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Wnt7b/β-catenin signaling pathway mediated by retinoid acid involved in the transdifferentiation of primary fetal alveolar epithelial type II cells

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Abstract. This study was designed to investigate the roles of retinoic acid (RA) in transdifferentiation of primary fetal alveolar epithelial type II cells (AECIIs) into alveolar epithelial type I cells (AECIs). Primary fetal AECIIs isolated from rats at a gestational of 19 days were divided into: (i) DMSO group treated using 0.1% DMSO; (ii) RA group, treated with 1 μM RA; and (iii) RA+BMS493 group treated with 1 μM RA and 10^{-8} M BMS493 (served as a pan-RA receptor antagonist). Then we determined the roles of AQP5 (a specific marker of AECIs), SP-C (a specific marker for AECIIs) and Wnt7b/β-catenin signaling pathway in the transdifferentiation of AECIIs to AECIs. SP-C mRNA and protein expression was significantly down-regulated in AECIIs exposure to RA for 24 h and 48 h, however, significant up-regulation was noticed after exposure for 72 h. AQP5 mRNA and protein expression showed significant increase in RA group, but showed significant decline in the RA+BMS493 group. Wnt7b mRNA, nucleus β-catenin and cyclin D1 were significantly up-regulated in RA group compared with DMSO group. RA may promote fetal AECIIs transdifferentiation into AECIs through activating Wnt7b/β-catenin signaling pathway. Our study contributed to the understanding on the pulmonary regeneration in cases of pulmonary injuries, together with the prevention and treatment of neonatal respiratory distress syndrome.

Key words: Alveolar epithelial type II cells — Retinoic acid — Wnt7b — β-catenin — Cyclin D1

Introduction

Neonatal respiratory distress syndrome (NRDS), characterized by shortness of breath and respiratory failure, is primarily a disease of preterm infant associated with lung immaturity and a deficiency of endogenous surfactant (Ardell et al. 2015). Alveolar epithelium consisted of cubical alveolar epithelial type II cells (AECIIs) and squamous alveolar epithelial type I cells (AECIs), plays an important role in pulmonary homeostasis by serving as an extensive surface for air exchange and a regulator for water and ion transport (Guillot et al. 2013). AECIIs can secret pulmonary-associated surfactant protein (SP) to reduce surface tension, and regulate the stability and integrity of the lung tissues. In addition, the transdifferentiation of AECIIs into AECIs is essential in the repair of alveolar epithelial injury (Wu et al. 2017; Jia et al. 2021). On this basis, regulation of the transdifferentiation of AECIIs to AECIs may involve in the secretion of surfactant, which may contribute to the prevention and treatment of NRDS.

Wnt signaling pathway is a highly conserved regulator for cell pluripotency including proliferation, migration,

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apoptosis and differentiation (Papachristou et al. 2014; Xi and Chen 2014; Majidinia et al. 2018), depending on the activation of canonical β-catenin pathways. Moreover, Wnt signaling pathway plays an important role in lung endoderm development (Lehman et al. 2016). Besides, Wnt7b played a vital role in the development of airways and vessels, however, few reports focused on the roles of Wnt signaling pathway during transdifferentiation from AECIIs into AECIs.

Retinoic acid (RA), the bioactive metabolite of vitamin A, is essential for lung formation (Ng-Blichfeldt et al. 2017). It involved in pulmonary morphogenesis, such as embryonic development, epithelial reproduction, pulmonary angiogenesis and tissue repair (Love and Gudas 1994; Kayalar and Oztay 2014). In a previous study, RA regulated lung primordium formation through integrating with multiple pathways in foregut (Chen et al. 2014). In addition, in emphysema rats or hyperoxia mice induced by elastase, RA induced alveolar repair and formation (Kayalar and Oztay 2014; Fernandes-Silva et al. 2020). Our previous study showed that RA promoted fetal AECII proliferation by enhancing cell viability and inhibiting cell apoptosis. Meanwhile, RA promoted the transdifferentiation of AECIIs to ACEIs (Gao et al. 2015). RA regulates many specific gene expressions in different tissues and cells by means of RA-receptors (Lei et al. 2014). In this study, the transdifferentiation of AECIIs to AECIs was determined by measuring the expression of SP-C and AQP5 serving as a specific marker for the AECIIs and AECIs after treating with RA. In addition, to investigate the molecular mechanism of how RA induced AECIIs transdifferentiation to AECIs, we investigated the activity of Wnt signaling pathway in this process in the presence of RA and its inhibitor BMS493 (a pan-RAR antagonist), with an aim to further determine the roles of RA in the transdifferentiation of AECIIs to ACEIs.

