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Preliminary Study on Biological Effects of MiR-702-3p in Epithelial–mesenchymal Transition Induced by Nano-SiO₂ Particles

Abstract: Nano-sized SiO₂ particles are gradually applied in numerous fields, the biosecurity of nano-sized SiO₂ particles remains to be controversial. The objective of this study was investigating the biological effects of miR-702-3p in epithelial–mesenchymal transition (EMT) induced by nano-SiO₂ particles. In our previous study, results from Illumina Hiseq2000 high throughput sequencing has shown that miR-702-3p is an up-regulated miRNA in fibrotic pulmonary tissue induced by nano-SiO₂ particles on day 60 and 90. In this study, rats' lung tissue treated by nano-SiO₂ particles of 12.5 mg/ml on day 60 was chosen for the research while the control group was rats' lung tissue treated by equivalent saline. Target genes of miR-702-3p were predicted using the Target Scan (<http://www.targetscan.org/>). With the analysis of Gene Ontology and KEGG, BMP-7 was filtrated to be the target genes of miR-702-3p for further study. Dual-Luciferase Reporter Assay was used to verify the relationship between miR-702-3p and BMP-7. The expression level of miR-702-3p and BMP-7 in pulmonary fibrosis induced by nano-SiO₂ particles was detected by Quantitative real-time PCR and western blot analysis respectively. To analyze the correlation of EMT and pulmonary fibrosis induced by nano-SiO₂ particles, the expressions of TGF-β1, Smad3, E-cad, α-SMA and COL3 were determined by Quantitative real-time PCR and western blot analysis respectively. Result of Dual-Luciferase Reporter Assay indicated that miR-702-3p directly regulates the post-transcriptional manner of BMP-7. The expression of miR-702-3p in the lung tissue of nano-SiO₂ particles group was up-regulated (fold changes > 2.0) when compared with the saline group, which was consistent with the result of Illumina Hiseq2000 high throughput sequencing. The expression of BMP-7 mRNA and protein in the lung tissue of nano-SiO₂ particles group was down-regulated (fold changes < 0.5) when compared with the saline group (P<0.05). The expression of TGF-β1, Smad3, α-SMA, and COL3 were up-regulated (P<0.05) compared with the saline group both at mRNA and protein level. E-cad mRNA and protein were both down-regulated (P<0.05). In conclusion, miR-702-3p may be a positive regulator in pulmonary fibrosis induced by nano-SiO₂ particles, and BMP-7 was a target gene of miR-702-3p in regulating pulmonary fibrosis. MiR-

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702-3p may directly weaken the post-transcriptional manner of BMP-7 to promote the process of EMT which contributes to pulmonary fibrosis induced by nano-SiO₂ particles.

Keywords: component, MiR-702-3p, pulmonary fibrosis, epithelial-mesenchymal transition, Nanosized SiO₂, Bone morphogenetic protein-7.

1 Introduction

Silicosis is characterized by the silicosis nodules formation and development of pulmonary interstitial fibrosis with progressive impairment of pulmonary function which is caused by the persistent inhalation of silica particles [1,2]. Nano-sized SiO₂ particles are gradually applied in numerous fields like food industry, coating, rubber, cosmetics and pharmaceutical and health care and so on due to its special physical and chemical properties. Nano-SiO₂ particles have the same chemical composition as silica particles. Silica particles can lead to lung diseases such as silicosis and lung cancer [3,4], but the correlation of nano-SiO₂ particles and pulmonary fibrosis is still controversial. In recent years studies have shown that nano-SiO₂ particles can cause pulmonary fibrosis after sub-chronic or chronic exposure. Tracheal instillation of nano-SiO₂ particles in rats can lead to inflammation for 7 days and cause pulmonary fibrosis for 30 days [5]. Mice after pulmonary perfusion of nano-SiO₂ particles demonstrated significant lung inflammation and end up with the occurrence of pulmonary fibrosis [6]. Rats sacrificed at day 28 after exposure under continuous dynamic inhalation conditions presented with pulmonary fibrosis formation [7]. Our previous studies have also verified that rats treated with nano-SiO₂ particles through endotracheal instillation resulted in pulmonary injury, which mainly manifested in pulmonary inflammation at protophase (7d, 15d, 30d) and pulmonary interstitial fibrosis at anaphase (60d and 90d) [8].

