1α,25-Dihydroxyvitamin D₃ prevents the differentiation of human lung fibroblasts via microRNA-27b targeting the vitamin D receptor

FEI LI¹,², AIZHEN ZHANG², YIWEI SHI³, YUEHONG MA⁴ and YONGCHENG DU¹,²

¹Shanxi Medical University, Taiyuan, Shanxi 030001; ²Department of Respiratory Diseases, Shanxi Provincial People's Hospital, Taiyuan, Shanxi 030012; ³Department of Respiratory Diseases, The First Hospital of Shanxi Medical University, Taiyuan, Shanxi 030001; ⁴Central Laboratory, Shanxi Provincial People's Hospital, Taiyuan, Shanxi 030012, P.R. China

Received January 28, 2015; Accepted August 14, 2015

DOI: 10.3892/ijmm.2015.2318

Abstract. Pulmonary fibroblasts have key roles in the formation and maintenance of lung structure and function, and are involved in tissue repair and remodeling. Transforming growth factor-β1 (TGF-β1) induces differentiation of fibroblasts into myofibroblasts, the key effector cells in fibrotic states, which are characterized by the expression of α-smooth muscle actin (α-SMA) markers. 1α,25-Dihydroxyvitamin D₃ [1,25(OH)₂D₃] has been implicated in regulating TGF-β signaling. In addition, there is presently only limited information regarding microRNA (miRNA) regulation of lung fibroblast differentiation. To determine the role of 1,25(OH)₂D₃ in regulating the differentiation of fibroblasts induced by TGF-β1 and the functional importance of miR-27b, cell culture systems, cell transfection and the 3' untranslated region (3'UTR) luciferase assay were employed. 1,25(OH)₂D₃ inhibited differentiation and downregulated miR-27b expression in human lung fibroblasts induced by TGF-β1. In addition, human lung fibroblasts were transfected with miR-27b mimic or miR-27b inhibitor, and demonstrated that the overexpression of miR-27b decreased the VDR protein expression and increased the expression of α-SMA, while reducing levels of miR-27b had opposing effects. Finally, the luciferase reporter assays were performed to confirm that miR-27b directly targeted VDR 3'UTR. Taken together, these results suggest that 1,25(OH)₂D₃ inhibits lung fibroblast differentiation induced by TGF-β1 via miR-27b targeting VDR 3'UTR, which may be used as a novel treatment strategy in differentiation pathways.

Introduction

1α,25-Dihydroxyvitamin D₃ [1,25(OH)₂D₃], the active form of vitamin D, has long been known for its effects on bone mineralization and calcium homeostasis. However, its physiological importance outside of bone health and calcium homeostasis effects has received increasing attention and it has been recognized as a key regulator of cell proliferation, differentiation, apoptosis and immunomodulation (1-4). In this regard, several studies have identified that vitamin D may have a role in multiple chronic lung diseases, such as asthma, chronic obstructive pulmonary disease (COPD), respiratory infections, lung cancer, interstitial lung disease, cystic fibrosis and pulmonary arterial hypertension (PAH) (5-12).

Vitamin D3 is a steroid prehormone that requires two hydroxylation steps in the liver and kidney to generate the biological active form, 1,25(OH)₂D₃. The biological effects of 1,25(OH)₂D₃ are mediated through the vitamin D receptors (VDRs), which belong to the superfamily of steroid/thyroid nuclear hormone receptors and are expressed widely in the body, including the lungs and cells of the immune system (8). Following ligand binding, the VDR forms a heterodimer with retinoid X receptor (RXR) and binds to the vitamin D responsive element in the regulatory region of the target genes to activate or repress their transcription (13,14).

Pulmonary fibroblasts have key roles in the formation and maintenance of lung structure and function, and are involved in tissue repair and remodeling, which is a key feature of a number of chronic lung diseases such as asthma, COPD, pulmonary fibrosis and PAH (15-20). Transforming growth factor-β1 (TGF-β1) is secreted by numerous cell types and implicated in a wide range of cell functions, critically...
regulating cell proliferation, growth, differentiation, apoptosis, cell movement, and extracellular matrix secretion and deposition (21-24). Additionally, TGF-β1 is a crucial regulator of fibroblast phenotype and function. TGF-β1-stimulated fibroblasts undergo phenotypic transition and differentiate into myofibroblasts, the key effector cells in fibrotic states, which are characterized by the expression of α-smooth muscle actin (α-SMA) fibers, contributing to the progression of pulmonary fibrogenesis (25,26). Therefore, inhibition of fibroblast proliferation and differentiation may prove to be a common and effective approach to attenuate pulmonary fibrosis.

