Int J Clin Exp Pathol 2017;10(5):4941-4954
www.ijcep.com /ISSN:1936-2625/IJCEP0049544

Original Article

Characteristics of core fucosylation changes of TGF-β1-induced pericyte tranformation in mouse lungs

Wei Sun[1*], Lili Gao[1*], Haiying Tang[1], Xiuna Sun[1], Jia Liu[1], Weidong Wang[2], Taihua Wu[1], Hongli Lin[2]

Departments of [1]Respiratory Medicine, [2]Nephrology, The First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, P.R. China. [*]Equal contributors and co-first authors.

Received January 23, 2017; Accepted February 23, 2017; Epub May 1, 2017; Published May 15, 2017

Abstract: Recent studies have shown that during the development of pulmonary fibrosis, pericytes are a significant source of myofibroblasts. However, the specific mechanism involved in the transformation of pericytes into myofibroblasts remains unknown. Moreover, there is evidence that pericyte transdifferentiation is a complex process involving the activation of a multi-signaling pathway. Our previous studies found that core fucosylation (CF) alleviates the development of organ fibrosis by regulating the TGF/Smad2/3 signaling pathway. The objective of this study was to treat pericytes with TGF-β1 for different time to observe whether pericytes transform into myofibroblasts. Moreover, we sought to detect the surface expression of PDGFβR on pericytes and the changes in CF. We found that during pericyte transdifferentiation, α-1,6 core fucosyltransferase (FUT8) catalyzed CF, which was necessary for this process. Thus, we adopted a FUT8 small interference RNA technique to inhibit the expression of CF to block the activation of the PDGFβ/Erk signaling pathway. Furthermore, the inhibition of CF expression is an optional method for inhibiting pericytes from transdifferentiating into myofibroblasts. Our findings indicate that CF plays an important role in the transformation of pericytes into myofibroblast. This study provides a theoretical basis for understanding the penetration and intersection of glycobiology and pneumonopathy through in vitro experimental analysis.

Keywords: Pericyte, myofibroblasts, core fucosylation, FUT8

Introduction

Pulmonary fibrosis is the final stage of all interstitial lung diseases, resulting an average lifetime of less than five years after the diagnosis due to efficient treatment measures [1, 2]. Although the causes of pulmonary fibrosis are diverse, their final presentation is characterized by the aggregation and increment of myofibroblasts [3-6]. Therefore, the analysis of the major source of myofibroblasts in pulmonary fibrosis and the search for novel efficient targets for prevention of the disease will have a significant effect on mortality reduction [7].

The source of myofibroblasts remains unclear and is increasingly controversial. The mainstream opinion over the past decade has been challenged because epithelial-mesenchymal transition (EMT) could not be demonstrated in vivo [8, 9]. Recently, research supports the fact that pericytes are a major source of myofibroblasts through Genetic Fate Mapping [9].

Distributed between endothelial cells of the microvascular system and basement membrane, pericytes exhibit the physiological function of maintaining the stability of blood vessels [10]. Once stimulated, pericyte separate from the endothelial cells and migrate into the pulmonary interstitium and transform into myofibroblasts [10, 11]. It was suggested that pericyte activation was an important factor involved in the generation of myofibroblasts [10]. However, the mechanism of pericyte transformation remains unknown. It was reported in the literature that pericyte transformation involved a network pathway for the activation of PDGFβ/Erk and TGF-β/Smad2/3 multi-signaling pathways [12]. Finding their common targeting points is an efficient method of blocking pericyte transdifferentiation.

Core fucosylation (CF) is an important protein glycosylation modification, which has the physiological function of specifically and catalytically transferring fucose with α-1,6 fucosyltransfer-

ase (FUT8) to target protein [13, 14]. Our previous work demonstrated that TGF-β receptor (TGF-βR) was modified by CF such that inhibiting CF expression was an optional method of inhibiting the activation of the TGF-β/Smad2/3 pathway to inhibit the development of renal fibrosis [15, 16]. Thus, we considered that CF might be a potential target for regulating these signaling pathways and preventing pericyte transdifferentiation.

This study further determined the mechanism of pericytes transformation by observing whether pericytes could transform into myofibroblasts and change the characteristics of CF during the regulation of pericyte transformation. This is achieved via a typical fibrosing cytokine transforming growth factor-β1 (TGF-β1)-induced pericyte transformation model. This study is the first to propose and verify CF as the key factor involved in pericyte transdifferentiation.

Materials and methods

Pericyte isolation and culture

All experiments were performed in accordance with the Research Ethics Committees of Dalian Medical University, China. We isolated primary pericytes from C57BL/6J mice. Briefly, the fresh lung tissue was washed with Hanks' balanced salt solution three times; the lung tissues were removed and cut 0.5 × 0.5 mm small piece and the lung tissues were digested with a liberate TM (Roche Applied Science) for about 50 min at 37°C on an orbital shaker. The digested lung tissue was filtered through a 100 μm and 200 μm nylon mesh (Falcon, BD, US). Mouse lung pericytes were collected in a 50 mL centrifuge tube and centrifuged. We added 4 mL rabbit anti-PDGFβR antibody (ab93563, Abcam, US) for 15 min at 4°C. Then, the lung pericytes were suspended in 320 mL D-Hanks balanced salt solution and incubated them in 80 mL anti-mouse IgG beads (MiltenyiBiotec) for 15 min at 4°C. After washing three times, the bead-bound pericytes were seeded in gelatin-coated cell culture plates containing pericyte medium (Science Inc., US).

siRNA design, preparation, and transfection

Chemically synthesized FUT8-siRNAs were designed to target the *FUT8* gene. The siRNA sequence was identified using the mouse genome database to assess any potential cross-reactivity. Once the siRNAs (5' GCUACUGAUGAUCCUACUU dTdT3'; 3'dTdT CGAUGACUACUAGGAUGAA 5') were synthesized, the dried siRNA were reconstituted in DEPC-treated H_2O to a final concentration of 20 nM and stored at 20°C until use. The pericytes were plated in 12-well culture plates and incubated for 24 h. The siRNA and transfection reagents were complexed and added to the cell culture wells. The transfection efficiency of FUT8siRNA was evaluated by a Western blot.

Immunofluorescence of pericytes in mice

Pericytes cultured on cover slides were fixed with 4% paraformaldehyde and incubated in 0.3% H_2O_2 and 0.1% triton X-100 to quench endogenous peroxidase activity and penetrate the cytomembrane. Then, the cells were incubated in 3% blocking goat serum for 1 h and then incubated with anti-PDGFβR, CD73, desmin, and α-SMA overnight at 4°C. The following day, the pericytes were incubated with the relevant fluorescence conjugated secondary antibody for 2 h at room temperature.

Pericytes cultured on cover slides were fixed with 4% paraformaldehyde and incubated in 0.3% H_2O_2 and 0.1% triton X-100 to quench endogenous peroxidase activity and penetrate the cytomembrane. Then, the cells were incubated in 3% blocking goat serum for 1 h and then incubated with anti-PDGFR-β, CD73, desmin, and α-SMA overnight at 4°C. The following day, the pericytes were incubated with the relevant fluorescence conjugated secondary antibody for 2 h at room temperature, and then analyzed with a laser confocal microscope.

Immunofluorescence

Immunofluorescence was used to test CF and PDGFβR, α-SMA expression. The cells were applied with a rabbit anti-PDGFβR antibody (1:200), rat anti-α-SMA antibody (1:200), a rabbit anti-FUT8 antibody (1:200) and LCA-FITC (1:1000), followed by the incubation of a FITC-goat anti-rabbit or TRITC-goat anti-rat antibody (1:200) for 40 min at 23°C out of direct light. After washing the cells in 0.1 M PBS, and the expression of these antibodies can be observed by laser confocal microscopy.

