Clinical, Cosmetic and Investigational Dermatology

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Angiotensin (1-7) Inhibits Transforming Growth Factor-Β1–Induced Epithelial-Mesenchymal Transition of Human Keratinocyte Hacat Cells in vitro

Yueda Jihu[1,2,*], Ruobing Leng[1,*], Mengchang Liu[1,2], Hongjing Ren[1,2], Defu Xie[1,2], Chong Yao[1,2], Hong Yan[1,2]

[1]Clinical College of Medicine, Southwest Medical University, Lu zhou, People's Republic of China; [2]Department of Plastic and Burn Surgery, the Affiliated Hospital of Southwest Medical University, Lu zhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Hong Yan, Email zxsswk@163.com

Introduction: Angiotensin (1–7) (Ang-(1-7)) is an emerging component of the renin-angiotensin system (RAS) with effective anti-fibrosis properties and has been shown to interfere with epithelial-mesenchymal transition (EMT) by numerous studies. In recent years, EMT has been proposed as a new therapeutic target for skin fibrotic diseases such as keloids. However, the effect of Ang-(1-7) on EMT in skin is still unclear. Hence, the purpose of this study was to explore the effect of Ang-(1-7) on Transforming growth factor-β1 (TGF-β1)–induced EMT of human immortalized keratinocytes HaCaT in vitro.

Methods: The study involved the use of the human immortalized keratinocyte cell line (HaCaT). The cells were cultured in high-glucose DMEM medium with 10% fetal bovine serum and 1% penicillin-streptomycin. Four groups were created for experimentation: control group (Group C), TGF-β1-treated group (Group T), Ang-(1-7)-treated group (Group A), and a group treated with both TGF-β1 and Ang-(1-7) (Group A + T). Various assays were conducted, including a cell proliferation assay using CCK-8 solution, a scratch wound healing assay to evaluate cell migration, and Western blotting to detect protein expressions related to cell characteristics. Additionally, quantitative real-time polymerase chain reaction (PCR) was performed to analyze epithelial-mesenchymal transition (EMT) related gene expression levels. The study aimed to investigate the effects of TGF-β1 and Ang-(1-7) on HaCaT cells.

Results: We found that Ang-(1-7) not only reduced the migration of HaCaT cells induced by TGF-β1 in vitro but also reduced the expression of α-SMA and vimentin, and restored the protein expression of E-cadherin and claudin-1. Mechanistically, Ang-(1-7) inhibits the phosphorylation levels of Smad2 and Smad3 in the TGF-β1 canonical pathway, and suppresses the expression of EMT-related transcription factors (EMT-TFs) such as SNAI2, TWIST1, and ZEB1.

Discussion: Taken together, our findings suggest that Ang-(1-7) inhibits TGF-β1–induced EMT in HaCaT cells in vitro by disrupting the TGF-β1-Smad canonical signaling pathway. These results may be helpful in the treatment of EMT in skin fibrotic diseases such as keloids.

Keywords: angiotensin (1-7), epithelial-mesenchymal transition, transforming growth factor-β, keratinocytes

Introduction

Keloids are pathological scars formed by abnormal and excessive deposition of the extracellular matrix (ECM) during wound healing due to various reasons. In addition to manifesting as tumors or cords above the skin surface and localized pain, itching, burning, and other discomfort, it can also invade the surrounding skin or mucous membrane tissues,[1,2] and even spontaneously form on healthy skin,[3] which considerably affects the patients' physical health and even causes

Received: 22 September 2023
Accepted: 24 March 2024
Published: 8 May 2024

psychological disorders.[3] Thus far, the main prevention and treatment methods for keloids have not achieved satisfactory results, and improved treatment options are thus urgently needed.[4]

At present, the research on the pathogenesis of keloids is not comprehensive. Although many previous studies[5–7] have mainly focused on fibroblasts, an increasing number of studies[8–10] have found that epithelial-mesenchymal transition (EMT) may play an important role in the development of keloids.