The epithelial–mesenchymal transition (EMT) is a prevailing phenomenon that plays a crucial role in organic fibrosis and cancer metastasis [9]. EMT is a process of trans-differentiation from the epithelial cells of mature differentiation into the mesenchymal cells of complete differentiation, mainly transforming into fibroblasts and myofibroblasts [10]. During this process, epithelial cells will lose their markers, such as E-cadherin (E-cad) and cytokeratin, as well as mesenchymal cells acquisition of markers, such as vimentin and α -smooth muscle actin (α -SMA) [11,12], meanwhile a large number of extracellular matrix (including COL1 and COL3) was secreted. EMT is a critical process in pulmonary fibrosis. TGF- β 1 is a member of the transforming growth factor superfamily, which can be secreted by neutrophils, eosinophils, macrophages, fibroblasts and myofibroblasts, and studies have reported that TGF- β 1 plays an important role in regulating the process of EMT [13,14]. TGF- β signaling has been reported to be mediators of EMT, induced

by TGF- β through Smad2/3 [15,16]. TGF- β 1 can promote the formation of fibrosis by stimulating fibroblast translate into myofibroblasts and secreting a large amount of collagen to make the extracellular matrix depositing [17] and inducing the EMT process [16]. The canonical Smads-dependent pathway is one of the main pathways in the process of TGF- β 1-induced EMT. [18]

With the development of epigenetics, microRNAs (miRNAs) are gradually attracting more and more attention on account of their extensive regulating effect in organism. MiRNAs are short non-coding endogenous RNAs of approximately 23 nucleotides that mainly modulate gene expression through facilitating degradation of target mRNAs or suppressing translation by binding to the 3'Untranslated Region (UTR) [19,20]. MiRNAs have the characteristics of specificity of time and change of miRNAs occurs before the pathological change of a disease, based on gene expression profiling technology it provides new ideas and methods for the research of pulmonary toxicity effect and mechanism of nano-SiO₂ particles. MiRNAs play a momentous regulatory role in the process of cell apoptosis, tissue inflammation and fibrosis [21-23]. In recent years, some studies have reported that miRNAs play crucial roles in regulating the development of pulmonary fibrosis [24-28]. Results from Illumina Hiseq2000 high throughput sequencing in our previous study have shown that miR-702-3p is a dysregulated miRNA that up-regulated in fibrotic pulmonary tissue at 60 and 90 days [29]. Present studies have reported that miR-702 have the function of promoting the proliferation of embryonic stem cell [30] and inhibiting cell apoptosis [31]. Results of gene ontology analysis showed that miR-702-3p might participate in the development process of pulmonary fibrosis through regulating TGF- β signaling pathway, formation of connective tissue, smad-dependent pathway, metabolism of collagen, wnt receptor signaling pathway. It is speculated that miR-702-3p may play an important role in the development process of pulmonary fibrosis induced by nano-SiO₂ particles. Prediction of the database targets can.org displayed that, bone morphogenetic protein 7 (BMP-7) might be the downstream target genes of miR-702-3p. BMP-7 belonging to the TGF- β superfamily which is a negative regulator of pulmonary fibrosis. BMP-7 can reverse the process of pulmonary fibrosis by antagonizing TGF- β /Smad signaling pathway [32] and inhibiting EMT [33]. Considering the above, we assume that miR-702-3p and its target genes BMP-7 may contribute to pulmonary fibrosis induced by nano-SiO₂ particles through regulating EMT. In this study, we investigate the biological effects of miR-702-3p and the correlation of miR-702-3p and EMT in pulmonary fibrosis induced by nano-SiO₂ particles.

