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FUS-stabilized USP35 promotes growth, invasion and angiogenesis in NSCLC through deubiquitinating VEGFA

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Abstract. Vascular endothelial growth factor A (VEGFA) is an important regulator for non-small cell lung cancer (NSCLC). Our study aimed to reveal its upstream pathway to provide new ideas for developing the therapeutic targets of NSCLC. The mRNA and protein levels of VEGFA, ubiquitin-specific peptidase 35 (USP35), and FUS were determined by quantitative real-time PCR and Western blot. Cell proliferation, apoptosis, invasion and angiogenesis were detected using CCK8 assay, EdU assay, flow cytometry, transwell assay and tube formation assay. The interaction between USP35 and VEGFA was assessed by Co-IP assay and ubiquitination assay. Animal experiments were performed to assess USP35 and VEGFA roles *in vivo*. VEGFA had elevated expression in NSCLC tissues and cells. Interferences of VEGFA inhibited NSCLC cell proliferation, invasion, angiogenesis, and increased apoptosis. USP35 could stabilize VEGFA protein level by deubiquitination, and USP35 knockdown suppressed NSCLC cell growth, invasion and angiogenesis *via* reducing VEGFA expression. FUS interacted with USP35 to promote its mRNA stability, thereby positively regulating VEGFA expression. Also, USP35 silencing could reduce NSCLC tumorigenesis by downregulating VEGFA. FUS-stabilized USP35 facilitated NSCLC cell growth, invasion and angiogenesis through deubiquitinating VEGFA, providing a novel idea for NSCLC treatment.

Key words: Non-small cell lung cancer — VEGFA — USP35 — FUS

Abbreviations: Act D, actinomycin D; LUAD, lung adenocarcinoma; NSCLC, non-small cell lung cancer; qRT-PCR, quantitative real-time PCR; USPs, ubiquitin-specific peptidases; VEGFA, vascular endothelial growth factor A.

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Introduction

As a common type of lung cancer, non-small cell lung cancer (NSCLC) is characterized by micrometastasis, high recurrence rate and poor prognosis (Muthusamy et al. 2022; Sun et al. 2022). Surgery and chemotherapy are the main methods for the treatment of NSCLC, but the effect is limited and the physical damage to patients is great (Duma et al. 2019; Pirker 2020). In recent years, molecular targeted therapy has shown good results in NSCLC treatment with its advantages of low toxicity and high efficiency (Imyanitov et al. 2021; Salgia et al. 2021). Therefore, understanding the pathogenesis of NSCLC is expected to provide new potential targets for NSCLC treatment.

Vascular endothelial growth factor A (VEGFA), a member of the VEGF family, has an important role in angiogenesis and endothelial cell growth (Sun et al. 2020; White and Bix 2023). Abnormal expression of VEGFA is associated with a variety of disease processes (Braile et al. 2020; Huang et al. 2023). VEGFA overexpression stimulates angiogenesis to accelerate glioma cell proliferation and migration (Wang R et al. 2022). Also, increased VEGFA expression may alleviate osteoporosis process *via* promoting angiogenesis of osteoblasts (Wu W et al. 2021). Previous research showed that VEGFA was upregulated in NSCLC tissues, and activated VEGFA-related signals could enhance NSCLC tumorigenesis (Zhan et al. 2018; Cao et al. 2022). Moreover, targeted inhibition of VEGFA might be used for suppressing NSCLC cell growth, migration and angiogenesis (Yang et al. 2020; Ma et al. 2022). Thus, revealing VEGFA-related pathways may provide more ideas for the development of NSCLC therapeutic targets.

Ubiquitin-specific peptidases (USPs) are the members of the deubiquitinating enzyme family, which mainly regulate the stability of proteins through deubiquitination, thereby mediating cell biological processes (Pan et al. 2015; Kitamura 2023). Recently study suggested that USP35 inhibited NSCLC cell apoptosis *via* stabilizing BIRC3 and RRBP1 (Liu et al. 2022; Wang W et al. 2022). In this study, we conducted correlation analysis through TCGA database and found that USP35 was significantly positively correlated with VEGFA expression in lung adenocarcinoma (LUAD) tissues. However, whether USP35 regulates VEGFA stability through deubiquitination is still unclear. In addition, using the ENCORI website, we found that USP35 interacted with the RNA-binding protein FUS, and its expression was positively correlated with FUS expression in LUAD tissues. However, whether FUS interacts with USP35 to regulate VEGFA in NSCLC has not been revealed.

In this study, we aim to investigate VEGFA roles and underlying molecular mechanisms in NSCLC progression. Based on this analysis, we hypothesized that FUS-stabilized USP35 regulated VEGFA expression to promote NSCLC growth, invasion and angiogenesis through deubiquitination.

Materials and Methods

Tissues samples

NSCLC patients (n = 33) were recruited from Xianyang Hospital of Yan'an University and signed written informed consent for our research. A total of 33 paired NSCLC tumor tissues and adjacent normal tissues were stored at −80°C. Our study was approved by the Ethics Committee of Xianyang Hospital of Yan'an University.

Cell culture and transfection

Human NSCLC cells (Calu-6, H1299 and A549) and bronchial epithelial cells (16HBE) (Biovector NTCC, Beijing, China) were cultured in RPMI-1640 medium (11875119, including L-Glutamine and Phenol red, Gibco, Carlsbad, CA, USA) plus 10% FBS (10099141, Gibco) and 1% penicillin/streptomycin antibiotics (15140122, Invitrogen, Carlsbad, CA, USA). The siRNAs (si-VEGFA: 5'-AAUGAAUAUCAAAUUCCAGCA-3', R 5'-CUGGAAUUUGAUAUUCAUUGA-3'; si-USP35: F 5'-UCAAUCAUCCUGCUAAUGGCA-3'. R 5'-CCAUUAGCAGGAUGAUUGACU-3'; si-FUS: F 5'-UAUAACCACUGUAACUCUGCU-3', R 5'-CAGAGUUACAGUGGUUAUAGC-3') and pcDNA overexpression vector of VEGFA/USP35/FUS synthesized by RiboBio (Guangzhou, China) were transfected into cells using Lipofectamine 3000 (Invitrogen). For detecting the effect of USP35 on VEGFA protein synthesis, H1299 cells transfected with pcDNA/USP35 were treated with 20 μg/ml CHX (MedChemExpress Monmouth Junction, NJ, USA) for indicated time points. Besides, H1299 and A549 cells transfected with si-USP35 were then treated with proteasome inhibitor MG132 (10 μmol/l; MedChemExpress) for 24 h to investigate the effect of USP35 on VEGFA ubiquitination and expression.

Quantitative real-time PCR (qRT-PCR)

Total RNAs were isolated with TRIzol reagent (Invitrogen) and reverse-transcribed into cDNA by Reverse Transcriptase Kit (Invitrogen). SYBR Green mixed with cDNA and specific primers (Table 1) were used for PCR amplification. Relative expression of USP35 and VEGFA was normalized by GAPDH and calculated by the $2^{-\Delta\Delta Ct}$ methods.