EMT is the key cellular process during embryogenesis, wound healing, fibrosis, and tumor progression.[11] EMT is associated with loss of apical-basolateral polarity of epithelial cells, decreased expression of epithelial markers, increased expression of mesenchymal markers, and acquisition of invasiveness and migration.[12] The involvement of EMT in keloid formation has been extensively studied.[13–15] Do et al suggested that interleukin 18, its receptor, and its antagonists play an important role in the pathogenesis of keloids by inducing EMT,[16] while a study[8] has showed that the expression of vimentin in keloid epidermis was higher than that in normal skin epidermis, suggesting that EMT may be involved in the production of keloids.

Several growth factors including TGF-β and TNF-α are considered the main pro-fibrotic cytokines involved in EMT. Among them, transforming growth factor β1 (TGF-β1), a multifunctional pro-fibrotic cytokine, is one of the isoforms of TGF-β and the main inducer of EMT.[17,18] TGF-β–induced EMT plays an important role in tissue repair and fibrosis because high levels of TGF-β are associated with EMT changes in tissue fibrosis.[19] Furthermore, TGF-β–induced EMT is closely related to pro-fibrotic events in tissue fibrosis such as inflammation, epithelial cell migration, fibroblast recruitment, and ECM deposition.[20] In skin wounds, TGF-β is not only an inducer of keratinocyte migration but also a potent chemoattractant for endothelial cells and fibroblasts. In terms of fibrosis signaling pathways, the TGF-β1/Smad pathway is considered very critical.[21] This is because the TGF-β1 signaling pathway is overactive in keloid formation,[22] and enhanced TGF-β1 signaling and Smad3 phosphorylation levels and EMT-related markers have been found in keloid tissue.[23] A study on the contribution of EMT to renal fibrosis showed that epithelial cells undergoing EMT activate other genetic programs that lead to tissue fibrosis,[24] which again underscores the relevance of EMT to fibrotic diseases. Therefore, if the EMT process mediated by TGF-β1 can be effectively inhibited, it will be helpful in the treatment of keloids.

The components of the renin-angiotensin system (RAS) are expressed in the skin and function independently of the plasma RAS[25] and play regulatory roles in epidermal proliferation, wound healing, scar formation, skin thermal adaptation, and aging. RAS consists of two opposing axes. The classical axis produces vasoconstriction, pro-proliferation, pro-inflammatory and pro-fibrotic effects.[26] While the angiotensin-converting enzyme 2 (ACE2)/angiotensin(1–7) [Ang-(1-7)]/Mas receptor axis mainly transmits Ang-(1-7) vasodilator, anti- Proliferation, anti-inflammatory and anti-fibrotic effects.[27] Activation of this axis can reduce inflammatory cell function and fibrogenesis in various human disease models.[28] Many studies have demonstrated the anti-inflammatory effect of Ang-(1-7), eg, systemic administration of Ang-(1-7) effectively attenuates multi-organ fibrosis in bleomycin (BLM)-induced recessive dystrophic epidermolysis bullosa (RDEB) mice and increases the survival rate;[29] Ang-(1-7) inhibits the activated NLRP3 inflammasome and attenuates Angiotensin II–induced hepatocyte EMT;[30] and Ang-(1-7) inhibits TGF-β1/Smad2/3 signaling pathway, reduces the expression of Col-I, and inhibits fibroblast-myofibroblast transformation.[31] Therefore, the use of Ang-(1-7) in the treatment of chronic fibrotic diseases may achieve promising results.

The effect of Ang-(1–7) on TGF-β1–induced EMT of epithelial cells in skin is still unclear. Therefore, the purpose of this study was to investigate whether Ang-(1-7) has an effect on the EMT process in the human immortalized keratinocyte cell line HaCaT by affecting the TGF-β1 signaling pathway.

Materials and Methods

Cell Culture and Reagents

Human immortalized keratinocyte cell line (HaCaT), purchased from Meisen Cell Technology Co., Ltd. (Zhejiang, China), was maintained in high-glucose DMEM medium (Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin at 37°C in a 5% CO2 incubator.

Cells were divided into four groups: Group C: control group; Group T: cells treated with TGF-β1(10 ng/mL; MedChemExpress, USA) for 72 h; Group A: cells treated with Ang-(1-7) (1 μM, AbMole Bioscience, USA) for 72 h; Group A + T: Cells treated with TGF-β1 in the presence of Ang-(1-7) for 72 h.

