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RESEARCH PAPER

Galectin-1 overexpression induces normal fibroblasts translate into cancer-associated fibroblasts and attenuates the sensitivity of anlotinib in lung cancer

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ABSTRACT

We aimed to investigate galectin-1 overexpression induces normal fibroblasts (NFs) translates into cancer-associated fibroblasts (CAFs). Galectin-1 overexpression was conducted in Human embryonic lung fibroblasts (HFL1) cell. The motilities of H1299 and A549 cells were measured. Human umbilical vein endothelial cell (HUVEC) proliferation and tube formation ability were assessed. Tumor volume and tumor weight was recorded. Cells motilities were increased, while apoptosis rates were decreased after CMs co-cultured. B-cell lymphoma-2 (Bcl-2) expression level was increased, while Bcl2-associatedX (Bax) and cleaved-caspase3 decreased. CMs treatment enhanced HUVEC proliferation and tube formation. Tumor volume and weight in CMs treated mice were increased, and the sensitivity of anlotinib in co-cultured cells was decreased. Our results revealed that galectin-1 overexpression induced NFs translated into CAFs.

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Condition mediums; HUVEC; TGF-β1; tube formation; α-SMA

Introduction

Lung cancer is the highest cancer-related mortality in the world. Most patients with lung cancer were diagnosed as advanced and had no chance of surgery, the patients who can be operated have high postoperative metastasis rate and poor overall prognosis, the 5-year relative overall survival was less than 20% [1]. The occurrence and development of lung cancer not only depend on lung cancer cells, but also closely related to the environment of lung cancer cells (tumor microenvironment, TME) [2]. TME plays an important role in the occurrence and development of primary lung cancer [3]. TME is considered as a target rich environment for the development of new anticancer drugs [2]. As an important component of TME, cancer-associated fibroblasts (CAFs) promote tumor progression from tumor cell proliferation, invasion and metastasis, tumor angiogenesis, immune escape and other aspects [4]. Fibroblasts, endothelial cells, epithelial cells and mesenchymal stem cells can be transformed into CAFs, and fibroblasts in tumor surrounding tissues are the main source of CAFs transformation [5,6].

Galectin-1 is a member of galectin family. It has a simple structure and only one carbohydrate recognition domain (CRD) often exists in the form of dimer [7]. It is a typical prototype lectin. Galectin-1 was first found to have lectin binding activity in 1975 [8]. Galectin-1 is expressed in many normal tissues, including heart, lung, liver, kidney, and small intestine. Galectin-1 was also expressed in nervous system, endothelial cells, dendritic cells, macrophages and fibroblasts. Galectin-1 is highly expressed in lung cancer, breast cancer, liver cancer, pancreatic cancer, gastric cancer, and so on [9–12]. Studies have reported that the galectin-1 high expression in CAFs in a variety of tumor tissues may be related to the development of tumor [10,13].

In this study, we investigate the characteristic high level of α-smooth muscle actin (α-SMA) in human embryonic lung fibroblasts by overexpression of galectin-1, which proved the possibility of normal fibroblasts (NFs) transforming into CAFs. The interaction and influence between NFs and tumor cells in TME were determined by co culture NFs of overexpress galectin-1 and lung cancer cells in vitro. Cell proliferation assay, flow cytometry, transwell assay and tube formation assay were used to observe the effects of activated fibroblasts on the proliferation, invasion and migration of tumor cells. The effects of galectin-1 overexpression of NFs on the tumorigenicity and sensitivity to anlotinib were observed.

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Materials and methods

Regent

Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco BRL (Rockville, MA, USA). CCK-8 was purchased from Biomiky (Hefei, China). Annexin V-FITC and propidium iodide (PI) were purchased from AAT Bioquest (CA, USA). bicinchoninic acid (BCA) kit was purchased from Beyotime (Shanghai, China). Anlotinib was purchased from Chia Tai Tianqing Pharmaceutical Group Co.,Ltd. Immunohistochemistry (IHC) staining regents were purchased from Solarbio (Beijing, China).

Cell culture

Human embryonic lung fibroblasts (HFL1) and human lung cancer cell lines (H1299 and A549) were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China) and maintained in DMEM supplemented with 10% FBS, 100 units/mL penicillin, and streptomycin at 37°C with 5% CO_2. The shape of cells is regular and there is no clustering phenomenon. All experiments were performed with mycoplasma-free cells. Cells lines utilized in the research were tested by the Cell Bank of Chinese Academy of Sciences used STR method.

Establishment of stable galectin-1overexpression cells

Lentiviral-based carrying galectin-1 gene (Gal-1 OE) and the negative control lentiviral (Ctrl) were established (Genepharma, Shanghai, China). Lentivirus was then infected into HFL1 cells for 72 h in the presence of polybrene (5 μg/mL, Genepharma, Shanghai, China) and cells were selected using puromycin (5 μg/mL, Signa-Aldrich).

Preparation of conditioned medium (CMs) for CAFs

The galectin-1 overexpression HFL1 cell line was constructed by lentiviral vector. After the cells grew to about 80%, the culture medium was removed, washed with sterile PBS for three times, and cultured in serum-free DMEM medium for 48 h (CMs1), 72 h (CMs2), and 96 h (CMs3). CMs were processed according to previous study [14]. In the follow-up related experiments, the CMs collected at the corresponding time point were needed to continuously cultivate the cells.

Transwell migration and invasion assays

24-well transwell plates with 8 μm pore (Corning, NY, USA) were hired to detect the migratory and invasive ability of A549 and H1299 cells. For migration assay, 1×10^5 cells in 100 μL DMEM containing 0.2% FBS and 600 μL CMs were placed into the upper and lower chamber, respectively. After the cells stained with 0.1% crystal violet for 15 min, the photos were taken by microscope (Olympus, Japan).