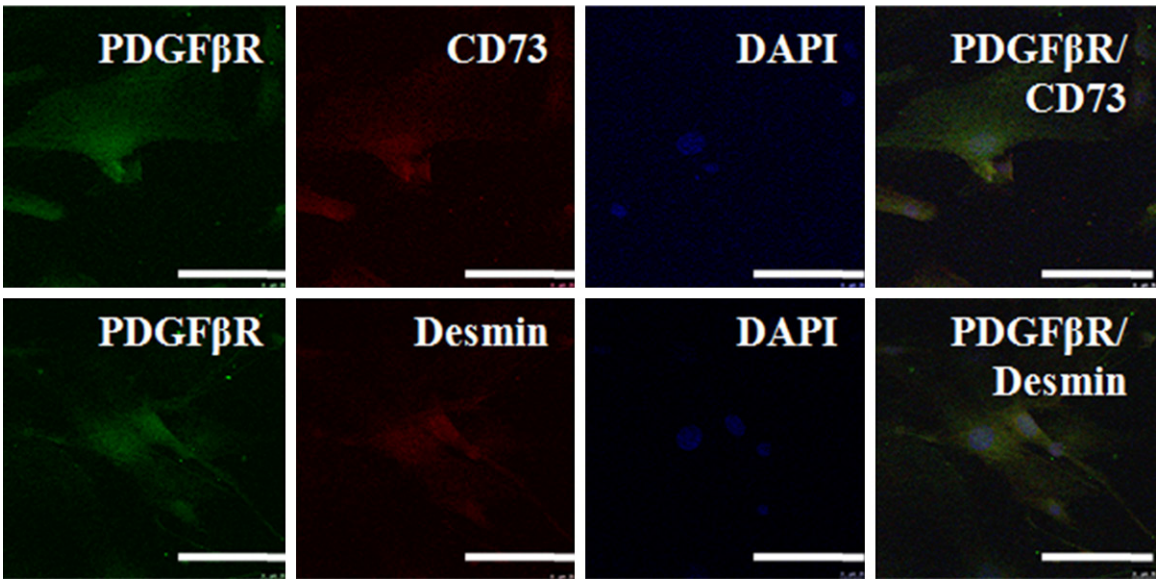


Figure 1. Verification of mice lung pericytes. Conduct double staining to detect the expression of pericyte markers through immunofluorescence with the amplification factor multiplied by 400. The experiment was repeated three times.

Lectin blotting

Immunoprecipitated PDGFβR was separated by 10% SDS-PAGE and electroblotted onto polyvinylidenedifluoride (PVDF) membranes (Bio-Rad). The membranes were blocked with 5% BSA (w/v) at 4°C overnight and then incubated for 2 h at 23°C in TBST containing LCA-Biotin (1:200), which preferentially recognizes Fuc-1, 6GlcNAc. After washing with PBST four times for 10 min, the lectin-reactive proteins were detected using an ECL kit (Amersham, Pittsburgh, PA).

Western blotting analysis

The protein samples were heated to 100°C for 5 min, and equal quantities of protein samples were separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto polyvinylidenedifluoride membranes (Millipore, Bedford, MA, USA). The membranes were incubated with primary antibodies against FUT8, PDGFβR, p-Erk, and α-SMA (1:200) overnight at 4°C. After washing, the membranes were incubated with an appropriate horseradish peroxidase-conjugated secondary antibody (1:5000 diluted; Zhongshan Biotechnology, Beijing, China) at room temperature for 2 h. A positive band on the membranes was detected using an ECL kit (Amersham). The band density was valued by using image analysis software (UVP, Upland, CA). The results are expressed as a ratio to β-actin.

Results

Verification of magnetic activated cell sorting of mouse lung pericytes

Currently, no specific marker has been identified for pericytes [17, 18]. Due to irregular cell expression, pericytes were verified by adopting various labels (PDGFβR, CD73, and Desmin). The immunofluorescent staining results demonstrate that pericytes were stained positively via monoclonal antibodies for PDGFβR, CD73, and Desmin (**Figure 1**).

FUT8siRNA inhibits the up-regulation of core fucosylation on the surface of pericytes by inhibiting TGF-β1

Since it remains unclear whether CF exists on the surface of lung pericytes in mice, to confirm the existence of CF, we used red fluorescence to label FUT8 and fluorescent Lens culinaris agglutinin-fluorescein complex (LCA-FITC) by using laser scanning confocal microscope. The results revealed that CF were expressed with moderate intensity and distributed on the pericyte membrane and endochylema, indicating

that core fucosylation expression exist in mouse pericytes. The level of CF modification gradually increased in the group of pericytes stimulated with TGF-β1.

FUT8siRNA successfully silences the endogenous FUT8 gene in pericytes

To understand the function of CF modification in the mechanism of TGF-β1-induced pericyte transdifferentiation, we adopted chemosynthetic FUT8siRNA interference fragments to transiently transfect pericytes for 24 h with 20 nM. We applied immunofluorescence to determine the expression of pericyte surface markers for each group. We applied Western blot method to detect FUT8siRNA interference effect and the results showed that FUT8siRNA could reduce FUT8 expression in pericytes, indicating that FUT8siRNA successfully interfered with the pericytes.

FUT8siRNA inhibits TGF-β1-induced pericyte morphological changes

During the process of stimulating lung pericyte transdifferentiation by TGF-β1, the pericyte morphology transformed from a multi-star shape into a fusiform myofibroblast shape. The results demonstrated that the pericytes in the control group were multi-star or medusiform shaped with a plump endochylema and clear outline. Following stimulation with TGF-β1 for 24 h, some of the cells began to stretch and separate from peripheral cells to induce a disordered arrangement. Following stimulation with TGF-β1 for 48 h, the shape of the pericytes changed significantly and formed into a strip. After a 20-nM concentration of FUT8siRNA was applied to the pericytes and the cells were incubated for 24 h, TGF-β1 was applied to stimulate pericytes for 24 h and 48 h; the majority of the pericytes under an optical microscope remained in a normal cell shape without obvious stretching or changes in the strip shape. This indicates that inhibiting core fucosylation by FUT8siRNA could suppress the development of the pericyte shape caused by TGF-β1 stimulation.

FUT8siRNA inhibits PDGFβR core fucosylation modification but not the expression of PDGFβR

Platelet-derived growth factor beta receptor (PDGFβR) is the primary protein involved in pericyte transformation following stimulation with TGF-β1 for 24 h and 48 h. The relationship between the core fucosylation and PDGFβR in pericytes remains unclear. The results of the immunofluorescence co-staining method found that CF and PDGFβR were expressed in pericytes, and started to increase after 24 h, peaking after 48 h. Moreover, the two markers overlapped completely, which indicated that PDGFβR was modified by core fucosylation. FUT8siRNA at a concentration of 20 nM was applied to the incubating pericytes for 24 h, and TGF-β1 was applied to stimulate the pericytes for 24 h and 48 h respectively. The core fucosylation expression of PDGFβR was inhibited; however, the PDGFβR protein was not. To verify this phenomenon, we adopted an immunoprecipitation and lectin blot method to detect the relationship between PDGFβR and the core fucosylation in pericytes, the results indicates that FUT8siRNA could not affect receptor protein expression. While the immunofluorescence result also revealed that FUT8siRNA inhibited the core fucosylation modification of PDGFβR but had no effect on the receptor protein expression.

FUT8siRNA inhibited PDGFβR core fucosylation and the activation of TGF-β1-induced PDGFβ/Erk signaling in pericytes

P-Erk indicates the activation of the PDGFβ/Erk pathway. To detect whether the PDGFβ/Erk pathway was activated by TGF-β1 during TGF-β-induced pericyte transformation, we applied immunoblotting and immunofluorescence methods to detect p-Erk changes. The results indicated that p-Erk expression increased significantly in the TGF-β1 24 h and 48 h. This indicates that the activation of PDGFβ/Erk involved pericyte transdifferentiation. In the above experiment, we found that FUT8siRNA did not affect receptor protein expression after interfering pericytes; thus, we further studied whether inhibiting core fucosylation by FUT8siRNA could inhibit the expression of phosphorylated protein p-Erk in the PDGFβ/Erk pathway. Moreover, the Western blot and immunofluorescence results showed that compared with the TGF 24 h and 48 h group, p-Erk expression in the pericytes decreased significantly after being treated with FUT8siRNA. This indicates that FUT8siRNA could inhibit p-Erk expression during TGF-β1-induced pericyte transdifferentiation.

