were collected from Tangshan People's Hospital according to protocols approved by ethical committee of Tangshan People's Hospital (RMYY-LLKS-2019-0620-1). Lung cancer cells (A549, NCI-H460, NCI-H520, PG-BE1 and SK-MES-1 cells) and BEAS-2B cells (human normal lung epithelial cells) were obtained from ATCC (USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) containing 10 percent fetal bovine serum (FBS, Gibco) and penicillin (100 U/mL)/streptomycin (100 U/mL) (Gibco) at 37^0C and 5 percent CO_2. Taxol (HY-B0015, MedChemExpress, USA) was acquired for treating lung cancer cells.

Cell Transfection

Short hairpin RNA of ESM1 (shR-ESM1-1, shR-ESM1-2, shR-ESM1-3) and KRT8 (shR-KRT8-1, shR-KRT8-2, shR-KRT8-3) and their respective negative controls (pSilencer-NC) were obtained from GenePharma (Shanghai, China). Overexpression of ESM1 or KRT8 were carried out using pcDNA3-Flag vector (Merck, Germany), which were named as pcDNA3-Flag-ESM1 and pcDNA3-Flag-KRT8. A549 and SK-MES-1 cells were plated onto 6-well plates and cell transfection was performed using the Lipofectamine 2000 (Invitrogen, USA) after the cell confluence reached 80 percent. After transfection, cells were treated with Taxol (0, 1.5, 3 and 6μM) for 24h. Protein expressions then were examined using western blot.

RT-qPCR

Total RNA in lung cancer tissues and cells was isolated using TRIzol reagent (Invitrogen, USA) followed by cDNA synthesis using iScript cDNA Synthesis Kit (Bio-Rad, USA). Afterwards, PCR was preformed using CFX Duet Real-Time PCR System (Bio-Rad) with SYBR Green Supermix (Bio-Rad). Sequences of primers were shown as below, which were ESM1: forward, 5'-AAGGCTGCTGATGTAGTTC-3' and reverse, 5'-GCTATTTATGGAAGTGTATGTGTTT-3' (Kano et al. 2020); KRT8: forward, 5'-CGAGGATATTGCCAACCGCAG-3' and reverse, 5'-CCTCAATCTCAGCCTGGAGCC-3' (Fang et al. 2017) and GAPDH: forward, 5'- GAGAAGGCTGGGGCTCATTT-3' and reverse, 5'- AGTGATGGCATGGACTGTGG-3' (Huang et al. 2020). RNA expressions were calculated using $2^{-\Delta\Delta Ct}$ methods.

CCK-8 Assay

A549 and SK-MES-1 cells were added into 96-well plates (4x10^3 cells/well) after the digestion with 0.05 percent trypsin (Gibco) and cultivated for 24h at 37^0C and 5 percent CO_2. Thereafter, A549 cells were transfected with pcDNA3-Flag, pcDNA3-Flag-ESM1 and pcDNA3-Flag-KRT8, respectively. Meanwhile, SK-MES-1 cells were transfected with pSilencer-NC, shR-ESM1-2 and shR-KRT8-3, respectively. Afterwards, Taxol (0, 1.5, 3 and 6μM) were applied for treating cells and 10μl of CCK-8 (Ameresco, USA) were added at 24h, 48h and 72h followed by cultivation of cells for another 2h. Finally, Synergy HTX (BioTek, USA) was used to evaluate the absorbance at 450nm.

TUNEL Detection

A549 and SK-MES-1 cells digested by 0.05 percent trypsin were seeded onto 24-well plates (810^4 cells/well) and incubated at 37^0C and 5 percent CO_2. Thereafter, A549 cells were transfected with pcDNA3-Flag, pcDNA3-Flag-ESM1 and pcDNA3-Flag-KRT8, respectively. Meanwhile, SK-MES-1 cells were transfected with pSilencer-NC, shR-ESM1-2 and shR-KRT8-3, respectively. Then, cells were treated by Taxol (0, 1.5, 3 and 6μM) and TUNEL detection was performed 48h after treatment. Briefly, cells after treatment were first rinsed using 1PBS (Gibco) and fixed by 4 percent paraformaldehyde (Beyotime, Shanghai, China). Thereafter, cells were washed by 1PBS three times, 5 min per session followed by incubating with PBS containing 0.3 percent Triton X-100 (Beyotime) for 5min. Later, 100μl TUNEL detection kit was added to incubate with cells for 60min at 37^0C in darkness. Then, 50μl DAPI buffer (0.1mg/ml DAPI, Beyotime) was added and cultured with cells for 5 min. Finally, results were recorded using a fluorescence microscope (Olympus, Japan).