Western blot

Cells and tissues were lysed with RIPA buffer (Beyotime, Shanghai, China) to isolate total proteins. After separated

and transferred, protein membrane was blocked with non-fat milk and incubated with indicated antibodies. Then, protein signals were visualized using ECL reagent (Beyotime) and analyzed by ImageJ software. Antibodies (Abcam, Cambridge, CA, USA) include anti-VEGFA (ab46154, 1:1000), anti-USP35 (ab86791, 1:10000), anti-FUS (an23439, 1:1000), anti-GAPDH (ab9485, 1:2500), and HRP-labeled Goat-anti-Rabbit IgG (ab205718, 1:50000).

Cell proliferation assay

H1299 and A549 cells were seeded into 96-well plates. For CCK8 assay, cells were treated with CCK8 reagent (Beyotime) for 2 h and then cell viability was analyzed under a microplate reader at 450 nm. For detecting EdU positive cells, cells were treated with EdU solution and DAPI solution according to the introductions of EdU Kit (RiboBio). Then, fluorescence signal was evaluated under a microscope.

Cell apoptosis assay

H1299 and A549 cells were washed with PBS and then collected into a tube. After suspended with binding buffer, cells were stained with Annexin V-FITC and PI solution (Abcam). Cell apoptosis rate was analyzed under a flow cytometer.

Cell invasion assay

The 24-well tranwell chambers were pre-coated with Matrigel (BD Biosciences, San Jose, CA, USA). H1299 and A549 cells in RPMI-1640 medium were seeded into the upper chamber, and completed medium were added into the lower chamber. After 24 h, invasive cells were fixed with paraformaldehyde and stained with crystal violet, followed by counted under a microscope.

Tube formation assay

H1299 and A549 cells were cultured in RPMI-1640 medium for 48 h and then the supernatant was collected for preparing conditioned medium. HUVECs suspended with conditioned medium were seeded into 96-well plates pre-coated with Matrigel. After 12 h, tube formation was observed under a microscope.

Co-IP assay

Co-Immunoprecipitation Kit (Pierce, Rockford, IL, USA) was used. Briefly, the lysates of H1299 and A549 cells were incubated with anti-IgG, anti-USP35 and anti-VEGFA overnight at 4°C, followed by treated with protein A/G agarose beads in Coupling Buffer (0.01 M sodium phosphate, 0.15 M NaCl and pH 7.2). Afterwards, the mixed suspension was centrifuged and the precipitated proteins were obtained for Western blot analysis.

Ubiquitination assay

H1299 and A549 cells were transfected with Flag-VEGFA, si-NC or si-USP35 for 24 h. Cell lysates were collected and then incubated with anti-USP35 and HA-Ub overnight. After incubated with protein A/G agarose beads, the immunoprecipitated protein was eluted and collected for Western blot assay.

mRNA stability assay

H1299 and A549 cells transfected with si-NC, si-FUS, pcDNA or FUS were treated with Actinomycin D (Act D) solution. At each time point, cells were collected for qRT-PCR to detect USP35 mRNA expression.

Animal experiments

Lentivirus encoding sh-USP35, sh-NC, SF3B4 overexpression and pcDNA vector were transduced into H1299 cells, and the stabled H1299 cells were selected using 2.0 μg/ml puromycin (Solarbio, Beijing, China). BALB/c nude mice (Vital River, Beijing, China) were subcutaneously injected with stabled H1299 cells (n = 6/group). After 7 days, tumor volume was measured every 3 days. After 22 days, mice were euthanized and tumors were collected. Part of tissues were prepared for paraffin sections to carry out immunohistochemistry (IHC) staining using anti-Ki67 (ab15580, 1:200, Abcam), anti-CD31 (ab28364, 1:50, Abcam) and anti-VEGFA (ab1316, 1:200, Abcam). Animal studies were approved by the Animal Ethics Committee of Xianyang Hospital of Yan'an University.

Statistical analysis

Data are expressed as mean ± SD by GraphPad Prism 7.0 software. Comparison was performed by Student's t-test or ANOVA. $p < 0.05$ was considered statistical significance.

Results

VEGFA was upregulated in NSCLC tissues and cells

Through TCGA database, VEGFA mRNA and protein levels were confirmed to be higher in LUAD tissues (Fig. 1A,B). Kaplan-Meier plotter predicted that VEGFA expression was associated with the prognosis of LUAD patients (Fig. 1C). Furthermore, qRT-PCR detected that

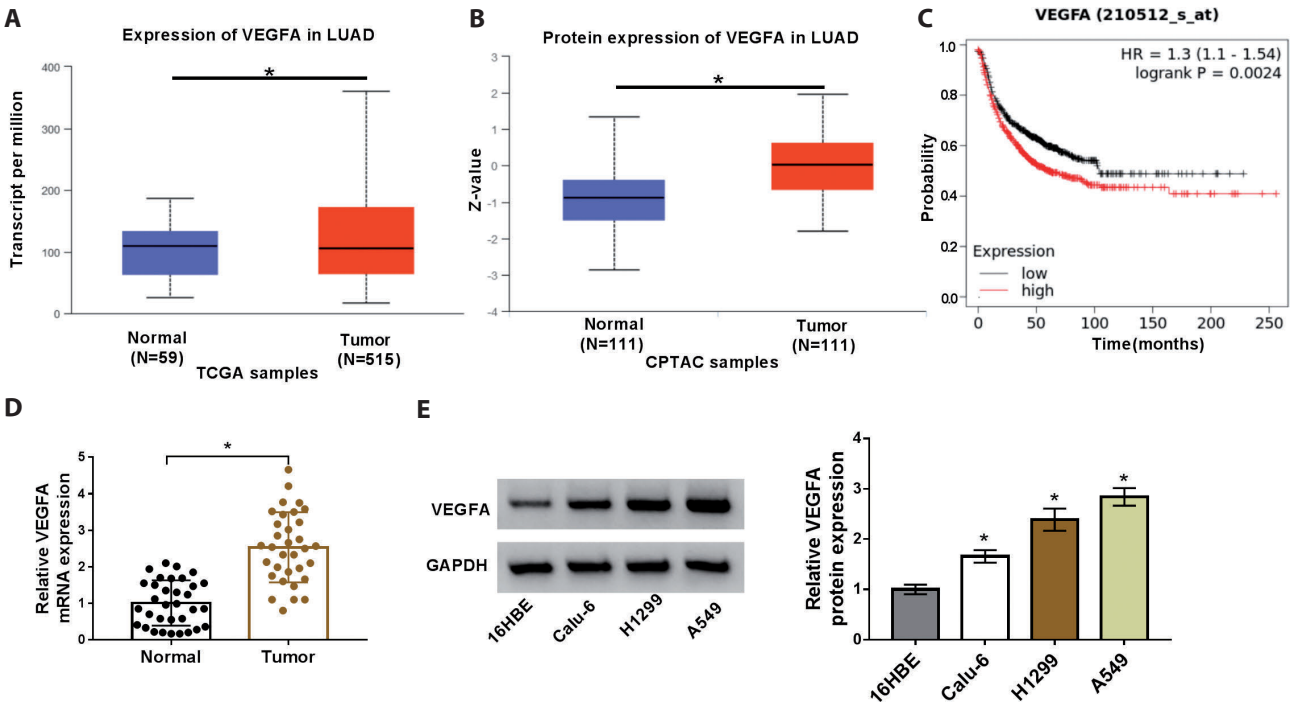

Figure 1. VEGFA expression in NSCLC tissues and cells. TCGA database predicted VEGFA mRNA (**A**) and protein levels (**B**) in LUAD tissues and normal tissues. **C.** Kaplan-Meier plotter predicted the association of VEGFA expression and the prognosis of LUAD patients. **D.** VEGFA mRNA expression was measured by qRT-PCR in NSCLC tissues (n = 33) and normal tissues (n = 33). **E.** VEGFA protein expression was examined by Western blot in bronchial epithelial cells (16HBE) and 3 NSCLC cells (Calu-6, H1299, A549) (n = 3). * $p < 0.05$.