Cell Proliferation Assay

HaCaT cells were seeded in 96-well plates at a density of 2×10^3 cells/well and treated with TGF-β1 (10 ng/mL), either with or without Ang-(1-7) (1 μM) for 48 h. Then, 10 μL of CCK-8 solution were added to each well and incubated at 37°C for 2 h, according to the manufacturer's protocol (Beyotime Institute of Biotechnology). The optical density of the cells was measured at a wavelength of 450 nm using a microplate reader (Omega Bio-Tek, Inc.).

Scratch Wound Healing Assay

HaCaT cells were seeded and grown in 6-well plates at a density of 1×10^4 cells/well until they were confluent. Then, the cells were cultured overnight in culture medium containing 2% fetal bovine serum before performing the scratch test to eliminate the interference caused by proliferation. The adherent cells were scratched horizontally using the tip of a 10 μL pipette, and then washed twice with phosphate buffered saline. The scratch was observed under a microscope (100×) and images were captured to record the position of the cells. The cells were treated with TGF-β1 (10 ng/mL), either with or without Ang-(1-7) (1 μM) for 36 h, and the position of the cells was again recorded. The area of the cell migration was measured by ImageJ 2.0 (Wayne Rasband, National Institute of Health, Bethesda, MD, USA) to evaluate the migration rate. The cell migration rate was calculated as a percentage of the cell migration area compared to the initial wound area.

Western Blotting

HaCaT cells were seeded in 6-well plates, treated with TGF-β1 (10 ng/mL), either with or without Ang-(1-7) (1 μM) for 72 h, following which the expression of E-cadherin/claudin-1, α-SMA/vimentin, Smad2/3, and pSmad2/3 were detected. Intracellular proteins were extracted with RIPA lysis buffer and quantified using the BCA protein quantification kit (Solarbio Science & Technology, Co., Ltd., Beijing, China). Equal concentrations of protein samples were electrophoresed on SDS-PAGE and transferred to PVDF membranes (Millipore, Billerica, MA, USA). Then, the membrane was blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 h at room temperature, and the corresponding primary antibodies (α-SMA, 1:2000; anti-vimentin, 1:1000; anti-E-cadherin, 1:1000; anti-claudin-1, 1:1000; Smad2, 1:1000; p-Smad2, 1:1000; Smad3, 1:1000; and p-Smad3, 1:1000) (all AbMole Bioscience) were incubated overnight at 4°C. PVDF membranes were washed four times with TBST the next day and incubated with the corresponding rabbit antibody (1:3000; AbMole Bioscience) or mouse antibody (1:3000; AbMole Bioscience) for 1 h at room temperature. Protein content was detected with an imaging system (Liuyi Biotechnology Co., Ltd., Beijing, China). Band gray values were quantified and normalized to β-actin values using ImageJ software.

Quantitative Real-Time Polymerase Chain Reaction (PCR)

Cells were seeded in 6-well plates and treated with TGF-β1 (10 ng/mL) with or without Ang-(1-7) (1 μM) for 72 h (There were 5*106 cells in each 6-well plate). Cell pellets were washed with PBS buffer, and resuspended in Trizol, which is then frozen in liquid nitrogen before homogenization.Total RNA was extracted using chloroform and precipitated with isopropanol. cDNA was then synthesized by a reverse transcription kit (Roche Applied Science, UK). RT-qPCR was carried out using SYBR GREEN PCR Master Mix (Roche Diagnostics, Germany). Relative gene expression levels were calculated using the 2-DDCt method. The primer sequences used are shown in Table 1.

Statistical Analyses

The experimental data was analyzed by Graph Pad Prism 9 (Graph Pad Inc., La Jolla, CA, USA), and the comparison between different groups was performed by one-way analysis of variance (ANOVA), and the data were expressed as the mean ± SEM. $P<0.05$ was considered to indicate statistically significant differences. Each experiment was repeated at least three times.

