Exosomes secreted from mesenchymal stem cells carry miRNA-486-5p to inhibit cell proliferation and EMT process to treat human lung cancer by down-regulating MIER3

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Research Article

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Abstract

Exosomes are membrane-enclosed nanovesicles that shuttle active cargoes, such as mRNAs and microRNAs (miRNAs), between different cells. Mesenchymal stem cells (MSCs) are able to migrate to the tumor sites and exert complex functions over tumor progress. We investigated the effect of human bone marrow-derived MSCs (BMSCs)-derived exosomal miRNA-486-5p on lung cancer. Frist, during the co-culture experiments, we found that both MSCs and MSCs-derived exosomal can inhibit the proliferation and EMT process of A549 cells.Then we verified that the exosomes carry miRNA-486-5p, and overexpression of miRNA-486-5p inhibits the proliferation and EMT process of A549 cells. In addition, miRNA-486-5p carried by MSCs-derived exosomes regulates MIER3 expression through binding to its 3'UTR. Furthermore, the silenced MIER3 inhibits the proliferation of A549 cells and the EMT process. Finally, we verified that the tumor necrosis area of mice was reduced, miRNA-486-5p expression increased, and the EMT process was inhibited after exosome treatment in vivo. In conclusion, MSC-derived exosomal miRNA-486-5p directly and negatively targets MIER3 to suppress lung cancer.

Introduction

Lung cancer is the first cause of malignant tumorrelated death, accounting for ~20% of tumor death cases. Non-small cell lung cancer (NSCLC), including adenocarcinoma, squamous cell carcinoma and large cell carcinoma, accounts for about 80–85% of all lung cancer cases([1],[2]). And metastasis is the main cause of its high mortality rate in patients([3],[4]).

Epithelial-mesenchymal transition (EMT) is generally considered to be the key progression promoting the metastasis of lung cancer([5]). The epithelial-to-mesenchymal transition (EMT) process is the phenotypic depolarisation of epithelial cells to elongated mesenchymal cells to enhance their migration potential, which is manifested by reduction of intercellular connection and adhesion, the enhancement of cell vitality and the changes of various related molecules([6],[7]). The EMT process can be determined by the loss of E-cadherin along with the up-regulation of N-cadherin, fibronectin and vimentin([8]).

In recent years, studies have found that exosomes can act as regulators of tumor microenvironment to influence tumor cell invasion and EMT([9],[10],[11]). However, whether exosomes promote EMT of lung cancer cells and the underlying mechanisms remain elusive. Among many studies have shown that microRNAs (miRNAs) have been identified in exosomes, which inhibit tumorigenesis, tumor metastasis, angiogenesis, chemoresistance and immune escape. Such as miR-146b, miR-124 and miR-145 have been reported to participate in inhibiting glioma growth and reducing the tumor cells migration and the stem cell properties of glioma cells([12],[13]). miR-124 inhibit breast cancer metastasis inhibits bone metastasis of breast cancer by repressing Interleukin-11([14]). miR-21-5p and miR-621 have also been found to suppress cancer in breast cancer([15],[16]). Meanwhile, the high expression of exosomal miR-122 can increase tumor cell sensitivity to chemotherapeutic agents([17]). However, horgtw MSCs-derived exosomal miRNA is involved in NSCLC tumorigenesis has not been elucidated clearly.

In this report, we carried a systematic study of the role of MSCs and MSC-exos in A549 cells proliferation and EMT process. We explored the changes in malignant features of A549 cells incubated with MSC-derived exosomes, and we studied the role of miR-486-5p in exosome secretion and the consequent effect on biological functions of A549 cells. Finally, we studies validated the role of MSC-exos in EMT process in vitro, which supports a novel mechanism by inhibit lung cancer metastasis through an MSC-secreted exosome.

Materials And Methods

Cell lines and cell culture

The human NSCLC cell lines A549, HLF-A (human lung epithelial cells), and MSCs (human bone marrow mesenchymal stem cells) were obtained from the Fenghbio Biological Technology (China). A549 cells were maintained in Ham's F12K (F12K) medium, HLF-A were maintained in alpha-minimal essential medium (α-MEM) medium and MSCs were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco BRL, USA), containing 10% fetal bovine serum (FBS) (Gibco, USA) and 1% penicillin-streptomycin solution (Hyclone), at 37 °C with 5% CO_2.

Exosome isolation and characterization

MSCs were cultured in media with 10% exosome-free FBS for 48h. Cell culture media were collected, and exosomes were isolated from the supernatant by differential centrifugation using a ultracentrifuge as described by Liu et al([18]). Briefly, the medium was collected and centrifuged at 1000 g for 15 min, at 3000 g for 30 min, at 10,000 g for 1h, at 100,000 g for 4 h. All centrifugal steps were performed at 4°C. The final pellet containing exosomes was re-suspended in PBS. The representative marker of MSCs, CD44, CD29, CD34 and CD45 were assayed by immunofluorescence staining and flow cytometry analysis.

Transient transfection

According to the manufacturer's instructions for Invitrogen Lipofectamine 2000 (Thermo Fisher Scientific, USA), A549 cells were transiently transfected with miRNA-486-5p mimic/inhibitor, pcDNA3.1-MIER3/ASO-MIER3, or the corresponding NC ((NC mimic/inhibitor, empty vector (pcDNA3.1) and ASO-NC)) (RiboBio, Guangzhou, China). The level of RNA or protein was measured using Western blot at 48 h after transfection.

Co-culture experiments and exosomes culture

MSCs and A549 cells (or HLF-A cells) were co-cultured by the Transwell chamber (0.4 μm, 6-well plates, Corning, USA). MSCs (1.0×10^3 cells in 500 μL medium) were seeded in the upper chamber, and A549 cells (1.0×10^4 cells in 1500 μL medium) were seeded in the lower chamber, using DMEM and F12K medium containing 10% exosome-free FBS for two chambers, respectively. MSCs and

A549 cells were co-cultured for 48 h and then proceeded to follow-up experiments. MSC-Exos (100 mg/mL) were added to A549 cells/HLF-A.

MTT assay

The MTT assay was performed following the manufacturer's instructions. Briefly, A549 cells were cultured in 96-well plates (5× 10^3 cells per well). After incubation for 48h, MTT solution (5 mg/mL) was added into each well and incubated with the cells at 37 °C for 4 h. The medium was then removed, and 150 μL DMSO was added to dissolve the crystal adequately. The absorbance was measured at 570 nm using a microplate reader (Perlong, China).