VEGFA mRNA expression was upregulated in NSCLC tumor tissues (Fig. 1D). Besides, we also measured VEGFA protein level in 3 NSCLC cell lines, and confirmed that it was significantly increased compared to 16HBE cells (Fig. 1E).

Interference of VEGFA inhibited NSCLC cell growth, invasion and angiogenesis

After transfected with si-VEGFA into H1299 and A549 cells, we explored the role of VEGFA in NSCLC. The decreased VEGFA protein expression confirmed the success of transfection (Fig. 2A). In function experiments, EdU assay and CCK8 assay were performed to assess cell proliferation, and flow cytometry was used to evaluate cell apoptosis. VEGFA knockdown reduced the viability, EdU positive cells and enhanced apoptosis rate in H1299 and A549 cells (Fig. 2B–D). Moreover, cell invasion was detected using transwell assay, and angiogenesis was analyzed by tube formation assay. The results showed that silencing of VEGFA also repressed the invasive cell number and tube formation rate of H1299 and A549 cells (Fig. 2E–G). All data suggested that VEGFA might promote NSCLC cell growth, invasion and angiogenesis.

Deubiquitinating enzyme USP35 stabilized VEGFA expression

TCGA database correlation analysis showed that only USP35 and VEGFA had a significant positive correlation in LUAD tissues (Fig. 3A). Also, the GEPIA database and ENCORI software predicted that USP35 was positively correlated with VEGFA in LUAD tissues (Fig. 3B,C). Moreover, TCGA and ENCORI websites analyzed that USP35 was highly expressed in LUAD tissues (Fig. 3D,E). Through qRT-PCR, we confirmed that USP35 was upregulated in NSCLC tissues and was positively correlated with VEGFA expression (Fig. 3F,G). Furthermore, USP35 protein expression was reduced by si-USP35 and promoted by USP35 overexpression vector (Fig. 3H). The detection of VEGFA expression showed that USP35 knockdown suppressed VEGFA protein expression and its overexpression enhanced VEGFA protein expression, while both of them had not effect on VEGFA mRNA expression (Fig. 3I,J). Co-IP assay showed that VEGFA was enriched in anti-USP35, and USP35 was enriched in anti-VEGFA (Fig. 3K). Under CHX treatment, we confirmed that USP35 overexpression could stabilize VEGFA protein synthesis (Fig. 3L). In addition, ubiquitin proteasome inhibitor MG132 could abolish the

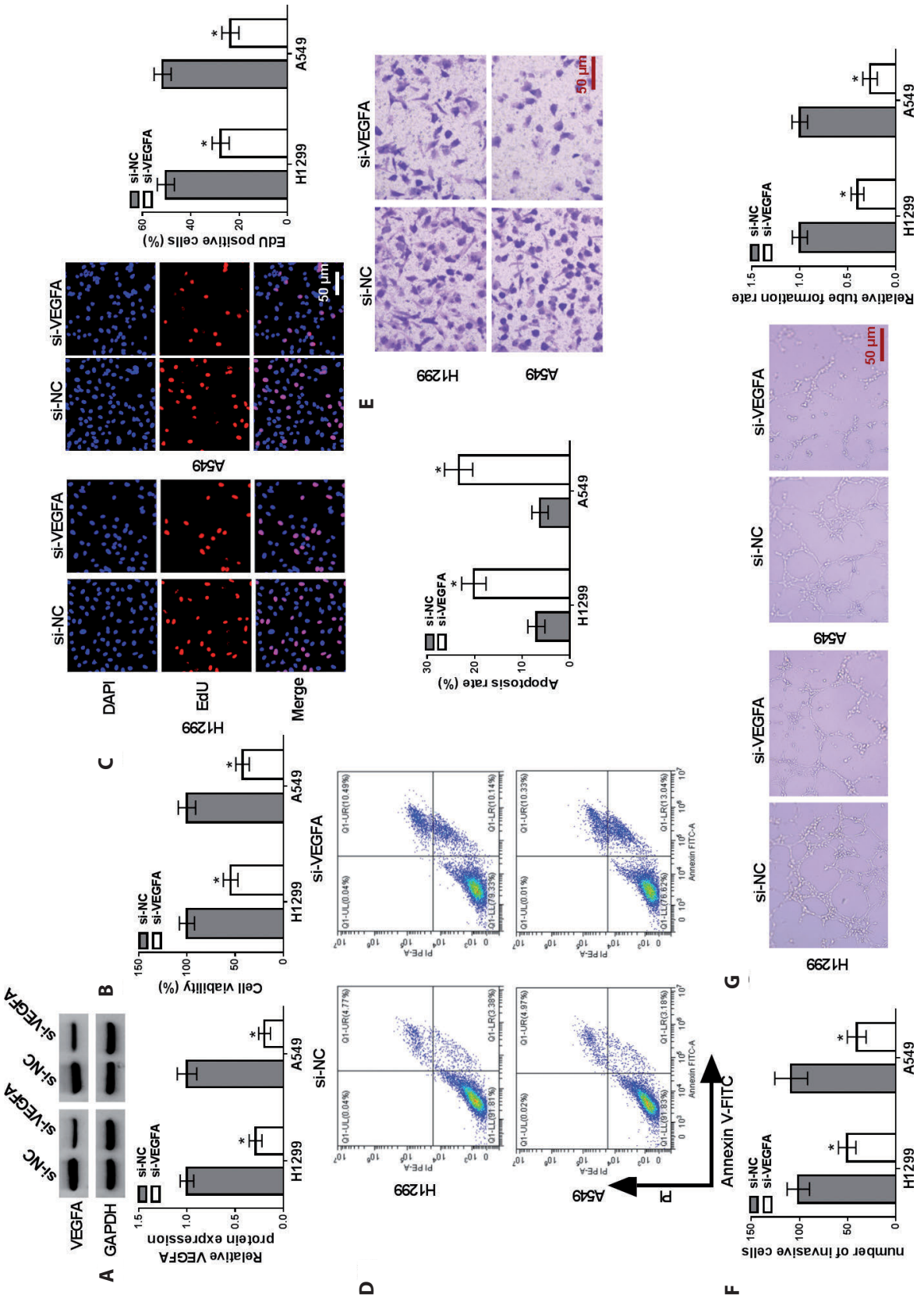

Figure 2. Effects of si-VEGFA on NSCLC progression. H1299 and A549 cells were transfected with si-NC and si-VEGFA ($n = 3$). **A.** VEGFA protein expression was tested by Western blot. CCK8 assay (**B**), EdU assay (**C**), flow cytometry (**D**), transwell assay (**E, F**) and tube formation assay (**G**) were used to measure cell proliferation, apoptosis, invasion and angiogenesis. * $p < 0.05$.