Transwell

Migratory and invasive abilities of lung cancer cells were examined using transwell assay. The upper compartment (Corning, USA) with or without matrigel (Corning) was used for invasiveness and migration detection. Cells with serum-free DMEM were cultured in the upper chamber and the lower chamber was filled with DMEM with 20 percent FBS. After cells were incubated for 24h, cells were fixed using 4 percent paraformaldehyde for 15 min and stained by crystal violet for 10 min. Images were taken randomly from six fields by a microscope and cell numbers were counted.

Mass Spectrometry Analysis

The Orbitrap Exploris 240 Mass Spectrometer was applied for mass spectrometry detec-

tion. Peptides were isolated using a IntegraFrit column (15cm, New Objective, USA) with a gradient of 0.1 percent formic acid (Thermo Scientific, USA) with 95 percent acetonitrile (Thermo Scientific) changing from 5 percent to 40 percent (300nl/min for 150min). In positive-ion mode, LC eluent was sprayed into mass spectrometry instrument using a voltage of 2.0 kV. Full scan spectra from m/z 300 to 2000 at resolution of 60000 were acquired.

Western Blot

A549 cells and SK-MES-1 cells after transfection were lysed by RIPA Lysis Buffer (Beyotime, Shanghai, China). Then, protein was isolated by SDS-PAGE and shifted onto PVDF membranes. Later, 5 percent skimmed milk powder was used for blocking membranes and primary antibodies were incubated with membranes overnight at 4^{0}C. Primary antibodies were listed, which were anti-ESM1 (1:1000; ab103590, Abcam, UK), anti-AKT (1:1000; ab32505), anti-p-AKT (1:1000; ab81283), anti-Bax (1:1000; ab32503), anti-Bcl2 (1:1000; ab182858), anti-caspase-3 (1:1000; ab32351), anti-E-cadherin (1:1000; ab233611), anti-N-cadherin (1:1000; ab254512), anti-Snail (1:1000; ab216347), anti-KRT8 (1:1000; ab9023) and anti-GAPDH (1:2000; ab9484). Then, membranes were cultivated with goat anti-mouse IgG H&L (HRP) (1:1000; ab97023) for 2h at 25^{0}C. BeyoECL Moon (Beyotime) was used for developing and Image J (NIH, USA) was applied for analyzing gray bands.

Statistical Analysis

All experiments were run in a triplicate and data were displayed as meanSD. SPSS 19.0 (USA) and GraphPad Prism 9 (USA) were applied for data analyzing. Differences in two groups were examined using Student's t-test while differences in groups over two were evaluated by one-way or two-way ANOVA. $P<0.05$ was meaningful statistically.

RESULTS

ESM1 was Upregulated in Lung Cancer Tissue Samples and Cells

To investigate impacts of ESM1 on lung cancer, the mRNA expressions in tissue samples and cells were evaluated. Results of RT-qPCR indicated that ESM1 mRNA expressions were higher in cancer tissue samples than normal ones (Fig. 1A). Moreover, ESM1 mRNA expressions were detected in cells, revealing that the mRNA expressions were downregulated in A549, NCI-H520 and PG-BE1 cells while ESM1 mRNA expressions were upregulated in NCI-H460 and SK-MES-1 cells (Fig. 1B, $^{**}P<0.05$). Hence, ESM1 might be differentially expressed in lung cancer cells.

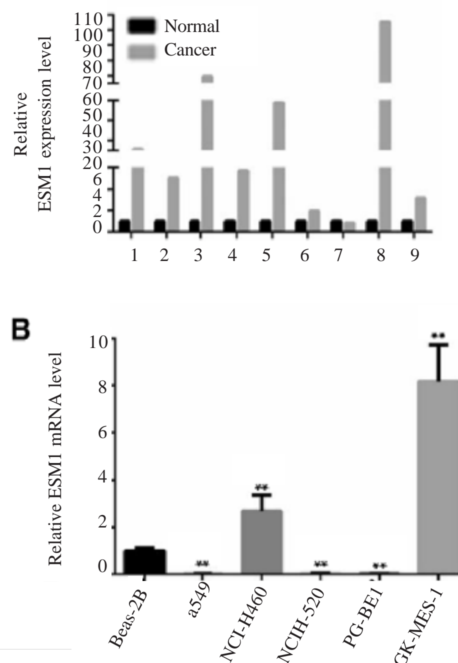

Fig. 1. ESM1 was differentially expressed in lung cancer tissue samples and cells A: ELISA was applied for examining ESM1 protein levels in normal and cancer tissues. B: ESM1 mRNA expression in BEAS-2B, A549, NCI-H460, NCI-H520, PG-BE1 and SK-MES-1 cells were evaluated using RT-qPCR, $^{**}P<0.05$