Materials and Methods

Ethics statement

The study protocols were approved by Animal Ethics Committee of Binzhou Medical University Hospital (No. 20190104-21).

Animals

Adult Sprague Dawley (SD) rats were purchased from Lukang Pharmacy (Jining, China), and were housed in stainless steel cages in a 12-h light/12-h dark cycle. Female rats (230–280 g) and male rats (260–320 g) were housed in the same cage with a ratio of 2:1 from 7 p.m. to 8 p.m, and were separated at the other time points. All the animals were free access to tap water and food. Positive mating was defined as presence of spermatozoids under a microscope at 8 o'clock on the next day. Then the day was marked as gestational day 0. Pregnant rats were fed to a gestational day of 19. The animal handling was complied with all the relevant national regulations and institutional policies for the care and use of animals.

Isolation and culture of fetal AECIIs

Pregnant rats were anesthetized at a gestational day of 19 and fetuses were carefully separated from the uterus. Primary fetal AECIIs were isolated and purified as previously described Gao et al. 2015). Briefly, purified fetal AECIIs were resuspended in α-modified Eagle's medium (α-MEM, Hyclone, UK) supplemented with 13% fetal bovine serum (FBS, Gibco, USA) and incubated at 37°C in 5% CO_2–95% air. Non-adherent cells were washed away after 36 h. The cell purity was 97.1 ± 2.6% as determined by modified Papanicolaou staining, and the viability was 96.8 ± 3.2% as determined by trypan blue staining.

Transmission electron microscopy (TEM)

Primary AECIIs were cultured in complete alpha-MEM medium (CMEM) for 36 h. Then cells were fixed by fresh glutaraldehyde (3%) at 4°C for more than 24 h, followed by fixing in 2% osmium tetroxide for 2 h, and dehydration in gradient acetone (50, 70, 90 and 100%). Subsequently, the cells were infiltrated with Epon812 and embedded at 37°C and 60°C for 24 h. Finally, the separated AECIIs were identified based on the ultramicrotome semi-thin sections using a JEM-1400 TEM system (Tokyo, Japan).

Experimental design

Primary fetal AECIIs were divided into the following groups: (i) DMSO group, cells treated using 0.1% DMSO; (ii) RA group, cells treated using 1 μM RA dissolved in DMSO; and (iii) RA+BMS493 group, cells treated using 1 μM RA and BMS493 (10^{-8} M, R&D Systems, Wiesbaden, Germany). For the BMS493 concentration selection, four concentrations (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} M) were set at first, and the dose of 10^{-8} M was selected as it caused inhibitory effects without affecting the cellular viability (data not shown).

Cell proliferation and viability assay

The proliferation and viability assay were based on MTT method. Fetal AECIIs were plated in a 96-well plate and cultured in CMEM for 36 h. Afterwards, MTT (5 mg/ml, Sigmal-Aldrich) was added and incubated for 4 h. Finally, the media was removed and DMSO was added. The absorbance

was measured at 570 nm using a microplate reader (Thermo, Waltham, MA).

Real-time quantitative PCR

Total RNA was extracted using RNAiso Plus (Takara, Japan). To observe the transdifferentiation of AECIIs to AECIs at different time points, cells were incubated under the treatment conditions in each group for 24, 48 and 72 h, respectively. RNA was reverse transcribed to cDNA using PrimeScript^{TM}RT reagent Kit with gDNA Eraser (Takara, Japan). Real-time quantitative PCR (RT-PCR) was performed using the Rotor-Gene 3000 (Corbett, Australia) in combination with SYBR Premix Ex Taq, with the specific primer sets in Table 1. PCR reaction was performed at 95°C for 30 s, followed by 50 cycles at 95°C for 5 s; 60°C for 30 s. The results were analyzed using the comparative Ct method.