FUT8siRNA inhibits the transformation of TGF-β1-induced pericytes into myofibroblasts

The existence of myofibroblasts may cause α-SMA expression to increase. To determine whether myofibroblast transdifferentiation occurred and the effect of FUT8siRNA on α-SMA expression, we conducted western blot and immunofluorescence and found that stimulating the pericytes, the α-SMA protein expression level increased and peaked after 48 h. After incubating the pericytes for 24 h by applying 20 nM FUT8siRNA, then stimulating the pericytes with TGF-β1, the level of α-SMA protein expression obviously decreased. This indicates that after stimulating the pericyte for 48 h with TGF-β1, the pericytes obtained myofibroblast markers and FUT8siRNA could inhibit the transformation of pericytes into myofibroblasts.

Modification changes of core fucosylation during pericyte transdifferentiation

The above experimental results have shown that CF plays an important role in pericyte transdifferentiation; however, the changes in CF before and after pericyte transformation remain unclear. We adopted immunofluorescence and immunoprecipitation methods to detect the changes in FUT8, PDGFβR and α-SMA before and after pericyte transformation. The results revealed that the protein expression level of FUT8 was consistent with that of PDGFβR and α-SMA, increased gradually in TGF-β1 24 h group, reached the peak value in TGF-β1 48 h group; In addition, after applying FUT8siRNA before stimulating the pericytes with TGF-β1, the protein expression levels of FUT8 and α-SMA were obviously decreased. This indicates that FUT8, PDGFβR and α-SMA are important during the transformation of TGF-β1-induced pericytes into myofibroblasts. Moreover, FUT8siRNA could inhibit the transformation of pericytes into myofibroblasts after silencing CF expression.

Discussion

The key factor involved in the development of pulmonary fibrosis is the aggregation and activation of myofibroblasts [19]. Growing evidence supports pericytes as the major source of myofibroblasts [8, 9]. Currently, the specific mechanism of the transformation of pericytes into myofibroblasts remains unclear and it was reported in relevant literature that pericyte transdifferentiation is an activating process involving PDGFβ/Erk and TGF-β/Smad2/3 multi-signal pathways [20]. Moreover, PDGFβ and TGF-β chemokine were also found to be critical factors for the development of organ fibrosis [21, 22]. Therefore, a common targeting point for regulating the above signal pathways is an effective method of blocking pericyte transdifferentiation and thereby treats pulmonary fibrosis.

Glycosylation is one of the most important post-translational modifications of protein that generally occurs in an extracellular environment [15, 23]. In particular, N-linked glycosylation is involved in the pathological process of multiple diseases, plays an important role in protein folding, transportation, and localization [24, 25]. Furthermore, it participates in receptor activation, signal transduction, and other important biological processes [26]. Relevant literature reports and a previous study by our research group have demonstrated that the TGF-βR protein N-oligosaccharide was modified by CF [15, 16, 27]. Moreover, it further played a decisive role in the development of pulmonary fibrosis; therefore, we concluded that during pericyte transdifferentiation, PDGFβR and TGF-βR were also modified by CF. Currently, there have been no studies that have investigated the relationship between CF and pericyte activation in pneumonopathy. This study is the first to investigate CF modifications and the mechanism of pericyte transdifferentiation in the lung.

Currently, the literatures involving the in vitro study of lung pericytes are extremely rare since pericytes do not have specific markers, such that the in vitro isolation and cultivation of these cells are difficult [17]. The relevant literature reports that pericytes are a type of unstably expressing cells and there are a variety of antibodies used to analyze the expression of molecular markers on pericyte during different stages [28]. Pericytes have been found to express platelet-derived growth factor receptor-β (PDGFR-β), desmin, CD73, NG-2 and others [28]. The expression level of these labeled molecules changes in accordance with pericyte development, pathological reaction, and culture in vitro. Therefore, we employed an immunofluorescence method

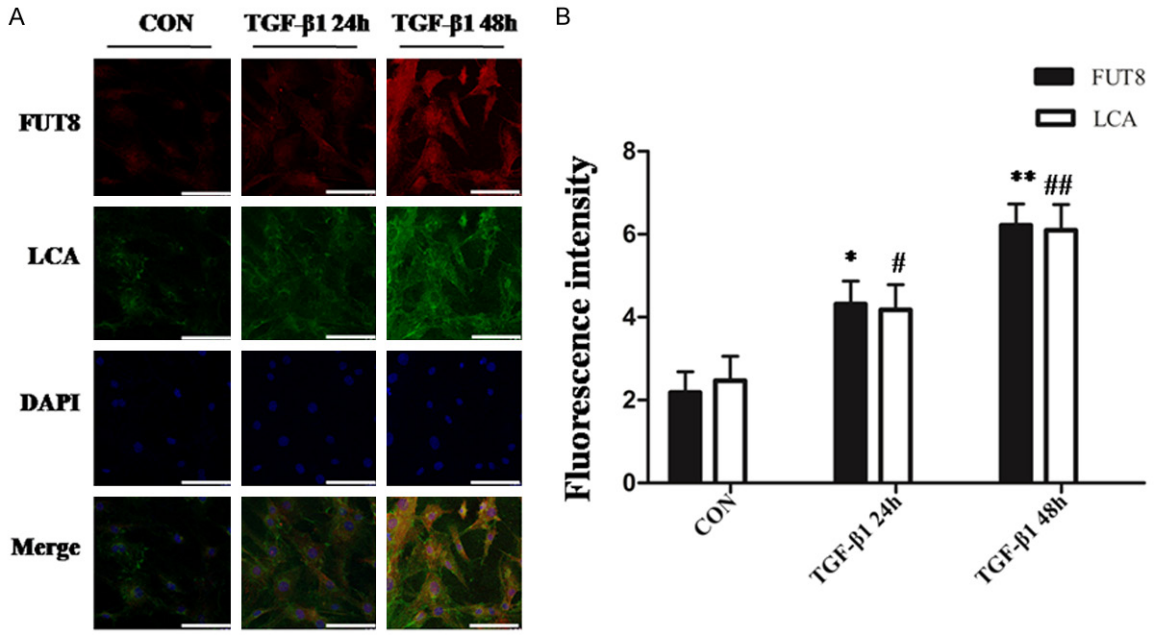


Figure 2. TGF-β1 induced the up-regulation of pericyte core fucosylation expression. A: The immunofluorescence method was applied to determine the expression of mouse lung pericyte core fucose links in each group with amplification times multiplied by 400. B: Quantitative analysis of the immunofluorescence results. All data was expressed as the mean ± standard deviation, each experiment was repeated three times. *P < 0.05, **P < 0.01 versus Control group, #P < 0.05, ##P < 0.01 versus TGF group.

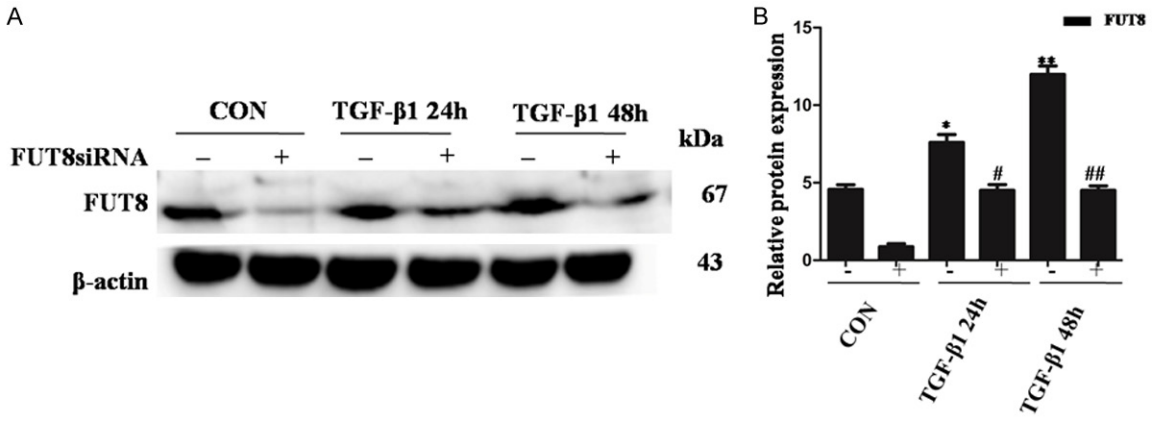


Figure 3. FUT8siRNA successfully silencing the FUT8 gene in pericytes. A: Apply immunoblotting to detect the efficiency of interference. B: Immunoblotting for a quantitative analysis of the interference efficiency results. All data is expressed as the mean ± standard deviation, each experiment was repeated three times; *P < 0.05, **P < 0.01 versus Control group, #P < 0.05, ##P < 0.01 versus TGF group.

to verify the expression of various markers after successfully extracting lung pericytes through the method of magnetic activated cell sorting (**Figure 1**).