Overexpressed ESM1 accelerated A549 Cell Viabilities, Invasiveness and Migration

After ESM1 mRNA expressions were examined, functions of ESM1 in regulating biological progressions of lung cancer cells were detected. After overexpressed transfection, ESM1 protein expression was elevated in A549 cells (Fig. 2A, $^{**}P<0.05$). Beyond that, A549 cell viabilities

were also facilitated by ESM1 overexpression (Fig. 2B, **P<0.05). Results of transwell migration test and scratch test indicated that the migratory ability of A549 cells was promoted by ESM1 upregulation (Fig. 2C, D, **P<0.05). Additionally, invasiveness of A549 cells was also facilitated by overexpressed ESM1 (Fig. 2E, **P<0.05). Therefore, ESM1 overexpression might facilitate lung cancer cell progression.

Knockdown of ESM1 Hampered SK-MES-1 Cell Viabilities, Invasiveness and Migration

After effects of ESM1 overexpression on lung cancer cells were examined, roles of downregulated ESM1 in SK-MES-1 cells were explored. RT-qPCR results showed that ESM1 protein expression was suppressed in SK-MES-1 cells (Fig. 3A, **P<0.05). Based on the results in transfection, shR-ESM1-2 was chosen for following experiments. Results of CCK-8 revealed that SK-MES-1 cell viabilities were inhibited with the knockdown of ESM1 (Fig. 3B, **P<0.05). Furthermore, migratory ability and invasiveness of SK-MES-1 cells were also restrained by ESM1 downregulation (Fig. 3C, D, E, **P<0.05). Based on these experiments, downregulated ESM1 might restrained viabilities, invasiveness and migration of lung cancer cells.

ESM1 Modulated Lung Cancer Cell Viabilities, Apoptosis and EMT after Taxol Treatment

After impacts of ESM1 on lung cancer cells were investigated, roles about this mRNA in modulating Taxol sensitivity in lung cancer were examined. After ESM1 upregulation, Taxol-treated A549 cell viabilities were facilitated while the knockdown of ESM1 suppressed Taxol-treated SK-MES-1 cell viabilities (Fig. $4A_{1-6}$, **P<0.05).

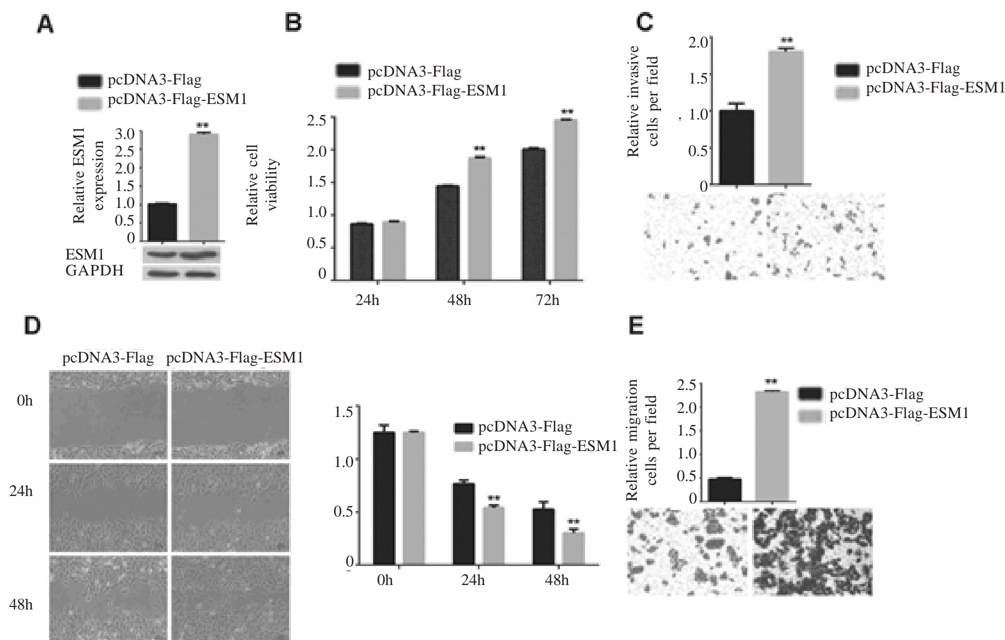

Fig. 2. ESM1 overexpression accelerated A549 cell viabilities, migration and invasiveness A: ESM1 protein expressions were examined with western blot, **P<0.05. B: CCK-8 was applied for analyzing A549 cell viabilities, **P<0.05. C, D: Transwell and scratch test was used to detect migratory abilities of A549 cells after ESM1 upregulation, **P<0.05. E: Transwell was applied to validate invasiveness of A549 cells with ESM1 overexpression, **P<0.05

