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Original Article

LncRNA OSER1-AS1 interacts with miR-612/FOXM1 axis to modulate gefitinib resistance of lung adenocarcinoma

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Abstract: Long noncoding RNAs (lncRNAs) play crucial roles in the acquired resistance to EGFR-directed therapies in lung cancer. LncRNA OSER1-AS1 has been reported to promote tumorigenesis of hepatocellular carcinoma. However, its functions and underlying molecular mechanisms remain unclear in the acquired gefitinib-resistance of lung cancer. Our study revealed that increased expression of OSER1-AS1 was correlated with gefitinib resistance in lung adenocarcinoma. Higher OSER1-AS1 expression predicted disease progression of lung adenocarcinoma patients. The *in vitro* assays indicated OSER1-AS1 contributed to gefitinib resistance of lung adenocarcinoma cells via inhibiting cell apoptosis and cell cycle arrest. *In vivo* experiments showed that the knockdown of OSER1-AS1 restored the sensitivity of lung cancer cells to gefitinib. Further studies showed that OSER1-AS1 functioned as a molecular sponge of miR-612. OSER1-AS1 down-regulated miR-612 to increase FOXM1 expression, suggesting that miR-612/FOXM1 axis was regulated by OSER1-AS1, which was partially responsible for gefitinib resistance of lung adenocarcinoma. In conclusion, OSER1-AS1 promoted gefitinib resistance of lung adenocarcinoma through the miR-612/FOXM1 axis.

Keywords: LncRNA OSER1-AS1, lung adenocarcinoma, gefitinib, miR-612, FOXM1

Introduction

Non-small-cell lung cancer (NSCLS) is globally the most frequent subtype of lung cancer, which is also featured withhigh cancer-associated mortalities [1]. Studies identified the oncogenic role of EGFR in the development and progression of lung cancer [2]. Then EGFR-tyrosine kinase inhibitors (TKIs) were developed and emerged as one of the most promising option for lung cancer, especially lung adenocarcinoma with EGFR-activating mutations [3]. However, clinical resistance impeded the long-term therapeutic efficiency of TKIs [4]. Thus, further investigation of the mechanism underlying TKI resistance is essential for improving the prognosis of lung adenocarcinoma patients.

Long non-coding RNAs (lncRNAs) showed critical roles in the regulation of carcinogenesis and progression of human cancer, although they could not be directly translated into proteins [5]. Various mechanisms were involved in the lncRNAs regulated process, such as sponging microRNA (miRNA) as competitive endogenous RNA (ceRNA) [6]. Previous investigation also indicated that some lncRNAs were involved in gefitinib resistance of lung cancer [7]. For instance, lncRNA MALAT1/ZEB1 axis promotes EGFR-TKI resistance through epithelial-to-mesenchymal transition (EMT) regulation [8]. Further studies for lncRNA in the regulation of TKI resistance will contribute to the development of novel strategies to improve therapeutic efficiency.

Recent study identified lncRNA OSER1-AS1 promoted tumorigenesis of hepatocellular carcinoma, which was correlated with its competition with miR-372-3p/Rab23 axis [9]. We also observed increased OSER1-AS1 expression in gefitinib resistance of lung adenocarcinoma.

However, the role of OSER1-AS1 was still unknown in lung adenocarcinoma. In present study, we explored the biological role of OSER1-AS1 in gefitinib resistance of lung adenocarcinoma. Detailed molecular mechanism was also studied in the regulation of miR-612/Forkhead box M1 (FOXM1) axis by OSER1-AS1.

Materials and methods

TCGA dataset analysis

We collected the RNA-seq data and clinical data of the lung adenocarcinoma patients from The Cancer Genome Atlas (TCGA) database website (http://cancergenome.nih.gov/). The statistical analysis for the correlations with clinical characteristics were performed with GraphPad 8. Receiver Operator Characteristic Curve (ROC) and Kaplan-Meier survival curves were analyzed for the prognostic value of OSER1-AS1.

Cell culture

The lung adenocarcinoma cell lines (A549, H1299 and SPCA1) and normal bronchial epithelial cells (16HBE) were purchased from ATCC (Rockville, MD). Cells were cultured in DMEM (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), and maintained in the 5% CO_2 and 37°C incubator. Gefitinib resistant A549 cells (A549/GR) were generated by continual gefitinib treatment for nine months.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay

Total RNA extraction was performed with Trizol reagent (Takara, Japan), and reverse transcription was performed with AMV reverse transcriptase (Takara, Otsu, Japan) and RevertAid™ H Minus First Strand cDNA Synthesis Kit (Takara). SYBR PrimeScript RT-PCR kit (Takara) was used for real-time PCR in BioRad CFX96 Sequence Detection System (BioRad company, Berkeley, CA). GAPDH and U6 were used as gene or microRNA internal control. The primers were synthesized by RiboBio (Guangzhou, China), and the sequences are shown as follows: OSER1-AS1: For, 5'-AATCACTGCAATTGAAGGAAAAA-3', Rev, 5'-CCTTGTTTTCCAACCCTTAGACT-3'; FOXM1: For, 5'-CGTCGGCCACTGATTCTCAAA-3', Rev, 5'-GGCAGGGGATCTCTTAGGTTC-3'; GAPDH: For, 5'-GGGAGCCAAAAGGGTCAT-3', Rev, 5'-GAGTCCTTCCACGATACCAA-3'; miR-612: For, 5'-GCAGGGCTTCTGAGCTCCTTAA-3'; Rev, 5'-GCGAGCACAGAATTAATACGAC; U6: For, 5'-CTCGCTTCGGCAGCAGCACATATA-3', Rev, 5'-AAATATGGAACGCTTCACGA-3'.

RNA oligoribonucleotides and cell transfection

The plasmids of OSER1-AS1 overexpressing or silencing (shOSER1) were designed and synthesized by GeneChem (Shanghai, China). The small interfering RNA against FOXM1, and miR-612 mimics were synthesized by Gene Pharma (Shanghai, China). Cells were transfected with Lipofectamine 3000 (Invitrogen, Paisley, UK). Cells transfection efficiency was evaluated with RT-qPCR assays 48 h after transfection.