For invasion assay, 500 μL serum-free medium was added to both the upper and lower chambers, and incubated in 37°C for 2 h to rehydrate the Matrigel matrix layer (BD, USA). 1×10^5 cells in 100 μL DMEM containing 0.2% FBS and 600 μL CMs containing 10% FBS were placed into the upper and lower chamber, respectively. The cells fixed by 4% paraformaldehyde for 30 min, then stained with 0.1% crystal violet for 30 min, the photos were taken by microscope (Olympus, Japan).

Cell apoptosis assays

After different treatment, the cells were collected, centrifuged at 1300 rpm for 5 min, then washed with 4°C phosphate buffer saline (PBS), 1×binding buffer washed. After centrifugation, cells were resuspended with 200 μL 1×binding buffer. Cells were stained with 2 μL Annexin V-FITC and PI, for 30 min at room temperature in dark. Cell apoptosis was analyzed by flow cytometry (BD FACSCanto, US).

Cell counting Kit-8 (CCK-8) assay

CCK-8 assay was performed according to the manufacture's protocol. HUVEC were seeded into 96-well plates at a density of 10^3 cells/well with 200 μL. After incubation 24 h, the culture medium was discarded, then added CMs1, CMs2 and CMs3, 10% DMEM was used as control (Ctrl). After incubation for 24 h, 48 h, 72 h, 96 h, and 120 h, 20 μL CCK-8 reagent was added into each well, continue to incubate at 37°C in the dark for 4 h. The optical density (OD) value was detected at 450 nm wavelength by a microplate reader (Tecan, Switzerland).

Tube formation assay

Human umbilical vein endothelial cell (HUVEC) was treated with CMs1, CMs2, and CMs3. 2×10^5 cells were placed in the 6-well plate. After adherent culture, cells washed twice with PBS, and then cultured in CMs for 24 h. The supernatant was collected. Images and data were obtained by microscope (Olympus, Japan).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA of A549 and H1299 cells was extracted via TRIzol Plus RNA Purification kit (Invitrogen, USA), and reversed to cDNA with SuperScript™ III First-Strand Synthesis SuperMix (Invitrogen). QRT-PCR was performed by Power SYBR® Green Master Mix (Bio-Rad, USA). All experiments were carried out based on the manufacture's protocol. PCR process: 95°C, 1 min, 95°C, 15 s, 63°C, 25 s for 40 cycle. The primers for genes were designed by Sangon Biotech Shanghai, galectin-1: forward, 5'-CCTGGAAGTGTTGCAGAGGT-3', reverse, 5'-CCGTCAGCTGCCATGTAGTT-3'; GAPDH: forward, 5'-CCATGACAACTTTGGTATCGTGGAA-3', reverse, 5'-GGCCATCACGCCACAGTTTC-3'. The cycle time (Ct) values of the genes detected were normalized to GAPDH and analyzed with the $2^{-\Delta\Delta Ct}$ method [15].

Western blotting

Total protein was extracted by tissue protein extraction reagent (TPRE, Thermo Pierce, USA) and quantified by bicinchoninic acid (BCA) kit (Beyotime, Shanghai, China). Proteins were transferred to polyvinylidene difluoride (PVDF, Millipore, USA) membranes after being separated with 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Membranes were incubated with primary antibodies at 4°C overnight after being blocked for 2 h. Secondary antibodies were then added to incubate for 2 h at 37°C. Proteins were visualized by the enhanced chemiluminescence (ECL) detection kit (ThermoFisher, USA). The antibodies for selected proteins were presented in Table 1.

Animal models

2×10^7 A549 cells were suspended in 200 μL PBS and then subcutaneously injected into the flanks of BALB/c-nude mice (4 weeks, $n = 7$). The mice were randomly divided into four groups, as follows: a model group was A549 group, a group receiving anlotinib, a group receiving Gal-1 OE @HFL1 : A549 (injected after mixing in the ratio of 3:1), a group receiving Gal-1 OE@HFL1 : A549 and anlotinib. Anlotinib (2 mg/kg) was administered orally once daily for 3 weeks. Tumor length and width were measured every three days. Tumor volume was calculated as follow: $\text{Volume } (mm^3) = 0.5 \times \text{Length } (mm) \times \text{Width}^2 \ (mm^2)$. Tumors were observed before xenograft tumors were resected. Tumor tissues were weighed before fixed in 4% phosphate buffered neutral formalin and subsequently embedded in paraffin for IHC and Masson staining. All research involving animals was conducted according the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press).

IHC analysis

IHC staining was performed on tumors tissue using DAB Horseradish Peroxidase Color Development Kit. 4 μm paraffin-embedded tissue sections were deparaffinized and rehydrated in xylene and alcohol, respectively. Sections were heated for 5 min to repair antigenicity and then treated for 10 min with 3% H_2O_2 to inactivate endogenous peroxidase activity. Sections were incubated with the primary antibody overnight and with secondary antibody for 1 h in turn.

α-SMA staining result: five different visual fields with better color development were observed in each section, and the staining intensity and staining range

Table 1. Antibodies used in western blot and IHC.

Protein	Cat.NO	dilution	Moleculer weight (kDa)
galectin	Abcam ab138513	1: 1000	14
TGF-β1	Abcam ab179695	1:1000	50
Alpha-SMA	Abcam ab21027	1: 500	45
MMP2	Abcam ab97779	1:500	74
MMP9	Abcam ab38898	1:1000	92
VEGFA	Abcam ab46154	1: 500	27
PDGFA	Abcam ab21669	1:500	24
Bcl-2	Proteintech12789–1-AP	1:2000	26
Bax	Proteintech50599–2-Ig	1:2000	21
Cleaved-caspase3	Affinity AF7022	1:1000	17
GAPDH	Abcam ab181602	1: 10000	36
Ki67	Abcam ab15580	1: 100	
Goat anti-Rabbit IgG	Thermo Pierce 31,210	1: 5000	
Goat anti-Mouse IgG	Thermo Pierce 31,160	1: 5000	

were recorded. The staining intensity was divided into no (0), weak (1), medium (2), and strong (3), and the staining range was divided into no (0), 1/4 (1), 1/4 to 1/2 (2), and more than 1/2 (3). Staining index = staining intensity × staining range.