Western blotting analysis

Proteins were extracted from cells using protein extraction kit (Beyotime Biotech, Haimen, China). To determine the expression of nuclear β-catenin, nuclear protein extraction kit (Beyotime Biotech, Haimen, China) was used. Protein (50 μg) was separated on 8% and 12% SDS-PAGE pre-cast gel, followed by transferring onto PVDF membranes. Then the PVDF membrane was blocked with 7% skimmed milk, and was incubated overnight at 4°C with pre-diluted rabbit monoclonal anti-cyclin D1 and anti-β-catenin antibody (1:1000, Cell Signaling Technology, MA, USA), rabbit polyclonal anti-SP-C (1:300, Santa Cruz, CA), anti-AQP5 (1:300, Santa Cruz, CA) and anti-β-actin antibody (1:1000, Santa Cruz, CA) followed by washing with TBST three times and incubation with horseradish peroxides-conjugated anti-rabbit secondary antibodies (1:5000). Finally the PVDF membrane was visualized on a Chemiluminescence Imaging System (3100 Mini, Clinx Science Instruments, USA).

Immunocytochemistry

To detect the expression of SP-C and β-catenin protein, fetal AECIIs were plated in Laser confocal culture dish and cultured in CMEM for 36 h. The cultured cells in each group were fixed with 4% paraformaldehyde for 30 min at room temperature. Upon washing with PBS (0.01 M), samples were permeabilized with 0.5% Triton X-100 for 10 min and blocked with 20% Donkey serum (Sigma, Aldrich, St. Louis, Mssouri) for 1 h at 37°C. Samples were then incubated with goat polyclonal anti-SPC antibody (1:100, Santa Cruz, CA), and rabbit monoclonal anti-β-catenin antibody (1:100, Danvers, MA) overnight at 4°C, followed by incubation with Alexa 488-conjugated anti-goat and Alexa 532-conjugated anti-rabbit secondary antibodies (1:300, Danvers, MA) for 1 h at 37°C in dark. Afterwards, the samples were stained with Hoechst 33342 (1:1000, Sigma-Aldrich, CA) for 15 min to identify cellular nuclei. Finally, the cells were observed under a laser scanning confocal microscope (LEICA TCS SPE, Germany).

Statistical analysis

Statistical analysis was conducted by SPSS 13.0 software. The data were presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) was utilized for the intergroup comparison, combining with the Bonferroni correction. All experiments were performed at least in triplicates. A value $p < 0.05$ was considered to be statistically significant.

Results

Identification of fetal AECII phenotype

Fetal AECIIs were identified under an inverted microscope, TEM and laser confocal microscope. Under inverted microscope, the cluster of adherent cells showed a typical insular-shaped morphology, with obvious cell nucleus and good refraction (Fig. 1A). For TEM findings, there were characteristic

Table 1. Sequences of primers used in the quantitative RT-PCR

Genes	Sequences of primers (5'→3')	GenBank accession number	Product sizes (bp)
Wnt7b	F: CCTTCACATACGCCATCACC R: GCCTTCTGCCTGGTTGTAGT	NM_001009695.1	125
SP-C	F: TGAGATGGTCCTTGAGATGAG R: TAGCGATGGTGTCTGTGTGTT	NM_017342.2	88
AQP5	F:CTCCCAACCCACTATCTCAACT R:GCCATCTATCCCTCTCCTGAAG	NM_012779.1	92
β-actin	F: CTGGGACGACATGGAGAAAA R: AAGGAAGGCTGGAAGAGTGC	NM_031144.3	564

F, forward primer; R, reverse primer.