Cell cycle analysis

A549 cells were seeded in 6-well plates (200,000 cells/well), transfected or co-cultured. At the end of transfection and/or treatments, the cells were collected by trypsinization, washed twice with cold PBS, and fixed with 75% ethanol overnight at 4˚C. Following fixation, the cells were washed again with PBS, incubated with PI at 4˚C for 30min. Cell cycle phase distribution was analysed using a BD FACSCantoII and ModFit LT software (BD, USA).

Colony formation assay

The A549 cells (1× 10^3 cells per well) were plated into a six-well plate (Corning, USA) continuously cultured for about 2 weeks. After that, the colonies were successively fixed with absolute ethanol for 15 min and stained with crystal violet (0.5% w/v) for 30 min. Finally, the colonies per well were counted and imaged.

Luciferase reporter assays

Luciferase reporter plasmids that contained the wild-type and mutant of the MIER3 promoter was constructed. The wild-type (pmirGLO-lncRNA MIER3 3'UTR wt) and mutant (pmirGLO-lncRNA MIER3 3'UTR mut) of luciferase reporter plasmids were co-transfected with miRNA-486-5p mimic or NC into A549 cells for 48 h. Luciferase activity was detected using the Dual-Luciferase Reporter Assay System (Promega, USA).

Immunofluorescence Staining

MSCs were fixed with 4% paraformaldehyde for 15 min at room temperature. Following blocking with 5% FBS for 1 h at room temperature. Then the cells were incubated with primary antibody at 4˚C overnight. Then, the MSCs were stained with secondary antibody at 37˚C for 1 h. The primary antibody (1:200 dilution) and secondary antibody (1:400 dilution) were purchased from Abcam (UK).DAPI was used to stain the nucleus at room temperature for 3-5 min. Images were captured using a laser confocal microscope (Olympus, Japan).

RNA fluorescence in situ hybridization (FISH) assay

FISH assay was performed on the tissue according to the manufacturer's instructions (Boster, USA). Briefly, the dewaxed sections were fixed with 4% paraformaldehyde for 20 minutes and dehydrated in an ascending series of ethanol solutions. Then, the dewaxed sections were prehybridized in hybridization buffer (formamide, 50 mM Tris-HCl, 5 mol NaCl, and 0.05% sodium dodecyl sulfate). The dewaxed sections were hybridized overnight at 42 ℃ with probe (probe of miRNA-486-5:)(Servicebio, China). Finally, the nucleus were stained with DAPI (Solarbio, China) and the images were acquired using a confocal microscope (Olympus, Japan).

HE staining

HE staining was performed on the tissue according to the manufacturer's instructions (Solarbio, China). Briefly, the dewaxed sections were firstly incubated with hematoxylin to stain the nucleus for 5 min, then 1% ethanol-hydrochloric acid for 30 s and eosin solution for 3 min. Finally, the sections were dehydrated in graded alcohol following by clearing in xylene. Images were acquired on an Olympus BH2 microscope (Olympus Corporation, Japan).

Immunohistochemicalstaining

Sections were deparaffinized, rehydrated, retrieved the antigens, then incubated with 1% H_2O_2 in methanol for 15-20 min to block endogenous peroxidase. After blocked with 5% bovine serum albumin, sections were incubated overnight with antibody (1:100 dilution) at 4°C. Then, incubating sequentially with a biotinylated secondary antibody and horseradish peroxidase conjugated streptavidin (1:400 dilution) for 30 min at 37℃. Immunoreactivity was visualized using diaminobenzidine (DAB). Then a light hematoxylin counterstain was applied. Images were captured by Olympus BX61 microscope (Olympus, Japan).

Quantitative real-time polymerase chain reactions (qRT-PCR)

Cultured cells were lysed by TRIzol (Invitrogen), and RNA was extracted according to the manufacturer’s instruction. 1 μg of total RNA from each sample was reverse transcribed using M-MLV (Takara) in a final volume of 20 μL. qRT-PCR was performed by the kitspecification of SYBR@ Premix Ex Taqm II (TakaRa, Japan) on iQ5 Real-Time PCR System (BioRad, USA). All quantitative real-time PCR (qRT-PCR) results were carried out in duplicate and normalized to U6.

Western blot

This experiment was accordance with previous study([19]). In briefly, tissue or A549 cells lysates were prepared in Protein Lysis Buffer (Beyotime, China) according to the manufacturer’s directions. The primary antibody (1:1000 dilution) and secondary antibody (1:8000 dilution) were purchased from Abcam (UK). GAPDH was used as an endogenous control to normalize the protein expression data.

Animal model

All animal procedures were performed according to national guidelines and approved by the Animal Care Ethics Committee. Twenty male mice (4–6 weeks old, Laboratory Animal Center of Shanghai, Academy of Science) were procured. All mice received subcutaneous injections of A549 cells in the right armpit (1×10^7 cells in 200 μL of PBS per mouse). Twenty mice were randomly divided into two groups (the control group and MSCs-exosome group) when the tumors reached a volume of 50–100 mm^3 (3 weeks after subcutaneous injections of tumor cells); exosomes (100 μg of total protein) were injected into the implanted tumors every 2 days for 10 times; the control group was injected with equal volume PBS. The mice were examined every 2 days, and all mice were sacrificed by cervical dislocation under general anesthesia with chloral hydrate (5%, 100 μL/10 g).

Statistical analysis

All data were analyzed using the InStat software (GraphPad, CA, USA) and displayed as mean ± SD. Two-tailed Student's t-test was used for statistical analysis, and significance was defined at *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

Results

1. Characterization of MSCs

To investigate the role of MSC-exos in tumor development, we first identified the MSC-like characteristics of human bone marrow MSCs. Flow cytometry analysis and immunofluorescence staining conveyed that human bone marrow MSCs were positive for CD29, CD44, but negative for CD34 and CD45 (Figure 1a and 1b), which displayed the characteristic surface markers of MSCs.