Ki67 staining result: The positive staining results were presented as brown granules in the nucleus. The average percentage of positive cells was calculated (5 visual fields were selected for each section).

Statistical analysis

All data were shown as means and standard errors. SPSS 21.0 (IBM SPSS software, NY, USA) and Prism 7 (GraphPad Software, La Jolla, CA, USA) were hired for the Student's t-test. The comparison among multiple groups was evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. p value < 0.05 were regarded as statistically significant.

Results

High expression of galectin-1 promoted NFs transferred to CAFs

We stably overexpression Gal-1 in HFL1 cells by lentivirus, and divided cells into Ctrl and galectin-1-OE groups. After 72 h of lentivirus infection, the cells were observed by fluorescence microscope. The results showed that the infection rate of cells was more than 90%, and the cells were in normal state (Figure 1(a)). QRT-PCR and western blotting were used to detected galectin-1expression level at different times. As shown in Figure 1(b,c) after transfection 48 h, 72 h, 96 h, the Gal-1 expression at both mRNA and protein were up-regulated in both cells. To clarify whether galectin-1 overexpression promoted NFs transferred to CAFs, we investigated the expression level of TGF-β1 and α-SMA. Western blot results presented that, after galectin-1 overexpression, the levels of TGF-β1 and α-SMA also increased (Figure 1c,d). α-SMA is common used

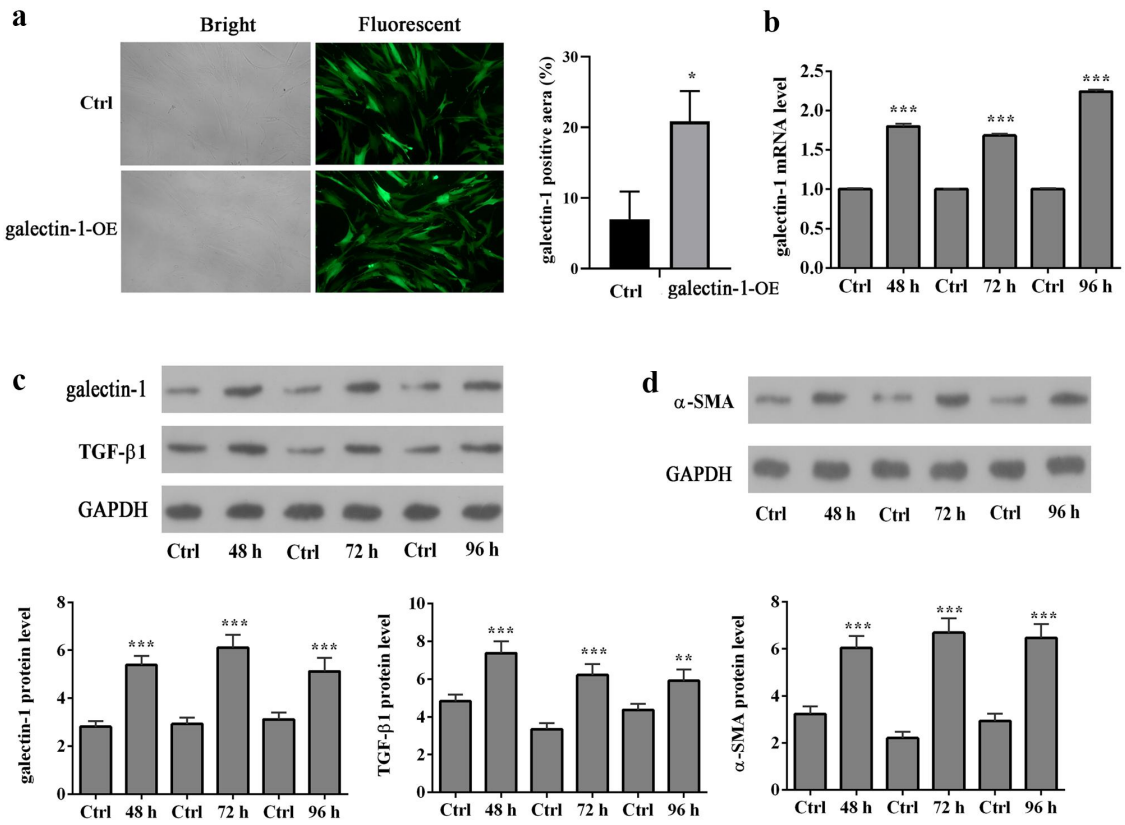

Figure 1. Galectin-1 overexpression in HFL1 cells. (a) Fluorescence images presented that galectin-1 was successfully overexpression in HFL1 cells (magnification: ×100). (b) qRT-PCR was conducted to measure galectin-1 mRnas expression. (c) Western blotting was conducted to measure galectin-1, TGF-β1 proteins. (d) The qualification of galectin-1, TGF-β1 proteins expression in ctrl and galectin-1-OE groups at different time. (e) Western blotting was conducted to measure α-SMA proteins expression in ctrl and galectin-1-OE groups at different time. The quantitative analysis of galectin-1 (f), TGF-β1 (g), and α-SMA (h) proteins. *p < .05, **p < .01, ***p < .001.

marker to identify CAFs [16]. High expression of α-SMA in NFs suggested that NFs have been transferred into CAFs.

High galectin-1 expression in NFs promotes cancer cell migration and invasion in vitro

To clarify whether NFs with galectin-1 overexpression functionally promoted lung cancer cells metastasis, we compared the ability of migration and invasion in lung cancer cells treated with Ctrl and CMs. The results of transwell migration assay indicated that both A549 and H1299 cells in CMs groups display an increased migration assay compared to those in Ctrl group (Figure 2a,b, $p < .05$). Further, transwell invasion assay demonstrated that cell invasion was enhanced in CMs groups compared to Ctrl group (Figure 2c,d, $p < .05$). In addition, the effect of CMs3 was stronger than CMs1 and CMs2 ($p < .05$). Taken together, galectin-1 overexpression CMs could promote lung cancer cell migration and invasion.