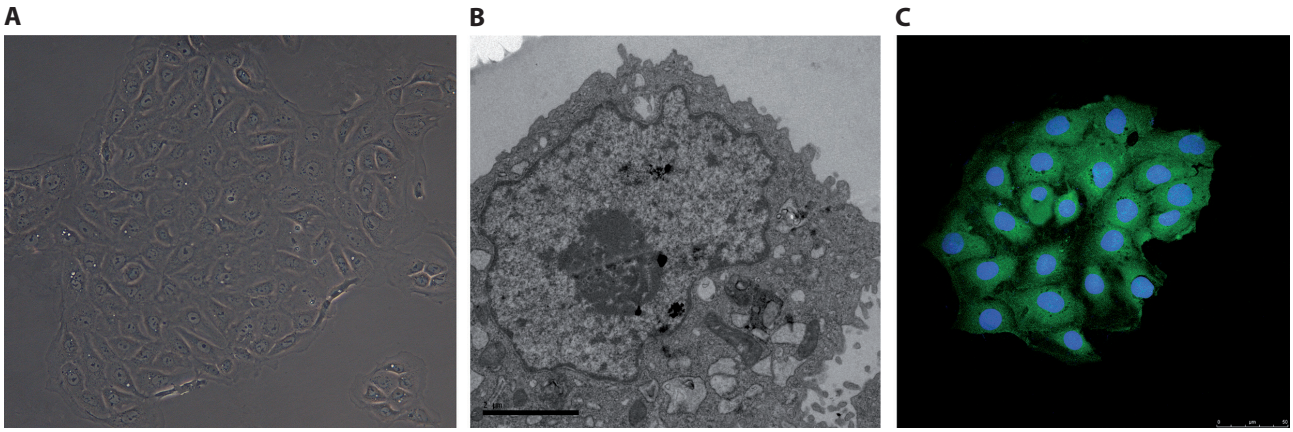

Figure 1. Identification of fetal AECIIs phenotype. **A.** Freshly isolated primary fetal AECIIs showed a typical insular-shaped morphology, with obvious cell nuclear and good refraction under a magnification of ×200. **B.** Ultra-structure of fetal AECIIs was characterized by lamellar bodies and microvilli under TEM at a magnification of ×8000. **C.** A single labeling of cells was incubated with anti-SP-C antibodies, and the protein was visualized by corresponding with Alexa 488-conjugated anti-rabbit secondary antibody using laser confocal microscope under a magnification of ×630. AECIIs, alveolar epithelial type II cells.

ultrastructures of AECIIs with microvilli and lamellar bodies (Fig. 1B), while laser confocal microscope indicated strong green fluorescence of SP-C in cytoplasm (Fig. 1C).

Cell proliferation and viability assay

Compared with DMSO group, RA triggered significant elevation of fetal AECIIs proliferation at 24, 48 and 72 h, respectively ($p < 0.01$, Fig. 2). Compared with RA group, significant decline was noticed in the cellular proliferation and viability in the cells treated with the combination of BMS493 and RA at 24, 48 and 72 h, respectively ($p < 0.01$, Fig. 2). The proliferation and viability of fetal AECIIs exposure to RA and BMS493 showed no significant difference compared with that of DMSO group.

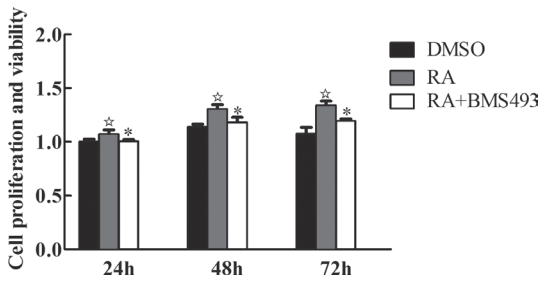

Figure 2. Effects of RA and pan-RAR antagonist BMS493 on proliferation and viability of fetal AECIIs. Freshly isolated primary fetal AECIIs were cultured in CMEM for 36 h. Then the cells were transferred into different groups followed by cultivation for additional 24 h, 48 h and 72 h. ☆ $p < 0.05$ *vs*. DMSO group; * $p < 0.05$ *vs*. RA group. RA, retinoic acid; AECIIs, alveolar epithelial type II cells; CMEM, complete alpha-MEM medium; DMSO, dimethylsulfoxide.

RA facilitated fetal AECIIs transdifferentiation into AECIs

As shown in Figure 3A and 3C, RA resulted in significant decrease in SP-C mRNA and protein expression at 24 h and 48 h compared with DMSO group ($p < 0.01$). In the group treated with the combination of RA and BMS493, significant elevation was noticed in SP-C mRNA compared with the RA group at 24 h and 48 h, respectively ($p < 0.05$). In addition, significant increase was noticed in the SP-C protein expression in RA+BMS493 group compared with the RA group at 48 h ($p < 0.05$). At 72 h, RA induced significant elevation of SP-C mRNA and protein compared with the DMSO group, while the combination of RA and BMS493 triggered significant decline in SP-C mRNA and protein compared with the RA group ($p < 0.05$).