Previous studies demonstrated that 1,25(OH)₂D₃ was capable of inhibiting the TGF-β1-mediated tissue remodeling responses in cultured lung fibroblasts and blocking myofibroblastic transformation of fibroblasts by TGF-β1 (18,27). In addition, several studies showed cross-talk between the TGF-β1 and vitamin D signaling pathways. VDR binds to the MHI domain of Smad3, which belongs to a receptor of the TGF-β superfamily, thereby enhancing Smad3 ligand-induced transactivation (28-31). However, the molecular mechanisms are far from understood. MicroRNAs (miRNAs) have attracted increasing attention due to their significant roles in diverse biological processes, including developmental processes, cell proliferation, differentiation, apoptosis, stress responses, and cancer initiation and progression (32-37). miRNAs are short non-coding RNAs with wide gene regulatory activity at the post-transcriptional level, which forms RNA silencing complexes with several proteins to cause mRNA degradation or translation inhibition, or both processes (32,33). The relatively few studies of miRNAs in lung fibrogenesis include miR-155, Let-7 family, miR-29 family, miR-21, miR-30 family, miR-145 and miR-27b (38-42). The present study aimed to evaluate the ability of 1,25(OH)₂D₃ to inhibit the differentiation of lung fibroblasts and the participation of miR-27b in this process. The first aim of the study was to determine the role of 1,25(OH)₂D₃ in regulating the differentiation of human lung fibroblasts induced by TGF-β1. Furthermore, whether miR-27b may be relevant for the regulating effect of 1,25(OH)₂D₃ was also determined.

Materials and methods

Materials and reagents. The α-SMA (cat. no. sc-53015), VDR (cat. no. sc-13133) and β-actin antibodies (cat. no. sc-47778) were all purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Secondary antibodies for goat anti-mouse immunoglobulin (IgG) conjugated with horseradish peroxidase (HRP) and goat anti-mouse IgG labeled with Cy3 were sourced from Sigma-Aldrich (St. Louis, MI, USA) and R&D Systems (Minneapolis, MN, USA), respectively. Scrambles, miRNA mimics and inhibitors were from Ambion (Austin, TX, USA). α-SMA, VDR and β-actin primers were produced by BGI-Beijing (Beijing, China).

Cell culture. MRC5 human lung fibroblasts and 293A cells were obtained from the Cell Resource Center of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). These two types of cells were incubated in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/l of glucose supplemented (Gibco, Carlsbad, CA, USA), supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin and 10% fetal bovine serum (FBS; Gibco) at 37°C in a humidified atmosphere with 5% CO₂. MRC5 cells were cultured to ~80% confluence, serum-starved for 24 h, and were treated for 48 h in 2% FBS medium with an ethanol vehicle or 1,25(OH)₂D₃ (100 nM) in the absence or presence of rhTGF-β1 (10 ng/ml).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. cDNA was generated from total RNA using PrimeScript RT Master mix (5X) (Takara, Dalian, China), according to the manufacturing instructions. To quantify mRNA expression, SYBR Premix Ex Taq (2X) (Takara) was used. The qPCR thermal cycling protocol was programmed in the CFX96™ Real-Time PCR Detection system (Bio-Rad, Hercules, CA, USA) and consisted of an initial denaturation step at 95°C for 30 sec, followed by 40 cycles of denaturation for 5 sec at 95°C, and annealing and extension for 30 sec at 60°C. A primer pair for the detection of human β-actin was used as the internal control. RT-qPCR primers were based on GenBank published sequences and were as follows: α-SMA sense, 5'-GGC GGT GCT GTC TCT CTA TG-3' and antisense, 5'-CCC ATC AGG CAA CTC GTA AC-3'; VDR sense, 5'-GGC CGG ACC AGA AGC CTT T-3' and antisense, 5'-ACT GGA ACG GTG AAG GTG AC-3'. Western blotting. Cells were plated in 60-mm dishes in 10% FBS-DMEM, cultured until nearly 80% confluence and serum-starved for 24 h, and subsequently the cells were treated as indicated. After 48 h culture, cells were washed twice with ice-cold phosphate-buffered saline (PBS) and were subsequently harvested with cell lysis buffer with protease inhibitors [20 mmol/l Tris-HCl (pH 7.5), 150 mmol/l NaCl, 1% Triton X-100, 1 mmol/l EDTA, 1 mmol/l EGTA, 20 mol/l NaPO₃O₂, 2 mol/l NaVO₃. 0.1% SDS, 10% glycerol, 2 µg/ml leupeptin and 1 mmol/l PMSF]. Upon centrifugation at 13,000 x g for 5 min at 4°C, the supernatants were collected and the total protein concentrations were determined by the bicinchoninic acid protein assay kit (Beyotime, Shanghai, China) following the manufacturer's instructions. SDS-PAGE (10%) gels were prepared and 10-20 µg/lane of cellular proteins was loaded. The resolved proteins were transferred to a polyvinyliden difluoride membrane and incubated with primary antibodies (α-SMA, 1:100 dilution; VDR, 1:50 dilution) following the manufacturer's instructions. Following incubation with HRP-conjugated secondary antibodies