Table 1 Primer Sequences Used in This Study

Gene	Forward Primer Sequence	Reverse Primer Sequence
SNAI1	CCTCGCTGCCAATGCTCATCTG	GCTCTGCCACCCTGGGACTC
SNAI2	CGAACTGGACACACATACAGGT	CTGAGGATCTCTGGTTGTGGT
TWIST1	GTCCGCAGTCTTACGAGGAG	GCTTGAGGGTCTGAATCTTGCT
TWIST2	CGCAAGTGGAATTGGGATGC	CGATGTCACTGCTGTCCCTT
ZEB1	CAGGCAAAGTAAATATCCCTGC	GGTAAAACTGGGGAGTTAGTCA
GAPDH	CGACCACTTTGTCAAGCTCA	AGGGGTCTACATGGCAACTG

Results

TGF-β1–Induced HaCaT Cells Exhibit Unique Morphological and Reduced Proliferation but are Inhibited by Ang-(1-7)

The CCK8 assay showed that compared with the control group, the proliferation of HaCaT cells treated with TGF-β1 (10 ng/mL) for 48 h decreased significantly, while the A + T group weakened this trend (Figure 1B). TGF-β1 has been widely accepted as a key factor in the induction of cellular EMT. HaCaT cells undergo unique morphological changes when exposed to TGF-β1 conditions (Figure 1A). After 72 h of exposure to TGF-β1 (10 ng/mL), HaCaT cells lost their typical paving-stone morphology and showed mesenchymal-like cell morphology; however, this change was not observed when treated with Ang-(1-7) alone. When the cells received both Ang-(1-7) and TGF-β1 treatment for 72 h, the cellular morphology was similar to that of mesenchymal cells, but the degree of intercellular connection seemed tighter than group T.