2 Material and Methods

2.1 Nano-sized SiO₂ Particles and Animal Model of Pulmonary Fibrosis

The amorphous SiO₂ nanoparticles (SNPs) (Code Name: SP1, purity: 99%, average diameter: 20±5nm, surface area: 640±30 m²/g, hydroxyl concentration>45%) were provided by Zhejiang Hongsheng Nano-materials Technologies Co Ltd. Rats were instilled with intratracheal nano-SiO₂ particles suspension to establish the model of pulmonary fibrosis and the change of morphology and histopathology was investigated based on that model, as previously described by Zhang [8]. Briefly, rats were endotracheal instillation of 6.25 mg, 12.5 mg, 25 mg of nano-SiO₂ particles in 1 ml saline for 7d, 15d, 30d, 60d and 90d, while the rats of control group just endotracheal instillation of equivoluminal saline, then rats' lung tissue were removed. In this study, rats' lung tissue of nano-SiO₂ particles of 12.5 mg/mL on day 60 was chosen for further research.

2.2 Prediction of Target Genes Regulated by miR-702-3p

Target genes of miR-702-3p were predicted using the Target Scan (<http://www.targetscan.org/>). Gene set enrichment analysis of the predicted target genes was significantly predicted via the Gene Ontology (<http://www.geneontology.org>) and signaling pathway analysis of the target genes was filtrated through the database of KEGG pathway analysis (<http://www.genome.jp/kegg/pathway.html>). Meanwhile the genes may be involved in fibrosis were screened out and BMP-7 was selected for the further research.

2.3 Culture of A549 Cells and Dual-luciferase Reporter Gene Assay

Human type II alveolar epithelial carcinoma cell line (A549 cell) was purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). A549 cells (1.0 × 10⁵ per well) were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Invitrogen, USA) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and streptomycin in 5% carbon dioxide atmosphere at 37°C.

The 3'-UTR of the rat BMP-7 gene was amplified by PCR using rat genomic DNA as a template. And the rno-miR-702-3p target site in the BMP-7 3'-UTR was mutated from GGTGGGC to CCACCCG by PCR mutagenesis. The PCR primers of wild type or mutant type of BMP-7 3'-UTR were showed as Table 1. The PCR fragment was inserted into the firefly luciferase reporter vector pmiR-RB-REPORT™ (RiboBio, Guangzhou, China) between the restrictive sites Xho I and Not I downstream from coding region of Renilla luciferase gene. All the cloning was confirmed by sequencing. A549 cells

were plated at 3×10^3 cells/well in 96-well plates. After reaching 40-60%, cells were co-transfected with 100 ng/ μ L of the luciferase reporters vector containing the wild-type or mutant 3'-UTR and 50 nM of the miR-702-3p mimic or miR-702-3p Non-target Control (RiboBio, Guangzhou, China) with riboFECT™ CP Transfection Kit (RiboBio, Guangzhou, China). After 48 hour transfection, luciferase activities were measured in cell lysates using a Luc-Pair™ Duo-Luciferase Assay Kit 2.0 (Genecopoeia, USA). The Renilla luciferase signal was normalized to the firefly luciferase signal for each individual analysis.

2.4 Prediction of Target Genes Regulated by miR-702-3p

Total RNA of lung tissues stored at -80°C was extracted with TRIzol®Regent (Invitrogen, USA) according to the specification. The concentration and purity of the total RNA were determined with Nanodrop2000 Micro-spectrophotometer (Thermo Scientific, USA). The reverse transcription of miR-702-3p, BMP-7, TGF- β 1, Smad3, E-cad, α -SMA and COL3 were directly performed with miRNA specific stem-loop primers or Oligo d (T) and the Reverse Transcription System (Promega, USA) according to the manufacturer's specification and the newly synthetic cDNA template was used for further research.