RA significantly promoted the expression of AQP5 mRNA and protein at 24, 48 and 72 h compared with DMSO group (Fig. 3B and D). In RA+BMS493 group, expression of AQP5 mRNA showed significant decrease compared with RA group at 24, 48 and 72 h, respectively ($p < 0.05$). The expression of AQP5 protein in RA+BMS493 group showed significant decrease compared with RA group at 24 and 72 h, respectively ($p < 0.05$).

RA promoted the transdifferentiation of AECIIs to AECIs through modulating the Wnt signaling pathway

RA significantly increased the expression of Wnt7b mRNA at 24, 48 and 72 h compared with DMSO group ($p < 0.05$)

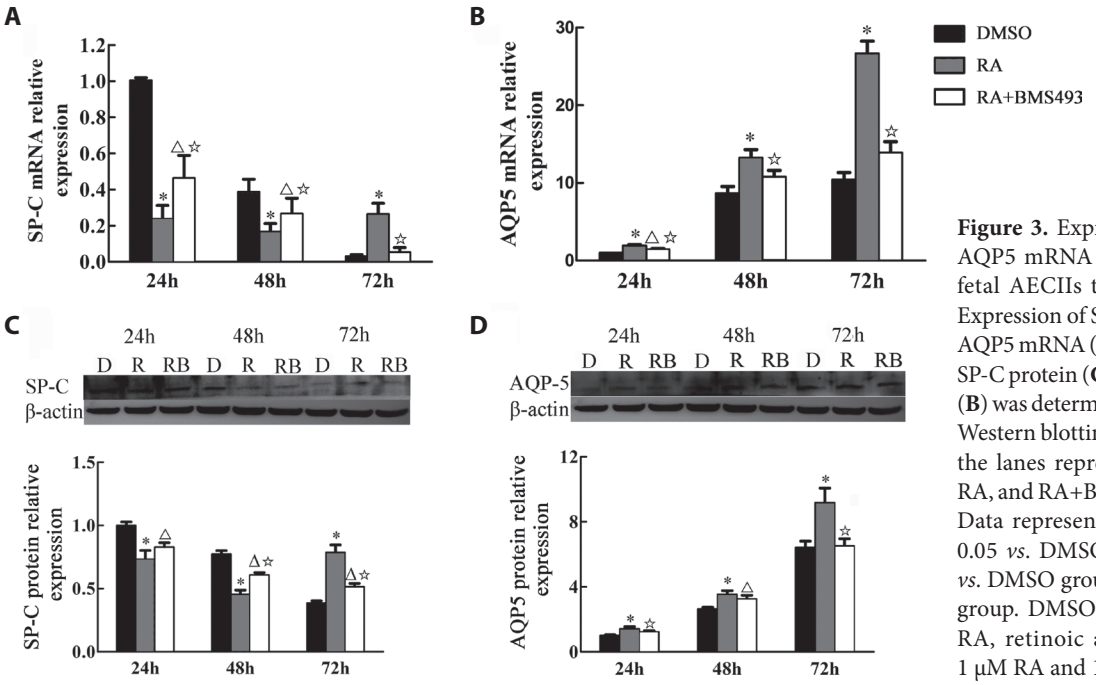

Figure 3. Expression of SP-C and AQP5 mRNA and protein during fetal AECIIs transdifferentiation. Expression of SP-C mRNA (**A**) and AQP5 mRNA (**B**), and expression of SP-C protein (**C**) and AQP5 protein (**B**) was determined by RT-PCR and Western blotting. Letter D, R, RB in the lanes represented the DMSO, RA, and RA+BMS493, respectively. Data represent mean ± SD. * $p < 0.05$ *vs.* DMSO group; $^{\triangle}$ $p < 0.05$ *vs.* DMSO group; * $p < 0.05$ *vs.* RA group. DMSO, dimethylsulfoxide; RA, retinoic acid; RA+BMS493, 1 μM RA and 10^{-8} M BMS493.