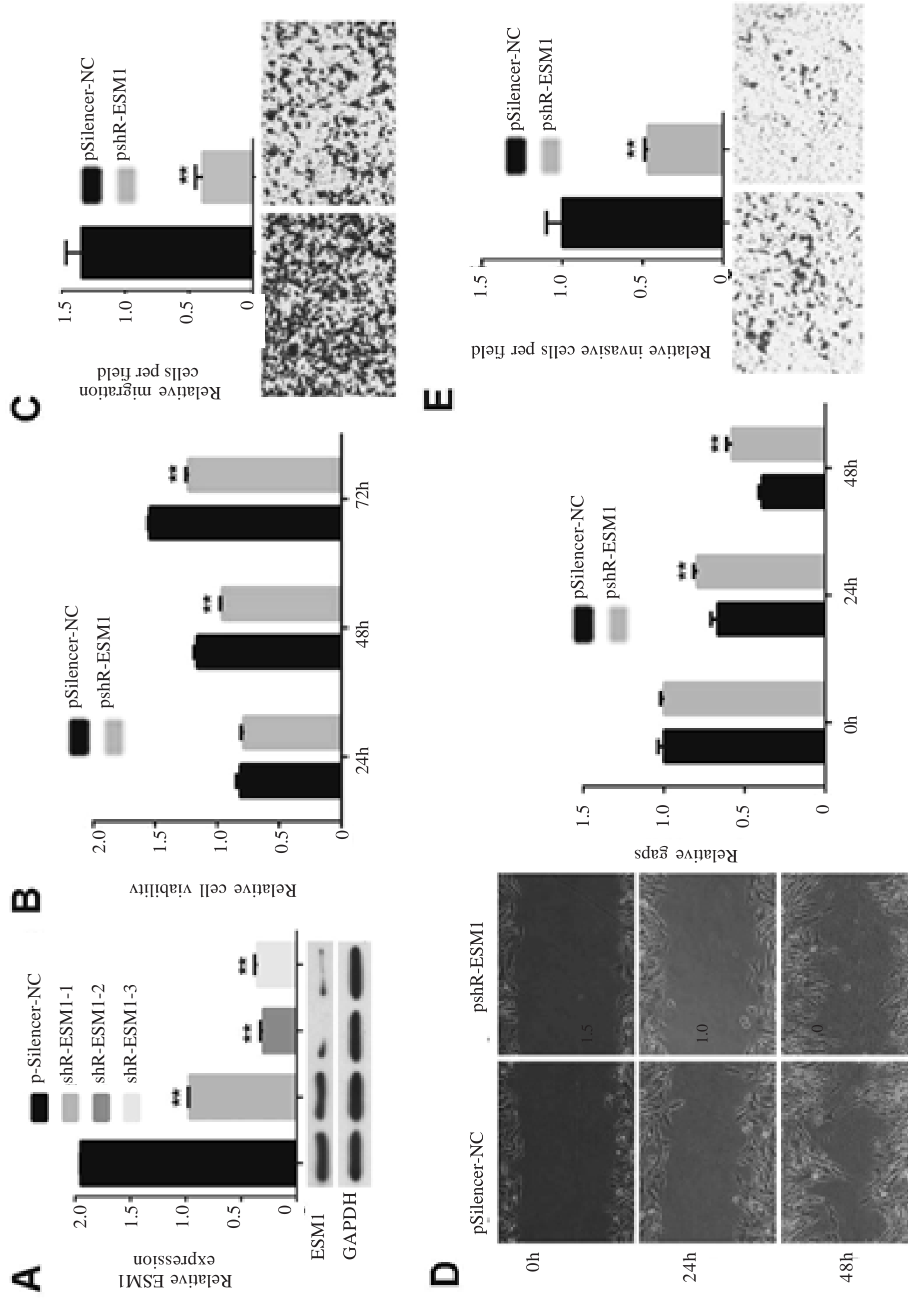

Fig. 3. ESM1 suppression hampered SK-MES-1 cell viabilities, migration and invasiveness A: ESM1 protein expressions were examined with western blot, **P<0.05. B: CCK-8 was applied for analyzing SK-MES-1 cell viabilities with ESM1 suppression, **P<0.05. C, D: Transwell and scratch test was used to detect migratory abilities of SK-MES-1 cells after ESM1 downregulation, **P<0.05. E: Transwell was applied to validate invasiveness of SK-MES-1 cells with the knockdown of ESM1, **P<0.05

