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Original Article

Effect of angiotensin-(1-7) and angiotensin II on the proliferation and activation of human endometrial stromal cells in vitro

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Abstract: Recent studies have shown that angiotensin II (Ang II) or angiotensin-(1-7) [Ang-(1-7)] has effect on the proliferation and activation of a variety of cells, however, the exact mechanisms that the role of Ang II or Ang-(1-7) in human endometrial stromal cell (ESCs) remains elusive. Here we demonstrated that Ang II could promote proliferation and activation of ESCs, up-regulated the expression of a-SMA, TGF-β1 and IGF-I, increased the secretion of extracellular matrix [Type I collagen (Col I) and fibronectin (FN)] of ESCs; Ang-(1-7) could inhibit Ang II induced the proliferation and activation of ESCs, down-regulated the expression of a-SMA, TGF-β1 and IGF-I, decreased the secretion of extracellular matrix (Col I and FN) of ESCs. These findings suggest that Ang-(1-7) can inhibits Ang II induced the proliferation of ESCs, Ang-(1-7) can inhibits the Ang II induced activation of ESCs and decreases secretion of Col I and FN by suppressing TGF-β1 and IGF-I expression.

Keywords: Angiotensin-(1-7), angiotensin II, human endometrial stromal cell, proliferation

Introduction

Intrauterine adhesions (IUA) refers to the whole or part of intrauterine adhesions, including uterine cavity, spondylolysis of uterus and the cervical tube, caused by irregular uterine cavity operation, the induced abortion technique, secondary infection, and so on. Patients often have the symptoms of menstrual reduce, amenorrhea and infertility. Asherman systematically describes the disease for the first time in 1948, so the clinical will be referred to as Asherman's syndrome [1]. Histologically, Asherman syndrome is a condition in which the endometrium becomes fibroses [2]; the endometrial stroma is largely replaced by fibrous tissue [3]; under hysteroscopy can be observed the connective tissue like as pale scar uterus sclerosis island shaped distribute among the normal endometrium, serious adhesion can cause thicknesses of band [4]. The incidence of IUA is 20%-30%. This adverse outcome is mainly manifested in the infertility crowd gradually increasing, so it has become very difficult clinical problem. Along with the development of assisted reproductive technology, many relevant problems are solved, but the endometrium abnormality has become a bottleneck problem in the treatment of infertility, the most failure cases of assisted pregnancy are caused by intrauterine adhesions or fibrosis. Now the mechanism of intrauterine fibrosis is unclear, there is still a lack of effective means of diagnosis and prevention both at home and abroad. Therefore relevant basic and clinical research is badly needed to clarify the mechanism of endometrial fibrosis, so as to develop new prevention and treatment technology.

Ang II as a main member of the renin angiotensin aldosterone system (RAS), has the roles of vasoconstriction, cell proliferation, fibrinolytic abate, elevated blood pressure, and so on, it has been confirmed that Ang II can participate in some organs' fibrosis formation such as heart [5], kidney [6, 7] liver [8, 9] so it plays an

important role in the process of organ fibrosis. Ang II not only has effect on promoting proliferation of various organizations mesenchymal cells, but also can cause the accumulation of extracellular matrix, leading to the occurrence of fibrosis [10-12]. Ang-(1-7), as a new member of RAS, is considered as a endogenous antagonism factor of Ang II, studies show that Ang-(l-7) play important physiological role in dilating blood vessels, lowering blood pressure, inhibiting the proliferation of vascular smooth muscle cells and cardiac fibroblasts [13], inhibiting myocardial cell hypertrophy and reducing ventricular remodeling [14, 15]. With the development of tissue fibrosis, the role of Ang II was repeatedly stated, as physiological antagonism of Ang II in vivo, Ang-(1-7) inhibiting the role of tissue fibrosis is increasingly valued.

IUA may due to the proliferation of ESCs, activation of ESCs and the accumulation of extracellular matrix, or caused by the number of myofibroblast increased, the exact mechanism of IUA is not clear, relevant reports are rare. The effect of Ang-(l-7) and Ang II on human ESCs is not clear now, so this study choose to human ESCs as the research object, to investigate the effect of Ang-(l-7) and Ang II on human ESCs so it can provide a new train of thought and theory basis for intrauterine adhesions and infertility prevention.

Materials and methods

Endometrial specimen collection

Endometrial tissues were obtained by from the hysterectomies of 20 women with uterine fibroids in the Second Hospital of Hebei Medical University from September 2012 to December 2012. This research had been approved by ethics committee of Hebei medical university, informed consent was obtained prior to hysterectomies of women with uterine fibroids. The women were aged from 30 to 45 years old, the menstrual cycle is 24 to 35 days (mean 28 days). All the women had not received any hormonal treatment for at least 3 months prior to hysterectomies. Endometrial tissues were confirmed in hyperplasia period and disease-free by postoperative pathology, endometrial was scraped immediately under aseptic conditions after the uterus in vitro, put into the Dulbecco's Modified Eagle's Media/Nutrient Mixture F-12 (DMEM/F12) (Gibco, USA) medium including 10% fetal calf serum, penicillin and streptomycin (100 mg/mL, Gibco, USA) with ice bath, and then quickly transported to the laboratory within 2 h.