(Fig. 4A). In the RA+BMS493 group, the expression of Wnt7b mRNA showed significant decline at 24, 48 and 72 h compared with the RA group ($p < 0.05$). Compared with DMSO group, the nuclear β-catenin was significantly up-regulated in RA group at 24, 48 and 72 h ($p < 0.05$, Fig. 4B). In addition, in RA+BMS493 group, the expression of nuclear β-catenin showed significant decline at 24, 48 and 72 h compared with RA group ($p < 0.05$, Fig. 4B). The expression of cyclin D1 showed significant up-regulation in RA group at 24, 48 and 72 h compared with DMSO group ($p < 0.001$). In the RA+BMS493 group, the expression of cyclin D1 showed significant down-regulation compared

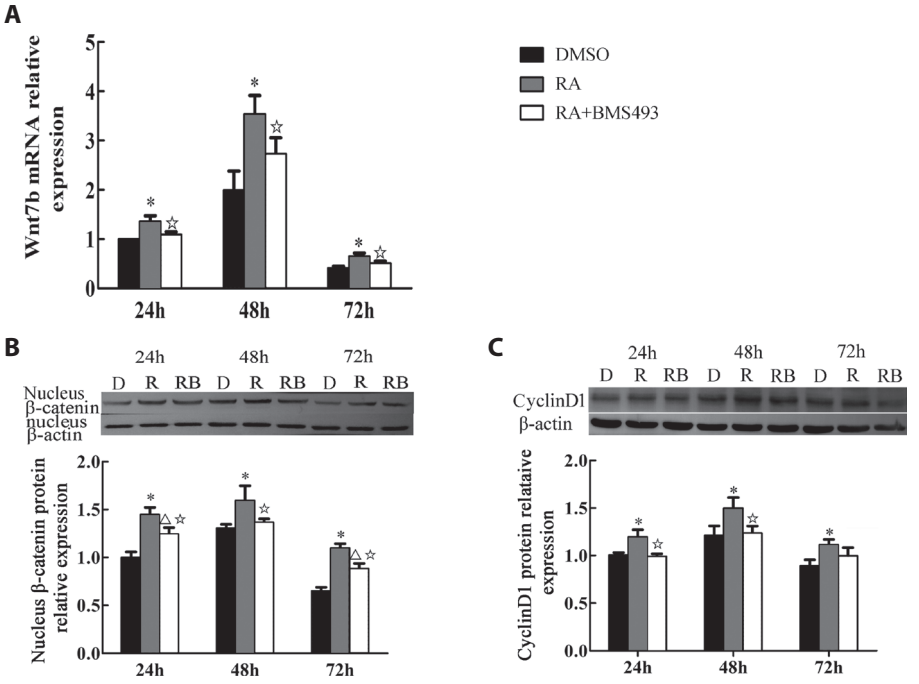

Figure 4. Effects of RA and BMS493 on the activation of Wnt7b/β-catenin pathway. **A.** Relative expression of Wnt7b mRNA was determined by RT-PCR. Expression of nucleus β-catenin protein (**B**) and cyclin D1 protein (**C**) was determined by Western blotting. Letter D, R, RB in the lanes represented the DMSO, RA, and RA+BMS493, respectively. Data were represented mean ± SD. * $p < 0.05$ *vs.* DMSO group; $^{\triangle}$ $p < 0.05$ *vs.* DMSO group; * $p < 0.05$ *vs.* RA group. DMSO, dimethylsulfoxide; RA, retinoic acid; RA+BMS493, 1 μM RA and 10^{-8} M BMS493.

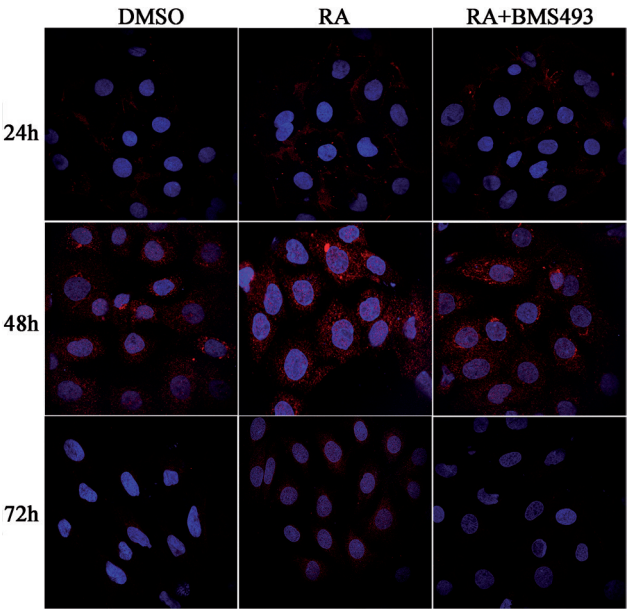

Figure 5. Expression of β-catenin during transdifferentiation of fetal AECIIs. Fetal AECIIs were exposed to DMSO, RA and the combination of RA and BMS493 for 24, 48 and 72 h. Expression of β-catenin was detected with immunofluorescent assay using Laser scanning confocal microscope. DMSO, dimethylsulfoxide; RA, retinoic acid; RA+BMS493, 1 μM RA and 10^{-8} M BMS493.

with RA group at 24 and 48 h ($p < 0.05$, Fig. 4C). No statistical differences were noticed in the expression of cyclin D1 between RA group and RA+BMS493 group at 72 h ($p > 0.05$).