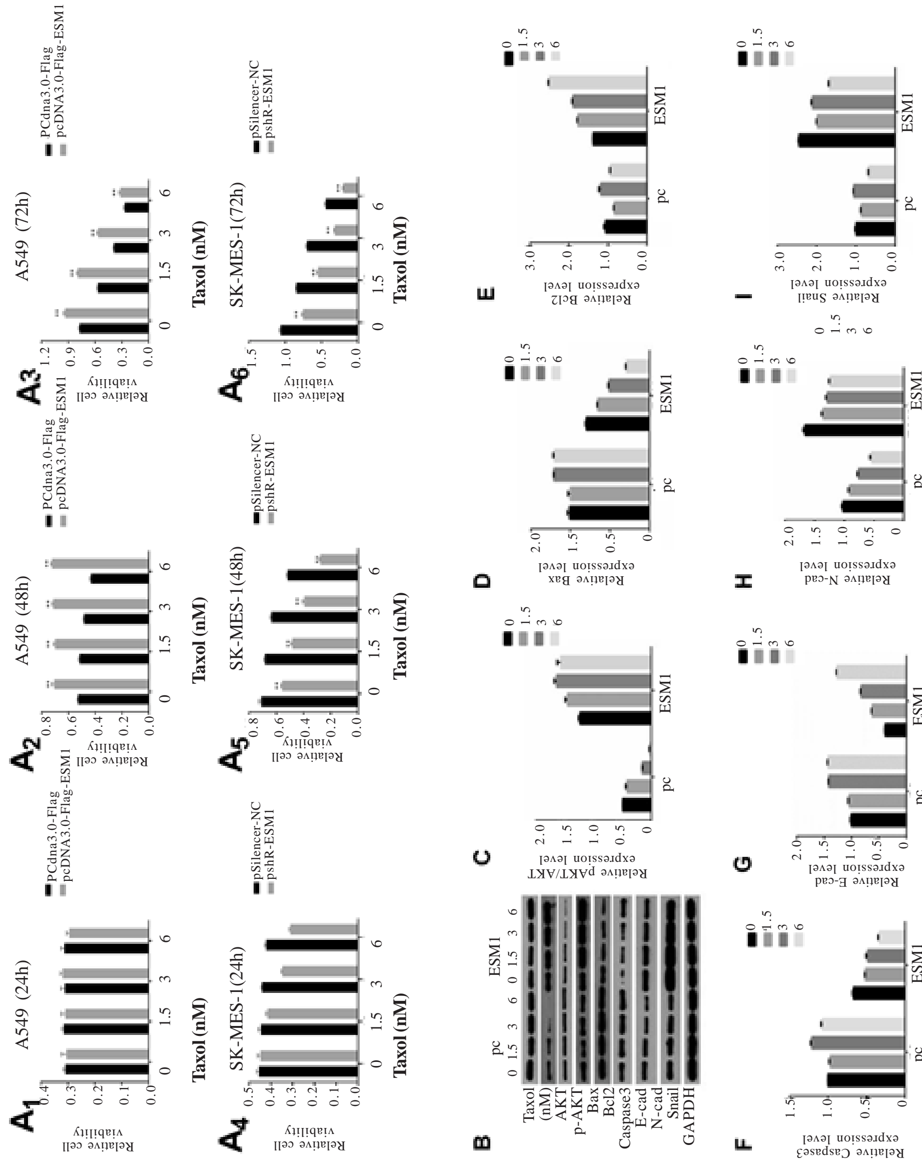

Fig. 4. ESM1 modulated Taxol-treated lung cancer cell viabilities, EMT and apoptosis. A1-3: CCK-8 was used to examine A549 cell viabilities with ESM1 overexpression, $^{**}P<0.05$. A4-6: CCK-8 was used to examine SK-MES-1 cell viabilities with ESM1 inhibition, $^{**}P<0.05$. B, C, D, E, F, G, H, I: Western blot was applied for validating AKT, phosphorylated AKT, Bax, Bcl2, caspase-3, E-cadherin, N-cadherin and Snail protein expressions

Moreover, functions of ESM1 in modulating biomarkers in apoptosis and EMT were examined as well (Fig. 4B). Results of western blot indicated that ESM1 overexpression elevated phosphorylated AKT protein expressions in Taxol-treated A549 cells (Fig. 4C). Meanwhile, Bax and caspase-3 protein expressions were suppressed while Bcl2 protein expressions were upregulated with ESM-1 upregulation (Fig. 4D, E, F). As for EMT, E-cadherin protein expressions were upregulated by upregulated ESM1 while N-cadherin and Snail protein expressions were decreased (Fig. 4G, H, I). Meanwhile, impacts of downregulated ESM1 on regulating viabilities, apoptosis and EMT in Taxol-treated SK-MES-1 cells were detected as well (Fig. 5A). After the knockdown of ESM1, phosphorylated AKT was suppressed (Fig. 5B). Besides that, Bcl2 protein expressions were decreased after ESM1 downregulation while Bax and caspase-3 protein expressions were elevated (Fig. 5C, D, E). E-cadherin protein expressions were also upregulated with ESM1 suppression, but N-cadherin and Snail protein expressions were inhibited (Fig. 5F, G, H). Therefore, ESM1 might facilitate lung cancer cell progression by suppressing apoptosis to restrain Taxol-sensitivity in lung cancer cells.