High galectin-1 expression in NFs inhibits cancer cell apoptosis

We investigated the lung cancer cells apoptosis treated with Ctrl and CMs. The results of flow cytometry assay indicated that both A549 and H1299 cells in CMs groups display a decreased apoptosis rate compared to those in Ctrl group (Figure 3, $p < .05$). We then detected the expression levels of Bcl-2, Bax, and cleaved-caspase3. The results confirmed that the expression level of Bcl-2 increased after CMs treatment, while the expression levels of Bax and cleaved-caspase -3 decreased. Furthermore, transwell invasion assay demonstrated that cell invasion was enhanced in CMs groups compared to Ctrl group ($p < .05$). The effect of CMs3 has no obvious difference compared with CMs1 and CMs2 ($p > .05$).

High galectin-1 expression in NFs promotes HUVEC proliferation and tube formation

To clarify whether NFs with galectin-1 overexpression are functionally promoted angiogenesis, we investigated the effect of CMs on HUVEC. As shown in Figure 4, CCK8 assay presented that the proliferation ability of HUVEV treated with CMs was increased, compared to Ctrl group ($p < .05$). Furthermore, we detected the angiogenesis by tube formation assay. The results indicated that, overexpressed galectin-1 CMs strongly accelerated the tubule formation of HUVECs ($p < .05$).

High galectin-1 expression in NFs promotes protein expression

We studied the effects of galectin-1 on the expression of MMP2, MMP9, and classical angiogenic factors (TGFβ1, PDGF, and VEGF). Western blot assay presented that the expression of MMP2, MMP9, TGFβ1, VEGF, and PDGF

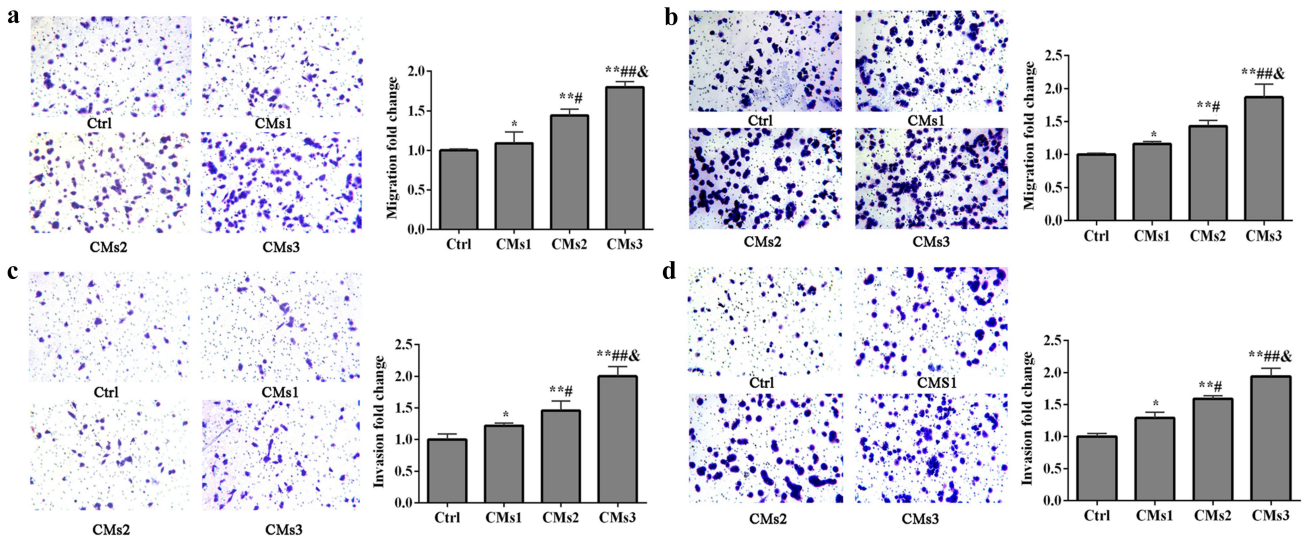

Figure 2. Galectin-1 overexpression CMs promotes cell migration and invasion *in vitro*. (a) The effect of galectin-1 overexpression CMs on A549 cell migration was analyzed by transwell migration assay. (b) The effect of galectin-1 overexpression CMs on H1299 cell migration was analyzed by transwell migration assay. (c) The effect of galectin-1 overexpression CMs on A549 cell invasion was analyzed by transwell invasion assay. (d) The effect of galectin-1 overexpression CMs on H1299 cell invasion was analyzed by transwell invasion assay. Magnification: × 100. vs ctrl group, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$; vs CMs1 group, $^{\#}p < .05$, $^{\#\#}p < .01$; vs CMs2 group, $^{\&}p < .05$, $^{\&\&}p < .01$.