After successfully extracting lung pericytes in mice, we first sought to determine if the surface of lung pericyte expresses core fucosylation. We utilized an immunofluorescence method to determine whether both the cytoplasm and cell surface exhibit the expression of the medium level of the core fucosylation (**Figure 2**). Next, we used 5 ng/mL TGF-β1 to stimulate mice lung pericytes for 24 h and 48 h respectivelyto establish a model of pericyte transdifferentiation and found that 24 h after TGF-β1 stimula-

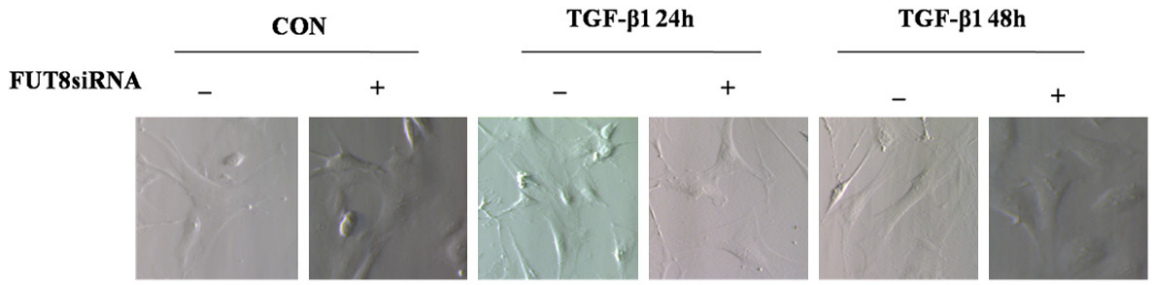


Figure 4. Observe the changes in the shape of TGF-β1-stimulated pericytes in each group. The test was repeated three times, with amplification times multiplied by 200.

tion, part of the cell shape began to change in TGF-β1 24 h group and shape of 80% of the pericytes changed from a multi-star shape to a long strip shape in TGF-β1 48 h (**Figure 4**). Immunofluorescence also revealed that the pericytes began to increase the expression of α-SMA while core fucosylation expression level also began to rise (**Figure 7**). Moreover, the immunofluorescence demonstrated that the pericytes exhibit a significant expression of α-SMA and core fucosylation (**Figure 8**). This indicates that TGF-β1 may be the factor causing the transformation of pericytes and suggests that the core fucosylation modification may also function in promoting the TGF-β1 induction of pericytes transdifferentiation.

To further study this mechanism, we inhibited the expression of pericytes surface core fucosylation. Since core fucosylation requires the α-1,6-fucosyltransferase (FUT8) catalytic protein N-oligonucleotide chitosan to complete the core fucosylation modification [29], we designed and synthesized a FUT8siRNA sequence and transfected pericytes. The experimental results demonstrate that the synthetic FUT8siRNA sequence successfully reduced the pericytes FUT8 protein expression (**Figure 3**). After we silenced FUT8 using gene silencing, 24 h and 48 h after TGF-β1 stimulation, it was found that over 80% of the pericytes did not change. In addition, the immunofluorescence test revealed that the expression of α-SMA as a marker of the transformation from to myofibroblasts was significantly lowered (**Figures 4**, **7**).

Since the relevant literature reports that PDGFβR and TGF-βRI are the key receptor proteins involved in the pericytes activation-related signaling pathways [30], we used the western blotting and precipitation methods to test whether these two proteins were modified by core fucosylation. Foreign Wang and our previous study conducted by our research team confirmed that TGF-βR is modified by core fucosylation [15, 16, 31]. However, our research team found that, in the percytes transdifferentiation process, PDGFβR is also modified by core fucosylation (**Figure 5**). We also found that during this process, TGF-β1 can induce pericytes transdifferentiation and the expression of PDGFβR was higher than in the control group. This indicates that the PDGF/Erk signaling pathway plays an important role in pericytes transdifferentiation. Interestingly, we found that FUT8siRNA can inhibit the core fucosylation modification level of PDGFR but does not influence the expression of the PDGFβR receptor itself (**Figure 5**). This means that the modification of the protein after translation is independent of the receptor protein expression.

The phosphorylation of Erk is an indicator of the activation of the PDGFβ/Erk signaling pathway [32]. To determine whether inhibiting core fucosylation can abolish periytes transdifferentiation by inhibiting PDGFβ/Erk signaling pathway, we assessed the expression of phosphorylated Erk. Our findings indicate that FUT8siRNA can inhibit the activation of the PDGFβ/Erk signaling pathway (**Figure 6**), indicating that core fucosylation modification is essential in the activation of the PDGFβ/Erk signaling pathway.

The experiment verified that the FUT8siRNA stops the lung pericytes from transforming into myofibroblasts by inhibiting the activation of the PDGFβ/Erk signaling pathway. To further clarify the characteristics involved in the CF