The isolation, purification and culture of ESCs

After several washes with PBS, the tissue was finely minced into 1-2 mm^3 pieces with sterile scissors and incubated with 5 mL of DMEM/F12 containing 0.2% collagenase I (Sigma, USA) in an incubator with atmosphere of 5% CO_2 at 37°C for 60 min. During the process of incubation, the tissue pieces were pipetted gently to disperse the cells. The whole cell suspension was centrifuged at 500× g for 5 min. The supernatant fluid containing ESCs was centrifuged at 1200× g for 5 min. The supernatant fluid was discarded, while the precipitation was re-suspended in culture bottle with 3 mL of complete cell-culture medium [DMEM/F12 + 10% fetal bovine serum (FBS) + 1% penicillin and streptomycin]. The ESCs attaching to a culture bottle were washed several times with serum-free DMEM/F12 to remove red blood cells.

Trypan blue exclusion assay was performed to assess the rate of living cell. Then, 1 ml cell suspension (1×10^5 cells/ml) were planted into a six-well plate containing coverslips and cultured in atmosphere of 5% CO_2 at 37°C for identification and purity assessment of cell.

Morphological observation of ESCs

ESCs were cultured with above medium for 0, 5 days respectively and were stained with hematoxylin and eosin (H&E), Morphology and structure of endometrial cells was observed with an inverted phase-contrast microscope and a light microscope.

Identification of ESCs

To identify ESCs and assess their purities, immunocytochemical staining was performed. Using PBS as a negative control instead of primary antibody. Cells which were cultured on the cover slides were fixed with 4% paraformaldehyde and treated with 0.25% Triton X-100. After blocking with 5% normal goat serum for 20 minutes at 37°C, the rabbit anti-human primary antibody vimentin (1:100) and cytokeratin (1:100) (Zhongshan Bio-Tech, Beijing, China)

were incubated with the cells at 4°C overnight. Then the cells were incubated with goat anti-rabbit IgG (1:100; Boster Corporation, Wuhan, China) for 20 minutes at 37°C and stained with DAB (5 mg/mL; Sigma) for 5 minutes at room temperature. The specimens were washed with PBS for 5 minutes three times and observed under light microscopy.

Experimental groups

ESCs were divided into four groups according to the different intervention factors. Control group: serum-free DMEM/F12; Ang II group: Ang II and serum-free DMEM/F12; Ang-(1-7) group: Ang-(1-7) and serum-free DMEM/F12; Ang II + Ang-(1-7) group: Ang II + Ang-(1-7) and serum-free DMEM/F12, the final concentration of Ang-(1-7) and Ang II was 10^{-5} mol/L and 10^{-6} mol/L respectively.

Cell proliferation assay

Cell proliferation was determined by MTT. ESCs (4×10^4/ml) were seeded in 96-well plates and cultured with serum-free DMEM/F12 for 24 h, so that it could make them growth synchronization. They were divided into above four groups, each group set up six holes, and cultured for 24 h, 48 h and 72 h. Thereafter, MTT (5 mg/ml) was added to each well, and plates were incubated at 37°C for 2 h. The medium was then replaced with 150 μl DMSO and agitated for 10 min. Absorbance at 560 nm was measured using a microplate reader (Packard, Meriden, CT, USA).

Immunocytochemistry analysis

The above four groups cells were cultured for 72 h. Using PBS instead of primary antibody as the negative control. Cells cultured on the cover slides were fixed with 4% paraformaldehyde and treated with 0.25% Triton X-100. After blocking with 5% normal goat serum for 20 minutes at 37°C, the rabbit anti-human monoclonal antibodies (Zhongshan Bio-Tech, Beijing, China) were incubated with the cells at 4°C overnight. Then the cells were incubated with goat anti-rabbit IgG (1:100; Boster Corporation, Wuhan, China) for 20 minutes at 37°C and stained with DAB (5 mg/mL; Sigma) for 5 minutes at room temperature. The specimens were washed with PBS for 5 minutes three times and observed under light microscopy.

ELISA

The above four groups cells were cultured for 72 h, absorbing the culture supernatant fluid, according to manual operation of ELISA kit (R&D, USA), A value of each hole was read on the full spectrum of the spectrophotometer of 450 nm wavelengths to detect the content of Col I, FN, TGF-β1 and IGF-I respectively. Each group was set up six holes, with its average as statistical data.