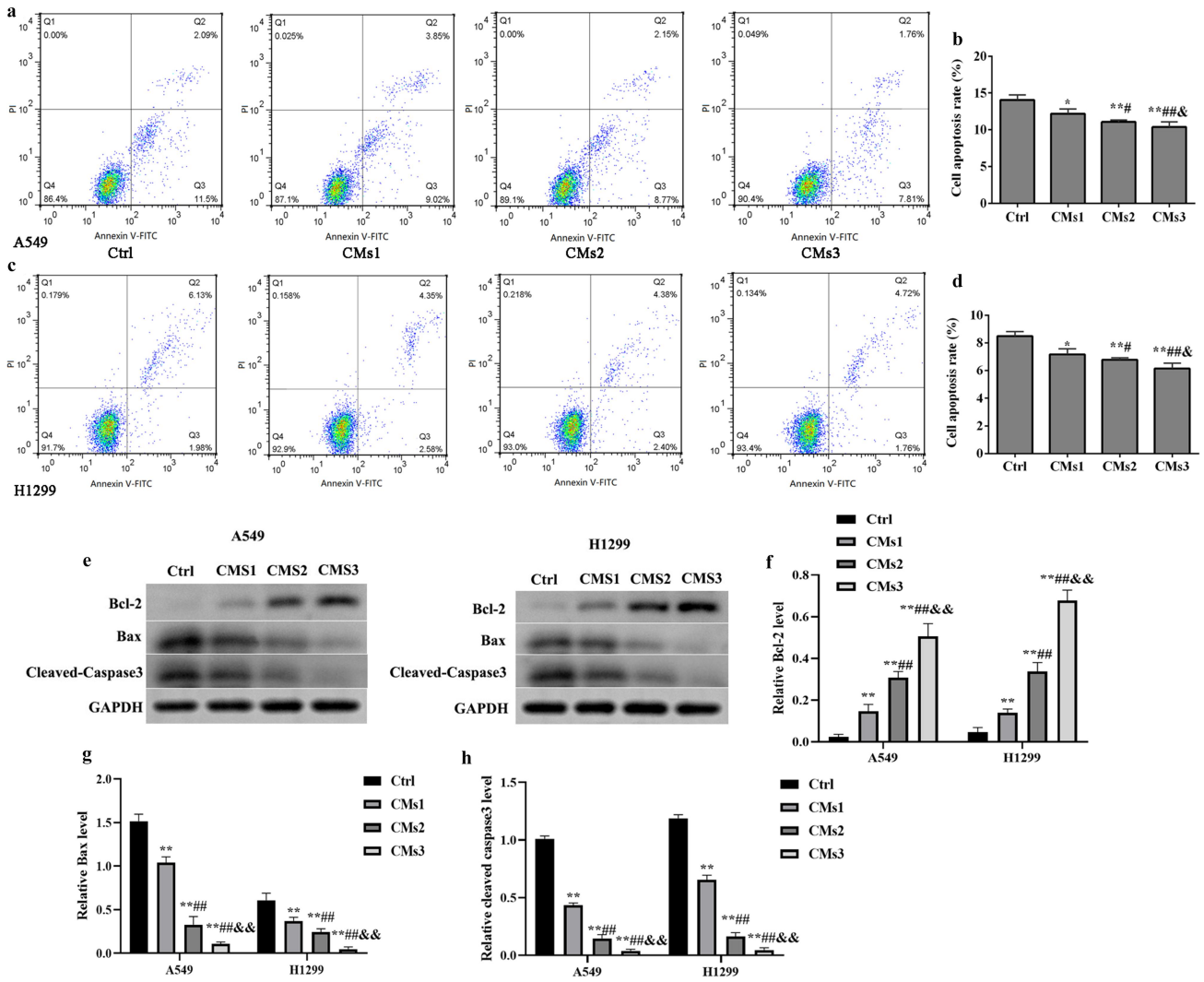

Figure 3. Galectin-1 overexpression CMs inhibits cell apoptosis *in vitro*. (a) Effects of galectin-1 overexpression CMs on the cell apoptosis of A549 cells. (b) The quantitative analysis of cell apoptosis rate of A549 cells. (c) Effects of galectin-1 overexpression CMs on the cell apoptosis of H1299 cells. (d) The quantitative analysis of cell apoptosis rate of H1299 cells. (e) The expression levels of bcl-2, Bax, and cleaved-caspase3 were detected by western blot. Quantitative analysis of bcl-2 (f), Bax (g), and cleaved-caspase3 (h) levels in A549 and H1299 cells vs ctrl group, $^{*}p < .05$, $^{**}p < .01$; vs CMs1 group, $^{\#}p < .05$, $^{\#\#}p < .01$; vs CMs2 group, $^{\&}p < .05$, $^{\&\&}p < .01$.

increased compared to Ctrl group, after treated with CMs (Figure 5, $p < .05$). However, the expression levels of PDGF and VEGF have no obvious difference between CMs2 and CMs3 ($p > .05$).

High galectin-1 expression in NFs promotes tumor growth *in vivo*

A549 cells with stable overexpression of galectin-1 CMs in a ratio of 1:3 were implanted into nude mice to verify the effect of CMs on tumor formation and the sensitivity of anlotinib. The average tumor volume and tumor weight was higher in mice inoculated with CMs and A549 cells (Gal-1 OE@HFL1:A549 group) than A549 group (Figure 6, $p < .05$). The average tumor volume and tumor weight was the lowest in A549 + anlotinib group. When A549 co-incubated with Gal-1 OE@HFL1, the tumor inhibitory effect of anlotinib was significantly decreased compared to A549 + anlotinib group (Figure 6, $p < .05$).

IHC was used to investigate the expression level of fibrotic-related protein α-SMA. Ki67 expression level could be employed to represent proliferative abilities of tumor cells in tumor tissues. As presented in Figure 7, compared to A549 group, α-SMA staining index and the number of Ki67 positive cells increased in Gal-1 OE@HFL1:A549 group, while they were decreased in A549+Anlotinib group ($p < .05$). Compared to Gal-1 OE@HFL1:A549 group, α-SMA staining index and the number of Ki67 positive cells decreased in Gal-1