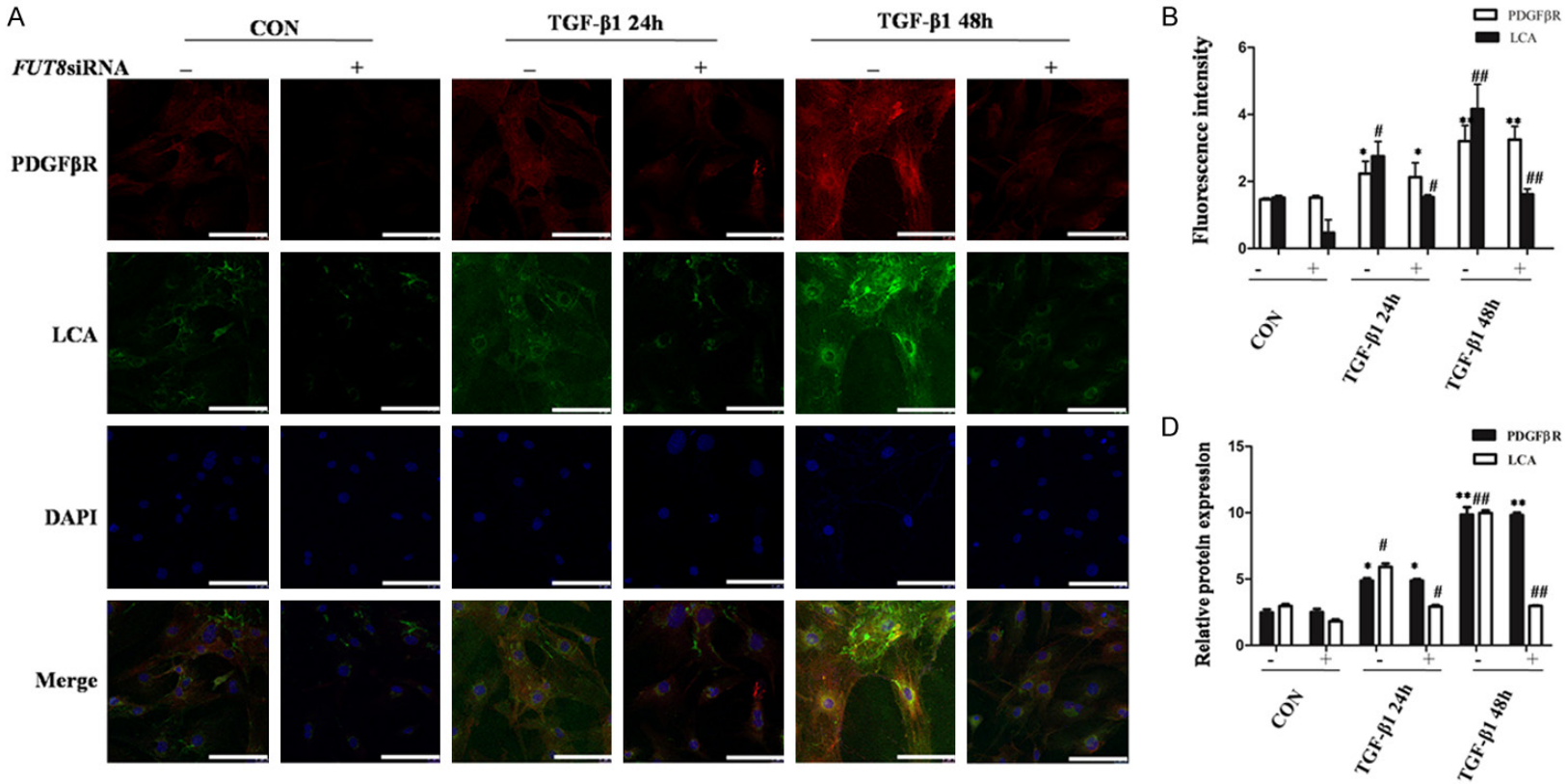


Figure 5. PDGFβR was modified by core fucosylation and FUT8siRNA blocked the core fucosylation modification of PDGFβR. A: An immunology double staining method to detect PDGFβR and core fucosylation expression in each group. B: Quantitative analysis of the immunofluorescence results. *P < 0.05, **P < 0.01 versus Control group, #P < 0.05, ##P < 0.01 versus TGF group. All data were expressed as the mean ± standard deviation, each experiment was repeated three times, with amplification times multiplied by 400. C: The immunoprecipitation and lectin blotting methods were applied to detect PDGFβR and core fucosylation expression in each group. D: An immunoprecipitation and lectin blotting method were applied for a quantitative analysis on PDGFβR protein and core fucosylation expression in each group. All data were expressed as the mean ± standard deviation and each experiment was repeated three times. *P < 0.05, **P < 0.01 versus Control group, #P < 0.05, ##P < 0.01 versus TGF group.

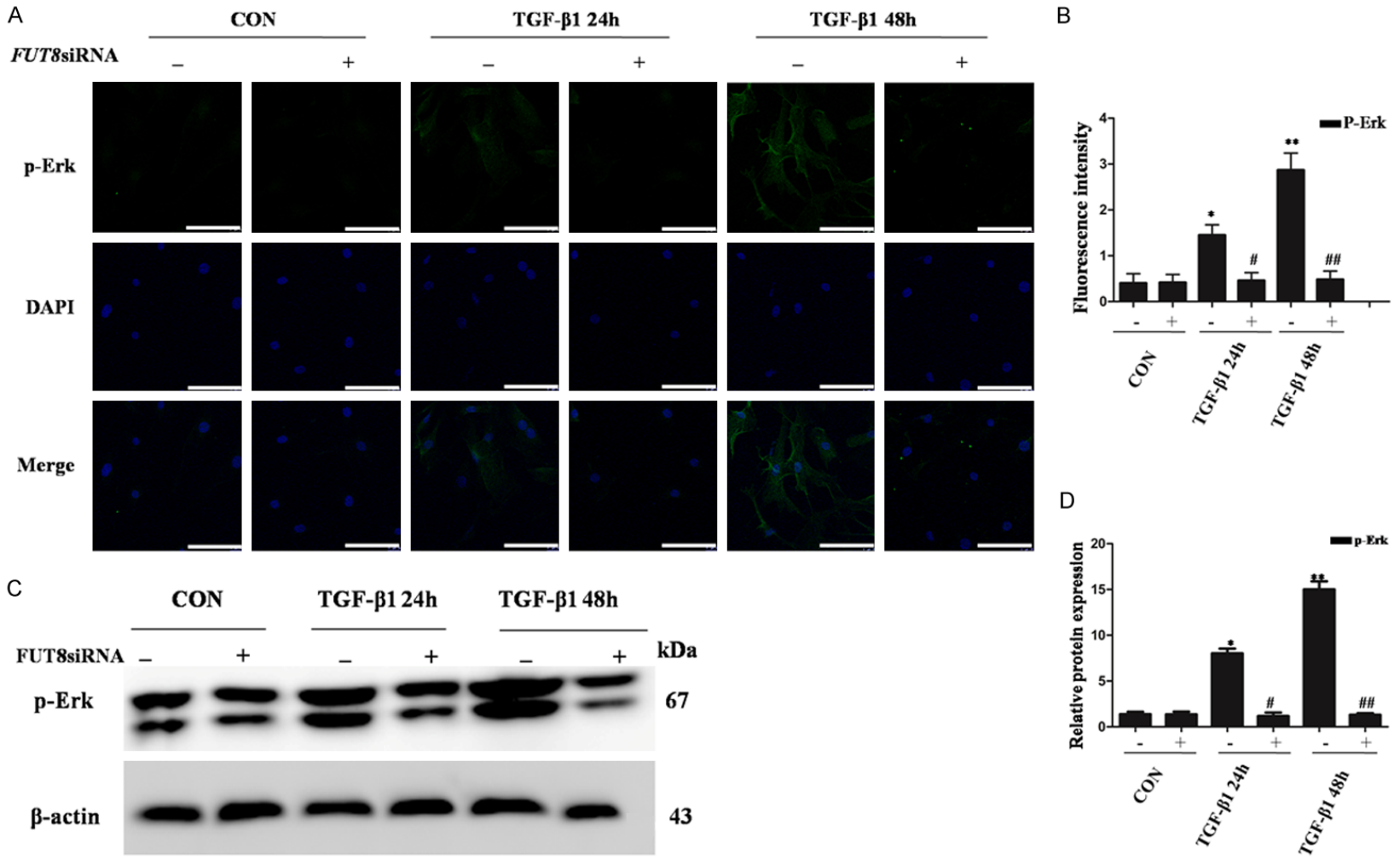


Figure 6. During the TGF-β1 induced pericyte transformation, p-Erk protein expression was up-regulated and FUT8siRNA blocked p-Erk protein expression. A: Immunofluorescence was used to detect changes in p-Erk protein expression levels in each group. B: Quantitative analysis of the immunofluorescence results. All data was expressed as the mean ± standard deviation, each experiment was repeated three times. *P < 0.05, **P < 0.01 versus Control group, #P < 0.05, ##P < 0.01 versus TGF group. C: Immunoblotting was used to detect the changes in the level of p-Erk protein expression in each group. D: Immunoblotting was used for a quantitative analysis on p-Erk protein expression in each group. All data were expressed as the mean ± standard deviation. *P < 0.05, **P < 0.01 versus Control group, #P < 0.05, ##P < 0.01 versus TGF group.