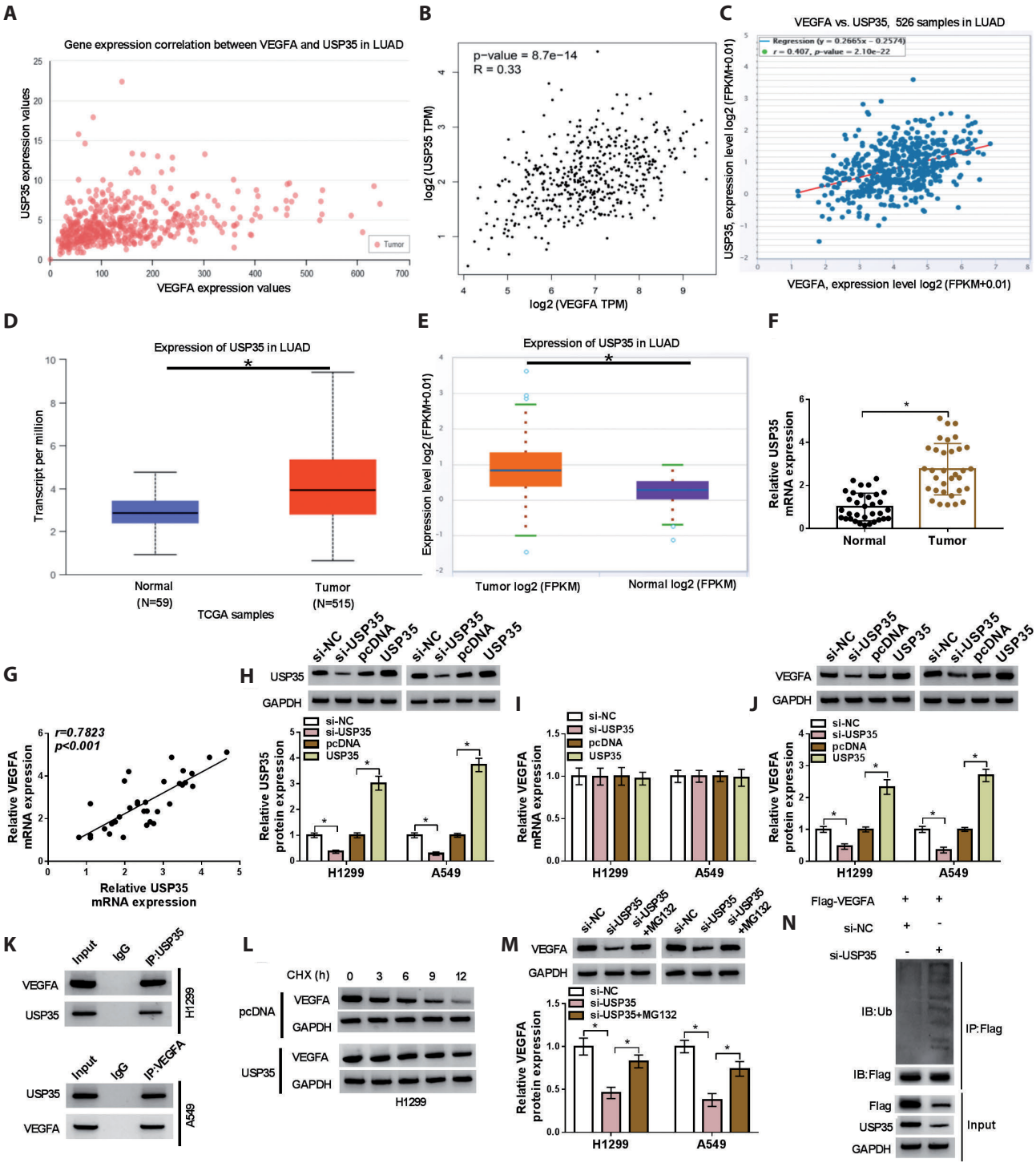

Figure 3. USP35 stabilized VEGFA expression. TCGA database (**A**), GEPIA database (**B**) and ENCORI software (**C**) predicted the correlation between USP35 and VEGFA expression in LUAD tissues. TCGA database (**D**) and ENCORI websites (**E**) analyzed USP35 expression in LUAD tissues and normal tissues. **F.** USP35 mRNA expression was measured by qRT-PCR in NSCLC tumor tissues ($n = 33$) and normal tissues ($n = 33$). **G.** Pearson correlation analysis revealed the correlation between USP35 and VEGFA expression in NSCLC tissues ($n = 33$). **H.** The transfection efficiencies of si-USP35 and USP35 overexpression vector were confirmed by Western blot ($n = 3$). VEGFA mRNA (**I**) and protein levels (**J**) were tested by qRT-PCR and Western blot ($n = 3$). **K.** Co-IP assay confirmed the interaction between USP35 and VEGFA. **L.** CHX treatment was used to explore the effect of USP35 overexpression on VEGFA protein synthesis. **M.** VEGFA protein level was detected by Western blot ($n = 3$). **N.** Detection of ubiquitinated proteins in cells transfected with si-NC and si-USP35. * $p < 0.05$.