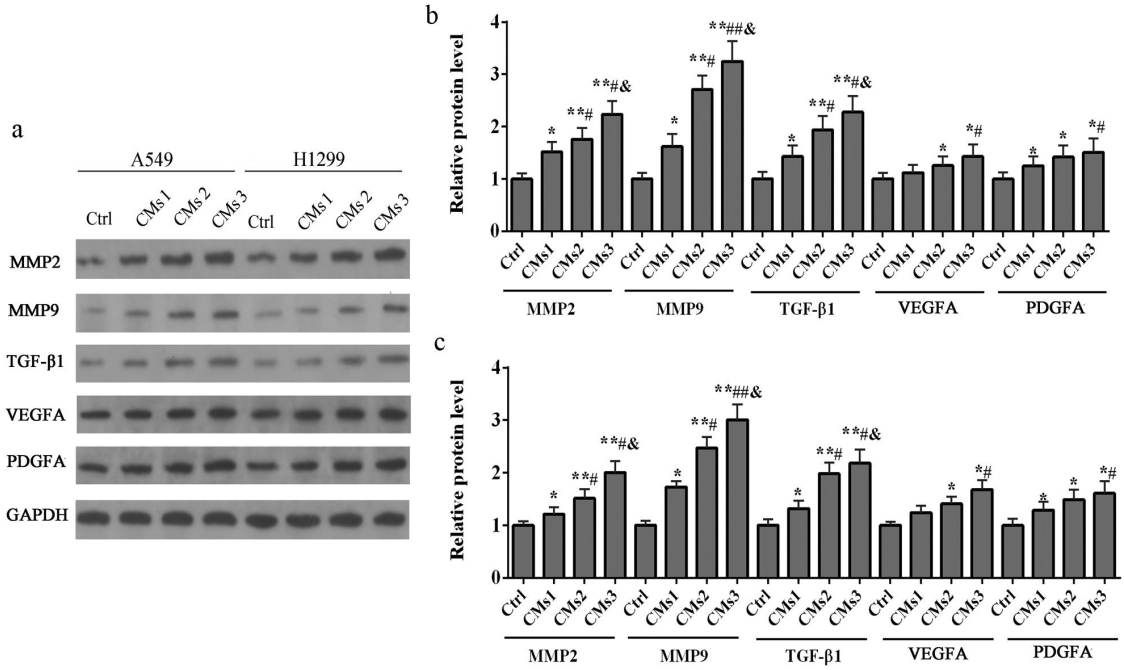

Figure 4. Effect of galectin-1 overexpression CMs on proteins expression. (a) Western blotting was conducted to measure MMP2, MMP9, TGF-β1, VEGFA, PDGFA. (b) The qualification of proteins expression in different groups in A549 cells. (c) The qualification of proteins expression in different groups in H1299 cells. vs ctrl group, $^{*}p < .05$, $^{**}p < .01$; vs CMs1 group, $^{\#}p < .05$, $^{\#\#}p < .01$; vs CMs2 group, $^{\&}p < .05$p.

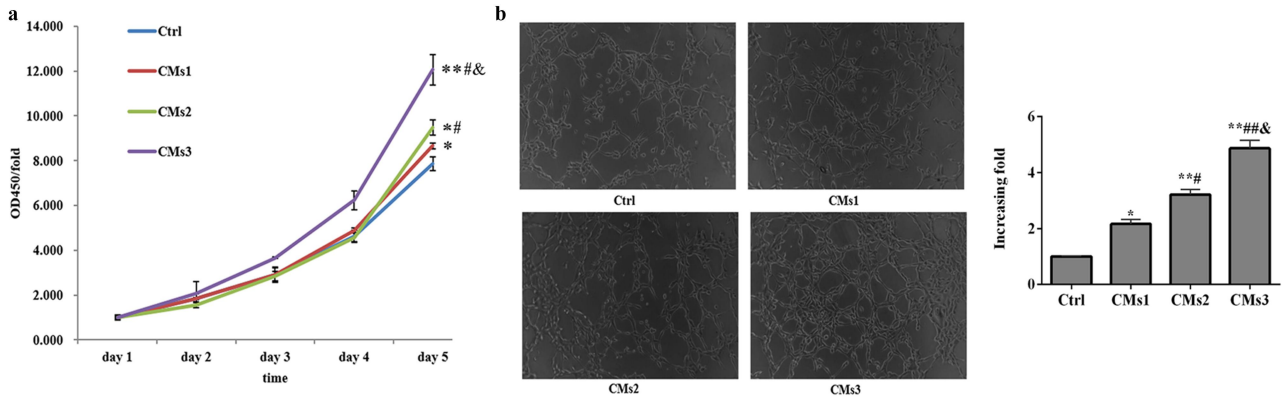

Figure 5. Galectin-1 overexpression CMs promotes HUVECs proliferation and tube formation. (a) Galectin-1 overexpression CMs promotes HUVECs proliferation was detected by CCK8 assay. (b) Galectin-1 overexpression promotes HUVECs proliferation and tube formation. vs ctrl group, $^{*}p < .05$, $^{**}p < .01$; vs CMs1 group, $^{\#}p < .05$, $^{\#\#}p < .01$; vs CMs2 group, $^{\&}P < .05$.

OE@HFL1:A549 + Anlotinib group ($p < .05$). However, the levels in Gal-1 OE@HFL1:A549 + Anlotinib group was higher than A549+Anlotinib group ($p < .05$). In addition, the expression of Gal-1, TGF-β1, MMP2, MMP9, TGFβ1, VEGF, and PDGF increased in Gal-1 OE@HFL1:A549 group, and the levels in Gal-1 OE@HFL1:A549 + Anlotinib group was higher than A549+Anlotinib group ($p < .05$).

Taken together, galectin-1 overexpression could promoted tumor progress *in vivo*, it also decreased the sensitivity of anlotinib to A549 cells.

Discussion

Previous studies indicated that galectin-1 induced NFs translate to CAFs, activated CAFs can secrete a variety of growth factors, cytokines and extracellular matrix, and then affect tumor cell proliferation, invasion, metastasis and angiogenesis [13,17]. TGF-β1 has a wide range of potential roles in tumor metastasis [18]. TGF-β1 can act on fibroblasts to enhance their proliferation, invasiveness and contractility, thus transforming them into CAFs [19], CAFs can secrete TGF-