Cell proliferation assays

Cell Counting Kit-8 (CCK-8) was purchased for Beyotime (Shanghai, China). Totally 5000 cells were planted in 96-well plates and treated with 0.1 μM gefitinib. We added 10 μl CCK-8 reagent into each well and incubated for 2 h at 37°C. Then cell proliferation was measured with the absorption at 450 nm using a microplate reader (Tecan, Switzerland).

Flowcytometry analysis for cell cycle and apoptosis

Flow cytometry analysis was performed with a FACS Calibur (BD Biosciences, CA). For the cell cycle analysis, gefitinib treated cells were collected and fixed with 75% cold ethanol overnight. Cells were stained with PI and RNase solution form Cell Cycle and Apoptosis Analysis Kit (C1052, Beyotime). For the analysis of cell apoptosis, all collected cells were stained with Annexin V-FITC Apoptosis Detection Kit (C1062S, Beyotime). Cell cycle distributions and cell apoptosis were analyzed with FlowJo 10 software (Tree Star, San Carlos, CA).

Tumorigenesis assays

The tumorigenesis assays were authorized by the animal center of PLA 960th Hospital. BALB/c nude mice were randomly grouped with shOSER1 and control groups (n = 6 each group). Stably infected cells were implanted at the right flank of the mice. Oral gavage gefitinib treat-

ment was performed after 15 days of xenograft implantation, which were administrated with 25 mg/kg in 1% Tween 80 (Sigma, St Louis, MO). Tumor volumes were measured every five days with the formula: Volume = length * width^2/2. The mice were sacrificed on the 30th day after implantation. The xenografts were excised and photographed.

Immunohistochemistry (IHC) staining

IHC staining of the xenografts were performed as our previously study [10]. The primary FOXM1 antibody was purchased from Abcam (ab232649, Cambridge, MA).

Luciferase activity analysis

Wild type and mutant OSER1-AS1 sequences (as shown) were subcloned into the pGL3-basic luciferase reporter vector (Promega, Madison, WI). A549 cells were transfected with the luciferase vector and miRNA mimics (miR-612) or negative control (NT). After cultured for 48 hours, cells were lysed to measure the luciferase activities with the Dual-Luciferase Reporter Assay System (Promega). Normalized firefly luciferase activity was compared between groups as previous report [11].

RNA immunoprecipitation (RIP) assays

We performed RIP assays with the EZ-Magna RIP Kit (Millipore, Billerica, MA). A549 cells were lysed with complete RNA lysis buffer. The magnetic beads conjugated with human anti-Argonaute2 (Ago2) antibody (Millipore) and control IgG (Millipore) were added into the cell lysis. TRIzol reagent was used to collect the RNAs contacted with immunoprecipitates. RT-qPCR assays were performed for the levels of lncRNA and micro RNA.

Western blot

Western blot assays of the transfected cells were performed as the standard conditions. The primary FOXM1 antibody (ab232649) were purchased from Abcam.

Statistics analysis

All *in vitro* experiments were independently performed at least in triplicate. The results were presented as mean ± SD, which were analyzed with SPSS v19.0 and GraphPad Prism 8. The comparison within groups were analyzed with student's t-test or one-way ANOVA. The correlation of RNA expression was analyzed with Pearson correlation test. $P < 0.05$ were considered statistically significant.

Results

OSER1-AS1 was correlated with gefitinib resistance of lung adenocarcinoma

We initially assessed the expression of OSER1-AS1 in adenocarcinoma with the dataset from TCGA database. The results showed higher OSER1-AS1 expression in tumor tissues than adjacent normal tissues ($P = 0.015$, **Figure 1A**). Patients with late clinical stage also showed higher levels of OSER1-AS1 than that with early stage ($P = 0.001$, **Figure 1B**). Notably, the patients with higher OSER1-AS1 expression showed worse response to gefitinib (2 years) (**Figure 1C**). ROC analysis indicated a prognostic value of OSER1-AS1 in disease progression of lung adenocarcinoma (cutoff value = 4.30, area under the curve = 0.666, $P < 0.001$, **Figure 1D**). Kaplan-Meier analysis indicated that higher OSER1-AS1 expression was correlated with shorter disease-free survival (DFS) (log rank test, $P = 0.005$; **Figure 1F**). However, no significant difference was observed between two groups in overall survival (OS) (log rank test, $P = 0.935$; **Figure 1E**). These results suggested that OSER1-AS1 might be involved in lung adenocarcinoma progression, especially gefitinib resistance.

OSER1-AS1 contributed to gefitinib resistance of lung adenocarcinoma cells

To explore the potential function of OSER1-AS1 in lung adenocarcinoma cells, OSER1-AS1 expression was measured in A549, H1299, and SPCA1 cell lines, as well as normal bronchial epithelial cells, 16HBE. RT-qPCR assays indicated low OSER1-AS1 expression in 16HBE cells. And higher OSER1-AS1 was observed in H1299 and SPCA1 cells than A549 cells (**Figure 2A**). a gefitinib resistant A549 cells. Moreover, RT-qPCR assays showed that OSER1-AS1 is significantly expressed in a higher level in gefitinib resistant A549 cells (A549/GR), which were generated with continuous gefitinib treatment, than their parental cells (**Figure 2B**). Then, OSER1-AS1 knockdown and overexpression were performed with gefitinib resistant or parental