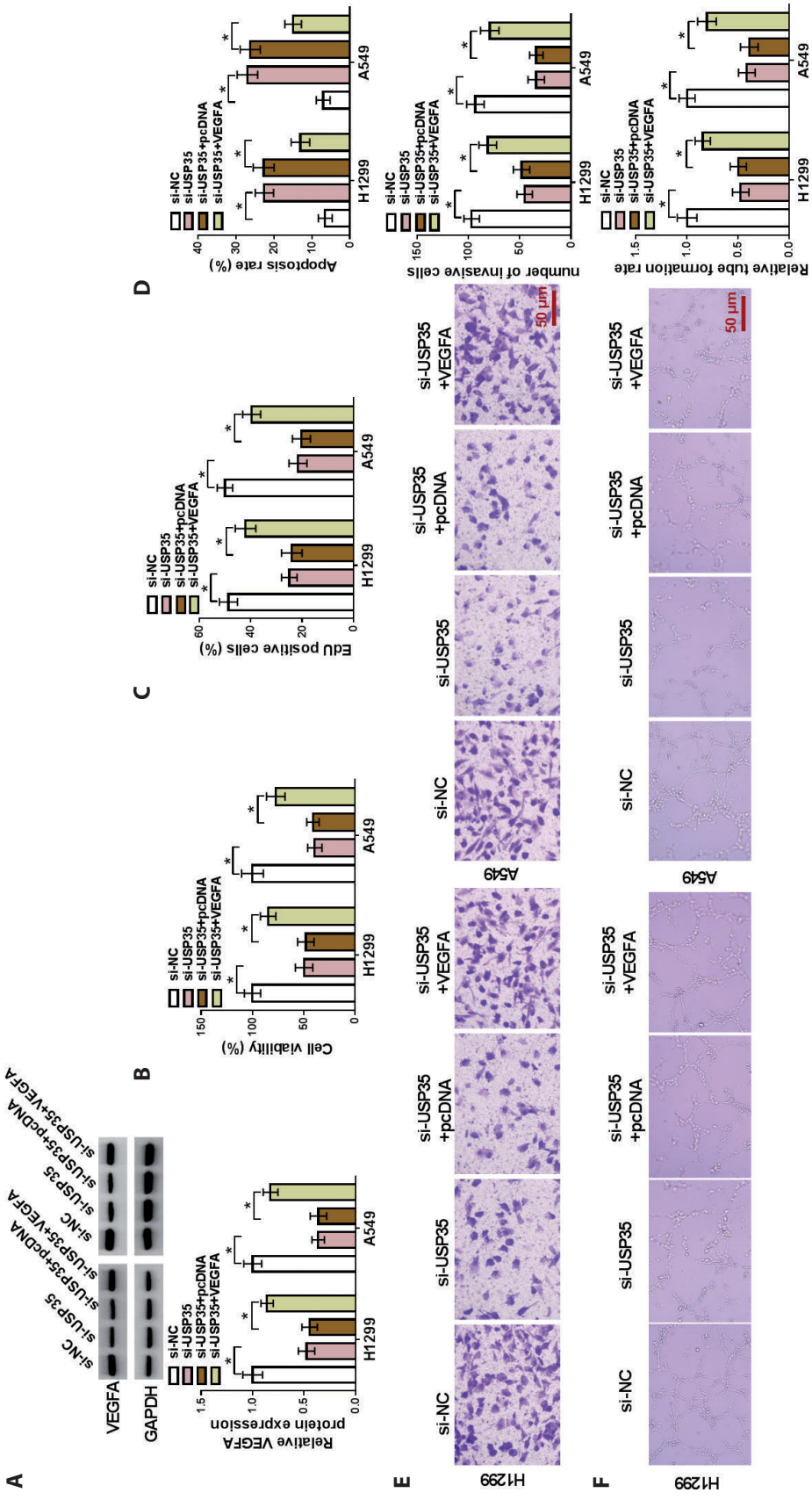

Figure 4. Effects of si-USP35 and VEGFA on NSCLC progression. H1299 and A549 cells were transfected with si-NC, si-USP35, pcDNA and VEGFA ($n = 3$). **A.** VEGFA protein expression was examined by Western blot. Cell proliferation, apoptosis, invasion and angiogenesis were determined using CCK8 assay (**B**), EdU assay (**C**), flow cytometry (**D**), transwell assay (**E**) and tube formation assay (**F**). * $p < 0.05$.

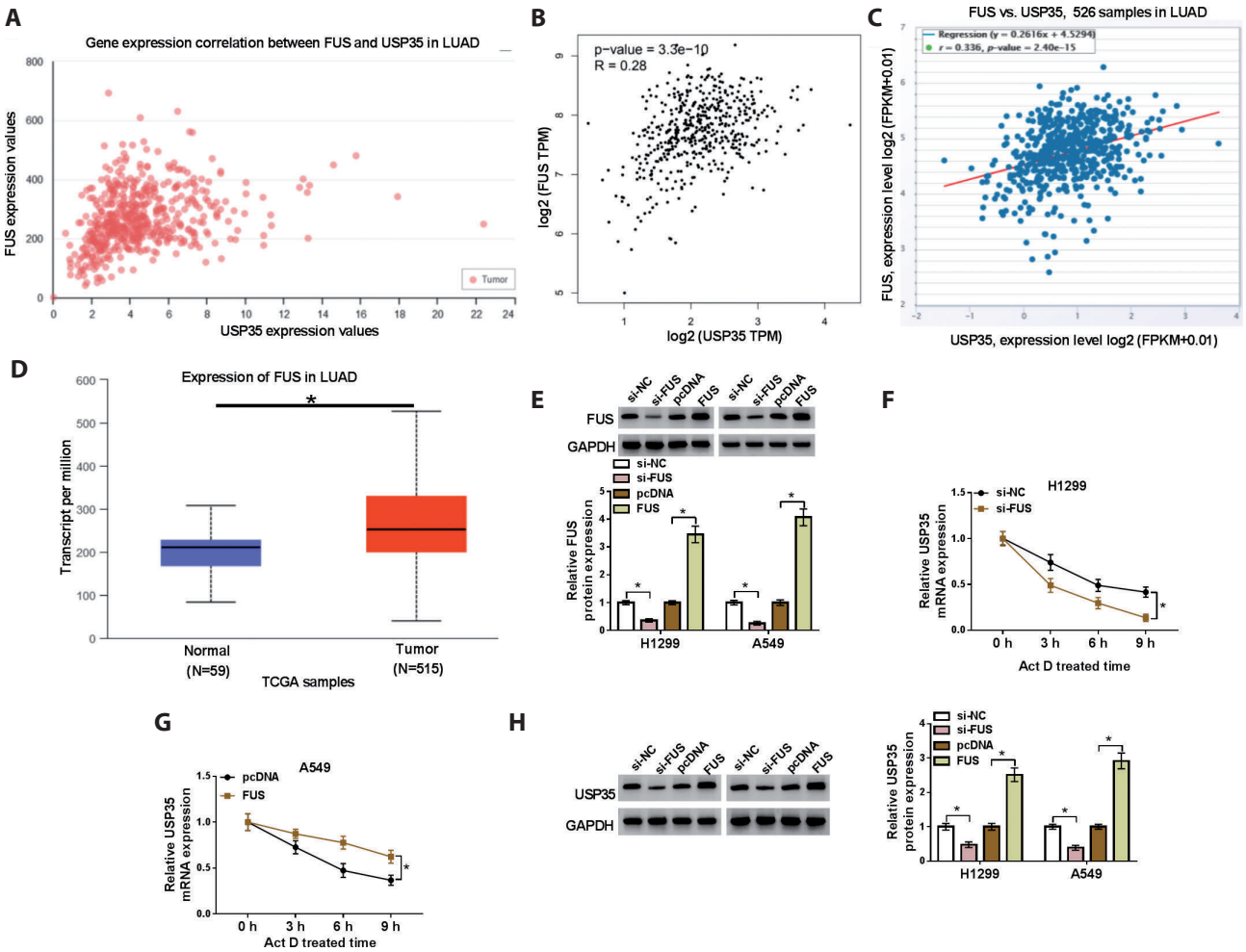

Figure 5. FUS interacted with USP35. TCGA (**A**), GEPIA (**B**), and ENCORI websites (**C**) analyzed the correlation between FUS and USP35. **D.** TCGA website analyzed FUS expression in LUAD tissues and normal tissues. **E.** The transfection efficiencies of si-FUS and FUS overexpression vector were confirmed by Western blot ($n = 3$). **F, G.** Act D assay was used to assess USP35 mRNA stability ($n = 3$). **H.** USP35 protein expression was examined by Western blot in cells transfected with si-FUS and FUS overexpression vector ($n = 3$). * $p < 0.05$.

decreasing effect of USP35 knockdown on VEGFA protein expression (Fig. 3M). Further analysis revealed that USP35 knockdown promotes the VEGFA ubiquitination (Fig. 3N). Above all, USP35 stabilized VEGFA protein expression by deubiquitination.