Selectedaffinedmadediscussion
(1:1,000 dilution), the blotting bands were visualized with ECL chemiluminescent kit (Thermo Scientific, Rockford, IL, USA) and quantified with a ChemiDoc™ XRS+ Imaging System (Bio-Rad).

Immunoﬂuorescence. Fibroblasts growing on cover slides were ﬁxed in 4% paraformaldehyde for 1 h. Following being permeabilized with 0.2% Triton X-100 in Tris-buffered saline (TBS) for 30 min, the cells were blocked in TBS containing 2% bovine serum albumin for 1 h. Cells were subsequently incubated with anti-α-SMA antibody (1:50 dilution) at 4°C overnight. Cells were washed 3 times and were incubated at room temperature with Cy3-conjugated goat anti-mouse secondary antibody (1:50 dilution) for 1 h. Following this, cells were counterstained with 4',6-diamidino-2-phenylindole (Boster) for nuclear staining. Cells were then washed 3 times. Negative controls were carried out by omitting the primary antibody. Fluorescent images were captured with a laser scanning confocal microscope (Olympus FV1000; Olympus, Tokyo, Japan).

Cell transfection. Transfection was performed with scramble, miR-27b mimic (50 nM) or miR-27b inhibitor (100 nM) (Ambion). All the transfection experiments were conducted with Lipofectamine 2000 (Invitrogen), following the manufacturer's instructions. Cells in medium containing 10% FBS were seeded in each well and incubated at 37°C. The transfection mixture was incubated at room temperature for 10 min, and was added to a 6-well plate. After 24 h, cells were serum-starved for an additional 24 h, following which they were treated with vehicle control, 1,25(OH)2D3 or TGF-β1 for 48 h in 2% FBS medium. Cells were subsequently harvested and total RNA, miRNA or proteins were extracted and analyzed by RT-qPCR or western blot analysis.

3′ Untranslated region (3′UTR) luciferase assay. A synthetic oligonucleotide was inserted at the 3′UTR region of the luciferase reporter gene of the pMIR-target vector. The wild-type and mutant 3′UTR of the VDR genes were ampliﬁed by PCR and subcloned in the pMIR-target vectors. The constructs were co-transfected into 293A cells along with scramble (50 nM), miR-27b mimic (50 nM) or miR-27b inhibitor (100 nM) using Lipofectamine 2000, according to the manufacturer’s instructions. Luciferase activities were measured with the Dual Luciferase Reporter Assay system (Promega, Madison, WI, USA) 48 h after transfection.

Statistical analysis. Values are expressed as means ± standard error of the mean derived from at least three independent experiments. Comparison of two groups was made with an unpaired, two-tailed Student's t-test. Comparison of multiple groups was made with a one-way analysis of variance followed by Dunnett or Tukey test. P<0.05 was considered to indicate a statistically signiﬁcant difference. All the statistical analyses were performed and graphs were plotted using the GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA).

