Cancer-associated mesenchymal stem cell exosomes facilitate non-small cell lung cancer cell viability and invasiveness by delivering miR-182 in a FBXW7-related AKT and ERK-dependent pathway

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Abstract. Cancer-associated mesenchymal stem cells (CA-MSCs) modulate the tumor microenvironment and promote tumor progression. The present study aimed to investigate the effects of CA-MSCs, CA-MSC-derived exosomes and CA-MSC exosome-derived microRNA (miR)-182 on non-small cell lung cancer (NSCLC) cell viability and invasiveness. CA-MSCs were established by treating MSCs with supernatant from NSCLC cells. Then, two NSCLC cell lines (A549 and H1299) were treated with CA-MSCs, CA-MSCs + GW4869 (inhibits exosomes) and CA-MSC exosomes. Additionally, miR-182 inhibitor was added to CA-MSCs and the related exosomes were used to treat NSCLC cells. Furthermore, miR-182 mimic and F-box and WD repeat domain containing 7 (FBXW7) overexpression vector were used to treat NSCLC cells. The results indicated that CA-MSCs promoted NSCLC cell viability and invasiveness and inhibited cell apoptosis, an effect that was attenuated following GW4869 treatment. The CA-MSC exosomes also enhanced NSCLC cell viability and invasiveness while inhibiting cell apoptosis. In addition, CA-MSC exosomes elevated miR-182 expression in NSCLC cells. Subsequently, CA-MSC exosomes with miR-182 expression knockdown exhibited a weakened effect on NSCLC cell viability, apoptosis and invasiveness compared with control CA-MSC exosomes. Direct miR-182 mimic transfection enhanced NSCLC cell viability and invasiveness and inhibited cell apoptosis, an effect that was attenuated by transfection with the FBXW7 overexpression vector. Furthermore, miR-182 negatively regulated and sponged FBXW7 expression in NSCLC cells. Finally, treatment of the cells with miR-182 mimic increased the phosphorylated (p-) AKT and p-ERK1/2 expression levels, while treatment with the FBXW7 overexpression vector decreased these levels in NSCLC cells. In summary, CA-MSCs facilitated NSCLC viability and invasiveness via transmitting exosomal miR-182 in a FBXW7-related AKT and ERK-dependent pathway.

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Introduction

Lung cancer is a critical malignancy with both high incidence and mortality. A 2020 world cancer statistical report estimated that 2.21 million new cases and 1.80 million new deaths occur due to lung cancer every year globally (1). Furthermore, according to an up-to-date Chinese cancer statistical report, 1.06 million new cases and 0.73 million new deaths due to lung cancer occur every year in China (2). As the major type of lung cancer, non-small cell lung cancer (NSCLC) comprises 80-85% of the total lung cancer cases (3). The exploration of the mechanism of NSCLC has enabled the discovery of various potential targets, such as EGFR, anaplastic lymphoma kinase, ROS proto-oncogene 1, ret proto-oncogene and BRAF; tyrosine kinase inhibitors that inhibit some of these targets have already been applied in clinical therapy and improve the prognosis of patients with NSCLC (4,5). This emphasizes the importance of identifying the mechanism of progression of NSCLC.

Cancer-associated mesenchymal stem cells (CA-MSCs) play a crucial role in modulating the tumor microenvironment and promoting tumor development (6,7). MSCs are abundant in the bone marrow, adipose tissue, placenta, umbilical cord and other tissues, and are pluripotent stem cells with multilineage differentiation potential, with the capability of differentiating into osteoblasts, chondrocytes and adipocytes (8). During tumor progression, MSCs are recruited into the tumor and are transformed into CA-MSCs, undergoing substantial changes in their phenotypes and functions (9-11). Recently, certain studies have demonstrated that CA-MSCs can also promote tumor progression by secreting exosomes and transferring small molecules, such as proteins and RNAs (12,13). For example, CA-MSCs are able to transfer transmembrane

BAX inhibitor motif containing 6 (TMBIM6) via exosomes to modify hepatocellular carcinoma viability, invasiveness and epithelial-to-mesenchymal transition (EMT), promoting its malignant progression (12). However, the involvement of CA-MSCs or CA-MSC exosomes in NSCLC pathogenesis has not been clearly defined. Moreover, microRNA (miR)-182 has been reported to be an oncogene in a number of cancer types, including NSCLC (14-19). Our previous study also uncovered a tumor-promoting role of miR-182 in NSCLC (20).

Therefore, the present study aimed to investigate the effect of CA-MSCs, CA-MSC exosomes and CA-MSC exosome-derived miR-182 on the viability and invasiveness of NSCLC cells.

Materials and methods

Cell culture. The NSCLC cell lines (A549 and H1299) were provided by Beyotime Institute of Biotechnology. The CA-MSCs were established by treating MSCs (Procell Life Science & Technology Co., Ltd.) with supernatant from A549 or H1299 cells for 14 days (21). The cells were cultured in Dulbecco's Modified Eagle's Medium (Procell Life Science & Technology Co., Ltd.) supplemented with 10% fetal bovine serum (Sigma-Aldrich; Merck KGaA) at 37˚C with 5% CO_2.

Isolation and identification of CA-MSC exosomes. CA-MSC exosomes were separated via an Exosome Isolation Kit (Shanghai Yeasen Biotechnology Co., Ltd.) in accordance with the standard procedure. The exosomes were identified with nanoparticle tracking analysis by Wuhan Servicebio Technology Co., Ltd., as shown in Fig. S1A. Western blotting was used to detect the marker proteins of the exosomes Fig. S1B.

Co-culture experiments. For the inhibition of exosome generation, CA-MSCs were treated with GW4869 for 24 h (10 μM; MedChemExpress) at 37˚C. The NSCLC cell and CA-MSC co-culture experiments were performed in a Transwell insert (Corning, Inc.). In brief, A549 ($5x10^4$) or H1299 cells ($5x10^4$) were added to the lower chamber and the CA-MSCs ($5x10^4$) or GW4869-treated CA-MSCs (CA-MSC-GW; $5x10^4$) were added to the upper chamber. The mock group was A549 or H1299 cells cultured alone, and the CA-MSC Exo group was A549 or H1299 cells cultured with exosomes isolated from CA-MSCs (10^6 particles/cell). Following culture for 24 h at 37˚C, the cells were collected to assess cell viability, invasion and apoptosis as well as to perform reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays.