Real-time PCR amplification (20 μ L reaction system) was performed under the condition of 95°C initial denaturation for 2 min, 95°C denaturation for 15 s, 60°C annealing for 60s on StepOne Plus Real-Time PCR System (Applied Biosystems, USA) with the GoTaq®qPCR Master Mix (Promega, USA) according to the manufacturer's specification and experiments were performed in triplicate following by 40 cycles. The mRNA sequences (Generay, Shanghai, China) and miRNA sequences (Ribobio, Guangzhou, China) used for detecting in PCR analysis were showed as Table 2. The data of PCR result was analyzed via the comparative threshold cycle (Ct) method. U6 snRNA and β -actin were chosen to be the endogenous control for miRNA and mRNA respectively. $2^{-\Delta\Delta\text{Ct}}$ denoted the fold change of target genes in the test group related to the control group ($\Delta\text{CT} = \text{CT}_{\text{target gene}} - \text{CT}_{\text{endogenous control}}$, $\Delta\Delta\text{Ct} = \Delta\text{Ct}_{\text{test}} - \Delta\text{Ct}_{\text{control}}$).

Table 1: Primer for the Wild-type or Mutant 3'-UTR of Rat BMP-7

primer name	sequences	Total length of PCR product
BMP-7_WT_F	GGCGG <u>CTCGAGA</u> AGTACAGAAACATGGTGGTC	793bp
BMP-7_WT_R	AAT <u>GCGGCCGC</u> ACACGTCCCTCCACCTTC	
BMP-7_MUT_F	ATAACCAG <u>CCACCCG</u> GCTTACGTCTGTGCTGAA	
BMP-7_MUT_R	ACGTAAGC <u>CGGGTGG</u> CTGTTATGCCCTTCCTC	

Table 2: The Gene Primer Sequences Used for Detection in PCR Analysis

Gene	Primer sequence
miR-702-3p and U6	Forward and Reverse: confidentiality
BMP-7	Sense: 5' GAGGGCTGGTTGGTATTGACAT 3' Antisense: 5' AGGGTTGCTTGTCTGGGGTC 3'
TGF- β 1	Sense: 5' CTAATGGTGGACCGCAACAAC 3' Antisense: 5' CACTGCTCCCGAATGTCTGA 3'
E-cad	Sense: 5' GCATCCGACCTTGCTAACG 3' Antisense: 5' CATCTCCAGAGTCCAGCACAAAT 3'
α -SMA	Sense: 5' TGAGGTCGGTGCCCGTATT 3' Antisense: 5' CGTTGGTCTTGGGGTCTGT 3'
COL3	Sense: 5' TTTAATGGATAGGGACTTGTGTGAA 3' Antisense: 5' GAGAGAGAGAGAAGCTGAGGGTAGG 3'
β -actin	Sense: 5' CTGAACCCTAAGGCCAACCG 3' Antisense: 5' GACCAGAGGCATACAGGGACAA 3'

2.5 Western Blot Analysis

Total protein was extracted with the RIPA (SunShine Biotechnology, Nanjing, China) from lung tissues according to the manufacturer's instructions. The concentration of total proteins was measured with BCA Protein Assay Kit (Pierce, Thermo, USA). The proteins were diluted with RIPA buffer to the desired concentration and then mixed with an appropriate amount of loading buffer (Thermo, Pittsburgh, USA) with the proportion of 5:1. The protein mixture was boiled for 5 min at the temperature of 100°C. The protein mixture (20 μ g each lane) were separated using 10% SDS-PAGE gel electrophoresis (Beyotime Biotechnology, China) at a constant voltage of 80 V for 2.5 h at 4°C and transferred to PVDF membranes (Millipore, USA) at a constant current of 250 mA for 1 h at 4°C. Then the PVDF membranes were blocked with 5% nonfat dry milk in TBST at room temperature for 1 h and incubated at 4°C overnight with the following primary antibodies: BMP-7, TGF- β 1, Smad3, E-cad, α -SMA, COL3 and GAPDH (Abcom, USA). The membranes were incubated with 1:2000 dilution of goat anti-rabbit HRP-conjugated secondary antibody (Abgent, USA) for 1 h at room temperature. Finally, the membranes were incubated in ECL reagent (Pierce, Thermo, USA) for HRP detection and then exposed to autoradiography film (Bio-Rad, USA) for band visualization.