Results

1,25(OH)2D3 inhibits differentiation and upregulates VDR protein expression of human lung ﬁbroblasts induced by TGF-β1. Previous studies have documented that 1,25(OH)2D3 opposed the effect of TGF-β1 on mouse lung ﬁbroblasts differentiation, as demonstrated by its ability to inhibit TGF-β1-induced expression of α-SMA (27). Consequently, whether 1,25(OH)2D3 could inhibit α-SMA expression of human lung ﬁbroblasts induced by TGF-β1 was examined. Based on previous experiments, the optimum concentration of 1,25(OH)2D3 (100 nM) was chosen as the dose for the present experiments. Notably, RT-qPCR and western blot analysis revealed that TGF-β1 signiﬁcantly upregulated α-SMA expression at the mRNA and protein levels in MRC5 cells; however, 1,25(OH)2D3 markedly inhibited this effect (Fig. 1A and C). Confocal immunofluorescence analysis of MRC5 ﬁbroblasts also revealed that TGF-β1 increased levels of α-SMA, and the process was inhibited by 1,25(OH)2D3 (Fig. 1D).

Several studies showed cross-talk between the TGF-β and vitamin D signaling pathways (28-31) and TGF-β contributed to the decreased expression of VDR in dermal ﬁbroblasts of healthy volunteers (43). Furthermore, 1,25(OH)2D3 had a signiﬁcant effect in vivo on the TGF-β signaling pathway by altering levels of VDR and Smad3, and subsequently affecting the bioactive of TGF-β1 (44). Based on this evidence, we speculated that VDR may be a negative regulator of ﬁbroblast differentiation induced by TGF-β. TGF-β signiﬁcantly decreased the protein expression of VDR, and treatment of TGF-β-stimulated ﬁbroblasts with 1,25(OH)2D3 effectively upregulated the VDR protein level (Fig. 1C). However, the levels of the VDR transcripts did not change (Fig. 1B).

1,25(OH)2D3 downregulates TGF-β1-induced miR-27b expression in human lung ﬁbroblasts. As TGF-β1 is a critical cytokine in the pathogenesis of human lung ﬁbroblast differentiation, and a previous study reported that miR-27b was downregulated in the lungs of mice that were administered with bleomycin, a widely used animal model of lung ﬁbrosis (45), we hypothesized that miR-27b participates in this process and could be relevant for the inhibitory effect of 1,25(OH)2D3 on the differentiation of lung ﬁbroblasts induced by TGF-β1. Therefore, the levels of miR-27b were examined in MRC5 cells treated with the ethanol vehicle or 1,25(OH)2D3 in the absence or presence of TGF-β1. In the present study, miR-27b expression levels were signiﬁcantly higher in MRC5 cells induced by TGF-β1. However, treatment of TGF-β-stimulated ﬁbroblasts with 1,25(OH)2D3 effectively decreased miR-27b expression (Fig. 2). These data suggest that miR-27b may have a role in regulating the differentiation phenotype of the pulmonary ﬁbroblasts.

miR-27b regulates differentiation and VDR expression of human lung ﬁbroblasts. To determine whether miR-27b regulates the differentiation phenotype of the pulmonary ﬁbroblasts, human lung ﬁbroblasts were transfected with scramble, miR-27b mimic or miR-27b inhibitor and evaluated α-SMA levels in these cells. Overexpression of miR-27b markedly increased the baseline levels of the α-SMA transcripts and α-SMA proteins in lung ﬁbroblasts (Fig. 3A-a and c). The miR-27b inhibitor decreased the α-SMA levels in lung ﬁbroblasts (Fig. 3B-a and c). Given that TGF-β1 upregulated miR-27b in lung ﬁbroblasts, these data suggest that miR-27b may mediate TGF-β1-induced α-SMA expression. TGF-β1-treated
Let al: 1,25(OH)2D3 PREVENTS DIFFERENTIATION VIA miR‑27b TARGETING VDR

Lung fibroblasts were transfected with miR‑27b inhibitor and subsequently evaluated α-SMA levels in the cells. miR‑27b inhibitor attenuated TGF‑β‑induced α-SMA expression at the mRNA and protein levels in lung fibroblasts (Fig. 3C-a and c). These data indicate that TGF‑β‑mediated α-SMA expression requires, at least in part, the induction of miR‑27b. In addition, these initial experiments have demonstrated that 1,25(OH)2D3 reduced α-SMA expression and downregulated miR‑27b expression in human lung fibroblasts induced by TGF‑β1. When the miR‑27b mimic was transfected into the human lung fibroblasts, the reduced expression of α-SMA protein in 1,25(OH)2D3‑treated cells was attenuated by the miR‑27b mimic (Fig. 3D-a and c).