Western bolt analysis

The above four groups cells grown in 10 cm dishes were cultured for 72 h, cells were washed with PBS and lysed with lysis buffer (pH 7.4; 1 M Tris-HCl, 1% Triton X-100, sodium deoxy cholate, 10% SDS). Solubilized proteins were centrifuged at 14,000 g in 4°C for 30 min. Extracted proteins were quantified by Coomassie Protein Assay Reagent (Sigma-Aldrich). Immunoblot detections for a-SMA, TGF-β1 and IGF-I were performed. In brief, 30 μg of isolated protein was electrophoresed on 8% sodium dodecyl sulphate polyacrylamide gel, and transferred (100 V for 1.5 h) onto a PVDF membrane. The membranes were treated with blocking solution (TBS pH 7.2, 0.1% Tween, 5% milk) for 1 h, incubated for 12 h at 4°C with rabbit anti-human monoclonal a-SMA, TGF-β1, IGF-I, Col I and FN antibodies (Invitrogen, USA), diluted 1/1000 in TBS (pH 7.2) 0.1% Tween. After 4 washings for 15 min with TBS (pH 7.2) 0.1% Tween, the membranes were incubated with the goat anti-rabbit IgG antibody horseradish peroxidase conjugate (Invitrogen, USA, diluted 1/1000 in TBS pH 7.2, 0.1% Tween) for 1 h. They were then washed 4 times for 15 min with TBS (pH 7.2), treated with enhanced chemiluminescent method according to the instruction of ECL-detection kit, exposed to Kodak X-ray film for 0.5-20 min as necessary to detect the signals. The relative intensity of immunoreactive band exposed on the films was quantified by a computer-assisted densitometry program (Smart view, Life Science Research Products and System Engineering). Proteins expression was quantified by comparison with internal-control GAPDH.

RNA extraction and reverse transcription

Total RNA was extracted from the above four groups cells that they were cultured for 72 h

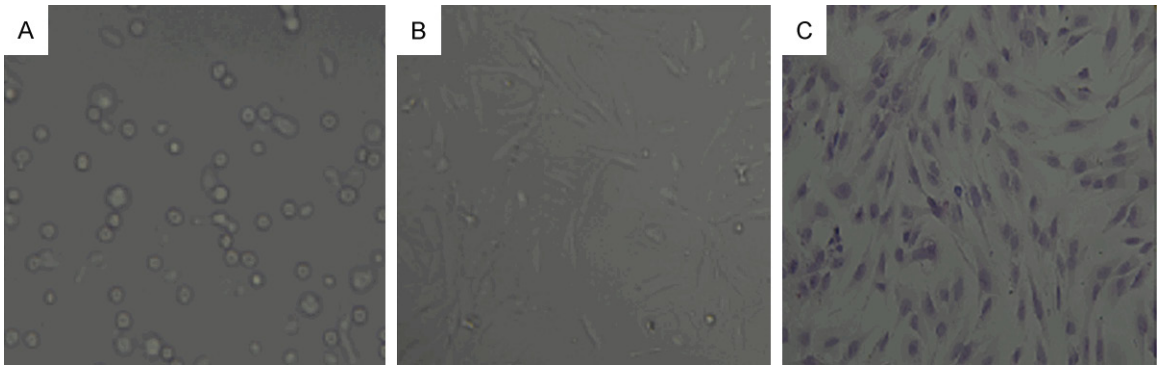

Figure 1. The observation of endometrial stromal cells under inverted phase-contrast microscope and after H&E staining (×400). A. The observation of ESCs remaining round for just isolated under inverted phase-contrast microscope. B. The observation of ESCs growing as spindle and polygonal-shaped cells with long cytoplasmic processes for 5 days under inverted phase-contrast microscope. C. The observation of ESCs for 5 days stained by H&E staining under light microscope.

with the TRIzol Reagent (Invitrogen, USA) according to the manufacturer's protocol, and the RNA was dissolved in RNase-free water. The integrity of the RNA was assessed by ethidium bromide agarose gel electrophoresis, and the quantity of RNA was determined by relative absorbance at 260 nm versus 280 nm. Complementary DNA (cDNA) was synthesized in a volume of 10 μl with a cDNA synthesis kit (Takara, Japan) according to the manufacturer's protocol. The generated cDNAs were stored at 20°C.

Primer preparation

For polymerase chain reaction amplification, primers (Invitrogen, Carlsbad, CA, USA) were derived from the Genbank database. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene.

SMA, 5'-GATGGGCATCTATCAGATAC-3' and 5'-AAGCATTTCTGATGGTGATG-3'; TGF-β1, 5'-ACCTGAACCCGTGTTGCTCT-3' and 5'-CTAAGGCGAAAGCCCTCAAT-3'; IGF-I, 5'-CCTCCTCGCATCTCTTCTACCTG-3' and 5'-CTGCTGGAGCCATACCCTGTG-3'; Col1, 5'-AGGGCCAAGACGAAGACATC-3' and 5'-GTCGGTGGGTGACTCTGAGC-3'; FN, 5'-TAGCCCTGTCCAGGAGTTCA-3' and 5'-CTGCAAGCCTTCAATAGTCA-3'; GAPDH, 5'-TGCACCACCAACTGCTTAGC-3' and 5'-GGCATGGACTGTGGTCATGAG-3'.