2.6 Statistical Analysis

In our study, the SPSS 21.0 software was used to evaluate statistical significance with the Student's t-test. All experimental results were expressed as means $\pm$ SD ($\bar{x} \pm s$). The values of $p < 0.05$ were statistically significant.

3 Results

3.1 BMP-7 was a Target Gene of miR-702-3p

There were 136 target genes identified that may be regulated by miR-702-3p predicted with the database TargetScan.org and BMP-7 was filtrated to be the target genes of miR-702-3p through the analysis of Gene Ontology and KEGG.

With the assistance of target-scan database, the predicted binding site of miR-702-3p and BMP-7 indicated that BMP-7 is probably a target gene regulated by miR-702-3p. The correlation with between miR-702-3p and BMP-7 was further verified with the Dual-Luciferase Reporter Assay. Co-transfection of miR-702-3p and the portion of the human BMP-7 3'-UTR carried by luciferase reporter significantly reduced luciferase expression by approximately 2-fold when compared with the control group. Simultaneously, co-transfection of miR-702-3p and the mutated BMP-7 3'-UTR at the predicted miR-702-3p site carried by the luciferase reporter failed to diminish the luciferase activities when compared with its control group (Fig. 1). This data indicated that miR-702-3p directly regulates the post-transcriptional manner of BMP-7.

Figure 1: BMP-7 was a target gene of miR-702-3p. (A) Predicted binding site of miR-702-3p and BMP-7. MiR-702-3p seed sequence is in bold. The representation is limited to the region around the miR-702-3p complementary site. The number of interactional region of BMP-7 3' UTR is 2090-2096. (B) Co-transfection of 50 nM miR-702-3p mimic or miR-702-3p NC and the portion of rat BMP-7 3'-UTR (WT or MUT) carried by the luciferase reporter operating with riboFECT™ CP Transfection Kit. After 48 h after transfection, the measured Renilla luciferase activity was normalized to the firefly luciferase signal for each individual analysis. *P<0.05.

3.2 Abnormal Expression of miR-702-3p and BMP-7 in Pulmonary Fibrosis Induced by Nano-SiO₂ Particles

After the experiments of Illumina Hiseq2000 high throughput sequencing and dual-luciferase reporter gene assay, the level expression of miR-702-3p and BMP-7 in pulmonary fibrosis induced by nano-SiO₂ particles was detected by Quantitative real-time PCR and western blot analysis. Results showed that the expression of miR-702-3p in the lung tissue of nano-SiO₂ particles group was up-regulated (fold changes > 2.0) when compared with the saline group (Fig. 2(A)), which was consistent with the result of Illumina Hiseq2000 high throughput sequencing. The expression of BMP-7 mRNA in the lung tissue of nano-SiO₂ particles group was down-regulated (fold changes < 0.5) when compared with the saline group (Fig. 2(A)) and the expression of BMP-7 protein in the lung tissue of nano-SiO₂ particles group was significantly reduced (fold changes < 0.5) than the saline group ($P < 0.05$) (Fig. 2(A) (B)).

Figure 2: Abnormal expression of miR-702-3p and BMP-7 in pulmonary fibrosis induced by nano-SiO₂ particles. (A) The expression of miR-702-3p and BMP-7 in pulmonary fibrosis induced by nano-SiO₂ particles compared with the saline group. (B) Western Blot analysis for BMP-7. “Student’s t-test” was used as statistical analysis. Values are expressed as mean + SD (n= 3). *p < 0.05: when compared with saline control group. BMP-7: bone morphogenetic protein 7.