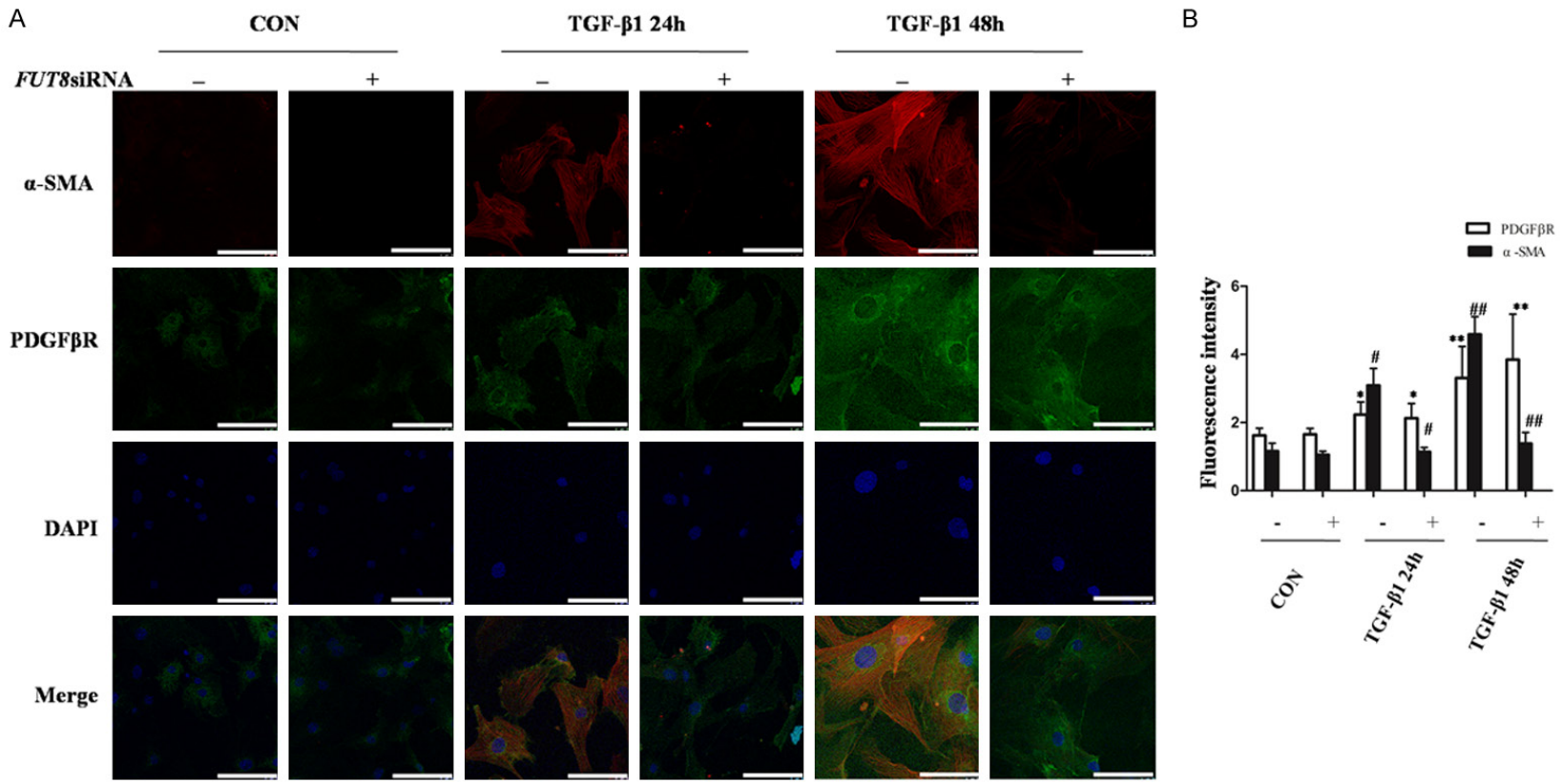
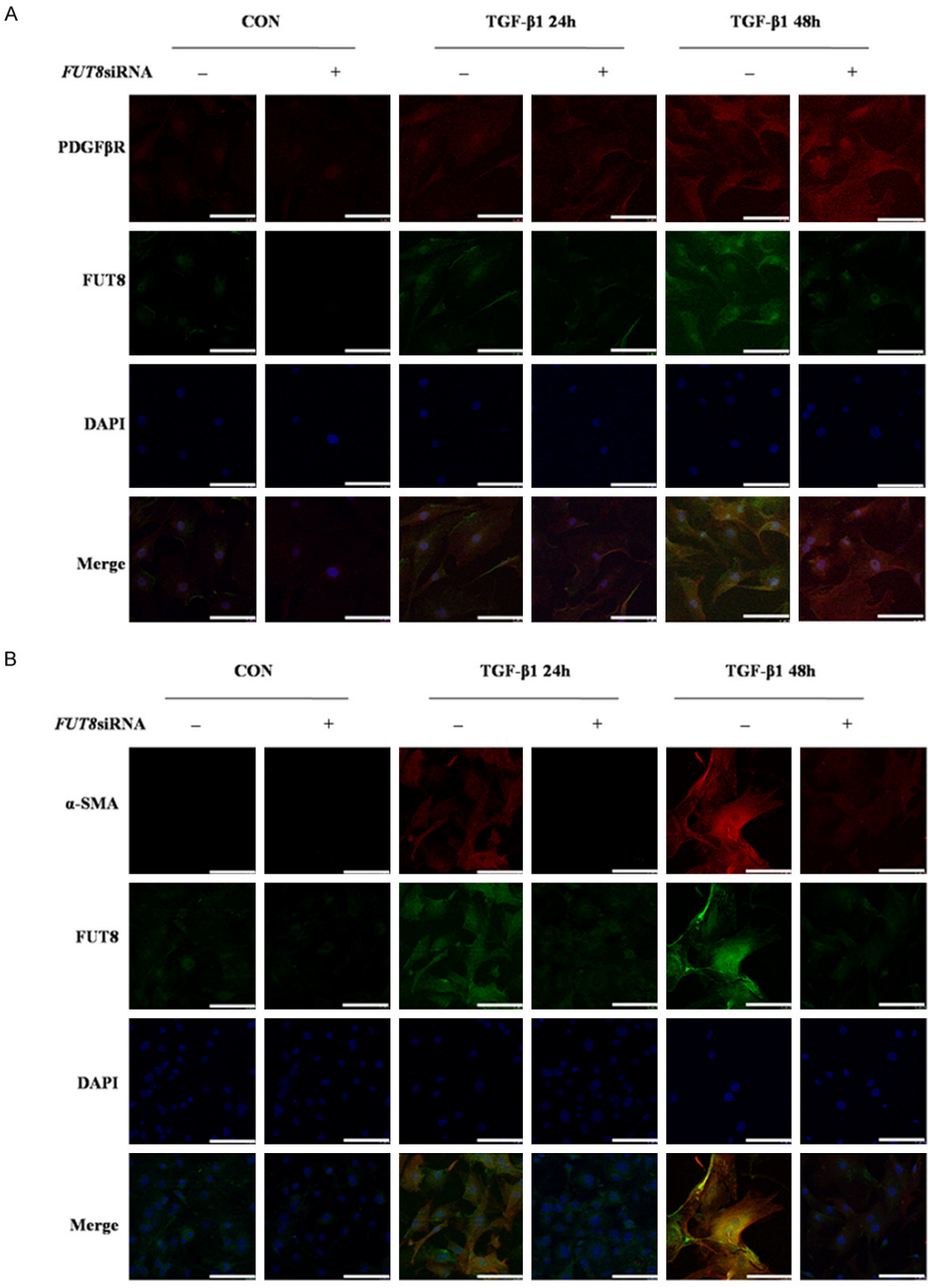


Figure 7. Effect of FUT8siRNA on TGF-β1-induced pericyte transformation. A: An immunofluorescence double staining method was used to detect PDGFβR and α-SMA protein expression in each group. The experiment was repeated three times, with the amplification times multiplied by 400. B: Quantitative analysis of the immunofluorescence results. All data were expressed with the mean ± standard deviation. *P < 0.05, **P < 0.01 versus Control group, #P < 0.05, ##P < 0.01 versus TGF group.