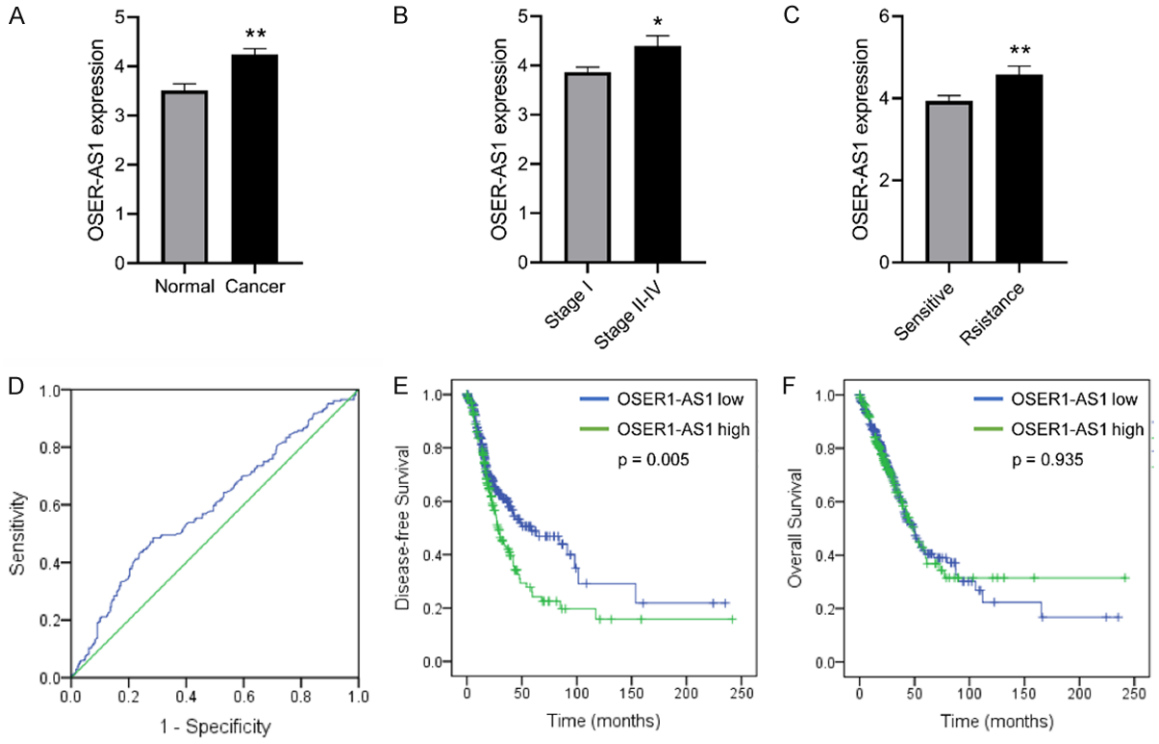
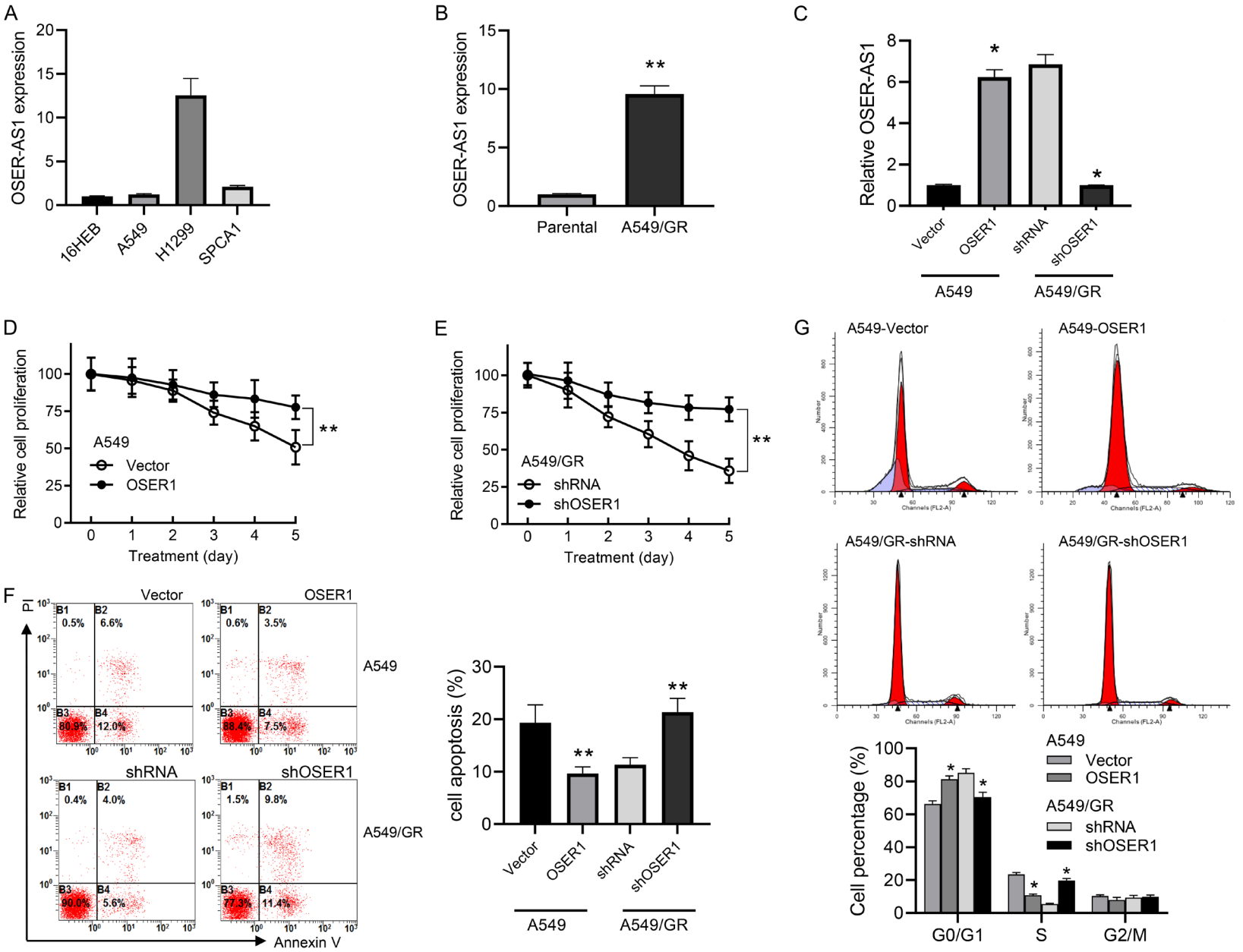


Figure 1. OSER1-AS1 was correlated with gefitinib resistance of lung adenocarcinoma. (A) OSER1-AS1 expression levels were compared between lung adenocarcinoma and adjacent normal tissues from the TCGA dataset. (B) The OSER1-AS1 expression was compared between early stage (Stage I, n = 349) and late stage patients (Stage II-IV, n = 242). (C) OSER1-AS1 expression levels were compared between the gefitinib-sensitive tumors (n = 35) and gefitinib resistance ones (n = 43). Data were shown as mean ± SD. *P < 0.01, **P < 0.001. (D) ROC analysis was performed for the prognostic value of OSER1-AS1 in lung adenocarcinoma. The cutoff value = 4.30, area under the curve = 0.666, P < 0.001. (E, F) Kaplan-Meier analyses for the disease-free survival (E) and overall survival (F) of the patients with high or low OSER1-AS1 expression with the TCGA dataset. *p* values were analyzed with a Log rank test.