USP35 knockdown decreased VEGFA expression to inhibit NSCLC progression

To explore USP35 roles in NSCLC and whether USP35 regulated VEGFA to mediate NSCLC progression, H1299 and A549 cells were co-transfected with si-USP35 and VEGFA overexpression vector. The transfection of VEGFA overexpression vector abolished si-USP35-mediated reduction on VEGFA protein expression (Fig. 4A). Functional experiments showed that USP35 knockdown repressed viability, EdU positive cell rate and promoted apoptosis rate in H1299 and A549 cells, while VEGFA overexpression could reverse these effects (Fig. 4B–D). Also, VEGFA overexpression eliminated the inhibitory effect of USP35 knockdown on invasive cell number and tube formation rate of H1299 and A549 cells (Fig. 4E,F). All data revealed that USP35 facilitated NSCLC cell growth, invasion and angiogenesis *via* enhancing VEGFA protein expression.

FUS promoted USP35 protein expression by stabilizing its mRNA level

TCGA, GEPIA, and ENCORI websites analyzed that there had positively correlation between FUS and USP35 (Fig.

5A–C). Through TCGA website analyzing, we confirmed that FUS was highly expressed in LUAD tissues (Fig. 5D). Besides, FUS was reduced by si-FUS and promoted by FUS overexpression vector (Fig. 5E). After Act D treatment, we found that USP35 mRNA stability could be decreased by FUS knockdown and increased by FUS overexpression (Fig. 5F,G). Moreover, si-FUS also could repress USP35 protein expression, and its overexpression had an opposite effect (Fig. 5H). These data suggested that FUS interacted with USP35 to stabilize its mRNA expression. Further analysis showed that FUS knockdown markedly decreased VEGFA protein expression, and this effect was reversed by USP35 overexpression (Fig. 6), confirming that FUS regulated VEGFA expression by interacting with USP35.

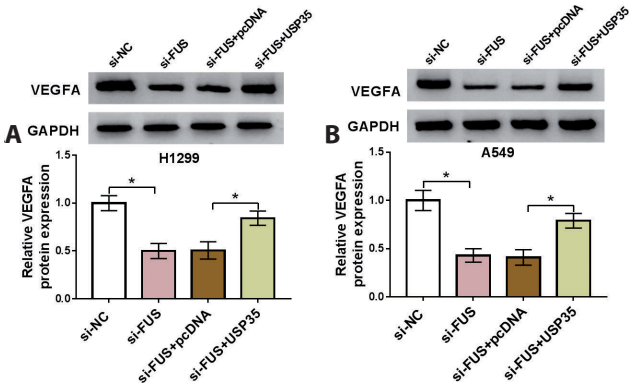

Figure 6. FUS regulated VEGFA by regulating USP35. VEGFA protein expression was examined by Western blot in H1299 cells (**A**) and A549 cells (**B**) transfected with si-NC, si-FUS, pcDNA and USP35 (RNA-binding protein FUS = 3). * $p < 0.05$.

USP35 knockdown reduced NSCLC tumor growth by VEGFA

To further confirm USP35/VEGFA roles in NSCLC progression, we performed *in vivo* experiments. Tumor volume and weight were reduced in the sh-USP35 group, and these effects could be abolished by VEGFA overexpression (Fig. 7A,B). USP35 and VEGFA protein levels were indeed decreased in the tumor tissues of sh-USP35 group, and VEGFA overexpression also could enhance VEGFA protein expression (Fig. 7C–E). Additionally, we detected the lower positive cells of proliferation marker Ki67, angiogenesis marker CD31 and VEGFA in the tumor tissues of sh-USP35 group, and these effects were eliminated by VEGFA overexpression (Fig. 7C–E). All data illuminated that USP35 might promote NSCLC tumorigenesis by regulating VEGFA.

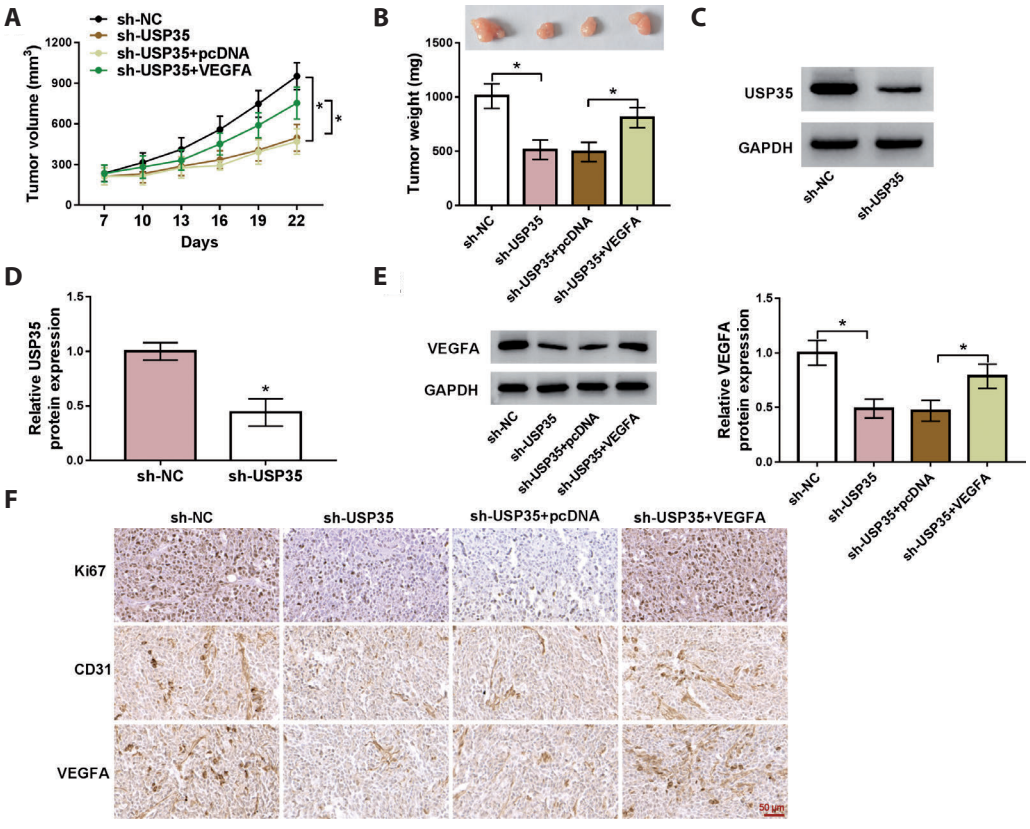

Figure 7. Effects of sh-USP35 and VEGFA on NSCLC tumor growth. Mice were injected with H1299 cells transfected with sh-NC, sh-USP35, pcDNA and VEGFA ($n = 6$). Tumor volume (**A**) and weight (**B**) were determined. USP35 expression (**C, D**) and VEGFA expression (**E**) were detected by Western blot in tumor tissues. **F.** IHC staining detected Ki67, CD31 and VEGFA positive cells. * $p < 0.05$.