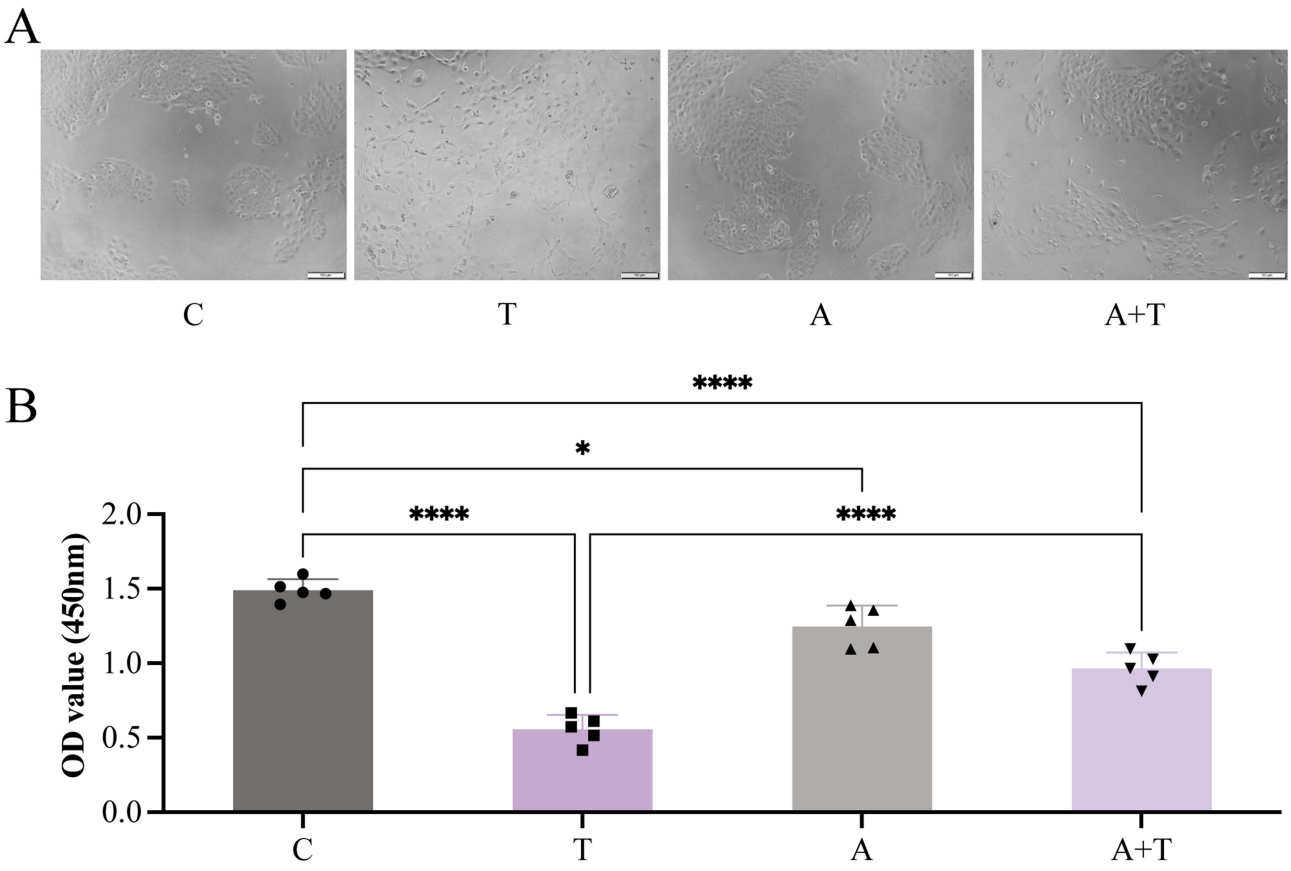

Figure 1 TGF-β1–induced HaCaT cells exhibit unique morphological changes and enhanced migration ability. (**A**) To examine the effect of Ang-(1-7) and TGF-β1 on the proliferation of human keratinocytes, HaCaT cells were treated with TGF-β1 (10 ng/mL), either with or without Ang-(1-7) (1 μM) for 48 h, and measured by CCK8 assay. Each bar represents the mean ± standard deviation (n=5). (C: Group C; T: Group T; A: Group A; A+T: Group A + T.) (**B**) Morphological changes after treatment in each group (100× magnification); *P <0.05, ****P < 0.0001 . (C: Group C; T: Group T; A: Group A; A+T: Group A + T).

TGF-β1–Induced HaCaT Cells Exhibit Enhanced Migration Ability but are Inhibited by Ang-(1-7)

The results of the scratch wound healing test showed that TGF-β1 treatment for 36 h significantly promoted the migration ability of HaCaT cells, and HaCaT cells in Group T also underwent pronounced morphological metamorphosis into fibroblast-like cells, concomitant with a decrease in proliferation levels. While similar alterations were observed in Group A + T, Ang-(1-7) ostensibly mitigated the intensity of these changes compared to Group T (Figure 2A and B).

TGF-β1–Induced Expression of EMT Marker Proteins in HaCaT Cells Was Inhibited by Ang-(1-7)

To explore the effect of Ang-(1-7) on the expression of EMT-related proteins in HaCaT cells induced by TGF-β1, Western blotting was performed on the proteins extracted from cells in each group. The results showed that after 72 h of TGF-β1 induction, HaCaT cells successfully underwent EMT (Figure 3A). This was confirmed by marked downregulation of epithelial