Given that VDR can be a negative regulator of fibroblast differentiation induced by TGF‑β, whether miR‑27b regulates VDR gene expression was further investigated. MRC5 cells were transfected with scramble, miR‑27b mimic or miR‑27b inhibitor, and VDR levels were evaluated in these cells. The miR‑27b mimic reduced VDR protein levels in lung
Figure 3. miR-27b regulates differentiation and vitamin D receptor (VDR) expression of human lung fibroblasts. (A) Human lung fibroblasts were transfected with 50 nM scramble, 50 nM miR-27b mimic (+) or 100 nM miR-27b mimic (++). At 48 h after transfection, levels of α-smooth muscle actin (α-SMA) and VDR were determined by (a and b) reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and (c) western blot analysis, normalized to β-actin expression. ***P<0.001 miR-27b mimic (+) vs. scramble. (B) Human lung fibroblasts were transfected with 100 nM scramble, 100 nM miR-27b inhibitor (+) or 200 nM miR-27b inhibitor (++), and 48 h post transfection, RNA or whole cell lysates were harvested. The expression levels of α-SMA and VDR were determined by (a and b) RT-qPCR and (c) western blot analysis, normalized to β-actin expression. **P<0.01 miR-27b inhibitor (+) vs. scramble. (C) Human lung fibroblasts were transfected with 100 nM control inhibitor (control inhib) or 100 nM miR-27b inhibitor (miR-27b inhib). At 24 h after transfection, cells were serum-starved for an additional 24 h, after which they were treated with transforming growth factor-β (TGF-β1) for 48 h. RNA or whole cell lysates were subsequently harvested. The expression levels of α-SMA and VDR were determined by (a and b) RT-qPCR and (c) western blot analysis, normalized to β-actin expression. ***P<0.001 TGF-β vs. control inhib; ***P<0.001 TGF-β + miR-27b inhib vs. TGF-β. (D) Human lung fibroblasts were transfected with 50 nM control mimic or 50 nM miR-27b mimic. At 24 h after transfection, cells were serum-starved for an additional 24 h, after which they were treated with 1α,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] (VD) for 48 h. RNAs or whole cell lysates were subsequently harvested. The expression levels of α-SMA and VDR were determined by (a and b) RT-qPCR and (c) western blot analysis, normalized to β-actin expression. *P<0.05 VD + miR-27b mimic vs. VD.
miR-27b directly targets vitamin D receptor (VDR) 3’ untranslated region (3’UTR). The miR-27b inhibitor increased the VDR protein levels in these cells (Fig. 3B-c). However, miR-27b had no effect on the levels of the VDR transcripts (Fig. 3A-b and B-b), suggesting that miR-27b may affect the VDR translation, but not the transcripts in lung fibroblasts. These initial experiments have demonstrated that TGF-β1 upregulates miR-27b and decreases VDR protein expression, but 1,25(OH)2D3 opposed the above effects of TGF-β1. The reduced expression of VDR protein in TGF-β1-treated cells was attenuated by the miR-27b inhibitor (Fig. 3C-c). Furthermore, the miR-27b mimic resulted in a decrease of VDR protein expression in lung fibroblasts treated with 1,25(OH)2D3 (Fig. 3D-c). Similarly, miR-27b had no effect on the levels of the VDR transcripts (Fig. 3C-b and D-b). Taken together, these data suggest that miR-27b promotes the differentiation of human lung fibroblasts by suppressing VDR protein expression.

Discussion

Pulmonary fibroblasts have important roles in lung tissue repair and remodeling. Fibroblasts often differentiate into
myofibroblasts that possess enhanced fibrotic, contractile and migratory activities. TGF-β1 stimulated the proliferation of lung fibroblasts and their differentiation, as highlighted by increased expression and organization of α-SMA, a marker of myofibroblasts and a primary contributor to the contractile force in myofibroblasts (46,47). New emerging studies regarding the roles of miRNAs in pulmonary fibrosis and in their regulation of TGF-β1 signaling are increasing (38,48-50). mir-27b has been studied in various cancer cells (51-55), however, mir-27b has not been well studied in lung fibroblasts. Previous studies suggested that mir-27b was identified as a pro-angiogenic miRNA (56) and modulated fibrotic responses (41). The aim of the present study was to investigate the role and mechanism of mir-27b regulating human lung fibroblasts differentiation induced by TGF-β1.