Co-culture of NSCLC cells with exosomes from miR-182-knockdown CA-MSCs. The CA-MSCs were transfected with miR-182 inhibitor (miR-inh; 5'-AGUGUGAGUUCUACC AUUGCCAAA-3'; 0.5 pmol) or negative control inhibitor (NC-inh; 5'-CAGUACUUUUGUGUAGUACAA-3'; 0.5 pmol) (Genepharm, Inc.) using Lipofectamine™ 2000 (Thermo Fisher Scientific., Inc.) at 37˚C for 6 h. The exosomes from the transfected or non-transfected CA-MSCs were subsequently isolated (48 h after transfection). The non-transfected CA-MSCs were set as a control. The miR-182 expression levels in CA-MSCs and their exosomes were detected via RT-qPCR assay (48 h after transfection). Subsequently, A549 and H1299 cells were treated with the exosomes (10^6 particles/cell) from non-transfected CA-MSCs (Control exo) or transfected CA-MSCs (NC-inh exo and miR-inh exo). The mock group was prepared alone without exosome treatment. Following culture for 24 h at 37˚C, A549 and H1299 cells were harvested for cell viability, invasion, apoptosis, RT-qPCR and western blotting assays.

miR-182 target gene validation. The target gene of miR-182 was analyzed using miRWalk (mirwalk.umm.uni-heidelberg.de). The wild type (WT) or mutant type (MT) plasmid was constructed by cloning FBXW7 3' untranslated region (UTR) wild sequence or mutant sequences into pGL6 vector (Beyotime Institute of Biotechnology). The 0.8 μg WT plasmid, 0.8 μg MT plasmid, 50 pmol NC mimics and 50 pmol miR-182 mimics were co-transfected into 293T cells (Beyotime Institute of Biotechnology) in the presence of Lipofectamine™ 2000 (Thermo Fisher Scientific., Inc.). The cells were harvested 48 h after transfection. Following the manufacturer's procedure, the transcriptional regulation between miR-182 and FBXW7 was analyzed using a Dual-Luciferase Reporter Gene Assay Kit (Beyotime Institute of Biotechnology) and normalized to *Renilla* activity.

miR-182 and FBXW7 transfection experiments in NSCLC cells. miR-182 mimic (sense, 5'-UUUGGCAAUGGUAGAACU CACACU-3'; and antisense, 5'-UGUGAGUUCUACCAUUGC CAAAUU-3'), negative control miR mimic (sense, 5'-UUC UCCGAACGUGUCACGUTT-3'; and antisense, 5'-ACG UGACACGUUCGGAGAATT-3'), the FBXW7 overexpression vector (OE-FBXW7) and the negative control vector were constructed by Shanghai GenePharma Co., Ltd. The pcDNA3.1 vectors were used for FBXW7 overexpression and the negative control. A549 and H1299 cells were transfected with the aforementioned mimics and vectors in the following grouping: i) Mock group, without transfection; ii) scramble group, transfected with 50 pmol negative control miR mimic and 50 pmol negative control vector; iii) miR-mimic group, transfected with 50 pmol miR-182 mimic and 0.8 μg negative control vector; iv) OE-FBXW7 group, transfected with 0.8 μg negative control 50 pmol miR mimic and 0.8 μg FBXW7 overexpression vector; and v) miR-mimic + OE-FBXW7 group, transfected with 50 pmol miR-182 mimic and 0.8 μg FBXW7 overexpression vector. Following transfection for 48 h according to the aforementioned protocol, RT-qPCR, western blotting, cell viability, cell invasion and cell apoptosis assays were performed. The transfection efficiency was assessed by RT-qPCR assays after transfection with miR-mimic, OE-FBXW7 and their respective negative controls.

Cell viability. The cell viability of A549 and H1299 cells was assessed using Super-Enhanced Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology). Briefly, 10 μl CCK-8 reagent was added to a 200 μl medium containing treated cells in a 96-well plate for 1 h. The optical density was detected using a microplate reader (Shanghai Flash Biotechnology Co., Ltd.) and the relative cell viability was measured (normalized to Mock group).

Cell apoptosis. The Annexin V-Alexa Fluor 488/PI Apoptosis Detection Kit (cat. no. 40305ES50; Shanghai Yeasen Biotechnology Co., Ltd.) was used to assess the cell apoptotic rate.

Briefly, A549 and H1299 cells were collected and washed with a binding solution. Subsequently, 5 μl Annexin V-fluorescein isothiocyanate and 10 μl propidium iodide were incubated with the sample for 20 min, successively. The apoptotic cells were examined by flow cytometry (FACSCalibur; BD Biosciences). The data was analyzed by FlowJo X (BD Biosciences).

Cell invasion. A Transwell assay was carried out to assess cell invasion. In brief, Transwell inserts were pre-coated with Matrigel (Corning, Inc.) at 37˚C for 1 h. A549 ($2x10^4$) and H1299 ($2x10^4$) cells were added to the upper chamber of a 24-well plate with Matrigel-coated inserts (Corning, Inc.), and the lower chamber was filled with complete medium (Dulbecco's modified Eagle's medium with 10% fetal bovine serum). Following treatment of the cells for 24 h at 37˚C, the invasive cells were stained with crystal violet (Wuhan Servicebio Technology Co., Ltd.) at room temperature for 10 min and subsequently counted manually using an inverted optical microscope (Keyence Corporation).

RT-qPCR. Total RNA was isolated from cells or exosomes with the TRIzol® reagent (Thermo Fisher Scientific, Inc.). RT-qPCR was performed using the SweScript One-Step reverse transcription-PCR Kit (Wuhan Servicebio Technology Co., Ltd.) according to the manufacturer's instructions. The primer sequences used were as follows: miR-182 [forward, GCGTTTGGCAATGGTAGAACT; and reverse, AGTGCA GGGTCCGAGGTATT (universal primer)], U6 (forward, CTC GCTTCGGCAGCACA; and reverse, AACGCTTCACGA ATTTGCGT), FBXW7 (forward, TTCACCAACTCTCCT CCCCATT; and reverse, GCTGAACATGGTACAAGCCCA) and GAPDH (forward, ACAACTTTGGTATCGTGGAAGG; and reverse, GCCATCACGCCACAGTTTC). The result was analyzed with $2^{-\Delta\Delta Cq}$ method (22). The U6 and GAPDH were the reference genes for miR-182 and FBXW7, respectively.

Western blotting. The proteins were separated from cells or exosomes with RIPA buffer (Beyotime Institute of Biotechnology) and quantified with a BCA kit (Wuhan Servicebio Technology Co., Ltd.). Electrophoresis (4-20% precast gel) was performed and 20 μg protein/lane was transferred to a nitrocellulose membrane (Wuhan Servicebio Technology Co., Ltd.). Subsequently, the membranes were blocked using 5% bovine serum albumin (Wuhan Servicebio Technology Co., Ltd.) at 37˚C for 1 h and incubated overnight at 4˚C with the following primary antibodies from Affinity Biosciences, Ltd.: Tumor susceptibility gene 101 (cat. no. DF8427; 1:1,000), cluster of differentiation (CD)9 (cat. no. AF5139; 1:500), CD81 (cat. no. DF2306; 1:500), Calnexin (cat. no. AF5362; 1:500), FBXW7 (cat. no. DF12400; 1:1,000), phosphorylated (p)-AKT (cat. no. AF0016; 1:500), AKT (cat. no. AF6261; 1:1,000), p-ERK (cat. no. AF1015; 1:500), ERK (cat. no. AF0155; 1:1,000) and GAPDH (cat. no. AF7021; 1:2,000). The membrane was subsequently incubated with secondary antibodies (cat. no. S0001; 1:5,000; Affinity Biosciences, Ltd.) at 37˚C for 1 h. The bands were visualized using an electrochemiluminescence (ECL) substrate (Affinity Biosciences, Ltd.). Density gray values were measured by ImageJ V1.8 (https://imagej.net/ij/).

For p-AKT/p-ERK and AKT/ERK detection, the membrane was first incubated with GAPDH antibody followed by visualization via ECL. Then, the GAPDH antibody was eluted using stripping buffer (Beyotime Institute of Biotechnology) and the membrane was incubated with p-AKT/p-ERK antibody followed by visualization via ECL. The p-AKT/p-ERK antibody was eluted using stripping buffer and the membrane was incubated with AKT/ERK antibody followed by visualization via ECL.