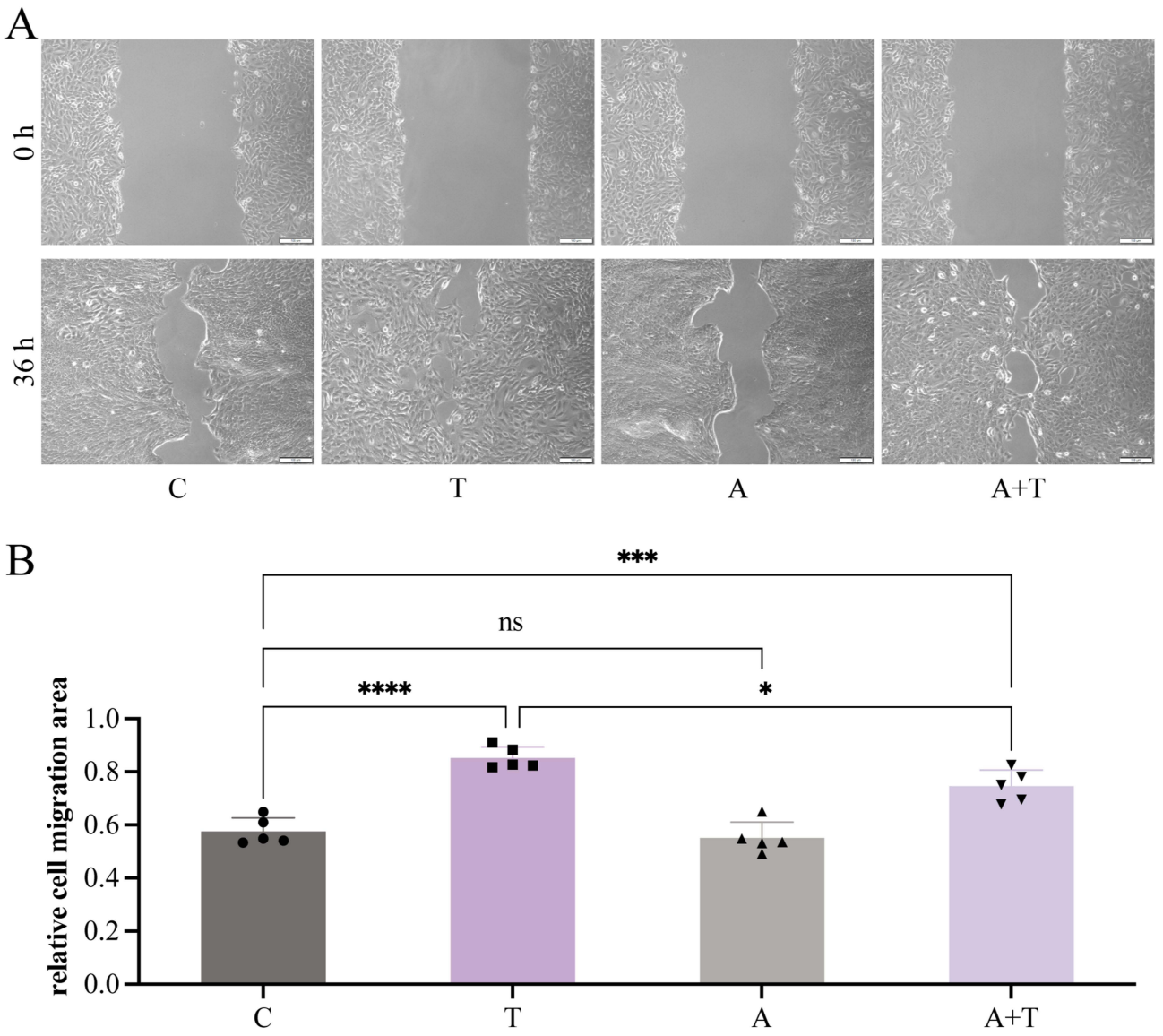

Figure 2 (**A**) Ang-(1-7) inhibits TGF-β1–induced cell migration in HaCaT cells (100× magnification). (C: Group C; T: Group T; A: Group A; A+T: Group A + T.) (**B**) The cell migration rate measured by Image J software (NIH). The outcomes are depicted as the mean values (± standard deviation) derived from quintuple biological replicates. ns, not significant. *P <0.05, ***P < 0.001 and ****P < 0.0001. (C: Group C; T: Group T; A: Group A; A+T: Group A + T).