ESM1 Interplayed with KRT8 in Lung Cancer Cells

To further exploring mechanism of ESM1 in lung cancer cells, underlying proteins that interacted with ESM1 were examined. Results of mass spectrometry and western blot showed that KRT8 was the protein that could interplay with ESM1 (Fig. 6A, B). After upregulated transfection, KRT8 mRNA expression was elevated in A549 cells (Fig. 6C, $^{**}P<0.05$). In addition to that, downregulated transfection of KRT8 suppressed its mRNA expressions in SK-MES-1 cells (Fig. 6D, $^{**}P<0.05$). As KRT8 were confirmed to be the target, impacts of this mRNA on modulating lung cancer cell progression were analyzed. Results of CCK-8 revealed that the Taxol-treated A549 cell viabilities were facilitated with KRT8 overexpression while Taxol-treated SK-MES-1 cell viabilities were decreased by KRT8 downregulation (Fig. $6E_{1-6}$, $^{**}P<0.05$).

ESM1 and KRT8 Modulated Lung Cancer Cell Apoptosis

Afterwards, we also examined lung cancer cell apoptosis with transfection of ESM1 or KRT8. TUNEL results indicated that Taxol-treated A549 cell apoptosis was inhibited by overexpression of ESM1 or KRT8 while Taxol-treated SK-MES-1 cell apoptosis were elevated by downregulation of ESM1 or KRT8 (Fig. 7).

DISCUSSION

In the present study, researchers have observed impacts of ESM1 on lung cancer cell progression and Taxol sensitivity through the interaction with KRT8. Researchers have discovered that ESM1 was differentially expressed in lung cancer cells with facilitating viabilities, migration, invasiveness and EMT but suppressing apoptosis and Taxol sensitivity. Moreover, ESM1 interacted with KRT8 to achieve oncogenic roles in lung cancer cells.

Evidence has revealed that dysregulated ESM1 facilitated cancer development through promoting cancer cell mobility, cell proliferation and cancer stemness properties (Pan et al. 2022). As a newly discovered tumor biomarker, ESM1 has been reported to not only accelerate tumor cell proliferation via HGF/SF signaling pathway, but also suppressed tumor cell apoptosis via NF-κB signaling pathway and promoted angiogenesis via VEGF and HIF signaling pathways (Li et al. 2019). Moreover, oncogenic roles of ESM1 have been verified in ovarian cancer (Laloglu et al. 2017), hepatocellular carcinoma (Baghy et al. 2016), prostatic cancer (Arslan et al. 2017) and so on. In this study, ESM1 expressions were detected to be increased in lung cancer tissues while its mRNA expressions were decreased in A549, NCI-H520 and PG-BE1 but upregulated in NCI-H460 and SK-MES-1 cells. Upregulated ESM1 also promoted A549 cell viabilities, migration and invasiveness. Moreover, ESM1 overexpression accelerated Taxol-treated A549 cells viabilities and EMT but suppressed apoptosis. Nevertheless, the knockdown of ESM1 resulted in opposite results in SK-MES-1 cells. Hence, ESM1 might be a promoter in lung cancer cells. Compared with previous studies, this detection explored effects of ESM1 on modulating biological functions of lung cancer cells.