Real-time polymerase chain reaction

The real-time PCR reactions were performed using Brilliant SYBR Green QRT-PCR Master Mixture according to the manufacturer's instructions (Invitrogen, USA). RNA for a-SMA, TGF-β1 or IGF-I were amplified using ABI Prism 7500 Sequence Detection System (Applied Biosystems, Forster City, CA, USA). The primers (Invitrogen, Carlsbad, CA, USA) are shown as above. For all real-time PCR studies, synthesizing by PCR procedure was performed with the following time courses: 95°C for 10 min, 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 32 min for amplification. The amplified products were subjected to a stepwise increase in temperature from 60°C to 95°C and dissociation curves were constructed.

Target mRNA was quantified by measuring the threshold cycle and reading against a calibration curve. The relative amount of each mRNA was normalized to the housekeeping gene, GAPDH mRNA. Results were analyzed using the relative standard curve method of analysis/ΔCt method of analysis.

Statistical analysis

All data presented as bar graphs were the means ± standard deviation (SD) of six independent experiments (the sample of each experiment comes from different subjects) and analyzed with SPSS 15.0 software. Statistical analysis was performed by one-way analysis of variance (ANOVA). The results were considered statistically significant at $P < 0.05$.

Results

Morphological observation of ESCs

The purity of ESCs can reach 95% by the use of differential centrifugation method, using above culture method, ESCs can preach 4-5 genera-

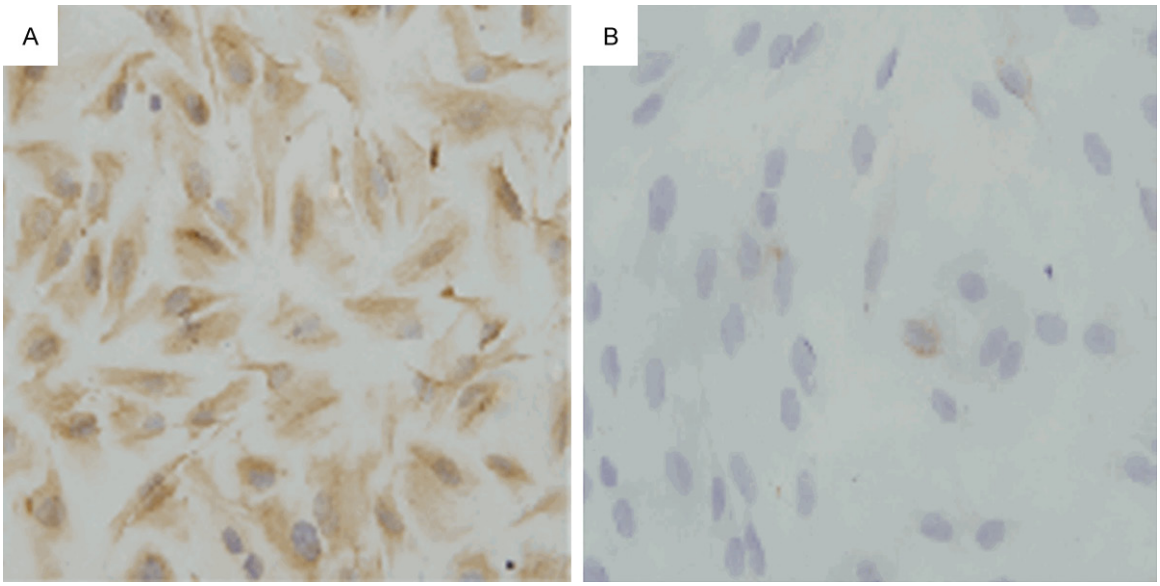

Figure 2. The identification of ESCs by immunocytochemical staining under light microscope (×400). ESCs were cultured for 5 day. A. The stromal cells were positively stained by vimentin. B. The stromal cells were negatively stained by Cytokeratin.