2. MSCs inhibit A549 cells proliferation and EMT process

We then evaluated MSCs effects on lung cancer cell proliferation and migration and characteristic abilities of tumor development. The cell viability of A549 cells treated with MSCs was significantly decreased compared with that of unstimulated A549 cells as showed by MTT assay (Figure 2a). There were no significant differences in HLA-A cells. And these were also verified by cell cycle assay (Figure 2b). Furthermore, MSCs-treated A549 cells showed lower average colony numbers and formed lager colonies than unstimulated A549 cells (Figure 2c), suggesting a markedly potent tumor-inhibiting effect of MSCs. It is known that epithelial cell can acquire its migratory capacity through epithelial-mesenchymal transition (EMT) that plays a crucial role in initiating cancer metastasis. We examined epithelial and mesenchymal markers in MSCs-treated A549 cells. The mesenchymal markers vimentin, N-cadherin and α-SMA were lower and the epithelial marker E-cadherin was higher after treated with MSCs in A549 cells. There were no significant differences in HLA-A cells (Figure 2d).

3. The exosomes derived from MSCs inhibit A549 cells proliferation and EMT process

We next studied the roles of MSC-derived exosomes in the proliferation of lung cancer cells. A549 cells were treated with exosomes and cellular proliferation was evaluated by MTT, cell cycle and colony formationassay. It revealed that compared to unstimulated A549 cells, MSC-secreted exosomes inhibit the cell viability of A549 cells and form a lower lager colonies numbers(Figure 3a,b and c). There were no significant differences in HLA-A cells. We examined epithelial and mesenchymal markers in exosome-treated A549 cells. The mesenchymal markers vimentin, N-cadherin and α-SMA were lower and the epithelial marker E-cadherin was higher after treatment with exosomes. Similarly, There were no significant differences in HLA-A cells (Figure 3d).

4. miRNA-486-5p in exosomes inhibit A549 cells proliferation and EMT process

To further explore the effects of exosomes on A549 cells proliferation and EMT, We screened out microRNAs that were abnormally expressed in cancer cells or tissus. Ultimately, 4 microRNAs (miRNA-let-7b-5p, miRNA-23a-3p, miRNA-486-5p, miRNA-30a-3p) were screened as candidates. The expression of microRNAs was verified by qPCR, showing that miRNA-let-7b-5p, miRNA-23a-3p, miRNA-486-5p and miRNA-30a-3p in A549 cells was down-regulated compared with HLA-A cells (Figure 4a). The expression of microRNAs in exosomes, showing that miRNA-let-7b-5p, miRNA-23a-3p, miRNA-486-5p and miRNA-30a-3p secreted by exosomes were up-regulated compared to exosome-free medium (Figure 4b). We selected miRNA-486-5p, which showed higher significant differences for further investigation. We next studied the roles of miRNA-486-5p in the proliferation and EMT of lung cancer cells. The MTT assay and cell cycle assay revealed that overexpression of miRNA-486-5p significantly reduced the cell viability of A549 cells and promoted cell apoptosis, while inhibition of miRNA-486-5p significantly increased cell proliferation (Figure 4c and d). The western blot assay results displayed that overexpression of miRNA-486-5p significantly reduced expression of vimentin, N-cadherin and α-SMA, while increased expression of E-cadherin, consistent with the inhibition of miRNA-486-5p (Figure 4e).

5. miRNA-486-5pinhibit A549 cells proliferation and EMTprocess through targeting 3'U of MIER3

We used the TargetScan website to identify MIER3 as a possible target gene for miRNA-486-5p (Figure 5a). To verify this prediction, we assessed the luciferase activities of the wild type or mutant promoter reporter gene of MIER3 in 293T cells. The overexpression of miRNA-486-5p markedly decreased the luciferase activities, whereas the activities of the mutant reporter gene were not affected (Figure 5b). The results suggest that MIER3 is the target gene of miRNA-486-5p. We next studied the roles of MIER3 in the proliferation and EMT of lung cancer cells. The MTT assay and cell cycle assay revealed that overexpression of MIER3 significantly increased the cell viability of A549 cells and inhibited cell apoptosis, while inhibition of MIER3 significantly reduced cell proliferation (Figure 5c and d). The western blot assay results showed that overexpression of MIER3 significantly increased expression of vimentin, N-cadherin and α-SMA, while reduced expression of E-cadherin, consistent with the inhibition of MIER3 (Figure 5e).

6. Exosomes derived from MSCs rescues tumor metastasis in vivo

To better understand the role of MSCs-derived exosomes in the lung metastasis, we established a subcutaneous xenograft and metastatic model and treated it with exosomes (100 μg of total protein) by tail vein injections. HE stain in the mice lung tissue of the control group showed that larger and more destructive or necrotic regions compared with exosomes group, which suggested that exosomes inhibited the lung colonization (Figure 6a). The FISH assay results showed that the expression of miRNA-486-5p in the exosomes group was higher than that in the control group (Figure 6b). Moreover, immunohistochemistry, qPCR and western blot assay in xenograft tumors of the exosomes group showed lower levels of epithelial-mesenchymal transition (EMT) marker vimentin, N-cadherin and α-SMA, along with higher levels of E-cadherin, compared with the control group (Figure 6c, d and e). Taken together, these results indicate that exosomes can suppression tumor growth and lung colonization in vivo via the up-regulate of miRNA-486-5p expression and the mediation of EMT.

Discussion

In recent years, increasing evidence has demonstrated thatmesenchymal stem cells (MSCs) are involved in repairing the tumor microenvironment and inhibiting tumor proliferation and metastasis. Ono et al. reported that bone marrow mesenchymal stem cells (BSMCs) secreted exosomes contain a microRNA that promotes dormancy in metastatic breast cancer cells(). Wen et al. reported that BMSCs promote prostate cancer progression via the conversion of normal fibroblasts to cancer-associated fibroblasts(). We first identified the characteristics of MSCs and co-cultured MSCs in HLA-1 and A549 cells. We found that after MSCs treatment, there was no significant difference in the cell viability, cell proliferation ability and EMT process markers vimentin, N-cadherin, α-SMA and E-cadherin of HLA-1 cells. The loss of E-cadherin, a key protein of intercellular adhesion, is one of the important reasons why EMT affects tumor metastasis by promoting the loss of intercellular adhesion and the ability of cells to migrate and invade. In A549 cells, MSCs treatment significantly reduced cell viability, proliferation capacity and EMT process. Based on these, we speculate that MSCs can save the development of lung cancer by inhibiting the proliferation of A549 cells. Therefore, we guessed that the substance in MSCs exerted a tumor suppressor effect.