Statistical analysis. Repeats were in triplicates and data are presented as the mean ± standard deviation. Unpaired students' t-test or one-way ANOVA followed by Tukey's test were used to analyze comparisons between two and multiple groups, respectively. Analyses were performed using GraphPad Prism 9 (Dotmatics). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effect of CA-MSCs and CA-MSC exosomes on NSCLC viability and invasiveness. CA-MSCs promoted the viability and invasiveness, whereas they inhibited the apoptosis of A549 cells (CA-MSC vs. Mock group; Fig. 1A and C). However, following the elimination of exosome production by GW4869, the effect of CA-MSCs was attenuated in A549 cells (CA-MSC-GW vs. CA-MSC group). In addition, following exosome isolation, CA-MSC exosomes facilitated the viability and invasiveness (Fig. 1B), whereas they repressed the apoptosis of A549 cells (CA-MSC exo vs. mock group). Subsequently, the aforementioned findings were further confirmed in H1299 cells (Fig. 1D-F).

Effect of miR-182-knockdown CA-MSC exosomes on NSCLC viability and invasiveness. CA-MSCs and CA-MSC exosomes increased the levels of miR-182 in both A549 and H1299 cells (CA-MSC and CA-MSC exo vs. Mock group; Fig. 2A and B). However, following the elimination of exosome production by GW4869, CA-MSCs had no effect on miR-182 levels (CA-MSC-GW vs. mock group), indicating that CA-MSCs may deliver miR-182 to A549 and H1299 cells. Based on previous studies that examined the significant role of miR-182 in the oncogenesis of lung cancer (14-16), the miR-182 levels were knocked down by a miR-182 inhibitor in CA-MSCs and the corresponding exosomes were collected to detect whether its diminishment would weaken the effect of CA-MSC exosomes on NSCLC viability and invasiveness. The miR-182 inhibitor significantly decreased miR-182 levels in both CA-MSCs and CA-MSC exosomes (miR-inh vs. NC-inh group; Fig. 2C-F).

CA-MSC exosomes increased the viability and invasiveness while suppressing the apoptosis of A549 cells (Control exo vs. Mock group); this effect was not altered following transfection with the NC inhibitor (NC-inh exo vs. Control exo group) (Fig. 3A-C). It is important to note that following transfection with the miR-182 inhibitor, the effect of CA-MSC exosomes on cell viability and apoptosis was attenuated, whereas the invasiveness of A549 cells was not affected (miR-inh exo vs. NC-inh exo group; Fig. 3A-C). Furthermore, similar results were observed in H1299 cells as those in A549 cells (Fig. 3D-F).

Effect of miR-182 knockdown in CA-MSC exosomes on FBXW7 expression in NSCLC cells. FBXW7 was previously

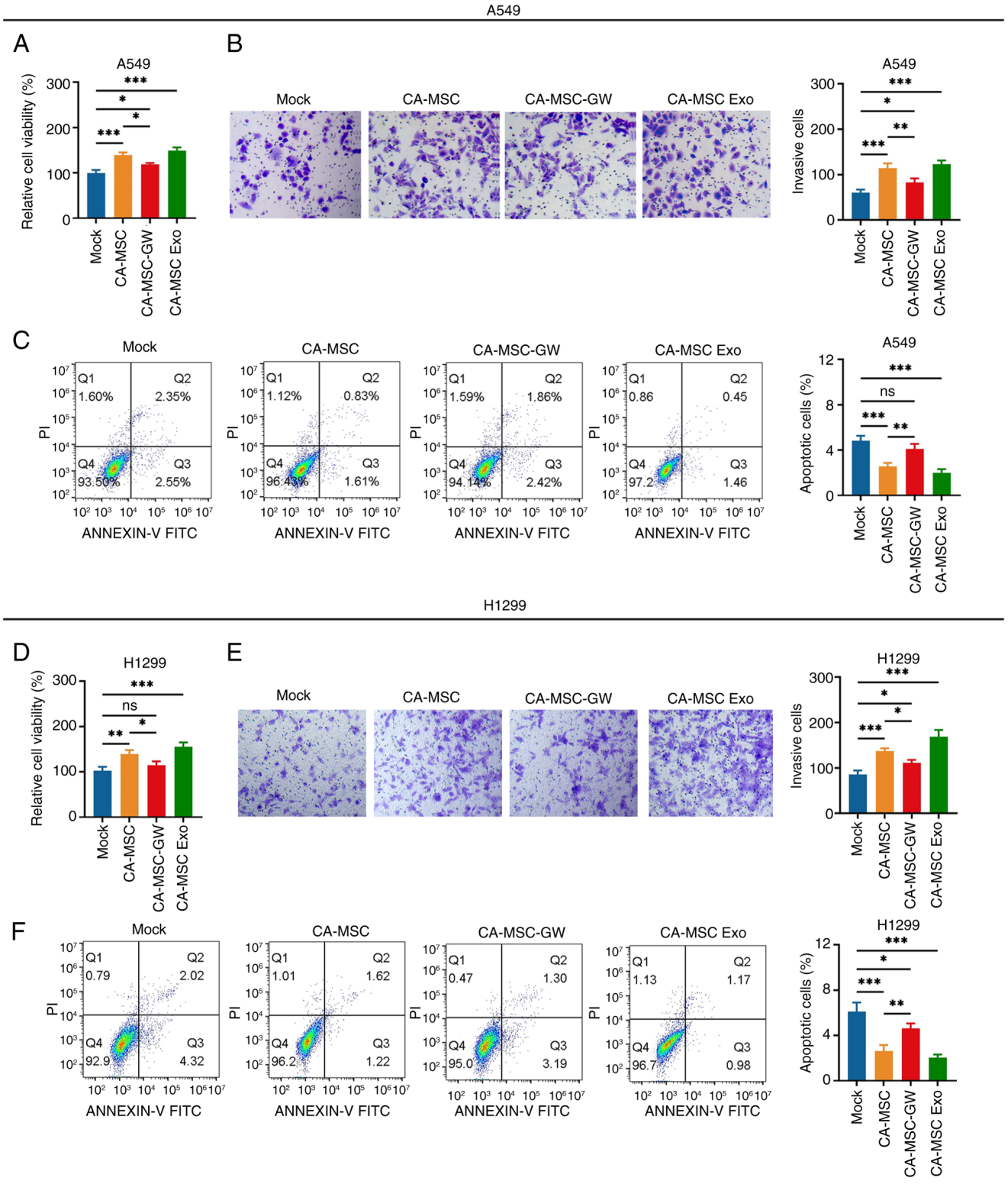

Figure 1. CA-MSCs promote non-small cell lung cancer cell viability and invasiveness via delivering exosomes. Comparison of the (A) viability, (B) number of invasive cells (magnification, x200) and (C) apoptosis rate of A549 cells in the Mock, CA-MSC, CA-MSC-GW and CA-MSC Exo groups. Comparison of the (D) viability, (E) number of invasive cells (magnification, x200) and (F) apoptosis rate of H1299 cells in the Mock, CA-MSC, CA-MSC-GW and CA-MSC Exo groups. *P<0.05, **P<0.01 and ***P<0.001; ns, no significance. CA-MSC, cancer-associated mesenchymal stem cell; CA-MSC-GW, GW4869-treated CA-MSCs; CA-MSC Exo, exosomes derived from CA-MSCs.

reported to be a direct target of miR-182 in various cancer types (23-25); therefore, it was further speculated that CA-MSC exosome-derived miR-182 promoted NSCLC viability and invasiveness via sponging FBXW7. Subsequently, it was observed that CA-MSC exosomes with miR-182 knockdown increased the FBXW7 expression levels compared with the CA-MSC exosomes with NC-knockdown (miR-inh exo vs. NC-inh exo group) in A549 (Fig. 4A and B) and H1299 cells (Fig. 4C and D).