A549 cells. Transfection efficacy was verified by RT-qPCR (**Figure 2C**). The transfected cells were treated with 0.1 μM gefitinib followed by cell viability assay. Accelerated cell proliferation was observed in OSER1-AS1 overexpressing cells compared with control cells (**Figure 2D**), whereas OSER1-AS1 knockdown in A549/GR cells showed decreased cell proliferation than corresponding control group (**Figure 2E**). Next, we assessed the function of OSER1-AS1 in cell cycle progression and apoptosis of parental and gefitinib resistant A549 cells. Decreased gefitinib-induced cell apoptosis was observed in OSER1-AS1 overexpressing A549 cells, whereas OSER1-AS1 silencing increased cell apoptosis (**Figure 2F**). Cell cycle analysis also indicated that OSER1-AS1 overexpression increased the percentage of G0/G1 cells and decreased the number of S phase cells with 0.1 μM gefitinib treatment, while opposite results were observed in OSER2-AS1 silencing A549/GR cells (**Figure 2G**). Our results suggested that OSER1-AS1 was involved in gefitinib-resistance by promoting cell survival and cell cycle progression.

OSER1-AS1 knockdown restored gefitinib sensitivity in vivo

We further assessed the role of OSER1-AS1 in gefitinib resistance *in vivo*. Stable OSER1-AS1 silencing cells of A549/GR were subcutaneously implanted into nude mice. The xenografts volumes were measured every five days after gefitinib treatment. We observed decreased growth of the tumors derived from OSER1-AS1 silencing cells than the counterpart group (**Figure 3A**). Decreased volume was notably observed in the OSER1-AS1 silencing tumors (**Figure 3B**). IHC staining was performed with the gefitinib treated tumors, which showed lower percentage of Ki67-positive cells in OSER1-AS1 silencing tumors than the control group (**Figure 3C**). Decreased levels of Bcl-2 expression was also observed in OSER1-AS1 silencing tumors (**Figure 3C**). Consistent with this, flowcytometry


A
OSER-AS1 expression
16HEB
A549
H1299
SPCA1
B
OSER-AS1 expression
Parental
A549/GR
C
Relative OSER-AS1
Vector
OSER1
shRNA
shOSER1
A549
A549/GR
D
Relative cell proliferation
A549
Vector
OSER1
Treatment (day)
E
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Treatment (day)
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OSER1
shRNA
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A549
A549/GR
PI
Annexin V
cell apoptosis (%)
Vector
OSER1
shRNA
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A549
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A549/GR-shOSER1
Cell percentage (%)
A549
Vector
OSER1
A549/GR
shRNA
shOSER1
G0/G1
S
G2/M

Figure 2. OSER1-AS1 contributed to gefitinib resistant lung adenocarcinoma cells. A. Relative expression of OSER1-AS1 was measured in a panel of lung adenocarcinoma cell lines A549, H1299, and SPCA1. Normal lung epithelial cells, 16HBE was used as a control. B. Increased OSER1-AS1 expression in gefitinib resistant A549 cells was observed compared with parental A549 cells. C. The transfection efficiency of OSER1-AS1 overexpression and silencing in gefitinib resistant or parental A549 cells were verified with RT-qPCR assays. D, E. CCK-8 assays were performed for the cell proliferation ability of transfected cells, which were treated with treated with 0.1 μM gefitinib. F. Cell apoptosis of the transfected cells was analyzed after gefitinib treatment, which was shown as scatter diagram (left) and histogram of Q2 and Q4 population (right). G. Cell cycle analysis was performed with the stably transduced cells which were treated with 0.1 μM gefitinib for 72 hours, which was shown as peak diagram (up) and histogram of G0/G1, S, and G2/M phases (down). At least three times were performed for all tests. Data were shown as mean ± SD. **P < 0.001; *P < 0.01.