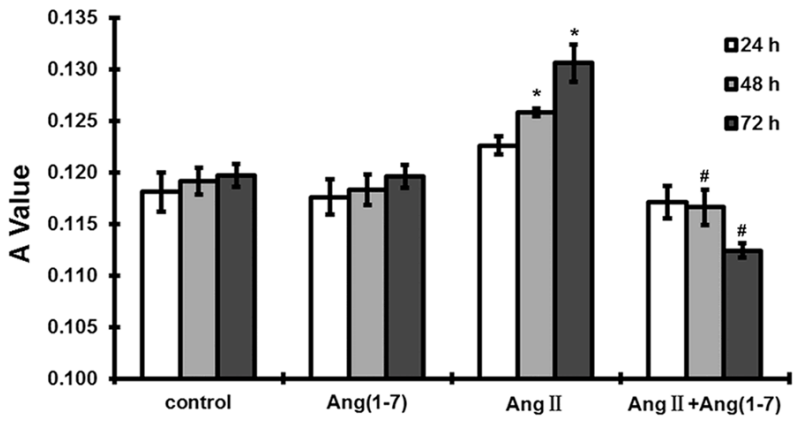

Figure 3. The proliferation of ESCs was evaluated by MTT. ESCs were incubated with different intervention factors at the indicated times (48 h-72 h). Control: ESCs incubated with serum-free DMEM/F12; Ang II: ESCs incubated with 10^{-6} mol/L Ang II and serum-free DMEM/F12; Ang-(1-7): ESCs incubated with 10^{-5} mol/L Ang-(1-7) and serum-free DMEM/F12; Ang II + Ang-(1-7): ESCs incubated with 10^{-6} mol/L Ang II + 10^{-5} mol/L Ang-(1-7) and serum-free DMEM/F12. Values are expressed as means ± SD, n = 6 (the repeats come from different subjects). *P < 0.05 versus control group, #P < 0.05 versus the Ang II group.

tions; The ESCs which were just isolated mostly remained round (**Figure 1A**), then they grew as spindle and polygonal-shaped cells with long cytoplasmic processes, they usually reached confluence after 5 days culture, displaying a single-cell monolayer growth pattern (**Figure 1B**). The ESCs were stained by H&E staining after 5 days cultured (**Figure 1C**). Trypan blue staining showed that the viability of ESCs was > 96%.

Identification of ESCs

The ESCs were detected by immunocytochemical staining. The cytoplasm of positive ESCs was stained claybank. The were positive for vimentin and negative for Cytokeratin (**Figure 2B**).

Ang-(1-7) inhibit Ang II-induced cell proliferation

After cells were cultured for 24 h, 48 h and 72 h, the number changes of ESCs were observed by the detecting purple crystal of each culture hole absorbance value (A) in 550 nm, the result showed that compared with control group, Ang II could promote the ESCs proliferation significantly (P < 0.05), while Ang-(1-7) could inhibit the ESCs proliferation significantly in the manner of time dependence (P < 0.05), the Ang II + Ang-(1-7) group has the role of inhibition significantly compared with Ang II group (**Figure 3**).