A
CON
TGF-β1 24h
TGF-β1 48h
FUT8siRNA – + – + – +
PDGFβR
FUT8
DAPI
Merge
B
CON
TGF-β1 24h
TGF-β1 48h
FUT8siRNA – + – + – +
α-SMA
FUT8
DAPI
Merge

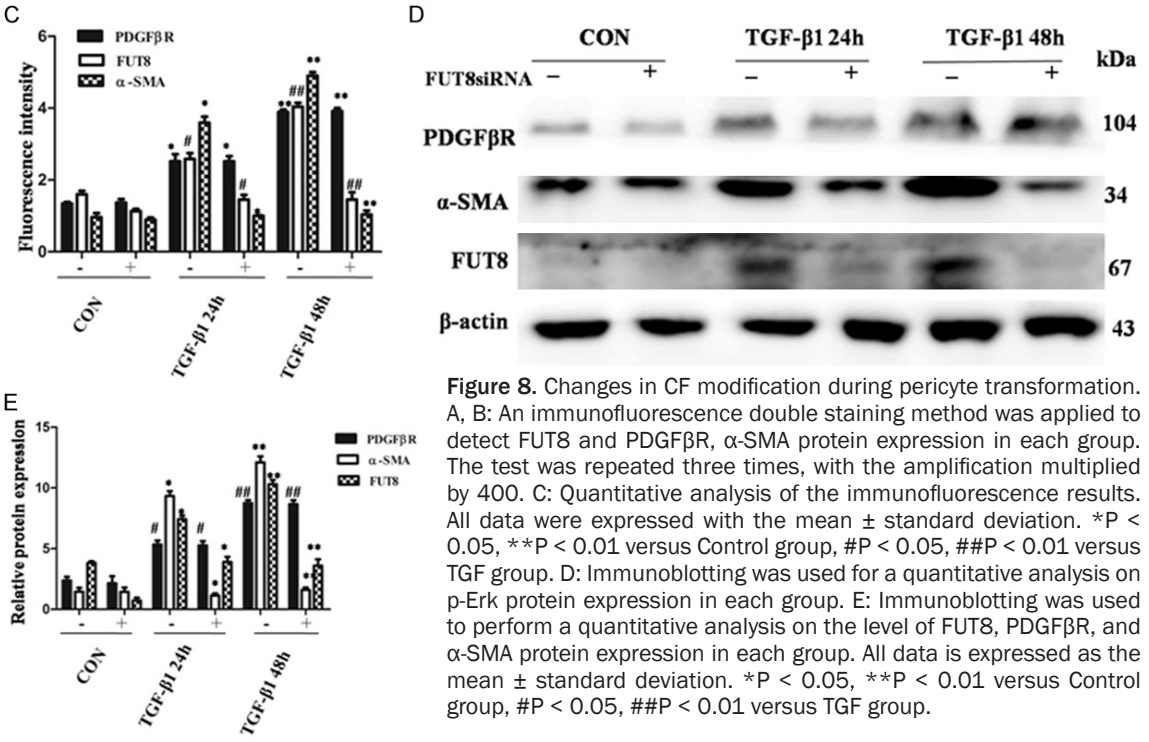


Figure 8. Changes in CF modification during pericyte transformation. A, B: An immunofluorescence double staining method was applied to detect FUT8 and PDGFβR, α-SMA protein expression in each group. The test was repeated three times, with the amplification multiplied by 400. C: Quantitative analysis of the immunofluorescence results. All data were expressed with the mean ± standard deviation. *P < 0.05, **P < 0.01 versus Control group, #P < 0.05, ##P < 0.01 versus TGF group. D: Immunoblotting was used for a quantitative analysis on p-Erk protein expression in each group. E: Immunoblotting was used to perform a quantitative analysis on the level of FUT8, PDGFβR, and α-SMA protein expression in each group. All data is expressed as the mean ± standard deviation. *P < 0.05, **P < 0.01 versus Control group, #P < 0.05, ##P < 0.01 versus TGF group.

changes that occur before and after pericytes transformation, this experiment involved change of PDGFβR as well as α-SMA and FUT8 before and after transformation. The results revealed that α-SMA exhibited minimal expression before the TGF-β1-induced transformation, and that PDGFβR and FUT8 are moderately expressed. After 24 and 48 h of TGF-β1 stimulation of the pericytes, the above index gradually increased and peaked at 48 h; however, FUT8siRNA cannot inhibit the increase in PDGFβR following stimulation with TGF-β1. Rather, it can inhibit the high expression of α-SMA after stimulation with TGF-β1 (**Figure 8**). Even during the process of pericytes transdifferentiation in which a high level of PDGFβR expression exists, if the PDGFβR was not modified by core fucosylation, the pericytes could not transform into myofibroblasts. Therefore, the change in CF may be an important mechanism involved in the pericytes transformation process.

TGF-βR and PDGFβR are also important proteins causing fibrosis [33-36]; thus, we speculated that the core fucosylation modification could regulate pericytes transdifferentiation and plays an important role in the process of pulmonary fibrosis. Our study shows lung pericytes transdifferentiation mechanisms through core fucosylation modifications for the first time, which provides a novel perspective for studying the molecular mechanisms of the pericytes that participate in pulmonary fibrosis, and indicates that the control of core fucosylation may be a potential target for the treatment of pulmonary fibrosis.

Acknowledgements

This work was supported by National Natural Science Foundation of China (NSFC) (No. 81273924 and 8153000127).

Disclosure of conflict of interest

None.

Address correspondence to: Taihua Wu, Department of Respiratory Medicine, The First Affiliated Hospital of Dalian Medical University, 222# Zhongshan Road, Dalian 116011, Liaoning, P.R. China. Tel: +86-411-83635963; Fax: +86-411-83635963; E-mail: wutaihuadoc@126.com; Hongli Lin, Department of Nephrology, The First Affiliated Hospital of Dalian Medical University, 222# Zhongshan Road, Dalian 116011, Liaoning, P.R. China. Tel: +86-411-83635963; Fax: +86-411-83635963; E-mail: linhonglidoc@126.com

References

[1] Wakamatsu K, Nagata N, Kumazoe H, Oda K, Ishimoto H, Yoshimi M, Takata S, Hamada M, Koreeda Y, Takakura K, Ishizu M, Hara M, Ise S, Izumi M, Akasaki T, Maki S, Kawabata M, Mukae H, Kawasaki M. Prognostic value of serial serum KL-6 measurements in patients with idiopathic pulmonary fibrosis. Respir Investig 2017; 55: 16-23.

[2] Li LC, Kan LD. Traditional Chinese medicine for pulmonary fibrosis therapy: progress and future. J Ethnopharmacol 2017; 198: 45-63.

[3] Hofmann K, Fiedler S, Vierkotten S, Weber J, Klee S, Jia J, Zwickenpflug W, Flockerzi V, Storch U, Yildirim AÖ, Gudermann T, Königshoff M, Dietrich A. Classical transient receptor potential 6 (TRPC6) channels support myofibroblast differentiation and development of experimental pulmonary fibrosis. Biochim Biophys Acta 2017; 1863: 560-568.

[4] Bindu S, Pillai VB, Kanwal A, Samant S, Mutlu GM, Verdin E, Dulin N, Gupta MP. SIRT3 blocks myofibroblast differentiation and pulmonary fibrosis by preventing mitochondrial DNA damage. Am J Physiol Lung Cell Mol Physiol 2017; 312: L68-L78.

[5] Wang C, Gu S, Cao H, Li Z, Xiang Z, Hu K, Han X. miR-877-3p targets Smad7 and is associated with myofibroblast differentiation and bleomycin-induced lung fibrosis. Sci Rep 2016; 6: 30122.

[6] Xie T, Liang J, Liu N, Huan C, Zhang Y, Liu W, Kumar M, Xiao R, D'Armiento J, Metzger D, Chambon P, Papaioannou VE, Stripp BR, Jiang D, Noble PW. Transcription factor TBX4 regulates myofibroblast accumulation and lung fibrosis. J Clin Invest 2016; 126: 3063-79.

[7] Zhao H, Bian H, Bu X, Zhang S, Zhang P, Yu J, Lai X, Li D, Zhu C, Yao L, Su J. Targeting of discoidin domain receptor 2 (DDR2) prevents myofibroblast activation and neovessel formation during pulmonary fibrosis. Mol Ther 2016; 24: 1734-1744.

[8] Rock JR, Barkauskas CE, Cronce MJ, Xue Y, Harris JR, Liang J, Noble PW, Hogan BL. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. Proc Natl Acad Sci U S A 2011; 108: E1475-83.

[9] Hung C, Linn G, Chow YH, Kobayashi A, Mittelsteadt K, Altemeier WA, Gharib SA, Schnapp LM, Duffield JS. Role of lung pericytes and resident fibroblasts in the pathogenesis of pulmonary fibrosis. Am J Respir Crit Care Med 2013; 188: 820-30.