Firstly, 1,25(OH)2D3 inhibited the effect of TGF-β1 on human lung fibroblast differentiation, as demonstrated by its ability to inhibit TGF-β1-induced expression of α-SMA. These results are consistent with findings from a study of vitamin D inhibition of pro-fibrotic effects of TGF-β1 in mouse lung fibroblasts (27). The present study identified that mir-27b expression levels were significantly higher in MRC5 cells induced by TGF-β1, however, treatment of TGF-β1-stimulated fibroblasts with 1,25(OH)2D3 effectively decreased mir-27b expression. This suggests that mir-27b may have a role in regulating the differentiation phenotype of the pulmonary fibroblasts. By contrast, two studies observed that TGF-β treatment downregulated mir-27b expression (41,57), which may be caused by different types of cells, and additional investigations are required to demonstrate the function.

There is evidence showing that VDR is involved in pathological fibrogenesis. VDR belongs to the superfamily of steroid/thyroid nuclear hormone receptors. Following ligand binding, the VDR forms a heterodimer with RXR, or Smad3, a receptor of TGF-β/Smad signalling. Previous studies showed cross-talk between the TGF-β and vitamin D signaling pathways and VDR binds to the MH1 domain of Smad3, enhancing Smad3 ligand-induced transactivation (28-31). 1,25(OH)2D3 had a significant effect in vivo on the TGF-β signaling pathway by altering levels of VDR and Smad3, and subsequently affecting the bioactive of TGF-β (44). Additionally, a recent study identified VDR as a negative regulator of fibroblast activation that interfered with the pro-fibrotic effects of TGF-β (43). The present study further showed that reduction of the VDR protein was mediated by TGF-β1 in human lung fibroblasts, whereas 1,25(OH)2D3 effectively upregulated VDR protein expression, which was in accordance with the study of activation of VDR by paricalcitol reducing the stimulatory effects of TGF-β on skin fibroblasts (43).

In addition, the experiments of lung fibroblasts transfected with mir-27b mimic or mir-27b inhibitor and luciferase reporter assays reveal that mir-27b directly targets VDR 3′UTR and inhibits VDR gene expression to promote differentiation of human lung fibroblasts characterized by expression of α-SMA. The present in vitro study showed that the over-expression of mir-27b decreased VDR protein expression and increased expression of fibroblast differentiation marker, α-SMA, while reducing levels of mir-27b had opposing effects. Studies of fibrosis have not reached a consensus on the importance of mir-27b in disease pathology. An in vitro study suggested that mir-27b may be profibrotic in activated hepatic stellate cells (58). Additionally, cardiomyocyte overexpression of mir-27b induces cardiac hypertrophy in mice (57). These two studies were similar to the present results. By contrast, a recent study suggested that mir-27b overexpression markedly repressed fibrotic responses in pulmonary epithelial cells (41).

In conclusion, 1,25(OH)2D3 inhibits differentiation and downregulates mir-27b expression in human lung fibroblasts induced by TGF-β1. Furthermore, mir-27b overexpression decreased the expression of VDR protein and increased the expression of α-SMA, while reducing levels of mir-27b had opposing effects. Notably, mir-27b has abilities for targeting the 3′UTR of VDR and negatively regulating VDR protein expression, which effects differentiation of human lung fibroblasts. Thus, 1,25(OH)2D3 inhibits lung fibroblast differentiation induced by TGF-β1 via mir-27b targeting VDR 3′UTR, and this may be used as a novel treatment strategy in differentiation pathways.