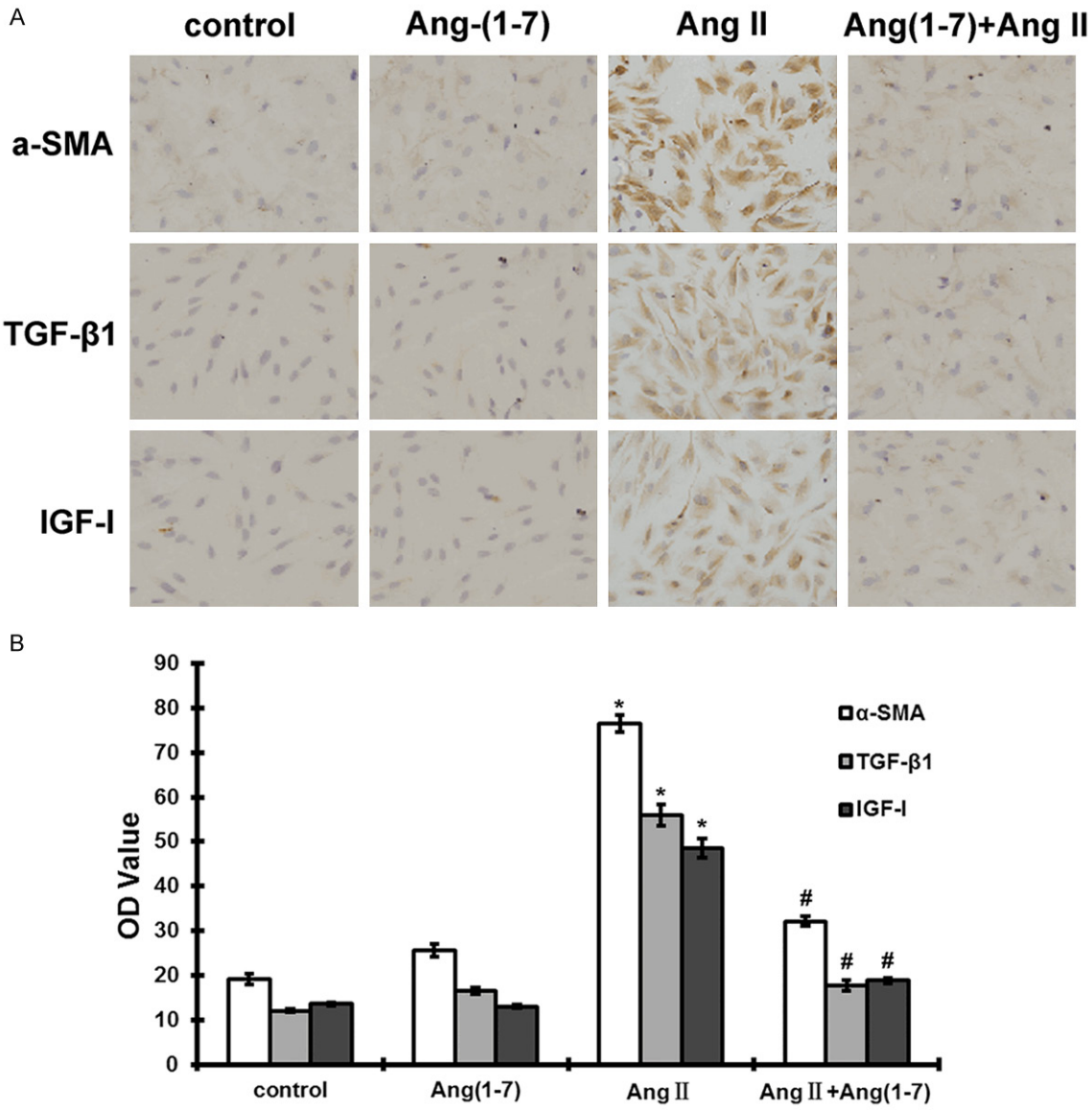

Figure 4. The protein expression of a-SMA, TGF-β1 and IGF-I in the ESCs. ESCs were incubated with different intervention factors for 72 h. (A) Immunocytochemistry staining showed Brownish-yellow granules in the cytoplasm of ESCs (at 400× magnification). (B) The levels of a-SMA, TGF-β1 and IGF-I in the ESCs were quantified by the average integral absorbance (A) values. Control: ESCs incubated with serum-free DMEM/F12; Ang II: ESCs incubated with 10^{-6} mol/L Ang II and serum-free DMEM/F12; Ang-(1-7): ESCs incubated with 10^{-5} mol/L Ang-(1-7) and serum-free DMEM/F12; Ang II + Ang-(1-7): ESCs incubated with 10^{-6} mol/L Ang II + 10^{-5} mol/L Ang-(1-7) and serum-free DMEM/F12. Values are expressed as means ± SD, n = 6 (the repeats come from different subjects). *P < 0.05 versus control group, #P < 0.05 versus the Ang II group.

Ang-(1-7) inhibit Ang II-induced cell activation of ESCs

The result of immunocytochemistry staining (**Figure 4A** and **4B**) and Western blot (**Figure 5A** and **5B**) showed: Compared with the control group, the protein expression of α-SMA, TGF-β1 and IGF-I in Ang-(1-7) group has no significant change, and significantly increased in Ang II group; Compared with the Ang II group, the protein expression of them in Ang II + Ang-(1-7) group decreased significantly. The results of ELISA (**Figure 5C**) showed: Compared with the control group, the protein content of TGF-β1, IGF-I, Col I and FN in the cultured supernatants in Ang-(1-7) group has no significant change, and increased significantly in Ang II group. Compared with the Ang II group, the protein