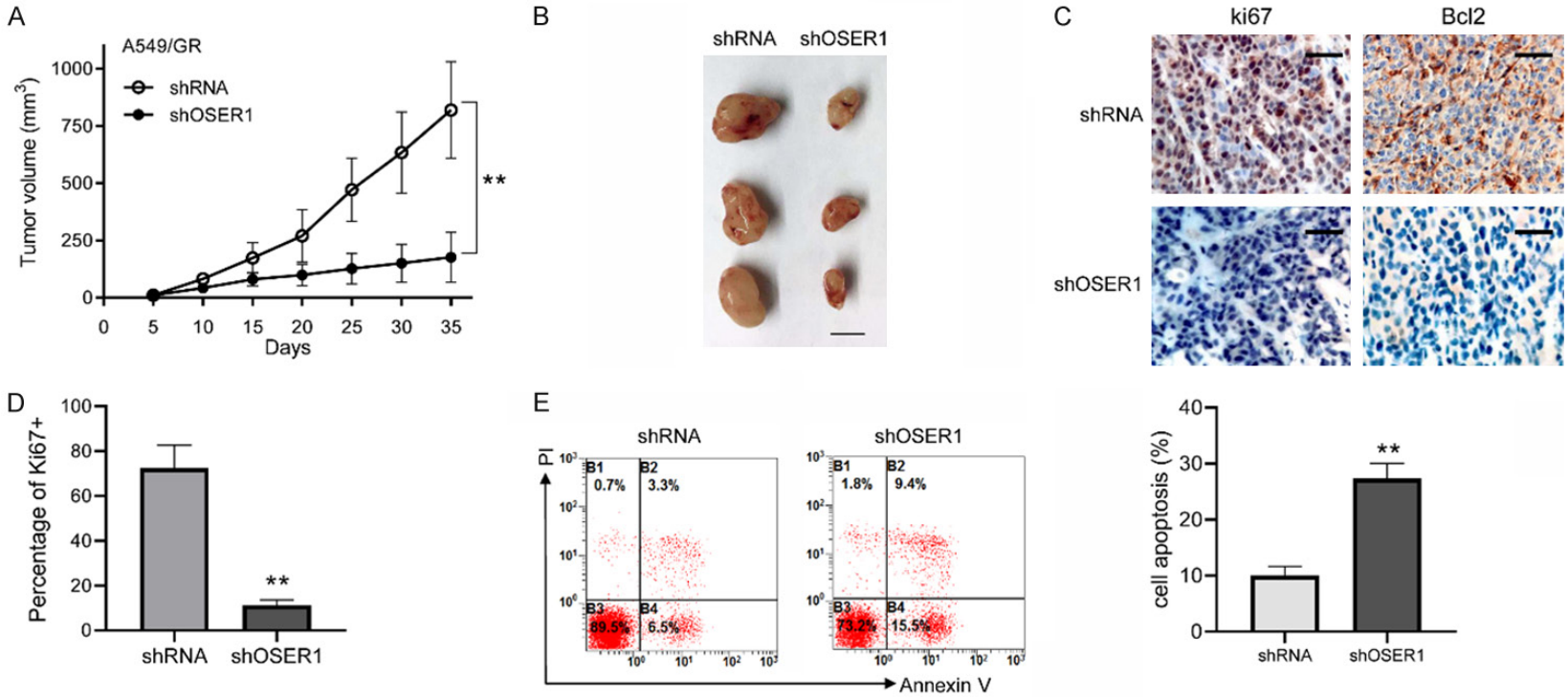

Figure 3. OSER1-AS1 knockdown restored gefitinib sensitivity *in vivo*. A. OSER1-AS1 expression were stably knocked-down in A549-GR cells, then subcutaneously implanted into the nude mice and treated with gefitinib. Tumor volumes were measured every five days. B. The tumors were dissected, photographed on the experimental endpoint. n = 6 each group. Bar = 1 cm. C. IHC staining was performed with the xenografts for the expression of ki67 and bcl2. Bar = 50 μm. D. The histogram showed the percentage of ki67 positive in five random sight of the xenografts. E. Cell apoptosis were analyzed with the fresh tumors of different groups. Data were shown as mean ± SD. *P < 0.01, **P < 0.001.