In recent years, much interest has been devoted to exosomes, which function as carriers of bioactive proteins, lipids, and nuclear acids and are increasingly regarded as crucial players in cell-cell communications(,,,). Next, we added the same amount of exosomes secreted by msc to HLA-1 and A549 cells for treatment. In the present study, we found the same result as after MSc treatment. That is, in HLA-1 cells, there is no significant difference in cell proliferation ability and EMT process markers after exosome treatment. After exosomal treatment of A549 cells, the levels of mesenchymal markers vimentin, N-cadherin and α-SMA decreased, and the levels of epithelial marker E-cadherin increased, thereby inhibiting the EMT process. Similarly, the proliferation of A549 cells was inhibited after exosome treatment. So far we have proved that the exosomes secreted by msc are an important factor affecting the vitality and migration of lung cancer cell A549.

Studies have shown that the proportion of miRNAs in exosomes is higher than their donor cells in cells(,). MiRNAs are involved in NSCLC pathogenesis and their expression profiles have been used to classify cancers(,). Therefore, we expected that miRNAs in exosomes may regulate the proliferation and metastasis of lung cancer cells. Li et al. showed that miRNA let-7b-5p is sponged by SNHG16 to regulate the proliferation of liver cancer and promote G2/M and epithelial-mesenchymal transition(). Let-7b-5p has also been reported to participate in the endoplasmic reticulum stress response in acute pulmonary embolism by up-regulating the expression of stress-related endoplasmic reticulum protein 1(). Hao et al. found that miRNA-23a-3p inhibits the proliferation and invasion of non-small cell lung cancer through sponge LncRNA MAGI2-AS3 and PTEN(). Wei et al. found that miRNA-30a-3p inhibits the progression of lung cancer by targeting DNA methyltransferase 3a(). miRNA-486-5p inhibits tumor growth in cervical cancer, bladder cancer, and breast cancer(,,). Finally, we selected miRNA-486-5p, which has the most significant difference between HLA-1 cells and A549 cells, and confirmed that miRNA-486-5p is highly expressed in exosomes. This indicates that carrying miRNA-486-5p in exosomes inhibits the proliferation of A549 cells, rather than up-regulating the expression of miRNA-486-5p in A549 cells through other cytokines. It was confirmed that miRNA overexpression inhibited the proliferation and EMT process of cancer cells. Furthermore, the results confirmed that MIER3 is a direct target of miRNA-486-5p. A previous study found that, up-regulation of MIER3 inhibits epithelial-mesenchymal transition and thus inhibits the progression of colorectal cancer(). However, Huang et al. reported that MIER3 is highly expressed in breast cancer, forming a co-inhibitor complex through HDAC1/HDAC2/Snail, and silencing E-cadherin to promote breast cancer cell invasion(). In this study, we found that that MIER3 overexpression inhibited the proliferation and EMT process of lung cancer cells, consistent with the results of Huang et al. These results confirm that the miRNA-486-5p/MIER3 axis can play a role in tumor suppression.

Finally, we conducted animal experiments, and the results showed that after exosomes treatment, the necrotic area of tumor tissue was reduced, the expression of miRNA-486-5p increased, and the EMT process was inhibited. It is verified that MSCs-derived exosomes inhibited the growth and metastasis of lung cancer.

In summary, we found that exosomes derived from MSCs can inhibit tumor growth through miRNA-486-5p and regulate the occurrence of tumor EMT. Therefore, it can be reasonably predicted that exosomes carry miRNA-486-5p, which may be helpful in improve the prognosis of patients with lung cancer.

Declarations

Conflict of interest

The authors have no conflicts of interest to disclose.