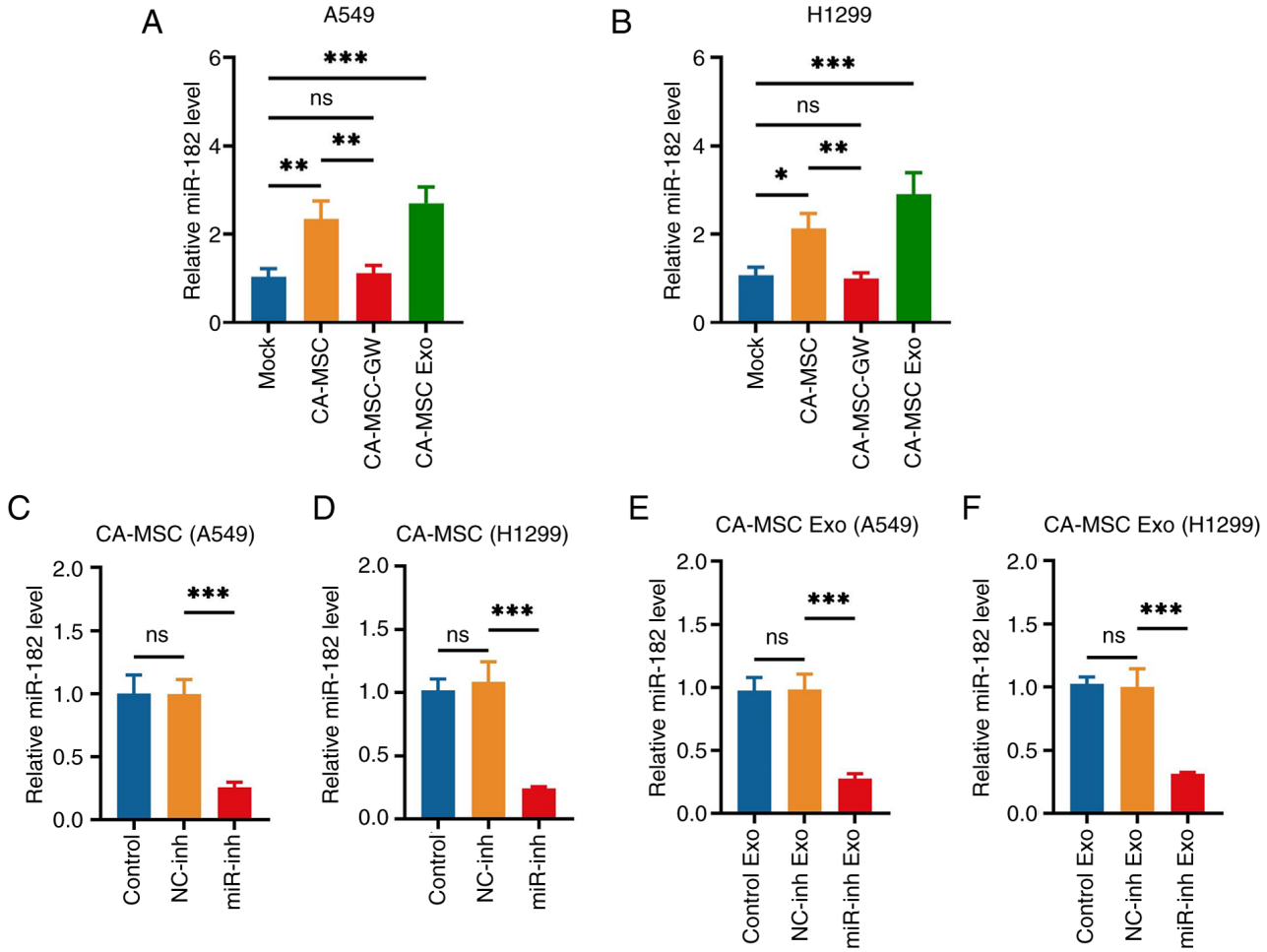

Figure 2. miR-182 expression quantifications. miR-182 expression in (A) A549 cells and (B) H1299 cells in the Mock, CA-MSC, CA-MSC-GW and CA-MSC Exo groups. After transfection, miR-182 expression in CA-MSCs cocultured with (C) A549 and (D) H1299 cells and the corresponding (E,F) CA-MSC exosomes, in the Control, NC-inh and miR-inh Exo groups. *P<0.05, **P<0.01 and ***P<0.001; ns, no significance. miR-182, microRNA-182; CA-MSC, cancer-associated mesenchymal stem cell; CA-MSC-GW, GW4869-treated CA-MSCs; CA-MSC Exo, exosomes derived from CA-MSCs; NC, negative control; inh, inhibitor.

In addition, miR-182 was confirmed to directly sponge FBXW7 via the Dual-Luciferase Reporter Gene assay (Fig. S2).

Effect of miR-182 and FBXW7 on NSCLC viability and invasiveness. miR-182 expression was increased after miR-182 mimic transfection (miR-mimic vs. NC-mimic group; Fig. S3A and B), and FBXW7 expression was elevated after OE-FBXW7 vector transfection (OE-FBXW7 vs. Control vector group; Fig. S3C and D), indicating transfection success. Following transfection in A549 cells, the miR-182 mimic decreased FBXW7 expression (miR-mimic vs. Scramble group), while FBXW7 overexpression increased the expression of this protein (OE-FBXW7 vs. Scramble group) and attenuated the impact of miR-182 mimic (miR-mimic + OE-FBXW7 vs. miR-mimic group) (Fig. 5A and B). The aforementioned observations were further confirmed in H1299 cells (Fig. 5C and D).

The miR-182 mimic elevated cell viability while reducing apoptosis, whereas it impacted the invasiveness of A549 cells to a lesser extent (miR-mimic vs. scramble group; Fig. 6A-C). FBXW7 overexpression decreased cell viability and invasiveness, whereas it enhanced the apoptosis of A549 cells (OE-FBXW7 vs. scramble group). In addition, FBXW7 overexpression weakened the effect of the miR-182 mimic on the aforementioned functions of A549 cells (miR-mimic + OE-FBXW7 vs. miR-mimic group). Furthermore, similar results were observed in H1299 cells as those in A549 cells (Fig. 6D-F).

Effect of miR-182 and FBXW7 on AKT and ERK expression in NSCLC cells. The miR-182 mimic elevated the p-AKT and p-ERK1/2 expression levels in A549 cells (miR-mimic vs. Scramble group; Fig. 7A). FBXW7 overexpression reduced the p-AKT and p-ERK1/2 expression levels in A549 cells (OE-FBXW7 vs. scramble group) and attenuated the effect of miR-182 mimic (miR-mimic + OE-FBXW7 vs. miR-mimic group). Subsequently, the aforementioned observations were also verified in H1299 cells (Fig. 7B).