References

1. Holick MF: Vitamin D deficiency. N Engl J Med 357: 266-281, 2007.
2. van Etten E, Stoffels K, Gysemans C, Mathieu C and Overbergh L: Regulation of vitamin D homeostasis: Implications for the immune system. Nutr Rev 66 (Suppl 2): S125-S134, 2008.
3. Haroon M and Fitzgerald O: Vitamin D and its emerging role in immunopathology. Clin Rheumatol 31: 199-202, 2012.
4. Messa P, Affieri C and Rastaldi MP: Recent insights into vitamin D and its receptor. J Nephrol 24 (Suppl 18): S30-S37, 2011.
5. Forli L, Bjortuft O and Boe J: Vitamin D status in relation to nutritional depletion and muscle function in patients with advanced pulmonary disease. Exp Lung Res 35: 524-538, 2009.
6. Black PN and Scragg R: Relationship between serum 25-hydroxyvitamin D and pulmonary function in the third national health and nutrition examination survey. Chest 128: 3792-3796, 2005.
7. Fosong RE and Zosky GR: Vitamin D deficiency and the lung: Disease initiator or disease modifier? Nutrients 5: 2880-2900, 2013.
8. Hansdottir S and Monick MM: Vitamin D effects on lung immunity and respiratory diseases. Vitam Horm 86: 217-237, 2011.
9. Herr C, Greulich T, Koczulla RA, Meyer S, Zakharkina T, Branscheidt M, Eschmann R and Bals R: The role of vitamin D in pulmonary disease: COPD, asthma, infection, and cancer. Respir Rev 12: 31, 2011.
10. Finklea JD, Grossmann RE and Tangpricha V: Vitamin D and chronic lung disease: A review of molecular mechanisms and clinical studies. Adv Nutr 2: 244-253, 2011.
11. Hughes DA and Norton R: Vitamin D and respiratory health. Clin Exp Immunol 158: 20-25, 2009.
12. Demir M, Uyan U, Keçecolu S and Demir C: The relationship between vitamin D deficiency and pulmonary hypertension. Prague Med Rep 114: 154-161, 2013.
13. Lips P: Vitamin D physiology. Prog Biophys Mol Biol 92: 4-8, 2006.
14. Haussler MR, Jurutka PW, Mizwicki M and Norman AW: Vitamin D receptor (VDR)-mediated actions of 1α,25(OH)2 vitamin D3: Genomic and non-genomic mechanisms. Best Pract Res Clin Endocrinol Metab 25: 543-559, 2011.
15. Dufﬁeld JS, Lupher M, Thannickal VJ and Wynn TA: Host responses in tissue repair and ﬁbrosis. Annu Rev Pathol 8: 241-276, 2013.
16. Hetzel M, Bachem M, Anders D, Trischler G and Faehling M: Different effects of growth factors on proliferation and matrix production of normal and ﬁbrotic human lung ﬁbroblasts. Lung 183: 225-237, 2005.
17. Coen M, Gabbiani G, Bochaton-Piallat ML and Chen YE: Vitamin/D-crosstalk: mediated apoptosis of lung fibroblast: remodeling: an underrefined player in arterial pathology. Arterioscler Thromb Vasc Biol 31: 2391-2396, 2011.
MicroRNA expression profiles classify human cancers. Open Rheumatol J 6: 130-139, 2012.

18. Sartore S, Chiavegato A, Faggini E, Franch R, Puato M, Auconi S and Pauletto P: Contribution of adventitial fibroblasts to neointima formation and vascular remodeling: From innocent bystander to participant. Circ Res 89: 1111-1121, 2001.

19. Liu X, Nelson A, Wang X, Farid M, Gunjan Y, Ikari J, Iwasawa S, Basma H, Feghali-Bostwick C and Rennard SI: Vitamin D modulates prostaglandin E2 synthesis and degradation in human lung fibroblasts. Am J Respir Cell Mol Biol 50: 40-50, 2014.

20. Stenmark KR, Frid MG and Yeager ME: Fibrocytocides: Potential new therapeutic targets for pulmonary hypertension? Eur Respir J 36: 1232-1235, 2010.

21. Kajdaniuk D, Marek B, Borgiel-Marek H and Kos-Kudla B: Transforming growth factor β1 (TGFβ1) in physiology and pathology. Endokrynol Pol 64: 384-396, 2013.

22. Ma L and Chung WK: The genetic basis of pulmonary arterial hypertension. Hum Genet 133: 471-479, 2014.

23. Biernacka A, Dobaczewski M and Frangiogiani NN: TGF-β signaling in fibrosis. Growth Factors 29: 196-202, 2011.

24. Suwanabol PA, Seedial SM, Zhang F, Shi X, Si Y, Liu B and Kent KC: TGF-β and Smad3 modulate PI3K/Akt signaling pathway in vascular smooth muscle cells. Am J Physiol Heart Circ Physiol 302: H2211-H2219, 2012.