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Figures

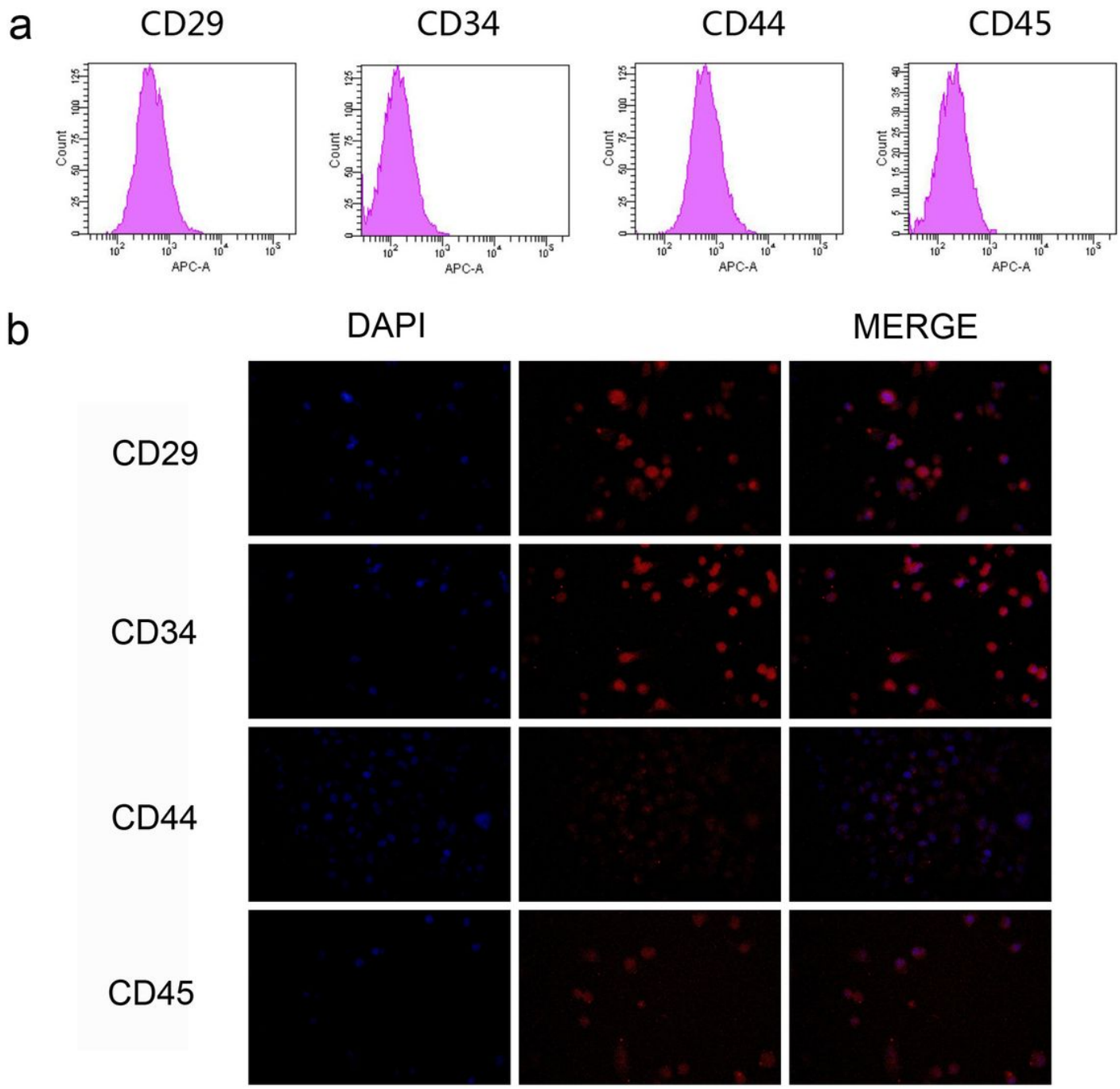

Figure 1

Characterization of MSCs.

(a) Flow cytometry analysis of MSCs positive marker (CD29 and CD44) and negative marker (CD34 and CD45). (b) Immunofluorescence staining analysis of MSCs positive marker (CD29 and CD44) and negative marker (CD34 and CD45). DAPI was used to stain nuclei (blue); Above red fluorescence intensity is positively correlated with the expression of protein; Under the merged image; Base magnification:×400.

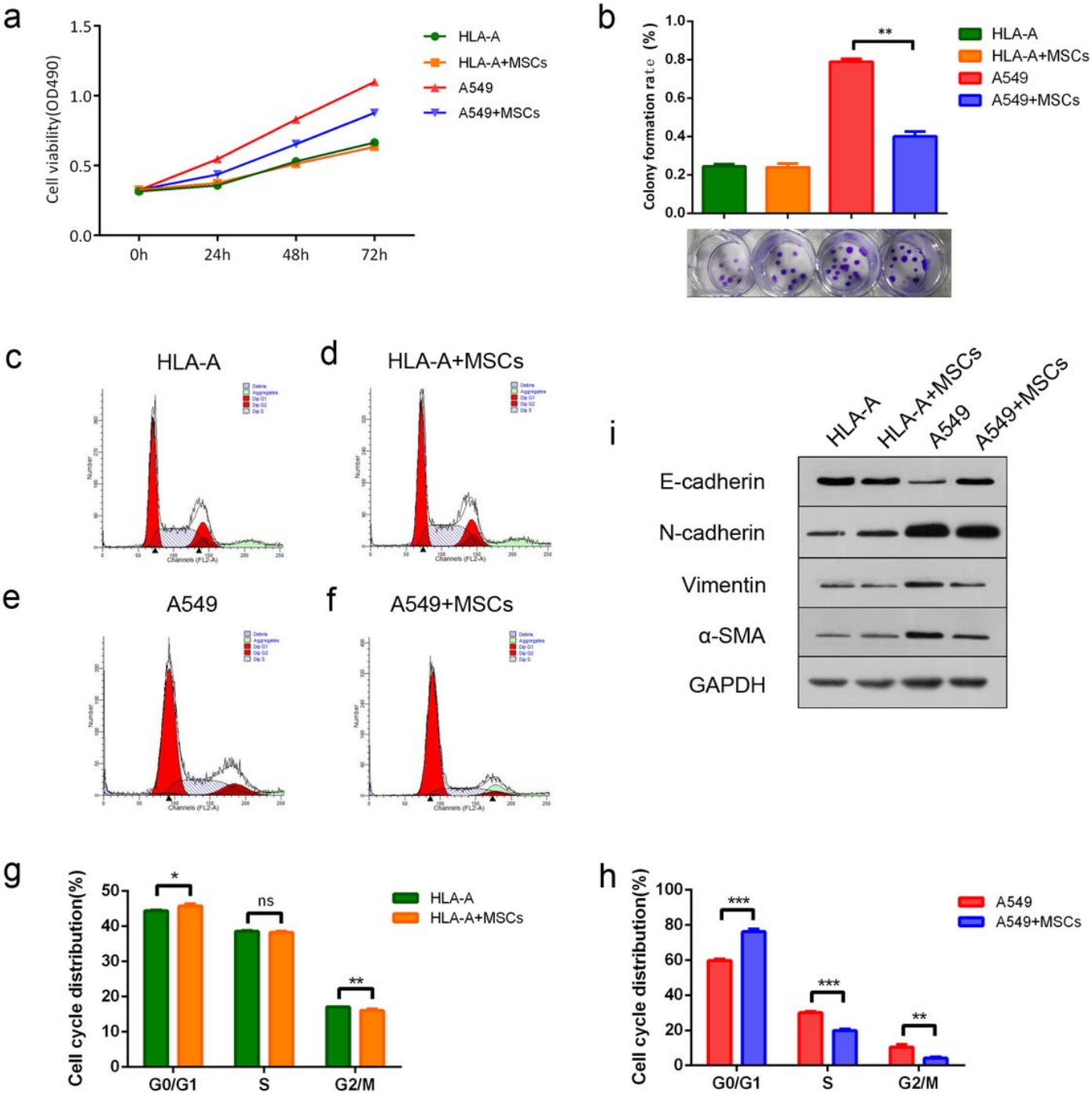

Figure 2

MSCs inhibit A549 cells proliferation and EMT process.