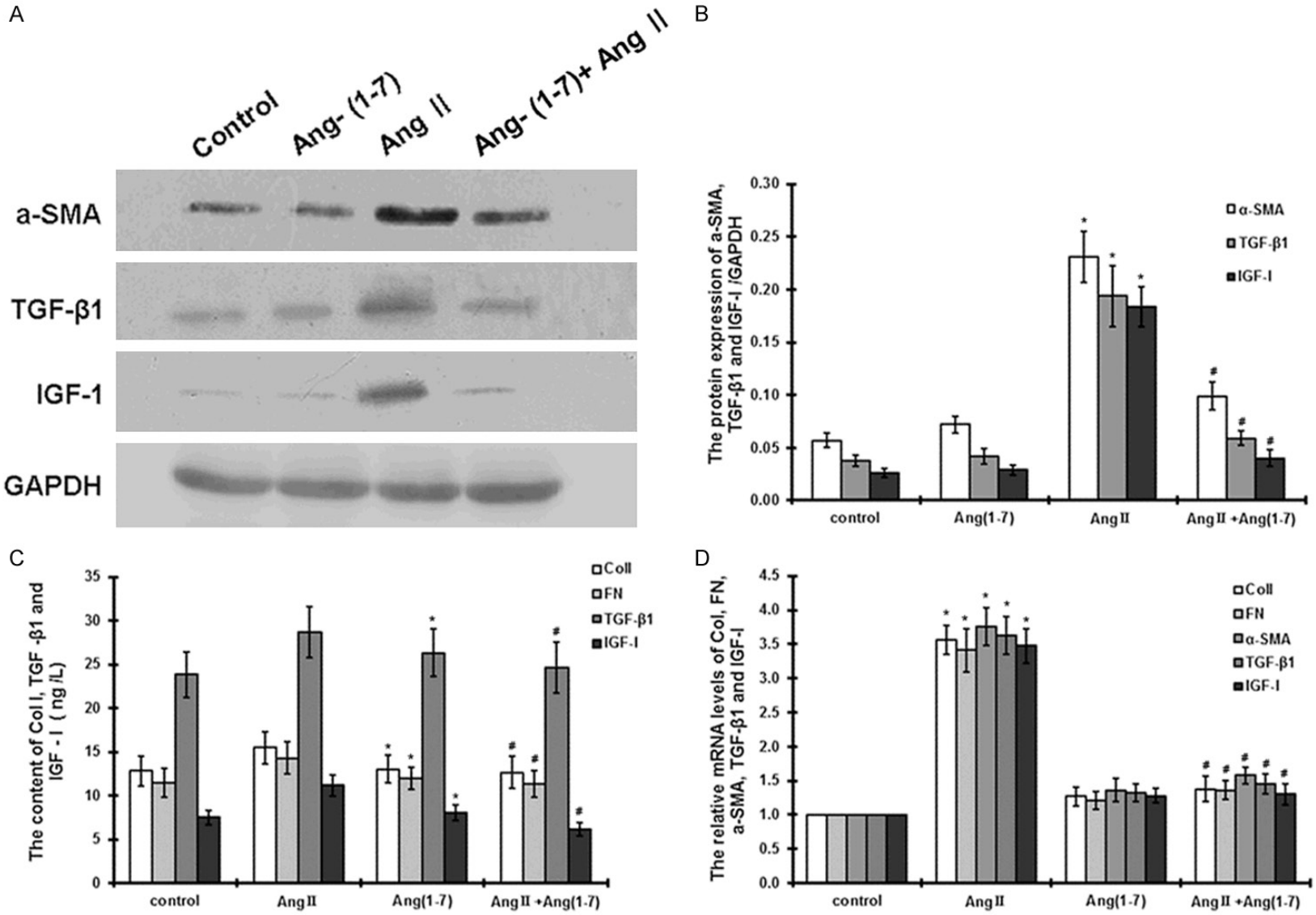

Figure 5. Ang-(1-7) inhibit Ang II -induced the activation of ESCs. ESCs were incubated with different intervention factors for 72 h. A. The protein expression of a-SMA, TGF-β1 and IGF-I was analyzed by Western blot. B. The protein levels of a-SMA, TGF-β1 and IGF-I were quantified by densitometry. C. The protein content of Col I, FN, TGF-β1 and IGF-I in the cultured supernatants of ESCs was detected by ELISA. D. The mRNA levels of Col I, FN, α-SMA, TGF-β1 and IGF-I were analyzed by

real-time PCR. Control: ESCs incubated with serum-free DMEM/F12; Ang II: ESCs incubated with 10^{-6} mol/L Ang II and serum-free DMEM/F12; Ang-(1-7): ESCs incubated with 10^{-5} mol/L Ang-(1-7) and serum-free DMEM/F12; Ang II + Ang-(1-7): ESCs incubated with 10^{-6} mol/L Ang II + 10^{-5} mol/L Ang-(1-7) and serum-free DMEM/F12. Values are expressed as means ± SD, n = 6 (the repeats come from different subjects). *P < 0.05 versus control group, #P < 0.05 versus the Ang II group.

content of them in Ang II + Ang-(1-7) group was decreased significantly. The result of real time PCR (**Figure 5D**) showed: Compared with the control group, the mRNA levels of α-SMA, TGF-β1, IGF-I, Col I and FN in Ang-(1-7) group has no significant change, and significantly increased in Ang II group; Compared with the Ang II group, the mRNA levels of them in Ang II + Ang-(1-7) group decreased significantly.

Discussion

Uterine adhesions is the pathological process caused by many factors, it is characterized by proliferation of endometrial stromal cells and excessive accumulation of extracellular matrix. Its mechanism may include: ① Under the stimulus of the inflammation, immune response and toxin, etc, endometrial stromal cells and endometrial epithelial cells are activated, proliferate and differentiate into myofibroblast; ② Extracellular matrix increase and degradation reduce; ③ There are the participation of some vascular active substances (Ang II), growth factor (TGF-β1) and cytokine.