Immunofluorescence staining confirmed that the β-catenin was expressed on cell membrane when fetal AECIIs were cultured for 24 h. Besides, the expression of β-catenin was enhanced in cytoplasm and nucleus at 48 h. In contrast, the expression of β-catenin in whole cells showed decline at 72 h (Fig. 5). These confirmed that Wnt-β-catenin played a crucial role in the transdifferentiation of fetal AECIIs into AECIs mediated by RA.

Discussion

The transdifferentiation of fetal AECIIs into AECI played important roles in the repair of lung injury. Our previous study showed that RA promoted fetal AECII proliferation by enhancing cell viability and inhibiting cell apoptosis (Gao et al. 2015). In this study, we found that RA promoted the fetal transdifferentiation of AECIIs into AECIs through Wnt7b/β-catenin signaling pathway, and such effect was partially reversed by the pan-RA receptor antagonist BMS493.

Fetal rats with an age of 19 days were in a canalicular stage, which were equivalent to the human fetus at the canalicular and saccular phases at a gestational age of 24 weeks and the time to birth (Williams 1977). Therefore, fetal AECIIs were isolated from lung tissues of pregnant rats at a gestational age of 19 days. Its growth and development were more consistent with lung epithelial cells in premature infants. After incubating for 24, 48, and 72 h, the morphology of fetal AECIIs was in an insular pattern to crazy-paving pattern. In addition, expression of AQP5 served as a special marker of AECI showed gradual increase. Expression of AQP5 mRNA and protein was detected in AECIIs cultured in CMEM for 36 h. Expression of SP-C, a special marker for AECII, was down-regulated, however, it began to elevate upon exposing in RA for 72 h. This indicated the transdifferentiation of AECIIs to AECI.

RA is a potential regulator for cell proliferation and differentiation. It has been well acknowledged to play important roles in the transdifferentiation of human bone marrow stromal stem cells (hBMSCs) to neural cells (Haratizadeh et al. 2017). It was reported to promote the differentiation of human fetal liver-derived mesenchymal stem cells (HFMSCs) into cardiomyocyte-like cells in presence of 5-azacytidine (Deng et al. 2016). Obinata and colleagues reported that RA induced transdifferentiation from epidermis to esophageal-like mucosal epithelium in rats, and the expression of Gbx1, TG2/Gh and TGF-β proteins was all up-regulated in that process (Obinata et al. 2011). Morphology of epithelial cells was highly depending on the cell signal transduction and cell communication. Signaling molecules (e.g. retinoid receptors) binded to nuclear receptors have been suggested to play essential roles in the development of various fetal tissues (Cunningham and Duester 2015). As members of steroid/thyroid hormone receptor super-family, retinoid receptors were classified into retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (Das et al. 2014). Each type of receptor was consisted of three known isoforms, designated α, β and γ (di Masi et al. 2015), respectively. Upon binding to their retinoid ligands, they may involve in gene transcription and then mediate cell growth, differentiation and apoptosis. In this study, a pan-RAR antagonist (i.e. BMS493) was utilized to further determinate the effects of RA on fetal AECIIs. However, BMS493 could only induce partial inhibition of the effects of RA. We speculated that RA exerted biological effects through combination with RAR and RXRs. To be specific, Wang and co-workers reported that the vasorelaxant effects of RA were partially inhibited by RAR or RXR antagonist. Nevertheless, the concurrent application of RAR and RXR antagonists completely blocked the vasorelaxant effects of RA (Wang et al. 2013). In future, further studies are needed to illustrate which receptor subtypes are activated in this process.