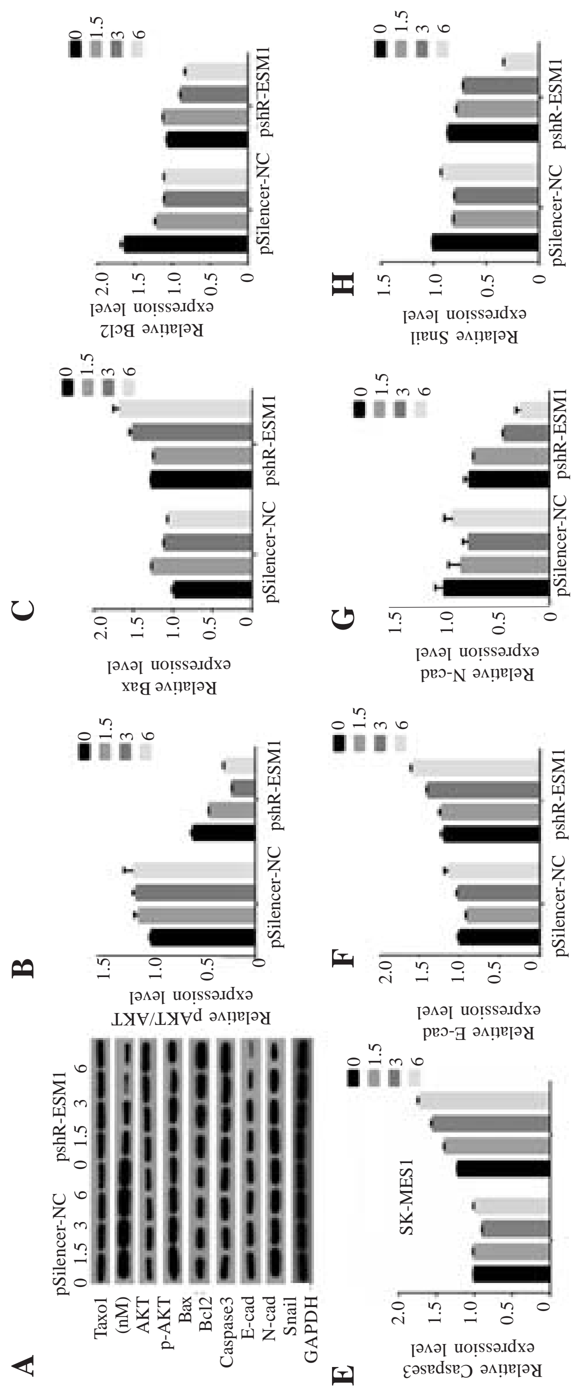

Fig. 5. ESM1 downregulation promoted apoptosis and inhibited EMT in SK-MES-1 cells after Taxol treatment A, B, C, D, E, F, G, H: Western blot was applied for validating AKT, phosphorylated AKT, Bax, Bcl2, caspase-3, E-cadherin, N-cadherin and Snail protein expressions

Fig. 6. KRT8 interplayed with ESM1 A, B: Mass spectrometry and western blot were used to examine KRT8. C, D: KRT8 expressions were examined after transfection, **P<0.05. E1-3: A549 cell viabilities with KRT8 upregulation were evaluated using CCK-8, **P<0.05. E4-6: SK-MES-1 cell viabilities with KRT8 suppression were validated by CCK-8, **P<0.05