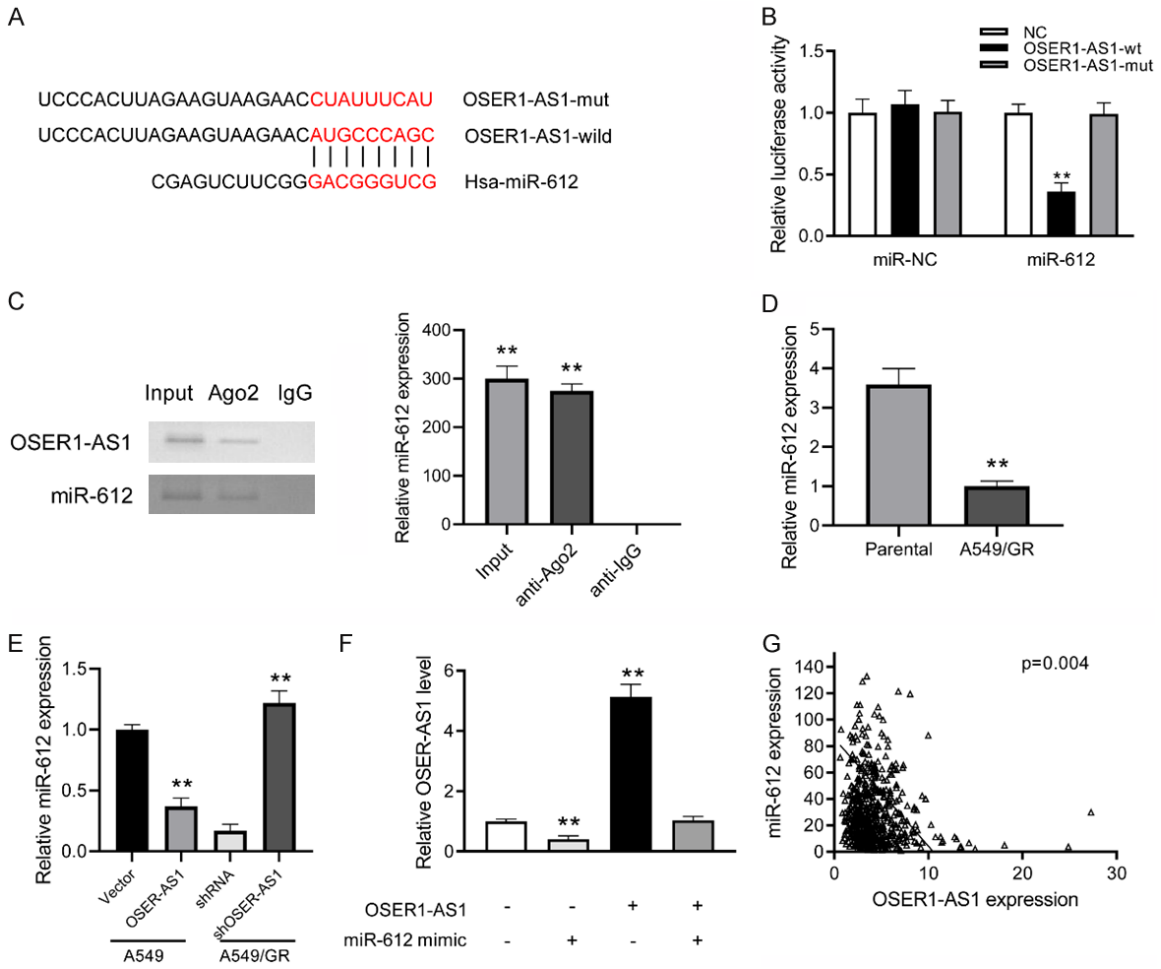

Figure 4. OSER1-AS1 functioned as a molecular sponge of miR-612. A. Putative complementary sites were analyzed between OSER1-AS1 and miR-612. Mutant OSER1-AS1 sequence was designed as shown for luciferase analysis. B. The luciferase activity was measured with A549 cells, which were transfected with luciferase reporters contained wild type or mutant OSER1-AS1, as well as miR-612 mimics or negative control (miR-NC). C. RIP assays were performed with control IgG, anti-Ago2. Then RT-qPCR assays were performed for miR-612 expression levels in the immunoprecipitates. D. The histogram showed that the relative expression of miR-612 in parental and gefitinib resistant A549 cells. E. RT-qPCR analyzed the expression of miR-612 in transfected A549 and A549/GR cells. F. H1299 cells were co-transfected miR-612 and OSER1-AS1 or respectively. The expression levels of OSER1-AS1 was measured with RT-qPCR assays. G. Scatter plots showed the negative linear correlation between the miR-612 and OSER1-AS1 expression in lung adenocarcinoma tissues from TCGA data. Data were shown as mean ± SD. **P < 0.001.

analysis also indicated a higher percentage of cell apoptosis in the OSER1-AS1 silencing tumor cells compared with the control group (**Figure 3D**). Collectively, our results indicated that OSER1-AS1 knockdown reversed gefitinib resistance of lung adenocarcinoma cells both *in vitro* and *in vivo*.

OSER1-AS1 functioned as a molecular sponge of miR-612

Previous studies indicated that lncRNAs could compete with certain miRNAs as an ceRNA, which was responsible for the upregulation of the miRNA-targeted genes [5]. Then, we analyzed the candidate miRNAs which may be interacted with OSER1-AS1 via DIANA tools. We found that miR-612 was predicted to bind to complementary sequences in OSER1-AS1 (**Figure 4A**). Then, the dual-luciferase reporter assays were performed to verify their direct interaction. We observed decreased luciferase activity in the cells which were transfected with wild-type OSER1-AS1 reporter together with miR-612 mimics, whereas there was no significant change in the control group (**Figure 4B**). Further RIP assays also indicated abundant OSER1-AS1 and miR-612 in Ago2-contained

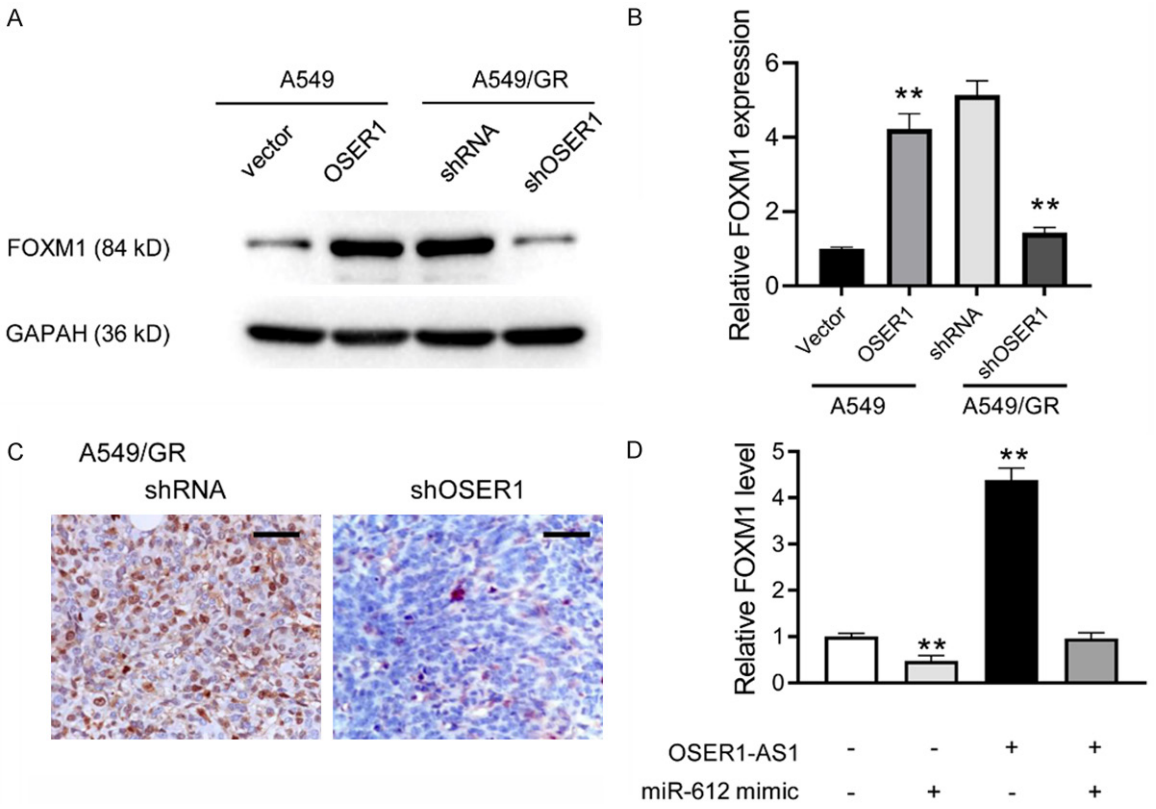

Figure 5. miR-612/FOXM1 axis was regulated by OSER1-AS1. A. Western blot of the expression of FOXM1 in transfected A549 and A549/GR cells. B. RT-qPCR analysis of FOXM1 in transfected A549 and A549/GR cells. C. IHC staining of FOXM1 with the xenografts form transfected A549/GR cells. Bar =50 μm. D. A549 cells were co-transfected with OSER1-AS1 and miR-612 mimics. The expression of FOXM1 was measured with RT-qPCR assays. Data were shown as mean ± SD. *P < 0.01, **P < 0.001.