(a) MTT was used to detect the activity of HLA-1 and A549 cells. (b) Flow cytometry was used to detect the cell cycle of HLA-1 and A549 cells after treatment of MSCs. (c) Colony formation assay was used to detect the cell cycle of proliferation HLA-1 and A549 cells after treatment of MSCs. (d) Western blot was

used to detect the mesenchymal markers vimentin, N-cadherin and α-SMA , the epithelial marker E-cadherin after treatment of MSCs. (*P<0.05, **P<0.01, ***P<0.001)

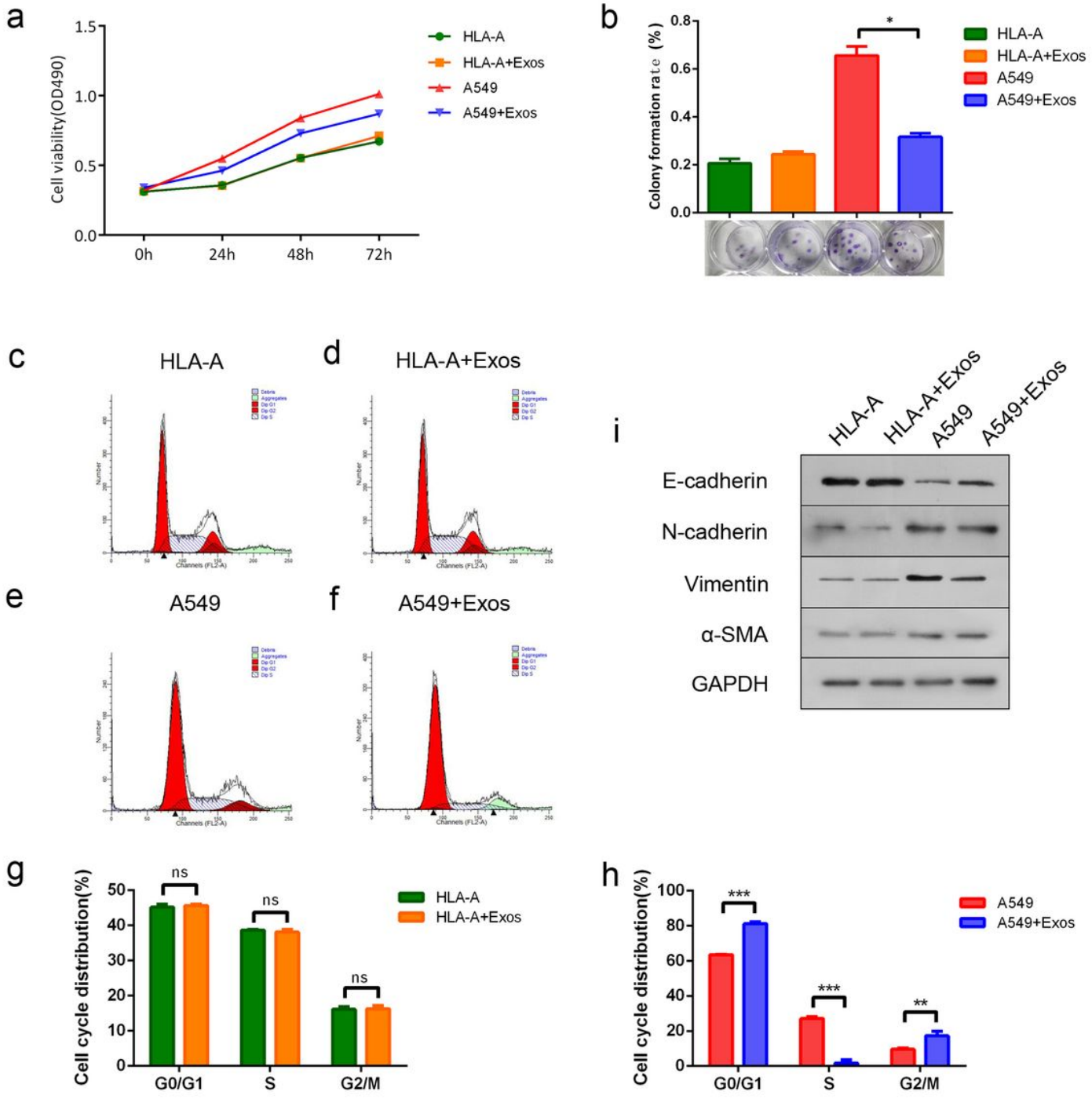

Figure 3

The exosomes derived from MSCs inhibit A549 cells proliferation and EMT process.

(a) MTT was used to detect the activity of HLA-1 and A549 cells. (b) Flow cytometry was used to detect the cell cycle of HLA-1 and A549 cells after treatment of exosomes. (c) Colony formation assay was used

to detect the cell cycle of proliferation HLA-1 and A549 cells after treatment of exosomes. (d) Western blot was used to detect the mesenchymal markers vimentin, N-cadherin and α-SMA , the epithelial marker E-cadherin after treatment of exosomes. (*P<0.05, **P<0.01, ***P<0.001)