3.3 EMT Contributed to Pulmonary Fibrosis Induced by Nano-SiO₂ Particles

Studies have demonstrated that EMT can contribute to bleomycin-induced lung fibrosis [33,35]. To analyze the correlation of EMT and pulmonary fibrosis induced

by nano-SiO₂ particles, the expressions of epithelial and mesenchymal markers were determined by Quantitative real-time PCR and western blot analysis. Results of Quantitative real-time PCR showed that the expression level of TGF- β 1 mRNA in the lung tissue of nano-SiO₂ particles group was up-regulated, Smad3 mRNA was up-regulated, E-cad mRNA was down-regulated, α -SMA mRNA was up-regulated and COL3 mRNA was up-regulated when compared with the saline group (Fig. 3(A)). Results from western blotting showed that the expression level of TGF- β 1 protein in the lung tissue of nano-SiO₂ particles group was significantly up-regulated ($P < 0.05$), Smad3 protein was significantly up-regulated ($P < 0.05$), E-cad protein was significantly reduced ($P < 0.05$), α -SMA protein was up-regulated and COL3 protein was significantly up-regulated ($P < 0.05$), when compared with the saline group (Fig. 3(B) (C)). This data revealed that rat lung epithelial cells lost epithelial characteristics and acquired mesenchymal markers, which became fibroblast-like cells after the treatment of nano-SiO₂ particles. EMT was contributed to pulmonary fibrosis induced by nano-SiO₂ particles.

Figure 3: The expression of TGF- β 1, Smad3, E-cad, α -SMA and COL3 in pulmonary fibrosis induced by nano-SiO₂ particles. (A) The expression of TGF- β 1, Smad3, E-cad, α -SMA and COL3 mRNA. (B) The expression of TGF- β 1, Smad3, E-cad, α -SMA and COL3 protein. (C) Western Blot analysis for TGF- β 1, Smad3, E-cad, α -SMA and COL3. “Student’s t-test” was used as statistical analysis. Values are expressed as mean + SD (n= 3). *p < 0.05: when compared with saline control group. BMP-7: bone morphogenetic protein 7.

Figure 3: The expression of TGF-β1, Smad3, E-cad, α-SMA and COL3 in pulmonary fibrosis induced by nano-SiO₂ particles. (A) The expression of TGF-β1, Smad3, E-cad, α-SMA and COL3 mRNA. (B) The expression of TGF-β1, Smad3, E-cad, α-SMA and COL3 protein. (C) Western Blot analysis for TGF-β1, Smad3, E-cad, α-SMA and COL3. "Student's t-test" was used as statistical analysis. Values are expressed as mean + SD (n= 3). *p < 0.05: when compared with saline control group. BMP-7: bone morphogenetic protein 7.

4 Discussion

Pneumoconiosis is a serious occupational disease characterized by fibrosing nodular lesions that eventually develop into progressive pulmonary fibrosis, which is induced by silicious dust, coal dust or other productive dusts [36]. Silicosis is an extremely serious pulmonary disease of pneumoconiosis, caused by the persistent inhalation of silica particles. Nano-SiO₂ particles have the same chemical composition with the micron-sized silica, but whether nano-SiO₂ particles can result in pulmonary fibrosis similarly still remains controversial. In recent years multiple studies have verified that nano-SiO₂ particles can induce cytotoxicity and lung injury of pulmonary inflammation and fibrosis in animal after intratracheal instillation of nano-SiO₂ particles [37,38]. Our previous studies have confirmed that rats had the symptom of pulmonary inflammation at protophase (7d, 15d and 30d) and pulmonary interstitial fibrosis at anaphase (60d and 90d) after treatment with nano-SiO₂ particles through endotracheal instillation [8]. Fibroblasts and myofibroblasts are effector cells to assemble abundance of ECM, which destroy the lung tissue structure and aggravate the fibrotic process [39]. Increasing evidence indicates that the mechanism of EMT may partially account for the origin of fibroblasts and myofibroblasts from alveolar epithelial cells [40]. TGF-β is a member of the superfamily of polypeptides which