Discussion

Tumor angiogenesis is a vital condition for tumorigenesis and metastasis. Therefore, the exploration of angiogenesis-related factors may provide new ideas for the development of anti-tumor drugs. As an important regulator of angiogenesis, the role of VEGFA in tumorigenesis has been largely revealed. Zhang et al. showed that inhibition of VEGFA restrained lung cancer cell proliferation, and *vice versa* (Zhang et al. 2022). Cao et al. reported that VEGFA upregulation promoted propagation and metastasis in bladder cancer cells (Cao et al. 2019). In NSCLC, high VEGFA expression was related to cell proliferation and EMT (Li et al. 2021). Also, reducing of VEGFA expression and secretion had been confirmed to inhibit NSCLC tumor growth *in vivo* (Xie et al. 2021). Consistent with these studies, we identified a significant upregulation of VEGFA in NSCLC tissues by database analysis and qRT-PCR validation. *In vitro* results showed that VEGFA knockdown inhibited NSCLC cell growth, invasion and angiogenesis. This provides new evidence for targeting VEGFA in NSCLC treatment.

The role of deubiquitinating enzyme USP35 in cancer has been studies in many researches. A recent study suggested that USP35 stabilized FUCA1 to accelerate colorectal cancer cell proliferation and chemoresistance (Xiao et al. 2023). Besides, BRPF1 was a direct deubiquitiation target of USP35, and USP35 promoted cell growth, stemness, and migration in prostate adenocarcinoma *via* increasing BRPF1 protein level (Lin et al. 2022). Moreover, USP35 overexpression suppressed PKM2 ubiquitination to restrain its degradation, thereby facilitating hepatocellular carcinoma proliferation and metastasis (Lv et al. 2023). Therefore, USP35-mediated deubiquitination is essential for tumor progression. In NSCLC, USP35 had been confirmed to stabilize RRBP1 and BIRC3 to impair cell apoptosis (Liu et al. 2022; Wang W et al. 2022). Through database analysis, we determined a positive correlation between USP35 and VEGFA expression in LUAD tissues. After further determining the high USP35 expression in NSCLC tissues, we found that USP35 stabilized VEGFA protein expression to enhance NSCLC cell growth, invasion and angiogenesis through deubiquitination. Animal study showed that USP35 knockdown also reduced NSCLC tumorigenesis by decreasing VEGFA protein level. These evidences suggested that USP35 was an upstream target of VEGFA, which mediated NSCLC malignant behavior by stabilizing VEGFA protein.

As an RNA-binding protein, FUS plays an important role in RNA transcription, splicing and microRNA processing (Tan and Manley 2012; Ghanbarpanah et al. 2018). SMURF1 promoted thyroid cancer proliferation and metastasis, which mRNA expression could be stabilized by FUS (Bian 2020). Also, FUS elevated EZH2 stabilization, thereby mediating laryngeal squamous cell cancer progression (Wu T et al. 2021). Previous studies had shown that FUS was overexpressed in NSCLC tissues and was associated with poor prognosis of NSCLC patients (Xiong et al. 2018). In this, we confirmed that FUS increased USP35 mRNA stability to promote its protein level. Besides, USP35 overexpression reversed the reducing effect of FUS knockdown on VEGFA protein level, confirming that FUS interacted with USP35 to positively regulate VEGFA expression.

A recent study showed that high VEGF expression was associated with histology type, low histology grade, clinical stage IV, smoking history and EGFR mutations, but there had no difference in survival for patients in advanced NSCLC grouped by VEGF status (Markovic et al. 2023). This indicates that VEGFA expression is associated with prognosis of NSCLC patients, but may not be an indicator to evaluate the survival rate of patients with advanced NSCLC. This does not conflict with our study. Of course, patient follow-up information may need to be collected in the future to further confirm the relationship between VEGFA and the survival rate of NSCLC patients.

Collectively, our study suggests a VEGFA-related pathway in NSCLC. Our study showed that FUS-stabilized USP35 enhanced VEGFA protein level through deubiquitination, thereby promoting NSCLC growth, invasion and angiogenesis. The discovery of FUS/USP35/VEGFA axis may provide new ideas for the treatment of NSCLC.

Conflict of interest. The authors declare that they have no conflict of interests.

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References

Bian S (2020): miR-4319 inhibited the development of thyroid cancer by modulating FUS-stabilized SMURF1. J. Cell. Biochem. **121,** 174-182
https://doi.org/10.1002/jcb.29026

Braile M, Marcella S, Cristinziano L, Galdiero MR, Modestino L, Ferrara AL, Varricchi G, Marone G, Loffredo S (2020): VEGF-A in cardiomyocytes and heart diseases. Int. J. Mol. Sci. **21,** 5294
https://doi.org/10.3390/ijms21155294

Cao W, Zhao Y, Wang L, Huang X (2019): Circ0001429 regulates progression of bladder cancer through binding miR-205-5p and promoting VEGFA expression. Cancer Biomark. **25,** 101-113
https://doi.org/10.3233/CBM-182380

Cao X, Wu W, Wang D, Sun W, Lai S (2022): Glycogen synthase kinase GSK3alpha promotes tumorigenesis by activating HIF1/VEGFA signaling pathway in NSCLC tumor. Cell. Commun. Signal. **20,** 32
https://doi.org/10.1186/s12964-022-00825-3

Duma N, Santana-Davila R, Molina JR (2019): Non-small cell lung cancer: Epidemiology, screening, diagnosis, and treatment. Mayo Clin. Proc. **94,** 1623-1640 https://doi.org/10.1016/j.mayocp.2019.01.013

Ghanbarpanah E, Kohanpour MA, Hosseini-Beheshti F, Yari L, Keshvari M (2018): Structure and function of FUS gene in prostate cancer. Bratisl. Lek. Listy **119,** 660-663 https://doi.org/10.4149/BLL_2018_118

Huang MJ, Ji YW, Chen JW, Li D, Zhou T, Qi P, Wang X, Li XF, Zhang YF, Yu X, et al. (2023): Targeted VEGFA therapy in regulating early acute kidney injury and late fibrosis. Acta Pharmacol. Sin. **44,** 1815-1825 https://doi.org/10.1038/s41401-023-01070-1

Imyanitov EN, Iyevleva AG, Levchenko EV (2021): Molecular testing and targeted therapy for non-small cell lung cancer: Current status and perspectives. Crit. Rev. Oncol. Hematol. **157,** 103194 https://doi.org/10.1016/j.critrevonc.2020.103194

Kitamura H (2023): Ubiquitin-specific proteases (USPs) and metabolic disorders. Int. J. Mol. Sci. **24,** 3219 https://doi.org/10.3390/ijms24043219

Li G, Li X, Yuan C, Zhou C, Li X, Li J, Guo B (2021): Long noncoding RNA JPX contributes to tumorigenesis by regulating miR-5195-3p/VEGFA in non-small cell lung cancer. Cancer Manag. Res. **13,** 1477-1489 https://doi.org/10.2147/CMAR.S255317