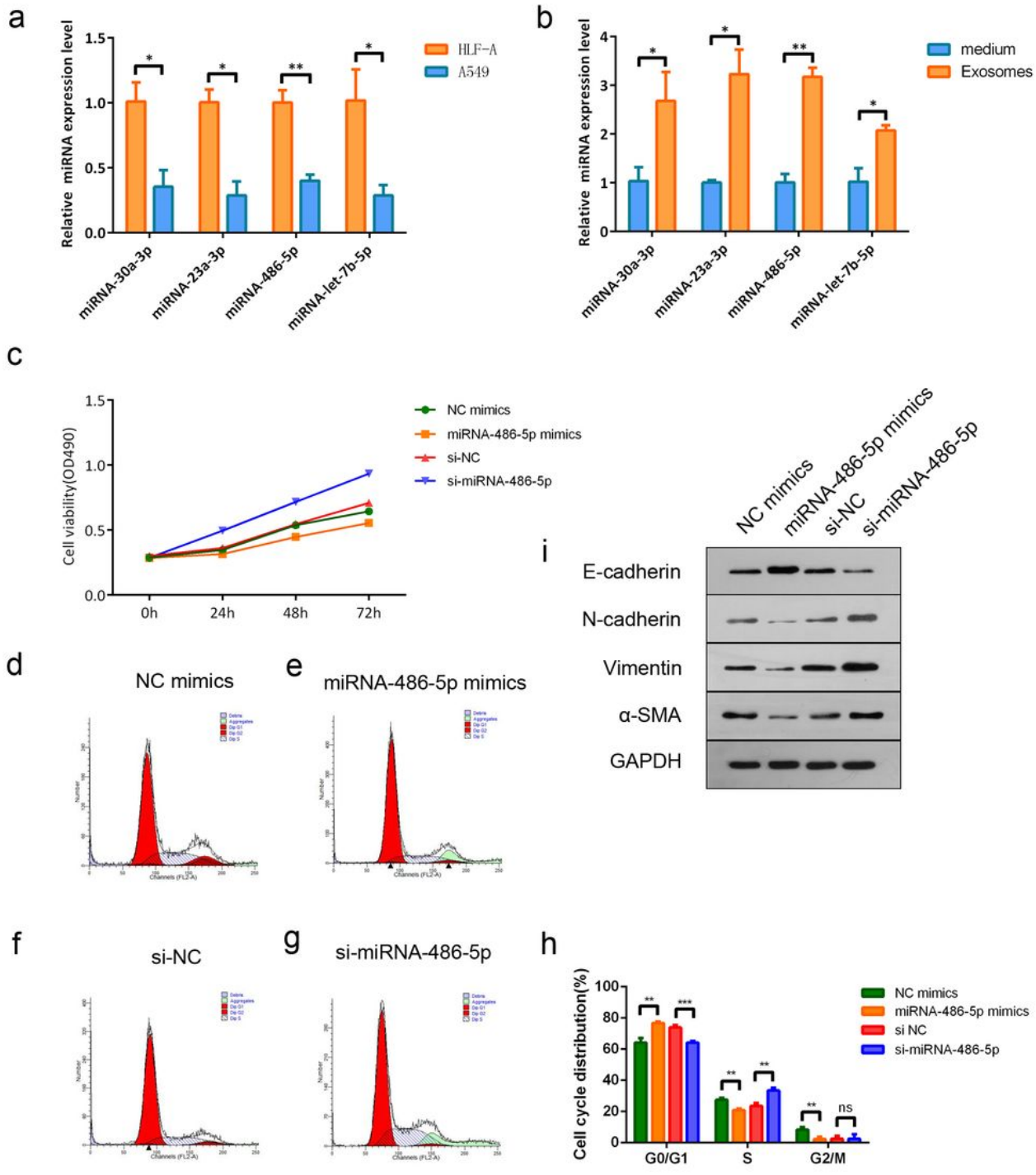

Figure 4

miRNA-486-5p in exosomes inhibit A549 cells proliferation and EMT process.

(a) qRT-PCR was used to detect the expression of miRNA-let-7b-5p, miRNA-23a-3p, miRNA-486-5p and miRNA-30a-3p in HLA-1 and A549 cells. (b) qRT-PCR was used to detect the expression of miRNA-let-7b-5p, miRNA-23a-3p, miRNA-486-5p and miRNA-30a-3p in exosomes and exosome-free medium. (c) MTT was used to detect the activity of A549 cells after overexpression or inhibition of miRNA-486-5p. (d) Flow cytometry was used to detect the cell cycle of A549 cells after overexpression or inhibition of miRNA-486-5p. (e) Western blot was used to detect the mesenchymal markers vimentin, N-cadherin and α-SMA , the epithelial marker E-cadherin after overexpression or inhibition of miRNA-486-5p. (*P<0.05, **P<0.01, ***P<0.001)

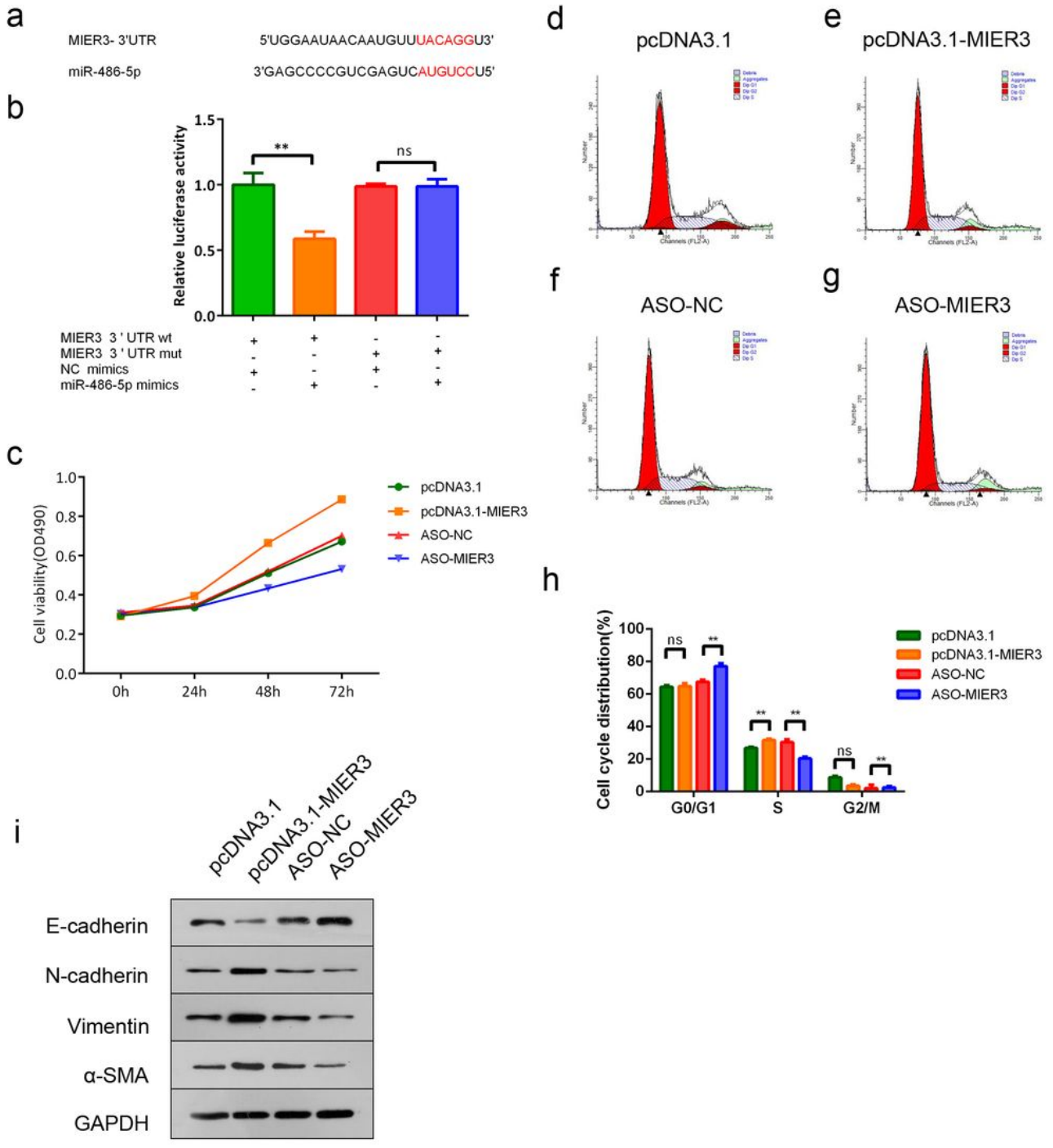

Figure 5

miRNA-486-5p inhibit A549 cells proliferation and EMT process through targeting 3'U of MIER3.