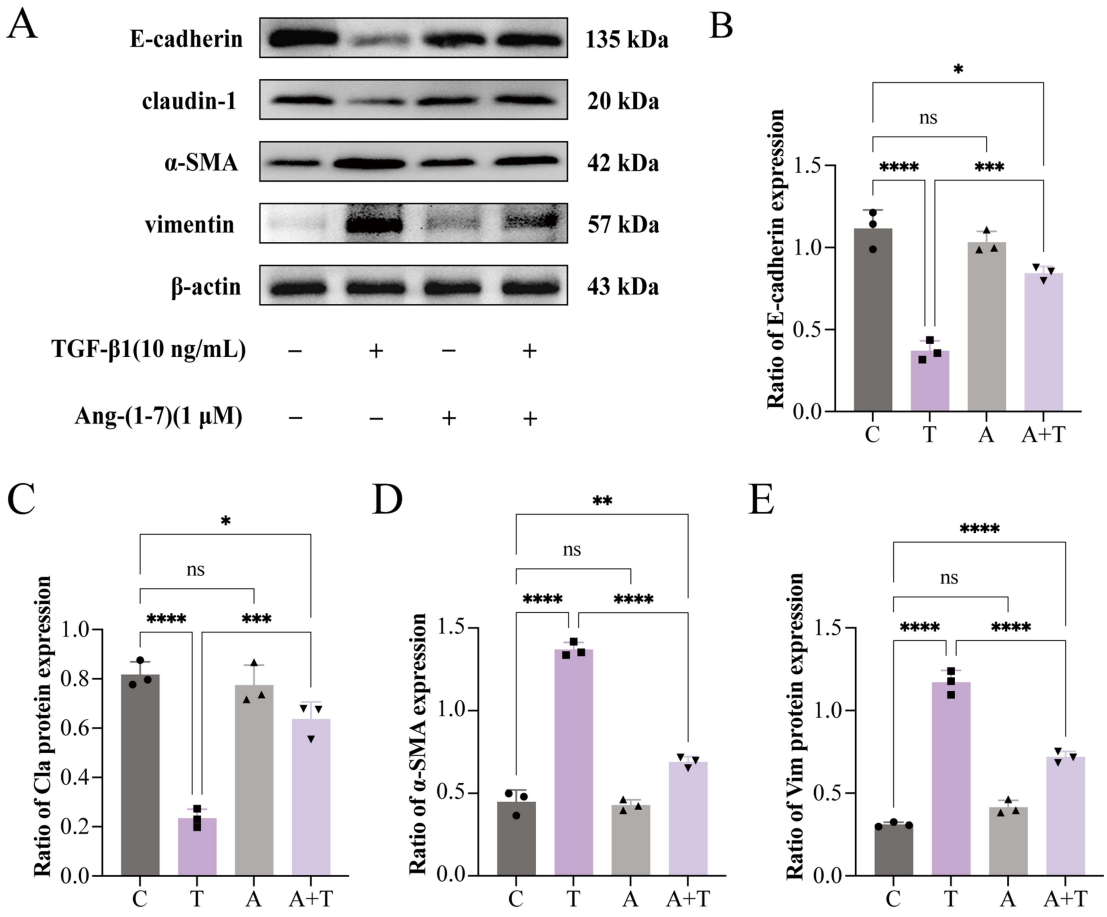

Figure 3 TGF-β1–induced expression of EMT marker proteins in HaCaT cells was inhibited by Ang-(1-7). (**A**) TGF-β1 induced EMT marker proteins' expression in HaCaT cells and was inhibited by Ang-(1-7). The protein was extracted and measured by Western blotting. The WB band image was evaluated by the imaging system. (**B**–**E**) Relative protein expression maps of E-cadherin, claudin-1, α-SMA, and vimentin respectively. GraphPad Prism 5 v5.01 software was used to analyze the relative gray value of each group of protein bands. Each bar represents the mean ± standard deviation (n=3). ns, not significant. *P <0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. (C: Group C; T: Group T; A: Group A; A+T: Group A + T.

marker proteins E-cadherin and claudin-1 and marked upregulation of mesenchymal markers α-SMA and vimentin. By comparing the relative expression data of proteins in each group (Figure 3B-E), it can be concluded that the cells of Group T which were treated with TGF-β1 (10 ng/mL) for 72 h compared with the cells of Group C: epithelial marker protein: E-cadherin and tight junction protein were down-regulated; while the Group A + T significantly weakened this trend; and mesenchymal cell marker proteins: α-SMA and vimentin were significantly up-regulated, and in Group A + T This trend was not obvious. This indicated that Ang-(1-7) could inhibit the expression of EMT-related proteins in HaCaT cells induced by TGF-β1.

TGF-β1-Smad Signaling Pathway in HaCaT Cells Was Inhibited by Ang-(1-7)

The above results showed that Ang-(1-7) has an influence on the EMT of HaCaT cells. To further study the related mechanism, we detected the expression of the classic TGF-β1-Smad signaling pathway. We found that the TGF-β–induced phosphorylation levels of Smad2 and Smad3 in HaCaT cells were significantly reduced with Ang-(1-7) treatment (Figure 4A-C). To detect the EMT-related transcription factors, the expressions of SNAI1, SNAI2, TWIST1, TWIST2, and ZEB1 were determined by RT-PCR, which are also genes downstream of the TGF-β1-Smad signaling pathway (Figure 4D-H). The results showed that compared with Group C, the expression of each transcription factor in Group T was significantly up-regulated, and those changes were inhibited after treatment with Ang-(1-7), which indicated that Ang-(1-7) inhibited EMT by disrupting the TGF-β1-Smad signaling pathway in HaCaT cells.