In the canonical Wnt/β-catenin pathway, Wnt ligands could bind to Frizzled and low-density lipoprotein receptor-related protein-5/6 (LRP5/6) to form a complex (Majidinia et al. 2018). Subsequently, it would activate intracellular Dishevelled protein, which then inhibited glycogen syntheses kinase 3 (GSK3). On this basis, the stabilized β-catenin could prevent the cells against degradation. Afterwards, it would accumulate in cytoplasm and then translocate into the nucleus (di Masi et al. 2015). In the nucleus, β-catenin modulated transcriptional programs through binding with T-cell factor/lymphoid-enhancing factor (TCF/LEF) family, followed by regulating the expression of downstream genes (e.g. *cyclin D1* and *c-myc*) (Wang et al. 2013). In the absence of an appropriate Wnt ligand, β-catenin was phosphorylated by a destructive complex containing Axin, anaphase-promoting complex (APC) and GSK3, and then was degraded by the proteasome (Clevers et al. 2014). Previous studies indicated that Wnt/β-catenin pathway was closely related to the AECIIs differentiation. In an adult rats AECII culture model, miR-375 inhibited AECII transdifferentiation through inhibiting the Wnt/β-catenin signaling pathway. Therefore, inhibition of Wnt/β-catenin pathway enhanced cell death, and the activation of Wnt/β-catenin pathway would accelerated the transdifferentiation of AECIIs (Wang et al. 2013).

To date, the roles of Wnt7b in the transdifferentiation of fetal AECIIs into AECIs are not well defined. As a type of signaling glycopeptide, Wnt7b played a vital role in the development of airways and vascellum by means of paracrine or autocrine, which subsequently coordinated the proliferation between adjacent epithelium and mesenchymal cells (Roker et al. 2017). In $Wnt7b^{-/-}$ mice, there was death induced by severe lung hypoplasia, which was associated with defects in branching morphogenesis, as well as abnormal development in lung epithelium and vessels (Su et al. 2002; Zhang et al. 2012). The bleomycin-induced airway epithelial injury would stimulate Wnt7b expression in ciliated cells, which then acted on the proliferation of parabronchial smooth muscle cells (PSMC) and the secretion of fibroblast growth factor 10 (Fgf10). Afterwards it would stimulate epithelial repair through a transient epithelial to mesenchymal transition (EMT) (Volckaert et al. 2011, 2013).

β-catenin is an important member of canonical Wnt signaling pathway, and is closely related to AECIIs differentiation, alveolarization, tissue remodeling and wound repair (Degryse et al. 2010). Deletion of β-catenin in epithelial cells was closely associated with branching morphogenesis interruption and alveolarization failure (Tanjore et al. 2013). In a previous study, Tanjore and colleagues reported elevation of β-catenin expression in the cytoplasm and nucleus of AECIIs, fibroblasts, endothelial cells and macrophages in lung parenchyma following bleomycin (Zhu et al. 2011). To investigate how RA regulated fetal AECIIs transdifferentiation through the Wnt/β-catenin pathway, we determined the expression of cyclin D1. As an element involving in regulating the activity of β-catenin in nucleus, cyclin D1 participated in the transition from G1 phase to S phase. Specifically, cyclin D1 was highly expressed in the bronchial epithelium and distal alveolar epithelial cells during the canalicular stage. This demonstrated that RA up-regulated the expression of cyclin D1 during fetal AECIIs transdifferentiation. We speculated the effects of RA on β-catenin may be associated with the activation of cyclin D1.

There are some limitations in our study. First, the sample size of the rats was not large, which may bring in a possibility of experimental bias. Second, we could not find out which protein in the Wnt7b/β-catenin signaling pathway was associated with the transdifferentiation of AECIIs into AECIs mediated by RA. In future, more studies are required to further illustrate such mechanism in it.

In summary, RA may promote fetal AECIIs transdifferentiation into AECIs through activating Wnt7b/β-catenin signaling pathway. Such effect was partially prevented by the pan-RAR antagonist BMS493. Our study provides new ideas for the understanding of the pathogenesis, prevention and treatment of pulmonary diseases, and contributed to the pulmonary regeneration in cases of pulmonary injuries, prevention and treatment of neonatal respiratory distress syndrome.

Conflicts of interest. Authors state no conflict of interest.

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Authors' contributions. Conceptualization, XL; methodology, JM; formal analysis, DZ; investigation, ML; data curation, QP; writing – original draft preparation, JM; writing – review and editing, XL; funding acquisition, XL. All authors have read and agreed to the published version of the manuscript.

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