participate in cell proliferation, induction of apoptosis and the excessive accumulation of collagens. TGF- β is considered to be the dominating stimulus and TGF- β /Smad signal pathway is crucial for EMT in pulmonary fibrosis [41]. Additionally, BMP-7 has been identified as an antagonist of EMT [42]. BMP-7 can be in the preservation of the epithelial phenotype and reverse EMT induced by TGF- β in renal epithelial cells [18]. BMP-7 has been confirmed to antagonize fibrosis through restraining TGF- β /Smad signaling pathway [43]. The counterbalance of BMP-7 and TGF- β can control the process of EMT [44].

MiRNAs play a momentous regulatory role in the process of cell apoptosis, tissue inflammation and fibrosis, and miRNAs may regulate the development of pulmonary fibrosis. In our previous study, results from Illumina HiSeq2000 high throughput sequencing have shown that miR-702-3p is an up-regulated miRNA in fibrotic pulmonary tissue at 60 and 90 days [28]. With the help of Target-Scan database, the Gene Ontology and KEGG pathway analysis, BMP-7 was speculated to be one of the target genes regulated by miR-702-3p in participating in pulmonary fibrosis induced by nano-SiO₂ particles. In this study, the expression of miR-702-3p in the lung tissue of nano-SiO₂ particles group was up-regulated when compared with the saline group, which was consistent with the result from Illumina HiSeq2000 high throughput sequencing. The result indicated that miR-702-3p may promote the development process of pulmonary fibrosis induced by nano-SiO₂ particles. BMP-7, an antagonist of EMT [41], can reverse EMT induced by TGF- β in renal epithelial cells [18]. The result of this study showed that the expression of BMP-7 mRNA and protein in the lung tissue of nano-SiO₂ particles group were both down-regulated when compared with the saline group, which implied that BMP-7 may be an inhibited factor of pulmonary fibrosis induced by nano-SiO₂ particles. From all of the above data, together with the result of the Dual-Luciferase Reporter Assay, we deduced that BMP-7 was a target gene of miR-702-3p in regulating pulmonary fibrosis.

EMT, a trans-differentiation process from epithelial cells into mesenchymal cells, plays a crucial role in pulmonary fibrosis [10]. In the process of EMT, epithelial cells lose their markers, such as E-cad and cytokeratin, as well as mesenchymal cells get acquisition of markers, such as vimentin and α -smooth muscle actin (α -SMA) [11]. BMP-7, an antagonist of EMT, can inhibit tissue fibrosis through restraining TGF- β /Smad signaling pathway [42]. In this study, results showed that the expression level of TGF- β 1 mRNA and protein in the lung tissue of nano-SiO₂ particles group were both up-regulated, Smad3 mRNA and protein were both up-regulated, E-cad mRNA and protein were both down-regulated, α -SMA mRNA and protein were both up-regulated and COL3 mRNA and protein were both up-regulated when compared with the saline group. The data revealed that in this process rat lung epithelial cells lost epithelial characteristics and acquired mesenchymal markers, which became fibroblast-like cells after the treatment of nano-SiO₂ particles. The above demonstrated that EMT contributed to pulmonary fibrosis induced by nano-SiO₂ particles.

5 Conclusions

MiR-702-3p may be a positive regulator in pulmonary fibrosis induced by nano-SiO₂ particles, and BMP-7 was a target gene of miR-702-3p in regulating pulmonary fibrosis. MiR-702-3p may directly weaken the post-transcriptional manner of BMP-7 to promote the process of EMT contributing to pulmonary fibrosis induced by nano-SiO₂ particles.

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