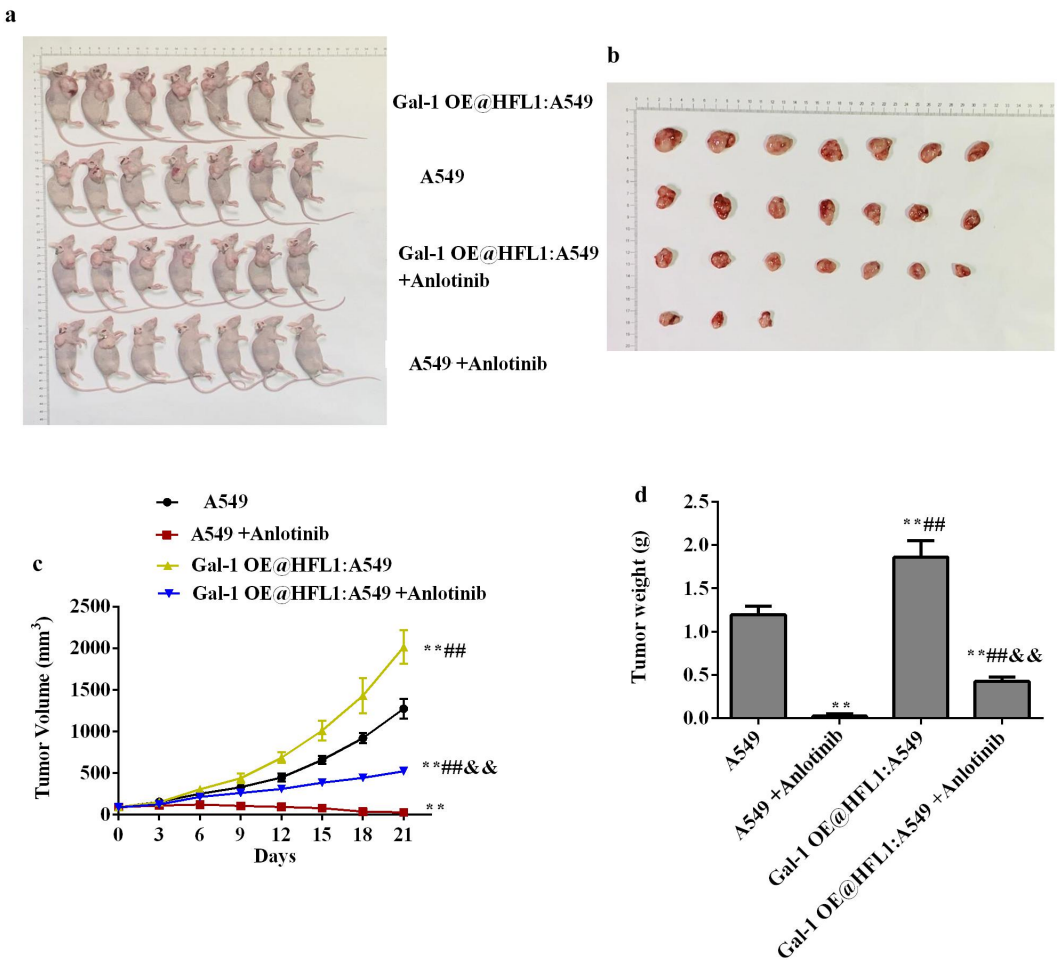

Figure 6. Galectin-1 overexpression promotes tumor growth *in vivo*. (a) Imaging technology to monitor the growth of A549 ×enograft tumors. (b)The growth of xenograft tumors was determined by volume. (c) The growth of xenograft tumors was determined by tumor weight. vs A549 group, $^{**}p < .01$; vs A549+Anlotinib group, $^{\#\#}p < .01$; vs gal-1 OE@HFL1:A549 group, $^{\&\&}p < .01$.

β1 and obtain the autocrine signal circuit mediated by TGF-β1, thus promoting and maintaining the differentiation of fibroblasts into CAFs [20]. In our study, we conducted galectin-1 overexpression in HFL1 cells. Our results indicated that, after galectin-1 overexpression, the expression levels of TGF-β1, and α-SMA were increased. The high expression level of TGF-β1 and α-SMA were used as NFs translated to CAFs. In addition, we collected the culture medium as CMs. Then the CMs were used to treat A549 and H1299 cells. We investigated the A549 and H1299 cells migration and invasion ability, and found that the cells migration and invasion ability were enhanced after incubation with CMs. The results was consistent with a previous study [10]. At the same time, we found that the expression of MMP2 and MMP9 increased in cancer cells. We thought that the expression of galectin-1 in CAFs promoted cell invasion and metastasis, which may be related to the degradation of extracellular matrix by MMP and the destruction of the histological barrier of tumor invasion and metastasis.

The effect of galectin-1 on apoptosis has different effects of promotion and inhibition. Researchers believe that the different effects of galectin-1 on apoptosis may be related to the expression of galectin-1 in different parts of cells and cell types [21]. Our study suggested that galectin-1 overexpression induced decreased apoptosis rate of cells cultured with CMs. The results were further confirmed by the detection of the expression levels of Bcl-2, Bax, and cleaved-caspase-3. The results confirmed that the expression level of Bcl-2 increased, while the expression levels of Bax and cleaved-caspase3 decreased after CMs treatment. This may be related to galectin-1 overexpression induced NFs translate into CAFs, and CAFs activation induce anti-apoptosis of cells.

The establishment of tumor vascular system depends on the budding and growth of neocapillaries induced by angiogenic factors in vivo, involving multiple cell signaling pathways, including growth factors