RAS is important endocrine regulation system in the body, uterine cavity operation is not standard, induced abortion operation and secondary infection, etc, all can cause uterine local RAS activation and a variety of pathological and physiological changes occur, so endometrial fibrosis may closely related to endometrial local RAS. Ang II is the main member of the RAS, as a growth factor and fibrosis factors, it can directly promote a variety of cytokines generate, cell hyperplasia, hypertrophy and matrix protein accumulation [16]. Ang II can induce cardiomyocyte hypertrophy and myocardial interstitial fibrosis, in vitro the myocardial fibroblasts of rat are isolated and stimulated with Ang II, the results show that the Ang II can make cardiac fibroblast activate, and promote the accumulation of extracellular matrix proteins, such as collagen protein and FN, which can lead to cardiac remodeling and myocardial fibrosis [5]. Ang II can also promote renal interstitial fibrosis through various channels, it can promote the proliferation of kidney interstitial fibroblasts, promote the protein expression of myofibroblast specific cytokines a-SMA in renal interstitial and accumulation of extracellular matrix. Myofibroblast as effector cells of the tissue fibrosis, compared with the ordinary fibroblasts, has stronger ability of proliferation and the secretion of ECM. Johnson RJ et al. [10], stimulate renal tubular epithelial cells produce TGF-β1 [12, 17]; The results of this study show that in vitro Ang II can promote proliferation and activation of ESCs, expression of myofibroblast specific protein a-SMA, and change its secretion, make the synthesis of Col I, FN, TGF-β1 and IGF-I increased, consistent with the above results. Therefore, Ang II may be relevant to the occurrence of endometrial fibrosis.

Ang-(1-7) is the endogenous factor of antagonism Ang II, can play a role of its anti fibrosis by inhibiting cell proliferation and extracellular matrix accumulation. Tallant et al. [14] study also show that Ang-(1-7) can inhibit fetal bovine serum and endothelin-1 induced the proliferation of newborn rat cardiac fibroblasts and the myocyte hypertrophy. Liu et al. [15] also find that Ang-(1-7) can inhibit Ang II on the proliferation of rat glomerular mesangial cell in the manner of dose dependence. Recently, the study also confirmed that Ang-(I-7) can inhibit Ang II on myocardial hypertrophy and fibrosis, thus improving myocardial remodeling, and can also reduce the level of TGF-β1 in plasma [18]. Burns, et al. [7] further confirmed Ang-(1-7) can inhibit Ang II induced transdifferentiation of renal tubular epithelial cells and the expression of extracellular matrix proteins with joint intervention of Ang II and Ang-(1-7) in rat renal tubular epithelial. The results of this study show that in vitro Ang-(1-7) can inhibit Ang II-induced the proliferation and activation of ESCs, decrease the expression of myofibroblast specific protein a-SMA, and change its secretion, make the synthesis of Col I, FN, TGF-β1 and IGF-I decreased, as result as the above results. Therefore, Ang-(1-7) may play an important role in inhibiting endometrial fibrosis.

Cytokines network plays a key role in the process of organ fibrosis formation, the TGF-β1

which are known is one of the most important cytokines of causing fibrosis, its play the role of promoting the synthesis of extracellular matrix proteins and inhibiting of its degradation, make the synthesis and degradation of ECM out of balance, so that the extracellular matrix deposition increased, eventually leading to tissue fibrosis. Liu C et al. [19] culturing NRK-49F cells in vitro, find that TGF-β1 can make NRK-49F cell activated and increase the expression level of a-SMA. Daian T et al. [20] reported that IGF-1 is mainly one of cytokines that promote fiber formation, it can strongly stimulate synthetic metabolism of cell, enhance obviously the synthesis of collagen protein, FN, and so on, secrete extracellular matrix increase, and eventually lead to fibrosis formation. Research has shown that IGF-1 has the effect on promoting fibrosis in pathogenesis of liver fibrosis, pulmonary fibrosis, and renal fibrosis [21, 22]. A large number of studies have shown that TGF-β1 and IGF-I can induce various cells to transform the fibroblasts, and change its function of secretion, accelerate organ fibrosis. Zheng et al. [23] cultured renal tubular epithelial cells with the intervention of IGF-I in vitro and confirmed that IGF-I also has the effects similar to that of TGF-β1, IGF-I can induce renal tubular epithelial cell trans-differentiation in the manner of dose dependence, the expression of a-SMA and secretion of extracellular matrix of FN, Col I increased. This study found that when Ang II promotes ESCs activation and increases the collagen secretion, the expression of cell factor TGF-β1 and IGF-I also increase. This study also observed that when Ang-(1-7) antagonism Ang II induced the role of above, the expression of TGF-β1 and IGF-I also decrease. Therefore, We speculate that Ang II inducing ESCs trans-differentiate into fibroblasts, expressing a-SMA, secreting Col I and FN increase might be related to the TGF-β1 and IGF-I increase, while Ang-(1-7) antagonism Ang II by down-regulating the expression of TGF-β1 and IGF-I. However, how do Ang-(1-7) and Ang II act on the ESCs? The exact mechanism is unclear, still need to further experiments to study.

In summary, Ang-(1-7) can inhibits Ang II induced the proliferation of ESCs, Ang-(1-7) can inhibits the Ang II induced activation of ESCs and decreases secretion of Col I and FN by suppressing TGF-β1 and IGF-I expression. It will have important guiding significance for the clinical prevention and treatment of intrauterine adhesions and infertility prevention.

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Disclosure of conflict of interest

None.

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