RNAs (**Figure 4C**). We analyzed the correlation between OSER1-AS1 and miR-612 expression in lung adenocarcinoma cells. RT-qPCR assays indicated lower levels of miR-612 expression in gefitinib resistant cells than parental ones (**Figure 4D**). Moreover, decreased miR-612 level was observed in OSER1-AS1 overexpressing cells, while its increased level in OSER1-AS1 silencing cells (**Figure 4E**). We transfected A549 cells with OSER1-AS1 and miR-612 together, and found that miR-612 down-regulated OSER1-AS1 expression levels in co-transfected cells (**Figure 4F**). Further analysis with TCGA data also indicated a significant reverse correlation between OSER1-AS1 and miR-612 expression in lung adenocarcinoma (**Figure 4G**). These data suggested OSER1-AS1 functioned as a molecular sponge of miR-612.

miR-612/FOXM1 axis was regulated by OSER1-AS1

Previous study indicated that miR-612/FOXM1 axis regulated cell apoptosis of gastric cancer [12]. Further investigation was performed for the correlation of OSER1-AS1 and miR-612/FOXM1 axis. We evaluated the FOXM1 mRNA and protein levels of OSER1-AS1 transfected cells. FOXM1 protein levels were obviously increased in OSER1-AS1 overexpressing cells, while decreased in OSER1-AS1 knockdown cells (**Figure 5A**). Similar results were also observed in FOXM1 mRNA expression levels (**Figure 5B**). IHC staining showed high FOXM1 expression in the xenografts from OSER1-AS1 overexpressing cells (**Figure 5C**). Moreover, A549 cells were co-transfected with OSER1-AS1 and miR-612, and we found that miR-612 induced FOXM1 down-regulation in OSER1-AS1 expressing cells (**Figure 5D**). These results supported miR-612/FOXM1 axis was a downstream cascade of OSER1-AS1.

miR-612/FOXM1 axis contributed to OSER1-AS1 induced gefitinib resistance

Rescue assays were performed for identifying the function of miR-612/FOXM1 axis in OSER1-

AS1 induced gefitinib resistance of lung adenocarcinoma. We knocked-down FOXM1 in OSER1-AS1 overexpressing cells, which was confirmed with RT-qPCR assays (**Figure 6A**). Cell viability was measured with CCK-8 assays, and the results showed that FOXM1 silencing decreased cell viability with 0.1 μM gefitinib treatment (**Figure 6B**). Cell cycle analysis indicated that FOXM1 silencing abrogated S phase accumulation but increased G0/G1 arrest with gefitinib treatment (**Figure 6C**). More importantly, we observed increased cell apoptosis in gefitinib treated and FOXM1 silencing cells than control cells (**Figure 6D**). To further verify the correlation of OSER1-AS1 with miR-612/FOXM1, we analyzed their expression with TCGA dataset. The results indicated higher FOXM1 expression in OSER1-AS1 positive tumors (**Figure 6E**). Moreover, higher percentage of FOXM1 positive patients showed disease progression for gefitinib resistance (**Figure 6F**). Overall, our data suggested that the OSER1-AS1 induced gefitinib resistance, at least partly, through the modulation of miR-612/FOXM1 axis.

Discussion

Our study revealed that increased OSER1-AS1 expression was correlated with gefitinib resistance, which also predicted disease progression of lung adenocarcinoma. Various studies had indicated that several lncRNAs were involved in the progression of lung cancer [13, 14]. For instance, LINC-PINT alleviated lung cancer progression [15], whereas LINC00094 participated in the tumorigenesis of smoking-related lung cancer [16]. Notably, accumulated evidences suggested some lncRNAs were involved in TKI-resistance, including MALAT1 [17], MIR31HG [18], UCA1 [19], and GAS5 [20]. Among them, the activation of EMT regulation pathways were involved in lncRNAs induced EGFR-TKI resistance [19, 21]. Besides, PI3K/AKT and MEK/ERK signaling activation by lncRNAs also contributed to EGFR-TKI resistance in NSCLC [22, 23]. Corresponding signaling inhibition indicated promising value in restoring gefitinib sensitivity in NSCLC cells [24]. A recent study revealed that lncRNA OSER1-AS1 promoted tumorigenesis of hepatocellular carcinoma [9]. In this study, we investigated the functional role of OSER1-AS1 in gefitinib resistance of lung adenocarcinoma. The *in vitro* and *in vivo* assays indicated OSER1-AS1 contributed to gefitinib resistance of lung adenocarcinoma cells via decreased cell apoptosis and rescued cell cycle arrest. More importantly, our investigation provided evidences to overcome gefitinib resistance by targeting OSER1-AS1.

Previous studies indicated that lncRNAs worked as a ceRNA to interact with miRNA, which allow the expression of miRNA targeted genes [6]. Our mechanism study showed that OSER1-AS1 functioned as a molecular sponge of miR-612. In particular, OSER1-AS1 inhibited miR-612 expression to upregulate FOXM1 expression. miR-612 has emerged as a tumor suppressor in multiple types of tumors, such as melanoma [25], bladder cancer [26], colorectal cancer [27] and ovarian cancer [28]. Decreased miR-612 expression in NSCLC was correlated with late clinical stage and lymph node metastasis [29]. Poor prognosis was also observed in NSCLC patients with low miR-612 expression [29]. In this study, we identified a reverse correlation between miR-612 and OSER1-AS1 expression in lung adenocarcinoma. Transfection with miR-612 decreased the luciferase activity of OSER1-AS1 reporter. Enrichment of miR-612 and OSER1-AS1 was observed in the immunoprecipitates of anti-Ago2. In this regard, we confirmed that OSER1-AS1 sponged miR-612 to promote gefitinib resistance in lung adenocarcinoma. Rescue experiments further indicated miR-612 partially reversed the overexpression of OSER1-AS1 of lung adenocarcinoma cells.