Discussion

CA-MSCs are a group of cells derived from MSCs that interact with cancer cells; they present essential roles in the development of the tumor microenvironment and are involved in oncogenesis (6). Since this concept was proposed in recent years and the related research studies are insufficient, the detailed mechanism

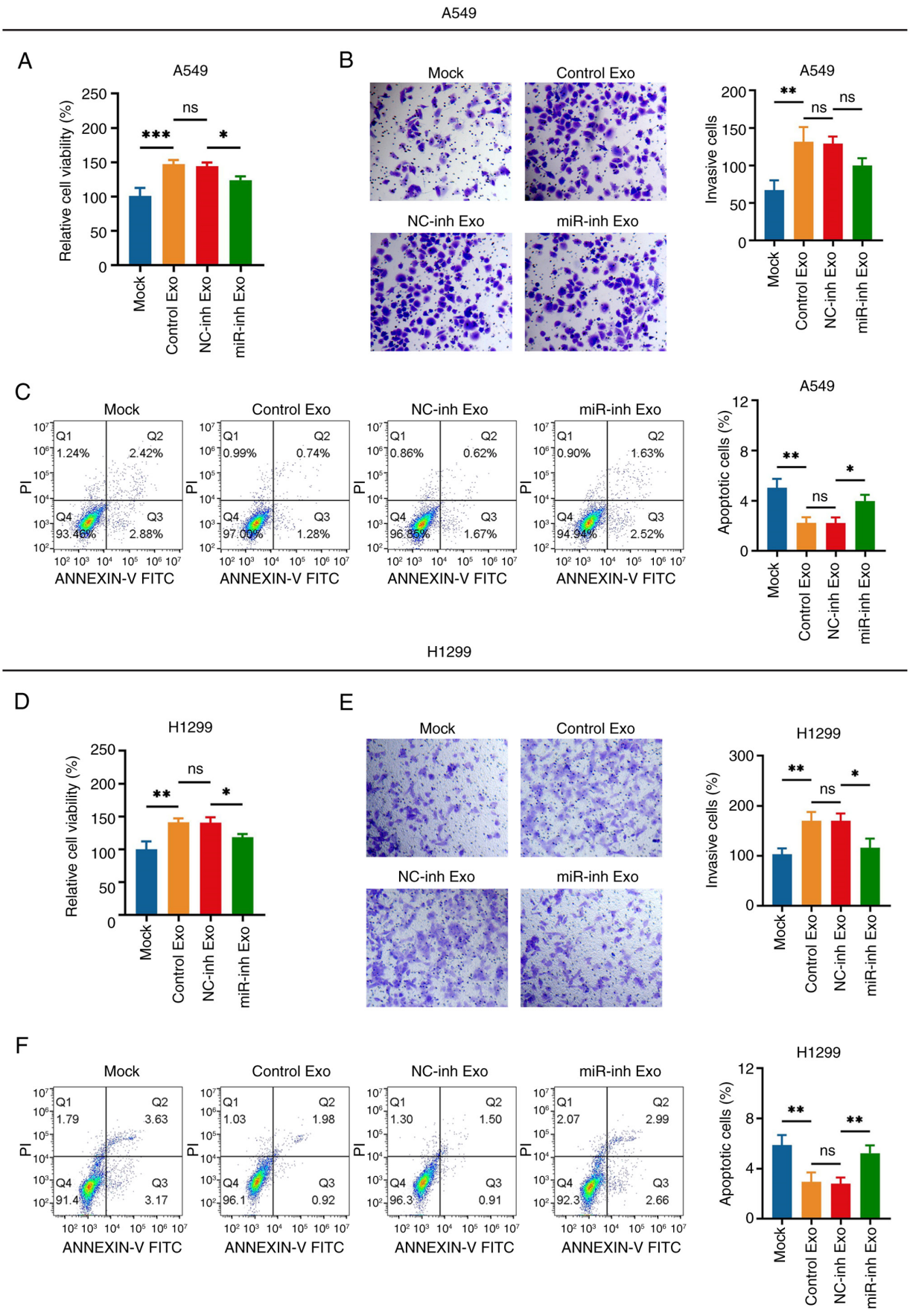

Figure 3. miR-182 knockdown attenuates the effect of CA-MSC exosomes on non-small cell lung cancer cell viability and invasiveness. Comparison of the (A) viability, (B) number of invasive cells (magnification, x200) and (C) apoptosis rate of A549 cells in the Mock, Control Exo, NC-inh Exo and miR-inh Exo groups. Comparison of the (D) viability, (E) number of invasive cells (magnification, x200) and (F) apoptosis rate of H1299 cells in the Mock, Control Exo, NC-inh Exo and miR-inh Exo groups. *P<0.05, **P<0.01 and ***P<0.001; ns, no significance. miR-182, microRNA-182; Exo, exosomes; NC, negative control; inh, inhibitor.