25. Scotton CJ and Chambers RC: Molecular targets in pulmonary fibrosis: The myofibroblast in focus. Chest 132: 1311-1321, 2007.

26. Guo W, Shan B, Klingsberg RC, Qin X and Lasky AP: Abrogation of TGF-β1-induced fibroblast-myofibroblast differentiation by histone deacetylase inhibition. Am J Physiol Lung Cell Mol Physiol 297: L684-L687, 2009.

27. Ranezić AM, Tomic-Canic M, Welch T, Steimann A, Zügel U and Roman J: Vitamin D inhibition of pro-fibrotic effects of transforming growth factor betal in lung fibroblasts and epithelial cells. J Steroid Biochem Mol Biol 118: 142-150, 2010.

28. Ding N, Yu RT, Subramaniam N, Sherman MH, Wilson C, Rao R, Leblanc M, Coulter S, He M, Scott C, et al: A vitamin D receptor/SMAD genomic circuit gates hepatic fibrotic response. Cell 153: 601-613, 2013.

29. Yanagi Y, Suzawa M, Kawabata M, Miyazono K, Yanagisawa J and Kato S: Positive and negative modulation of vitamin D receptor function by transforming growth factor-beta signaling through smad proteins. J Biol Chem 274: 12971-12974, 1999.

30. Yanagisawa J, Yanagi Y, Masuhiro Y, Suzawa M, Watanabe M, Kashihwagi K, Torigaye T, Kawabata M, Miyazono K and Kato S: Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. Science 283: 1317-1321, 1999.

31. Subramaniam N, Leong GM, Cock TA, Flanagan JL, Fong C, Eismann JA and Kouzenko AP: Cross-talk between 1,25-dihydroxyvitamin D3 and transforming growth factor-beta signaling requires binding of VDR and Smad3 proteins to their cognate DNA recognition elements. J Biol Chem 276: 15741-15746, 2001.

32. Ambros V: The functions of animal microRNAs. Nature 431: 350-355, 2004.

33. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, et al: MicroRNA expression profiles classify human cancers. Nature 435: 834-838, 2005.

34. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116: 281-297, 2004.

35. Stefani G and Slack FJ: Small non-coding RNAs in animal development. Nat Rev Mol Cell Biol 9: 219-230, 2008.

36. Gangaraju VK and Lin H: MicroRNAs: Key regulators of stem cells. Nat Rev Mol Cell Biol 10: 116-125, 2009.

37. Bauersachs J and Thum T: Biogenesis and regulation of cardio-vascular microRNAs. Circ Res 109: 334-347, 2011.

38. Lino Cardenas CL, Kaminski N and Kass DJ: Micromanaging vascular microRNAs. Circ Res 109: 474-487, 2011.

39. Tomasek JJ, Gabbiani G, Hinz B, Chapouard C and Brown RA: Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 3: 349-363, 2002.

40. Vettori S, Gay S and Distler O: Role of microRNAs in fibrosis. Open Rheumatol J 6: 130-139, 2012.

41. Graham JR, Williams CM and Yang Z: MicroRNA-27b targets gremlin1 to modulate fibroscopic responses in pulmonary cells. J Cell Biochem 115: 1539-1548, 2014.

42. Yang S, Cui H, Xie N, Icyuz M, Banerjee S, Antony VB, Abraham E, Thannickal VJ and Liu G: miR-145 regulates myofibroblast differentiation and lung fibrosis. FASEB J 27: 2582-2591, 2013.

43. Zerr P, Vollath L, Palumbo-Zerr K, Tomcik M, Huang J, Distler A, Beyer C, Dees C, Gela K, Distler O et al: Vitamin D receptor regulates TGF-β signalling in systemic sclerosis. Ann Rheum Dis 74: e20, 2015.

44. Aschenbrenner JK, Sollinger HW, Becker BN and Hullett DA: 1,25-(OH)2(D)3 alters the transforming growth factor beta signaling pathway in renal tissue. J Surg Res 100: 171-175, 2001.

45. Xie T, Liang J, Guo R, Liu N, Noble PW and Jiang D: Comprehensive microRNA analysis in bleomycin-induced pulmonary fibrosis identifies multiple sites of molecular regulation. Physiol Genomics 43: 479-487, 2011.

46. Tomasek JJ, Gabbiani G, Hinz B, Chapouard C and Brown RA: Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 3: 349-363, 2002.