We identified that FOXM1 was a downstream target of OSER1-AS1/miR-612, which partially mediated their effects on gefitinib resistance of lung adenocarcinoma. FOXM1 functions as a transcriptional factor to facilitate a various of tumors, including glioblastoma and lung cancer [30-32]. Active FOXM1 was correlated with Kras signaling cascade, which was crucial for the tumorigenesis of lung cancer [33]. FOXM1 transcription was regulated miR-612 in gastric cancer, which was correlated with gastric cancer radio-resistance [34]. Our study indicated an inverse relationship between miR-612 and FOXM1 in lung adenocarcinoma. Meanwhile, silencing FOXM1 restored the gefitinib sensitivity of OSER1-AS1 expressing lung cancer cells, which indicated the potential of targeting

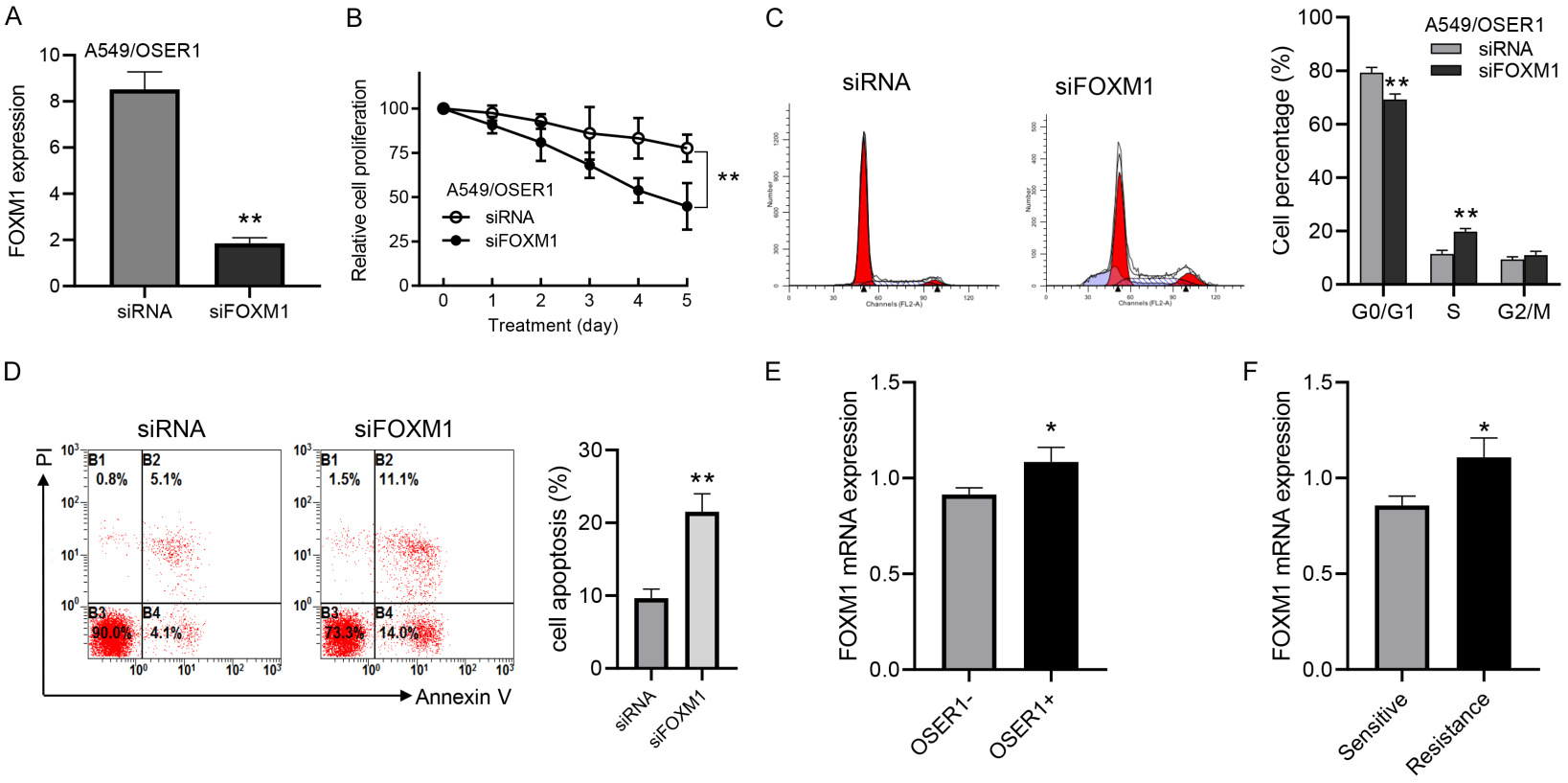

Figure 6. miR-612/FOXM1 axis contributed to OSER1-AS1 induced gefitinib resistance. A. FOXM1 silencing was performed with OSER1-AS1 overexpressing A549 cells. The transfection efficiency was verified with RT-qPCR assays. B. CCK-8 assays were performed for the cell proliferation ability of FOXM1 silencing cells, which were treated with 0.1 μM gefitinib. C. Cell cycle analysis was performed with FOXM1 silencing cells which were treated with 0.1 μM gefitinib for 72 hours, which was shown as peak diagram (left) and histogram of G0/G1, S, and G2/M phases (right). D. Cell apoptosis of the transfected cells was analyzed after gefitinib treatment, which was shown as scatter diagram (left) and histogram of Q2 and Q4 population (right). E. The FOXM1 mRNA expression was compared between high OSER1-AS1 expression and low expression tumors, which was analyzed with TCGA data. F. FOXM1 mRNA expression levels were compared between the gefitinib-sensitive tumors (n = 35) and gefitinib resistance ones (n = 43). Data were shown as mean ± SD. *P < 0.01, **P < 0.001.

FOXM1 to improve gefitinib therapeutic efficiency. Further molecular studies of FOXM1 in gefitinib resistance will shed light to restore therapeutic efficiency of TKIs.

Our work identifies OSER1-AS1 as a key component to promote gefitinib resistance in lung adenocarcinoma. By its interaction with miR-612, OSER1-AS1 facilitates FOXM1 expression in lung adenocarcinoma cells, consequently leading to gefitinib resistance. Our findings suggested that OSER1-AS1/miR-612/FOXM1 blockage may represent a promising therapeutic strategy for reversing gefitinib resistance of lung adenocarcinoma.

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Disclosure of conflict of interest

None.

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