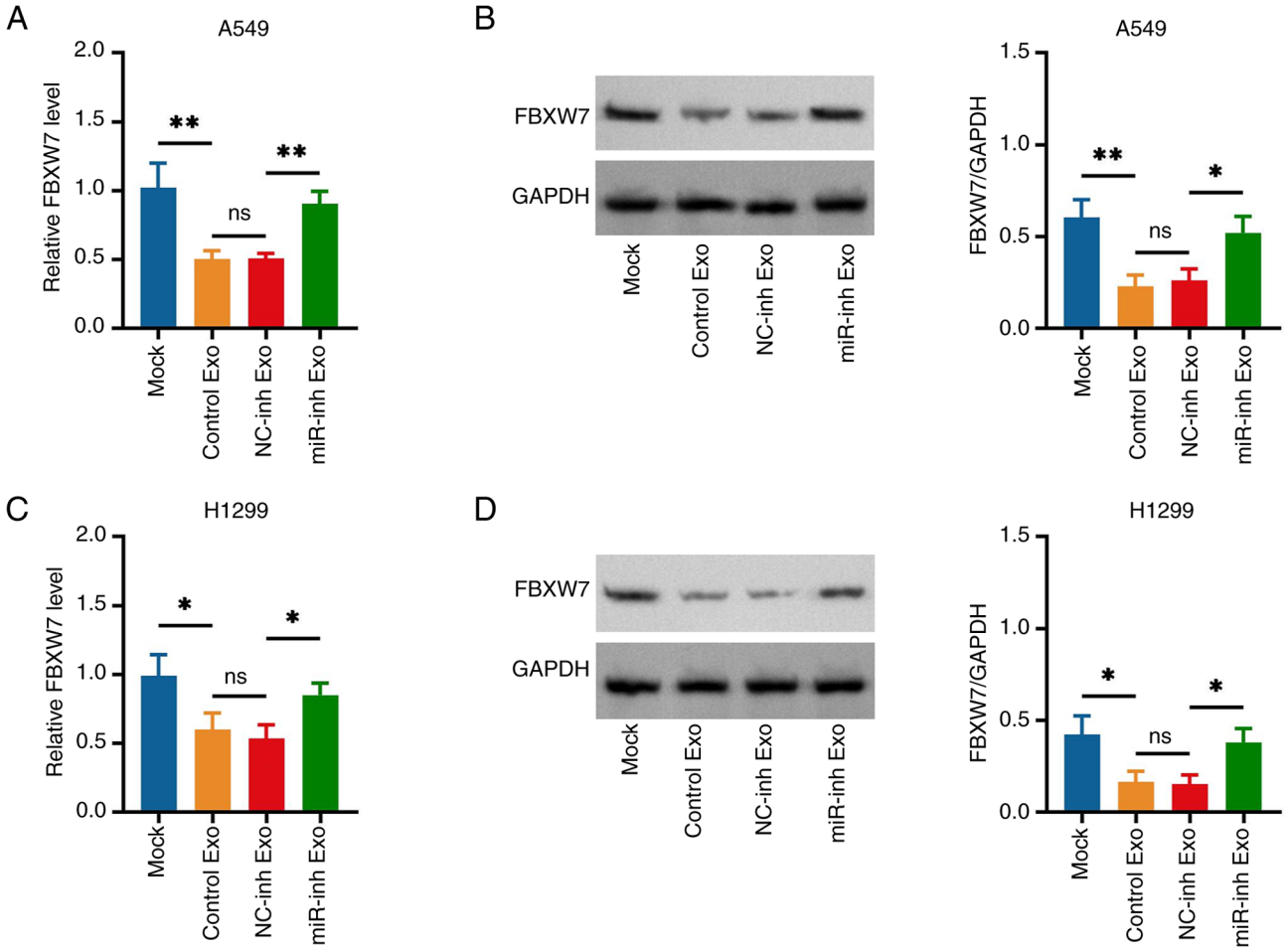

Figure 4. miR-182-knockdown CA-MSC exosomes increase FBXW7 expression in non-small cell lung cancer cells. Comparison of (A) gene and (B) protein expression of FBXW7 in A549 cells in the Mock, Control Exo, NC-inh Exo and miR-inh Exo groups. Comparison of (C) gene and (D) protein expression of FBXW7 in H1299 cells in the Mock, Control Exo, NC-inh Exo and miR-inh Exo groups. *P<0.05 and **P<0.01; ns, no significance. miR-182, microRNA-182; Exo, exosomes; NC, negative control; inh, inhibitor; FBXW7, F-box and WD repeat domain containing 7.

of CA-MSCs remains unclear. However, several reviews have preliminarily summarized the potential effects of CA-MSCs on regulating the microenvironment or modulating progression in various types of cancer, such as gastric, breast, ovarian and lung cancer (11,26,27). The possible effects of CA-MSCs can be categorized as follows: i) Differentiation into other stromal components that promote tumor development; ii) suppression of the immune responses; iii) facilitating angiogenesis; iv) promoting EMT in tumor cells; v) enhancing tumor cell stemness; and vi) inhibiting tumor cell death.

The present study was based on previous studies that examined the function of MSCs in cancer or other diseases via delivering exosomes and related molecules (28,29); moreover, a recent report indicated that CA-MSCs enhance hepatocellular carcinoma via transmitting exosomal TMBIM6 (12). It was therefore hypothesized that CA-MSCs may facilitate NSCLC progression via delivering exosomes. The present study observed that CA-MSCs promoted NSCLC cell viability and invasiveness; however, following repression of exosome secretion, this effect was weakened. Furthermore, direct addition of CA-MSC exosomes similarly enhanced NSCLC cell viability and invasiveness. These observations verified that CA-MSCs may enhance NSCLC cell viability and invasiveness by delivering exosomes. Although some studies have revealed that CA-MSCs promote lung cancer tumorigenesis and metastasis (10,30), to the best of our knowledge, the implication of exosomes in the function of CA-MSCs in lung cancer progression has not yet been reported. The possible explanations of the aforementioned findings are the following: MSCs present with tumor-promoting and antitumor functions simultaneously, which is determined by the source of MSC, tumor type and tumor features (31,32). Then, following the induction of NSCLC cells by co-culture with MSCs, the transformed CA-MSCs demonstrate sufficient/elevated levels of markers related to NSCLC progression (for instance, miR-182 in the present study). Subsequently, CA-MSCs transmit proteins, lipids and RNAs that demonstrate an oncogenic role in NSCLC cells (for instance, miR-182 in the present study), which results in promoting the viability and invasiveness of NSCLC cells.

miR-182 is a well-known oncogene identified in various cancer types and specifically in NSCLC (14-19). Notably, the tumor-promoting role of miR-182 in NSCLC was observed in our previous study (20). Besides, miR-182 was found to be enriched in CA-MSC exosomes in the present study. Therefore, miR-182 was selected for verification. The present study demonstrated that CA-MSCs and CA-MSC exosomes transmitted sufficient levels of miR-182 to NSCLC cells. miR-182 expression was knocked down in CA-MSCs to detect whether it was important for the