Lin G, Huang T, Zhang X, Wang G (2022): Deubiquitinase USP35 stabilizes BRPF1 to activate mevalonate (MVA) metabolism during prostate tumorigenesis. Cell Death Discov. **8,** 453 https://doi.org/10.1038/s41420-022-01231-x

Liu C, Chen Z, Ding X, Qiao Y, Li B (2022): Ubiquitin-specific protease 35 (USP35) mediates cisplatin-induced apoptosis by stabilizing BIRC3 in non-small cell lung cancer. Lab. Invest. **102,** 524-533 https://doi.org/10.1038/s41374-021-00725-z

Lv T, Zhang B, Jiang C, Zeng Q, Yang J, Zhou Y (2023): USP35 promotes hepatocellular carcinoma progression by protecting PKM2 from ubiquitination-mediated degradation. Int. J. Oncol. **63,** 113 https://doi.org/10.3892/ijo.2023.5561

Ma C, Tang X, Tang Q, Wang S, Zhang J, Lu Y, Wu J, Han L (2022): Curcumol repressed cell proliferation and angiogenesis via SP1/mir-125b-5p/VEGFA axis in non-small cell lung cancer. Front. Pharmacol. **13,** 1044115 https://doi.org/10.3389/fphar.2022.1044115

Markovic M, Mitrovic S, Dagovic A, Jovanovic D, Nikolic T, Ivosevic A, Milosavljevic MZ, Vojinovic R, Petrovic M (2023): Does the expression of vascular endothelial growth factor (VEGF) and Bcl-2 have a prognostic significance in advanced non-small cell lung cancer? Healthcare (Basel) **11,** 292 https://doi.org/10.3390/healthcare11030292

Muthusamy B, Patil PD, Pennell NA (2022): Perioperative systemic therapy for resectable non-small cell lung cancer. J. Natl. Compr. Canc. Netw. **20,** 953-961 https://doi.org/10.6004/jnccn.2022.7021

Pan J, Deng Q, Jiang C, Wang X, Niu T, Li H, Chen T, Jin J, Pan W, Cai X, et al. (2015): USP37 directly deubiquitinates and stabilizes c-Myc in lung cancer. Oncogene **34,** 3957-3967 https://doi.org/10.1038/onc.2014.327

Pirker R (2020): Chemotherapy remains a cornerstone in the treatment of nonsmall cell lung cancer. Curr. Opin. Oncol. **32,** 63-67 https://doi.org/10.1097/CCO.0000000000000592

Salgia R, Pharaon R, Mambetsariev I, Nam A, Sattler M (2021): The improbable targeted therapy: KRAS as an emerging target in non-small cell lung cancer (NSCLC). Cell. Rep. Med. **2,** 100186 https://doi.org/10.1016/j.xcrm.2020.100186

Sun J, Wu S, Jin Z, Ren S, Cho WC, Zhu C, Shen J (2022): Lymph node micrometastasis in non-small cell lung cancer. Biomed. Pharmacother. **149,** 112817 https://doi.org/10.1016/j.biopha.2022.112817

Sun Y, Xiong X, Wang X (2020): The miR-590-3p/VEGFA axis modulates secretion of VEGFA from adipose-derived stem cells, which acts as a paracrine regulator of human dermal microvascular endothelial cell angiogenesis. Hum. Cell. **33,** 479-489 https://doi.org/10.1007/s13577-019-00315-8

Tan AY, Manley JL (2012): TLS/FUS: a protein in cancer and ALS. Cell Cycle **11,** 3349-3350 https://doi.org/10.4161/cc.21875

Wang R, Sun S, Wang Z, Xu X, Jiang T, Liu H, Li X, Ren Z (2022): MCPIP1 promotes cell proliferation, migration and angiogenesis of glioma via VEGFA-mediated ERK pathway. Exp. Cell. Res. **418,** 113267 https://doi.org/10.1016/j.yexcr.2022.113267

Wang W, Wang M, Xiao Y, Wang Y, Ma L, Guo L, Wu X, Lin X, Zhang P (2022): USP35 mitigates endoplasmic reticulum stress-induced apoptosis by stabilizing RRBP1 in non-small cell lung cancer. Mol. Oncol. **16,** 1572-1590 https://doi.org/10.1002/1878-0261.13112

White AL, Bix GJ (2023): VEGFA isoforms as pro-angiogenic therapeutics for cerebrovascular diseases. Biomolecules **13,** 702 https://doi.org/10.3390/biom13040702

Wu T, Wang G, Zeng X, Sun Z, Li S, Wang W, Yu B (2021): Hsa_circ_0006232 promotes laryngeal squamous cell cancer progression through FUS-mediated EZH2 stabilization. Cell Cycle **20,** 1799-1811 https://doi.org/10.1080/15384101.2021.1959973

Wu W, Li Q, Liu YF, Li Y (2021): lncRNA GAS5 regulates angiogenesis by targeting miR-10a-3p/VEGFA in osteoporosis. Mol. Med. Rep. **24,** 711 https://doi.org/10.3892/mmr.2021.12350

Xiao Y, Jiang X, Yin K, Miao T, Lu H, Wang W, Ma L, Zhao Y, Liu C, Qiao Y, et al. (2023): USP35 promotes cell proliferation and chemotherapeutic resistance through stabilizing FUCA1 in colorectal cancer. Oncogenesis **12,** 12 https://doi.org/10.1038/s41389-023-00458-2

Xie X, Hu H, He J, Liu Y, Guo F, Luo F, Jiang M, Wang L (2021): Interleukin-36alpha suppresses growth of non-small cell lung cancer in vitro by reducing angiogenesis. FEBS Open Bio **11,** 1353-1363 https://doi.org/10.1002/2211-5463.13141

Xiong D, Wu YB, Jin C, Li JJ, Gu J, Liao YF, Long X, Zhu SQ, Wu HB, Xu JJ, et al. (2018): Elevated FUS/TLS expression is negatively associated with E-cadherin expression and prognosis of patients with non-small cell lung cancer. Oncol. Lett. **16,** 1791-1800 https://doi.org/10.3892/ol.2018.8816

Yang H, Yang W, Dai W, Ma Y, Zhang G (2020): LINC00667 promotes the proliferation, migration, and pathological angiogenesis in non-small cell lung cancer through stabilizing VEGFA by EIF4A3. Cell. Biol. Int. **44,** 1671-1680 https://doi.org/10.1002/cbin.11361

Zhan S, Wang C, Yin F (2018): MicroRNA-29c inhibits proliferation and promotes apoptosis in non-small cell lung cancer cells by targeting VEGFA. Mol. Med. Rep. **17,** 6705-6710 https://doi.org/10.3892/mmr.2018.8678

Zhang Y, Hu K, Qu Z, Xie Z, Tian F (2022): ADAMTS8 inhibited lung cancer progression through suppressing VEGFA. Biochem. Biophys. Res. Commun. **598,** 1-8 https://doi.org/10.1016/j.bbrc.2022.01.110

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