(a) Putative binding sites of miRNA-486-5p and MIER3 mRNA 3'UTR was predicted by DIANA-LncBase V2 and Targetscan. (b) Luciferase reporter analysis was performed to detect the bindings between miRNA-486-5p and MIER3 in A549 cells. (c) MTT was used to detect the activity of A549 cells after

overexpression or inhibition of MIER3. (d) Flow cytometry was used to detect the cell cycle of A549 cells after overexpression or inhibition of MIER3. (e) Western blot was used to detect the mesenchymal markers vimentin, N-cadherin and α-SMA , the epithelial marker E-cadherin after overexpression or inhibition of MIER3. (*P<0.05, **P<0.01, ***P<0.001)

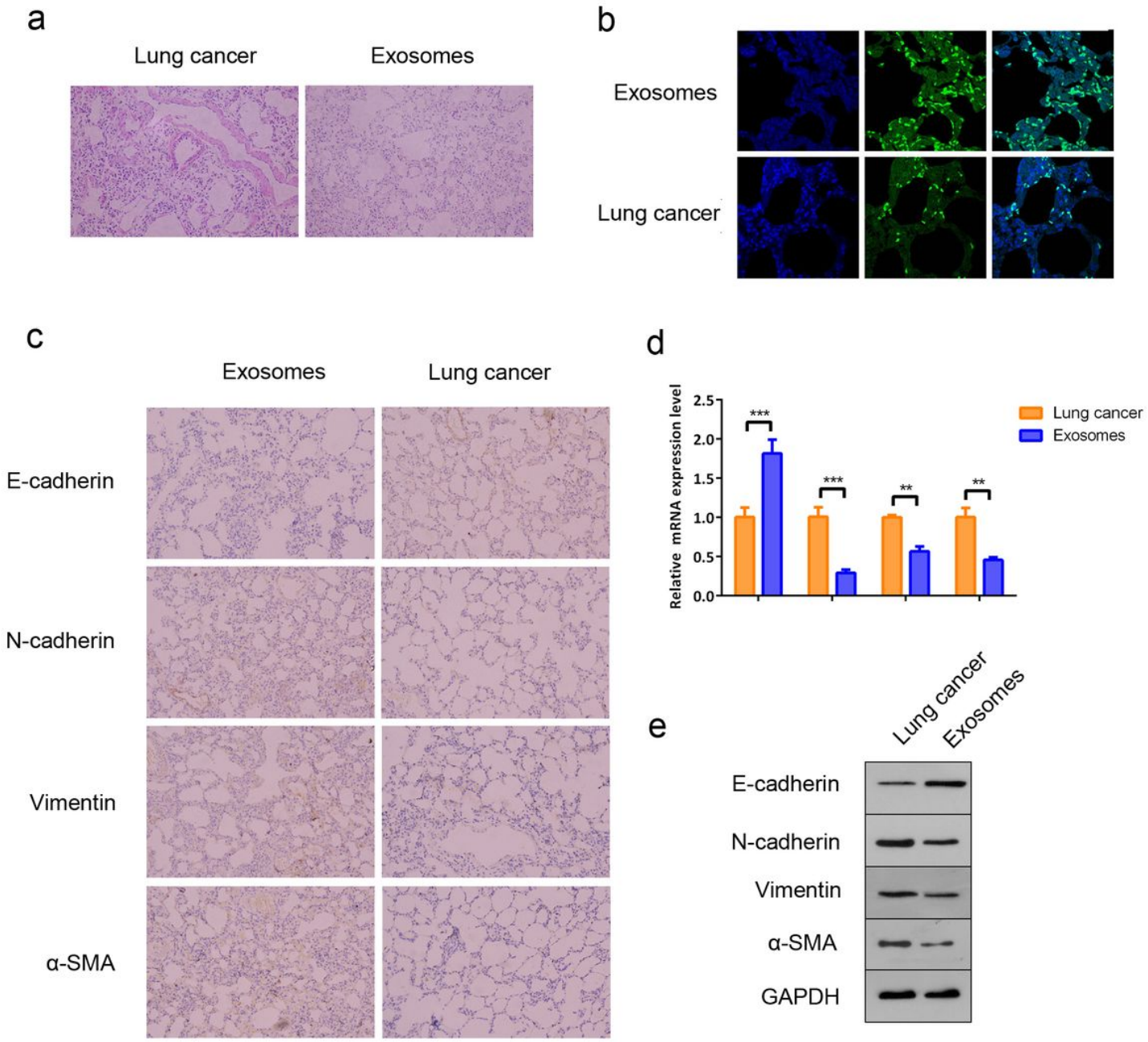

Figure 6

Exosomes derived from MSCs rescues tumor metastasis in vivo.

(a) HE staining was performed in control group and exosomes group. Base magnification:×200. (b) The result of a RNA-FISH assay showed that the expression of miRNA-486-5p in tumor tissues. DAPI was used to stain nuclei (blue); Left green fluorescence was from the biotin fusions; Right the merged image; Base magnification:×400. (c) Immunohistochemistry was performed to detect the mesenchymal markers vimentin, N-cadherin and α-SMA , the epithelial marker E-cadherin of tumor tissue. Base magnification:×200. (d) qRT-PCR was performed to detect the mesenchymal markers vimentin, N-cadherin and α-SMA , the epithelial marker E-cadherin of tumor tissue. (e) Western blot was performed to detect the mesenchymal markers vimentin, N-cadherin and α-SMA , the epithelial marker E-cadherin of tumor tissue. (*P<0.05, **P<0.01, ***P<0.001)