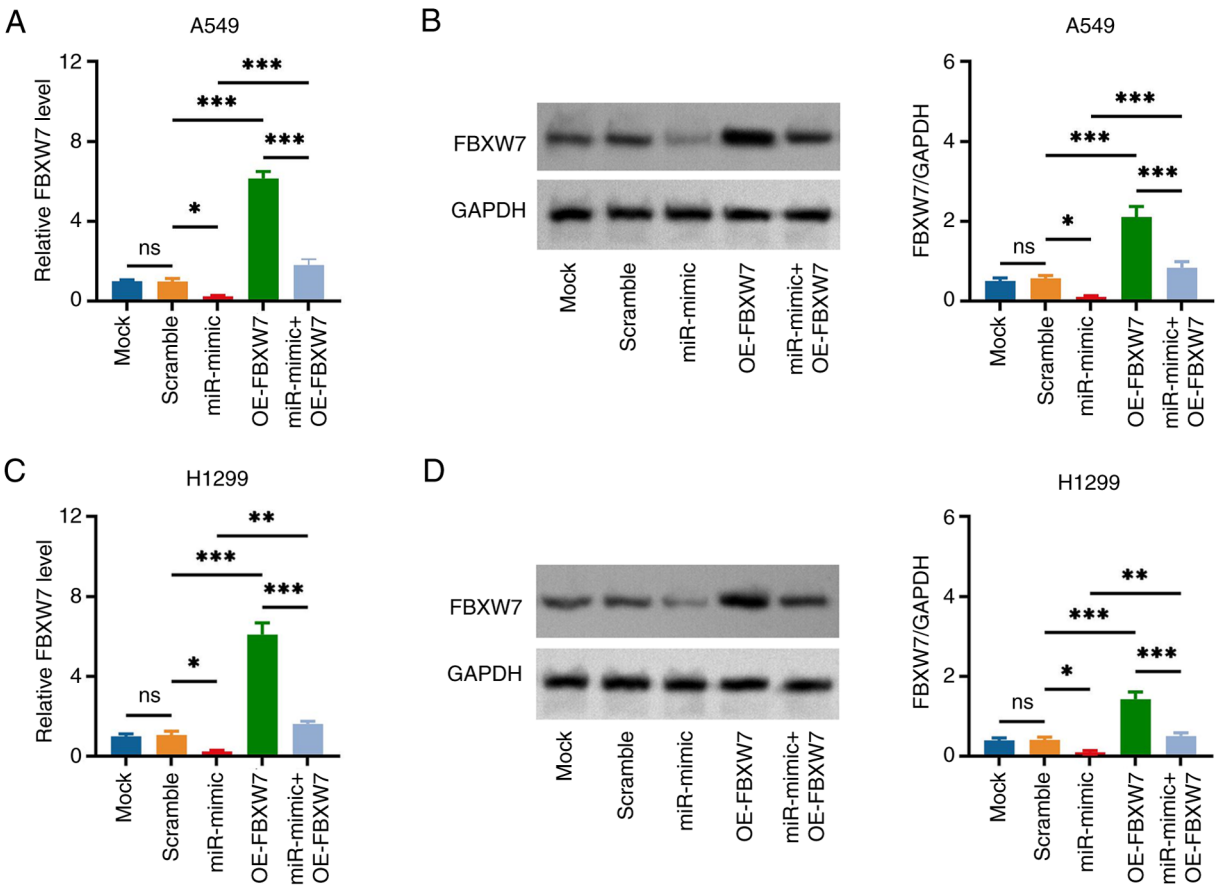

Figure 5. miR-182 mimic and FBXW7 OE vector modifies FBXW7 expression in non-small cell lung cancer cells. Comparison of (A) gene and (B) protein expression of FBXW7 in A549 cells in the Mock, Scramble, miR-mimic, OE-FBXW7 and miR-mimic + OE-FBXW7 groups. Comparison of (C) gene and (D) protein expression of FBXW7 in H1299 cells in the Mock, Scramble, miR-mimic, OE-FBXW7 and miR-mimic + OE-FBXW7 groups. *P<0.05, **P<0.01 and ***P<0.001; ns, no significance. miR-182, microRNA-182; FBXW7, F-box and WD repeat domain containing 7; OE, overexpression (vector).

carcinogenetic effect of CA-MSCs in NSCLC. The present study assessed whether knockdown of miR-182 expression attenuated the effect of CA-MSC exosomes on NSCLC viability and invasiveness, and the results indicated that CA-MSC exosomes may promote NSCLC progression by delivering miR-182. The explanations for this finding may be as follows: i) The co-culture of NSCLC cells and MSCs increased the expression levels of the oncogene, miR-182, in CA-MSCs, which was subsequently transmitted via exosomes; and ii) miR-182 promoted NSCLC viability and invasiveness via multiple mechanisms of action, such as the regulation of IL-8/STAT3, RNA binding motif protein 5, endothelial PAS domain-containing protein 1, FOXO3 and domain protein 13 (14-16,20,33). Besides, the findings regarding the effect of miR-182 on NSCLC progression in the present study were in-line with previous studies (16,20,24,34). Specifically, previous studies revealed that miR-182 induced metastasis and EMT in NSCLC (16), and it increased cell proliferation and colony formation of NSCLC (16); furthermore, miR-182 promotes the radioresistance of NSCLC (20), and its inhibition could repress NSCLC progression (34).

In the present study, to further identify the associated mechanism of action, the target of miR-182 was investigated. Using miRWalk database prediction, FBXW7 was identified as a candidate target of miR-182. Subsequently, by searching the related articles, it was discovered that several studies have reported that FBXW7 is a direct target of miR-182 in kidney, lung and breast cancer (23-25). In the present study, it was further confirmed that miR-182 negatively regulated FBXW7 in NSCLC and sponged FBXW7. This finding was in line with a previous study reporting that miR-182 represses FBXW7 in NSCLC cells (24). Moreover, the present study demonstrated that FBXW7 suppressed NSCLC viability and invasiveness and attenuated the impact of miR-182 on these two processes, indicating that miR-182 promoted NSCLC progression via targeting FBXW7. This finding was in accordance with a previous study that reported that miR-182 enhances NSCLC proliferation via suppressing FBXW7 (24). The explanation of the finding that FBXW7 suppressed NSCLC viability and invasiveness and attenuated the impact of miR-182 on these two processes may be as follows: i) FBXW7 suppresses NSCLC malignant growth and invasiveness via multiple mechanisms of action, such as coiled-coil domain containing 6-induced DNA damage, zinc finger protein SNAI1-mediated ubiquitin-dependent degradation and ERK3 degeneration (35-37); and ii) miR-182 directly binds to FBXW7 at the nucleotide sequence level to silence FBXW7 expression (23-25); therefore, FBXW7 attenuates the impact of miR-182 on NSCLC cell function. Furthermore, previous studies uncovered that FBXW7 negatively regulates the AKT and ERK pathways, which are important in NSCLC progression (38-40). The present study also found that miR-182 positively while FBXW7 negatively regulated the AKT and ERK pathways, which was in-line with previous findings in