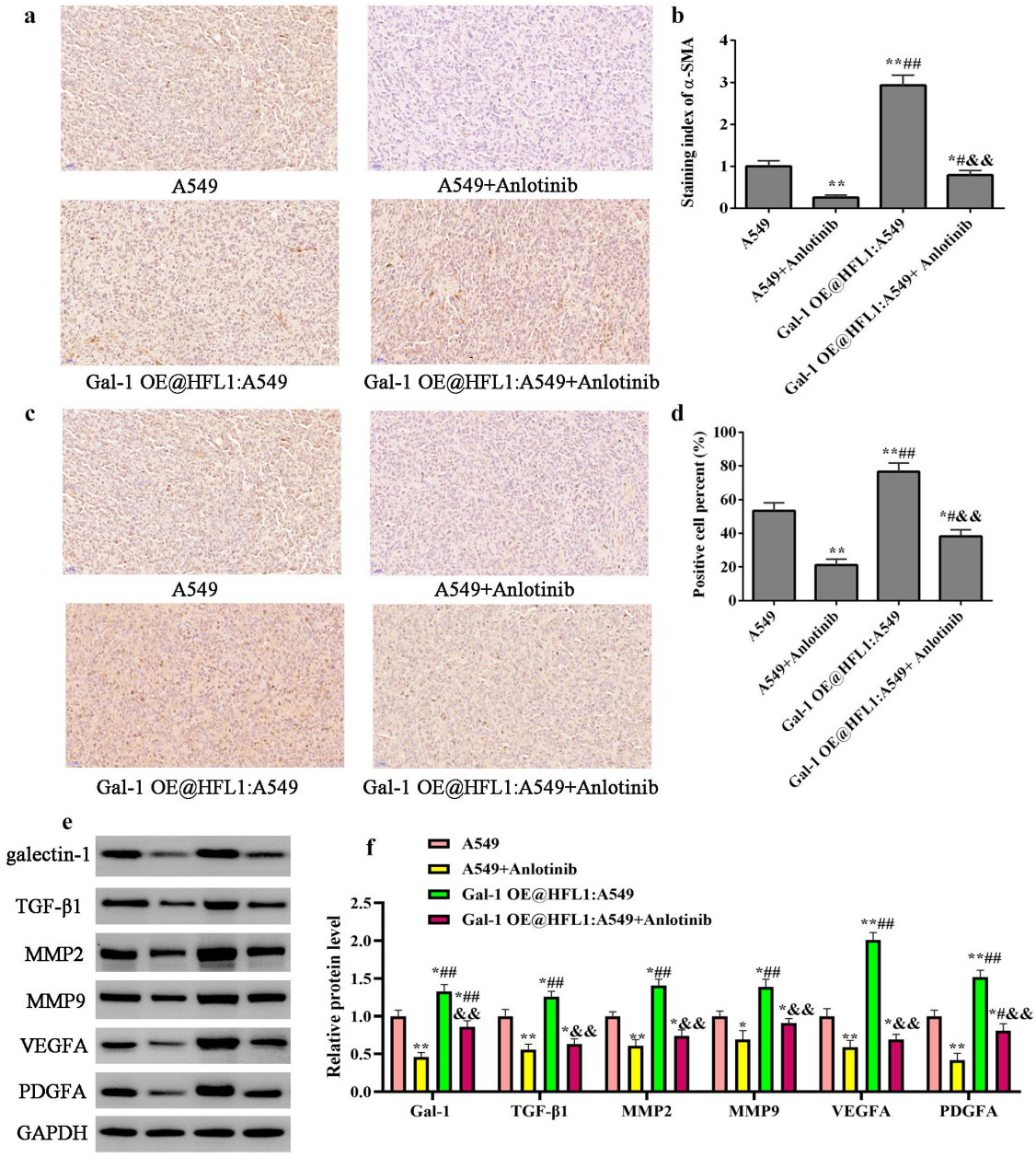

Figure 7. IHC detected α-SMA, and Ki67 protein expression *in vivo*. (a) IHC staining of α-SMA protein in xenograft tumors. (b) The staining index of α-SMA. (c) IHC staining of Ki67 protein in xenograft tumors. (d) Positive cell percent of Ki67. Scale bar: 50 μm. (e) Western blotting was conducted to measure MMP2, MMP9, TGF-β1, VEGFA, PDGFA. (f) The qualification of proteins expression in different groups. vs A549 group, $^{*}p < .05$, $^{**}p < .01$; vs A549+Anlotinib group, $^{\#}p < .05$, $^{\#\#}p < .01$; vs gal-1 OE@HFL1:A549 group, $^{\&}p < .05$, $^{\&\&}p < .01$.

(VEGF, PDGF, etc.) [22]. At present, it is considered that the most important regulatory factor of tumor angiogenesis is VEGF. The signal pathway mediated by VEGF plays a leading role in tumor angiogenesis [23]. Studies have shown that CAFs play a significant role in promoting angiogenesis in tumor tissues [17,24]. In our study, we investigated the effect of galectin-1 overexpression CMs on HUVEC proliferation and tube formation. After incubation with CMs, the proliferation ability of HUVEC was increased compared to control culture medium. Furthermore, CMs enhanced HUVEC tube formation ability. We also detected the expression levels of VEGF and PDGF in cancer cells, the results presented that the expression levels of VEGF and PDGF significantly increased, after CMs treatment. The above results

indicated that galectin-1 overexpression promoted angiogenesis. Our results were consistent with previous study [10,17,25].

Anlotinib is a novel multi-target small molecule tyrosine kinase inhibitor (TKI), which plays an anti-tumor angiogenesis role by inhibiting vascular endothelial growth factor receptor (VEGFR) [26]. Our previous study suggested that galectin-1 silencing can improve the sensitivity of A549/DDP cells to cisplatin [27]. In this study, we estimated the effect of galectin-1 overexpression CMs on tumor-bearing mice angiogenesis. The results indicated that anlotinib could effectively inhibited tumor growth. However, after the A549 co-culture with galectin-1 overexpression CMs, the tumors volume and weight were significantly increased than A549 inoculation alone. In addition, the sensitiveness of anlotinib was decreased. IHC results presented that, when the A549 co-culture with galectin-1 overexpression CMs, the staining index of α-SMA, and the positive cell number of Ki67 increased. There results further suggested that the activation of CAFs, and the sensitiveness of anlotinib was decreased.

In summary, our study demonstrated the galectin-1 overexpression induced NFs translated into CAFs, and the CMs co-culture with lung cancer cells could promote the cells migration and invasion ability, attenuate cells apoptosis rate. In addition, it also promoted HUVEC proliferation and tube formation *in vitro*. Galectin-1 overexpression attenuated the sensitiveness of anlotinib *in vivo*. However, our study was only confirmed the effect of galectin-1 in tumor microenvironment, the underlying mechanism is worthy of further study, which may become a potential target for the treatment of lung cancer. Our study provide a novel sight for the role of galectin-1 in lung cancer.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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