[10] Yuan K, Orcholski ME, Huang NF, de Jesus Perez VA. In vivo study of human endothelial-pericyte interaction using the matrix gel plug assay in mouse. J Vis Exp 2016.

[11] Stefanska A, Eng D, Kaverina N, Duffield JS, Pippin JW, Rabinovitch P, Shankland SJ. Interstitial pericytes decrease in aged mouse kidneys. Aging (Albany NY) 2015; 7: 370-82.

[12] Pozdzik AA, Giordano L, Li G, Antoine MH, Quellard N, Godet J, De Prez E, Husson C, Declèves AE, Arlt VM, Goujon JM, Brochériou-Spelle I, Ledbetter SR, Caron N, Nortier JL. Blocking TGF-β signaling pathway preserves mitochondrial proteostasis and reduces early activation of PDGFRβ+ pericytes in aristolochic acid induced acute kidney injury in wistar male rats. PLoS One 2016; 11: e0157288.

[13] Takamatsu S, Shimomura M, Kamada Y, Maeda H, Sobajima T, Hikita H, Iijima M, Okamoto Y, Misaki R, Fujiyama K, Nagamori S, Kanai Y, Takehara T, Ueda K, Kuroda S, Miyoshi E. Core-fucosylation plays a pivotal role in hepatitis B pseudo virus infection: a possible implication for HBV glycotherapy. Glycobiology 2016; 26: 1180-1189.

[14] Vainauskas S, Duke RM, McFarland J, McClung C, Ruse C, Taron CH. Profiling of core fucosylated N-glycans using a novel bacterial lectin that specifically recognizes α1,6 fucosylated chitobiose. Sci Rep 2016; 6: 34195.

[15] Shen N, Lin H, Wu T, Wang D, Wang W, Xie H, Zhang J, Feng Z. Inhibition of TGF-β1-receptor posttranslational core fucosylation attenuates rat renal interstitial fibrosis. Kidney Int 2013; 84: 64-77.

[16] Lin H, Wang D, Wu T, Dong C, Shen N, Sun Y, Sun Y, Xie H, Wang N, Shan L. Blocking core fucosylation of TGF-β1 receptors downregulates their functions and attenuates the epithelial-mesenchymal transition of renal tubular cells. Am J Physiol Renal Physiol 2011; 300: F1017-25.

[17] Meguro S, Akamatsu T, Matsushima S, Kosugi I, Kawasaki H, Arai Y, Baba S, Tsuchida T, Shido Y, Suda T, Iwashita T. Phenotypic characterization of perivascular myoid cell neoplasms, using myosin 1B, a newly identified human pericyte marker. Hum Pathol 2017; 11: S0046-8177.

[18] de Souza LE, Malta TM, Kashima Haddad S, Covas DT. Mesenchymal stem cells and pericytes: to what extent are they related? Stem Cells Dev 2016; 25: 1843-1852.

[19] Hofmann K, Fiedler S, Vierkotten S, Weber J, Klee S, Jia J, Zwickenpflug W, Flockerzi V, Storch U, Yildirim AÖ, Gudermann T, Königshoff M, Dietrich A. Classical transient receptor potential 6 (TRPC6) channels support myofibroblast differentiation and development of experimental pulmonary fibrosis. Biochim Biophys Acta 2017; 1863: 560-568.

[20] Ferland-McCollough D, Slater S, Richard J, Reni C, Mangialardi G. Pericytes, an overlooked

player in vascular pathobiology. Pharmacol Ther 2017; 171: 30-42.
[21] Dadrich M, Nicolay NH, Flechsig P, Bickelhaupt S, Hoeltgen L, Roeder F, Hauser K, Tietz A, Jenne J, Lopez R, Roehrich M, Wirkner U, Lahn M, Huber PE. Combined inhibition of TGFβ and PDGF signaling attenuates radiation-induced pulmonary fibrosis. Oncoimmunology 2015; 5: e1123366.
[22] Deng X, Jin K, Li Y, Gu W, Liu M, Zhou L. Platelet-derived growth factor and transforming growth factor β1 regulate ards-associated lung fibrosis through distinct signaling pathways. Cell Physiol Biochem 2015; 36: 937-46.
[23] Cuccui J, Terra VS, Bossé JT, Naegeli A, Abouelhadid S, Li Y, Lin CW, Vohra P, Tucker AW, Rycroft AN, Maskell DJ, Aebi M, Langford PR, Wren BW; BRaDP1T Consortium. The N-linking glycosylation system from Actinobacillus pleuropneumoniae is required for adhesion and has potential use in glycoengineering. Open Biol 2017; 7.
[24] Beriault DR, Dang VT, Zhong LH, Petlura CI, McAlpine CS, Shi Y, Werstuck GH. Glucosamine induces ER stress by disrupting lipid-linked oligosaccharide biosynthesis and N-linkedprotein glycosylation. Am J Physiol Endocrinol Metab 2017; 312: E48-E57.
[25] Gusakov AV, Dotsenko AS, Rozhkova AM, Sinitsyn AP. N-linked glycans are an important component of the processive machinery of cellobiohydrolases. Biochimie 2017; 132: 102-108.
[26] Matoba K, Mihara E, Tamura-Kawakami K, Miyazaki N, Maeda S, Hirai H, Thompson S, Iwasaki K, Takagi J. Conformational freedom of the LRP6 ectodomain is regulated by N-glycosylation and the binding of the Wnt antagonist Dkk1. Cell Rep 2017; 18: 32-40.
[27] Gu W, Fukuda T, Isaji T, Hashimoto H, Wang Y, Gu J. α1,6-Fucosylation regulates neurite formation via the activin/phospho-Smad2 pathway in PC12 cells: the implicated dual effects of Fut8 for TGF-β/activin-mediated signaling. FASEB J 2013; 27: 3947-58.
[28] Navarro R, Compte M, Álvarez-Vallina L, Sanz L. Immune regulation by pericytes: modulating innate and adaptive immunity. Front Immunol 2016; 7: 480.
[29] Yang Q, Wang LX. Mammalian α-1,6-fucosyltransferase (FUT8) is the sole enzyme responsible for the N-acetylglucosaminyltransferase I-independent core fucosylation of high-mannose N-glycans. J Biol Chem 2016; 291: 11064-71.
[30] Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. Circ Res 2005; 97: 512-23.
[31] Wang X, Gu J, Miyoshi E, Honke K, Taniguchi N. Phenotype changes of Fut8 knockout mouse: core fucosylation is crucial for the function of growth factor receptor(s). Methods Enzymol 2006; 417: 11-22.
[32] Guo S, Yu L, Cheng Y, Li C, Zhang J, An J, Wang H, Yan B, Zhan T, Cao Y, Zheng H, Li Z. PDGFRβ triggered by bFGF promotes the proliferation and migration of endothelial progenitor cells via p-ERK signalling. Cell Biol Int 2012; 36: 945-50.
[33] Walraven M, Akershoek JJ, Beelen RH, Ulrich MM. In vitro cultured fetal fibroblasts have myofibroblast-associated characteristics and produce a fibrotic-like environment upon stimulation with TGF-β1: is there a thin line between fetal scarless healing and fibrosis? Arch Dermatol Res 2017; 309: 111-121.
[34] Mueller AA, van Velthoven CT, Fukumoto KD, Cheung TH, Rando TA. Intronic polyadenylation of PDGFRα in resident stem cells attenuates muscle fibrosis. Nature 2016; 540: 276-279.
[35] Tang H, He H, Ji H, Gao L, Mao J, Liu J, Lin H, Wu T. Tanshinone IIA ameliorates bleomycin-induced pulmonary fibrosis and inhibits transforming growth factor-beta-β-dependent epithelial to mesenchymal transition. J Surg Res 2015; 197: 167-75.
[36] He H, Tang H, Gao L, Wu Y, Feng Z, Lin H, Wu T. Tanshinone IIA attenuates bleomycin-induced pulmonary fibrosis in rats. Mol Med Rep 2015; 11: 4190-6.