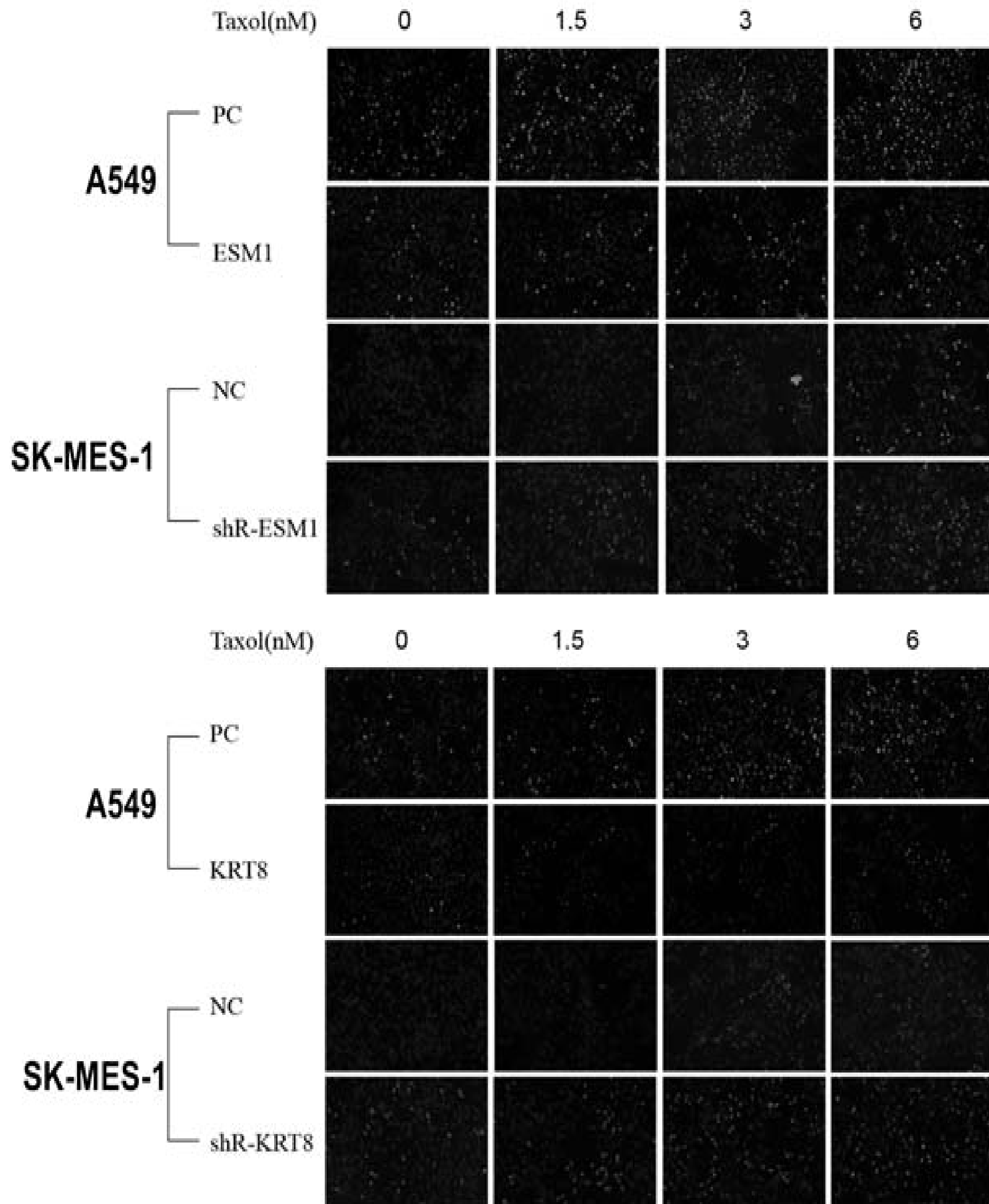

Fig.7. ESM1 and KRT8 modulated lung cancer cell apoptosis A: TUNEL was applied to examined Taxol-treated lung cancer cells

After roles of ESM1 were detected, underlying genes that interacted with ESM1 was examined. Using the mass spectrometry, KRT8 was confirmed to be the gene that interplayed with ESM1. KRT8 has been found to be upregulated in many kinds of malignancies including breast cancer (Dvoránková et al. 2012), colorectal cancer (Lu et al. 2021) and hepatocellular carcinoma (Golob-Schwarzl et al. 2019), etc. In this study, overexpressed KRT8 also accelerated viabilities and restrained apoptosis of Taxol-treated A549 cells. In contrast, KRT8 downregulation caused reversed results in Taxol-treated SK-MES-1 cells. Moreover, Taxol-treated A549 cell apoptosis was restrained with the overexpression of ESM1 or KRT8 while Taxol-treated SK-MES-1 cell apoptosis was facilitated after the knockdown of ESM1 or KRT8. Therefore, KRT8 might be the downstream biomarker that co-expressed with ESM1 in lung cancer cells.

CONCLUSION

ESM1 was dysregulated in lung cancer cells and accelerated viabilities, migration, invasiveness and EMT but restrained apoptosis and inhibited Taxol sensitivity in lung cancer cells through interplaying with KRT8. Hence, ESM1 might be a promising mRNA for lung cancer treatment.

RECOMMENDATIONS

ESM1 was upregulated in lung cancer tissues samples and differentially expressed in lung cancer cells. ESM1 also upregulated KRT8, leading to accelerated A549 cell migratory and invasive abilities, increased Taxol-treated A549 cell viabilities and EMT but inhibited apoptosis. ESM1 and KRT8 downregulation caused opposite effects. This study has demonstrated that ESM1 restrained Taxol sensitivity in lung cancer cells via interacting with KRT8, which provided a novel way for increasing Taxol efficacy.

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ABBREVIATION LIST

NSCLC: Non-Small Cell Lung Cancer;
Emt: Epithelial-Mesenchymal Transition;
ESM1: Endothelial Cell Specific Molecule 1;
KRT8: keratin 8;
ELISA: Enzyme-Linked Immunosorbent Assay;
DMEM: Dulbecco's Modified Eagle Medium;
FBS: Fetal Bovine Serum;
RIPA: Radio Immunoprecipitation Assay;
PBS: Phosphate Buffer Saline;
AKT: protein kinase B;
SCLC: Small Cell Lung Cancer;
EGFR: Epidermal Growth Factor Receptor;
LUAD: Lung Adenocarcinoma;
LUSC: Lung Squamous Cell Carcinoma;
HGF: Hepatocyte Growth Factor;
SF: Scatter factor NF-κB: nuclear factor kappa-B;
VEGF: Vascular Endothelial Growth Factor;
HIF: Hypoxia Inducible Factor

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