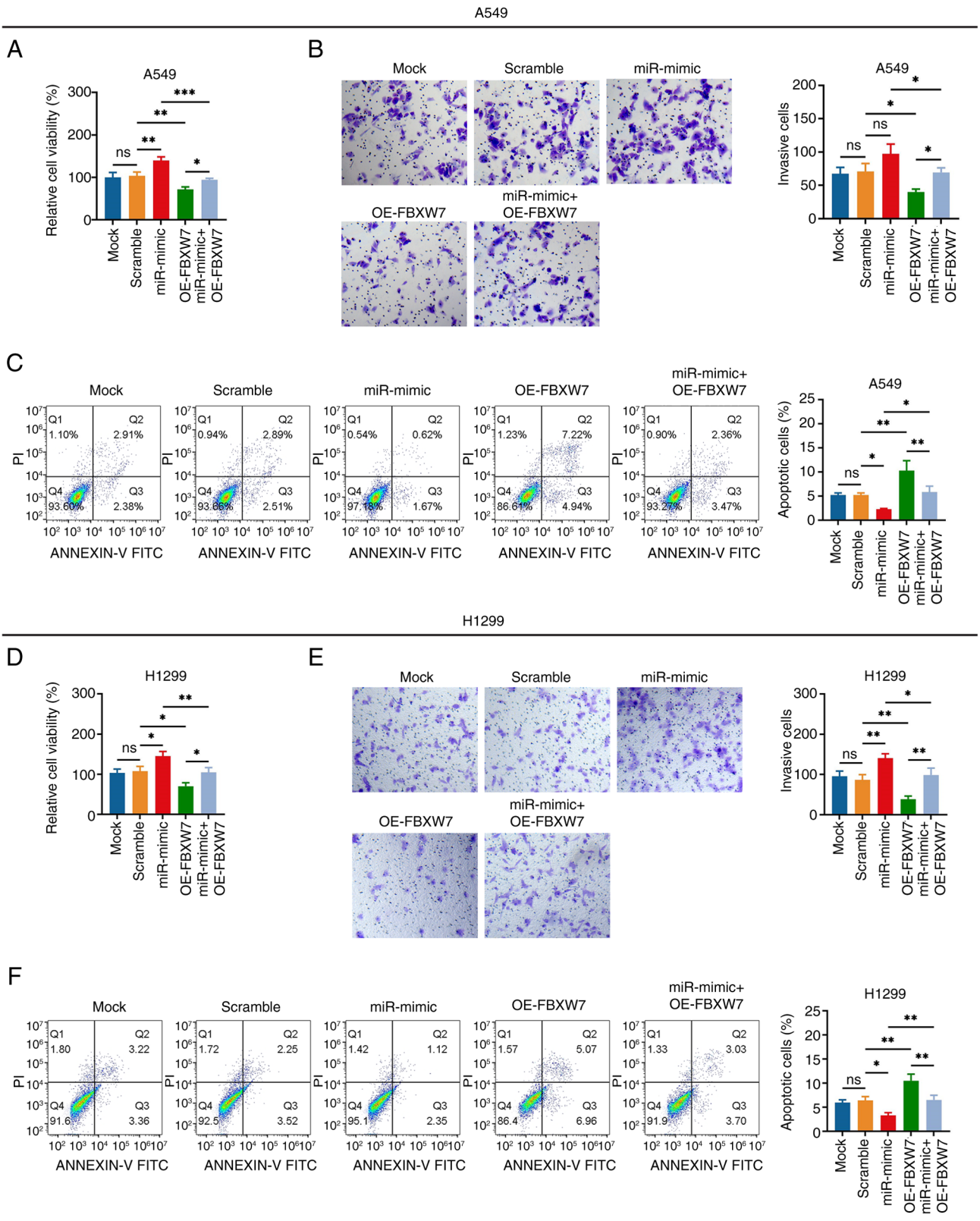

Figure 6. miR-182 mimic promotes non-small cell lung cancer cell viability and invasiveness via targeting FBXW7. Comparison of the (A) viability, (B) number of invasive cells (magnification, x200) and (C) apoptosis rate of A549 cells in the Mock, Scramble, miR-mimic, OE-FBXW7 and miR-mimic + OE-FBXW7 groups. Comparison of the (D) viability, (E) number of invasive cells (magnification, x200) and (F) apoptosis rate of H1299 cells in the Mock, Scramble, miR-mimic, OE-FBXW7 and miR-mimic + OE-FBXW7 groups. *P<0.05, **P<0.01 and ***P<0.001; ns, no significance. miR-182, microRNA-182; FBXW7, F-box and WD repeat domain containing 7; OE, overexpression (vector).

cancer (23-25,38-40). Specifically, previous studies revealed that miR-182 targets FBXW7 to promote kidney cancer growth and metastasis (23), NSCLC proliferation and colony formation ability (24) and breast cancer proliferation and invasion (25). Furthermore, previous studies also disclosed that FBXW7 inactivates the AKT and ERK pathways to regulate the progression

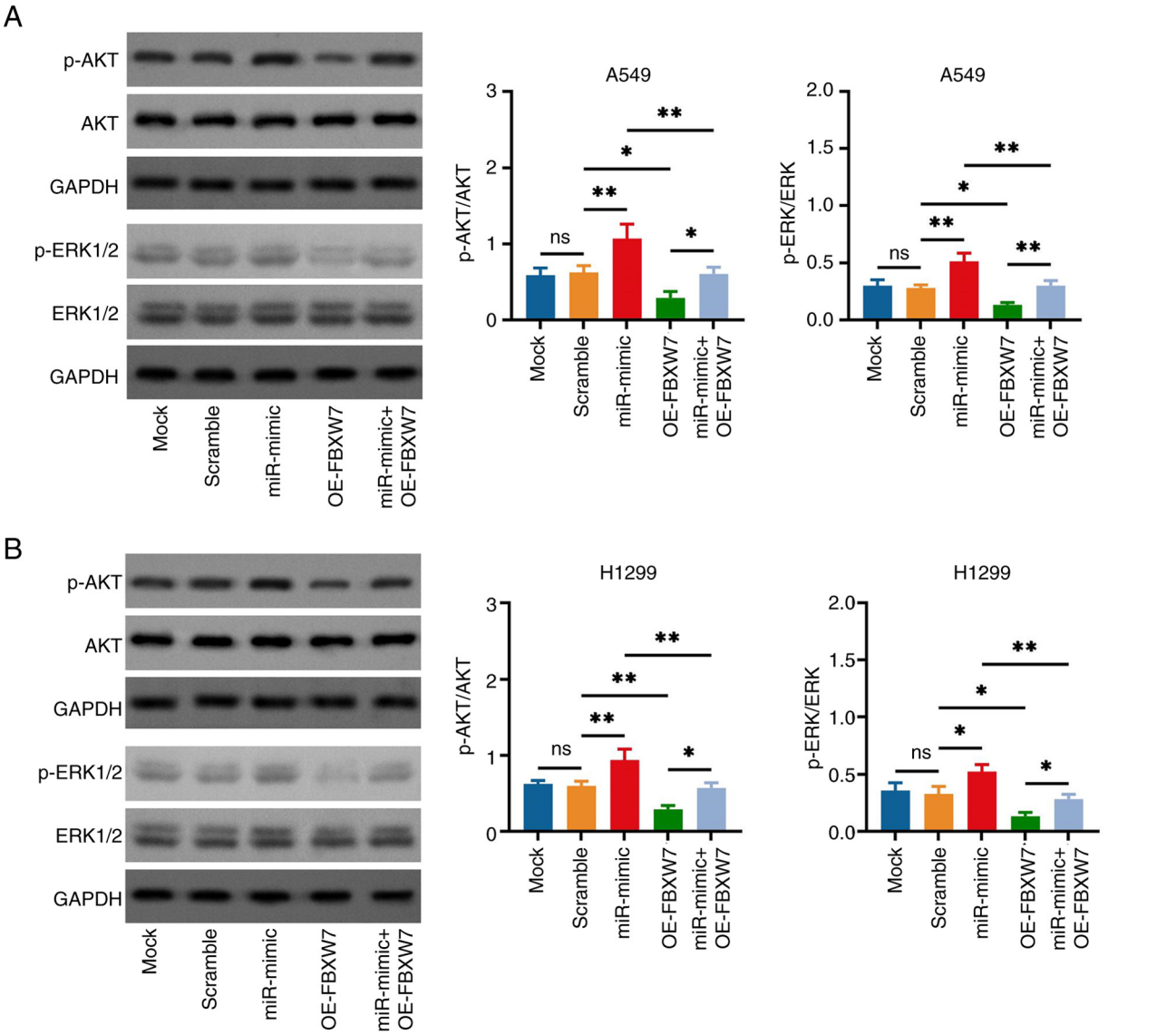

Figure 7. miR-182 mimic and FBXW7 OE vector modifies AKT and ERK signaling in non-small cell lung cancer cells. Comparison of p-AKT and p-ERK1/2 expression levels in (A) A549 and (B) H1299 cells in the Mock, Scramble, miR-mimic, OE-FBXW7 and miR-mimic + OE-FBXW7 groups. p-ERK1/2 was analyzed using ERK1/2 as a reference control, p-AKT was analyzed using AKT as a reference control, and the loading control (GAPDH) was set to ensure that the loading amount of all samples was consistent. *P<0.05 and **P<0.01; ns, no significance. miR-182, microRNA-182; FBXW7, F-box and WD repeat domain containing 7; OE, overexpression (vector); p-, phosphorylated.

of oral squamous cell carcinoma and esophageal squamous cell carcinoma, respectively (38,39), and that the AKT and ERK pathways are important in cancer oncogenesis (40,41).

The findings of the present study uncover the potency of CA-MSC-derived miR-182 as a treatment target for NSCLC treatment. However, some limitations of this study still exist. Firstly, the findings need further exploration in animal experiments. Secondly, further studies are needed to explore alternative pathways in addition to the FBXW7-mediated AKT and ERK pathway.

In conclusion, the present study demonstrated that CA-MSCs promote NSCLC viability and invasiveness by delivering exosomal miR-182 in a FBXW7-mediated AKT and ERK-dependent pathway.

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Availability of data and materials

The datasets generated in the present study may be requested from the corresponding author.

Authors' contributions

YS, XZ and ZL contributed to the study conception and design. Material preparation, data collection and analysis were performed by YS, XZ, LY, HD and ZL. The first draft of the manuscript was written by YS, XZ, LY, HD and ZL. YS and ZL confirm the authenticity of all the raw data. All

authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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