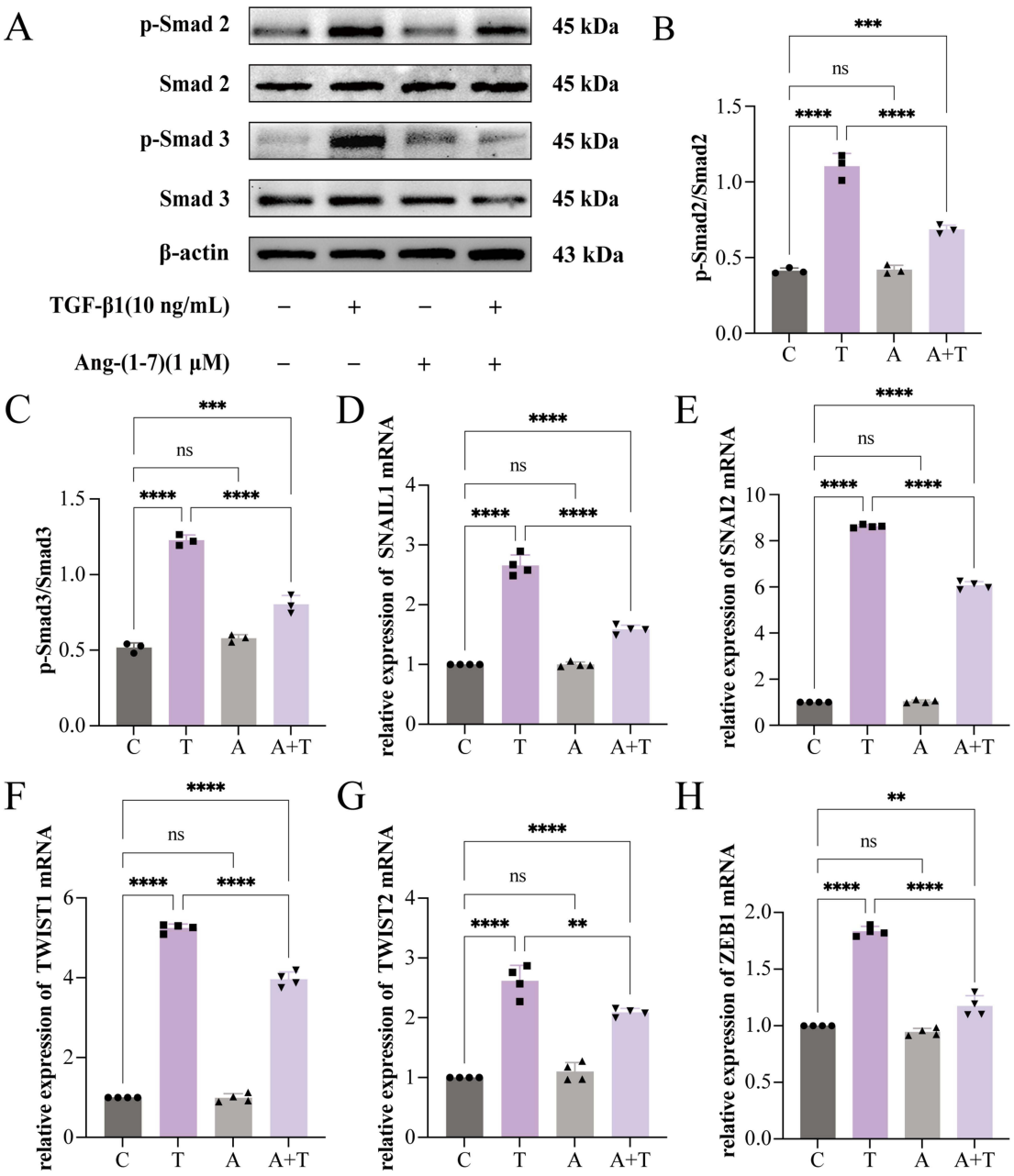

Figure 4 Ang-(1-7) disrupted the TGF-β1-Smad pathway in HaCaT cells. HaCaT cells were treated in groups, and the phosphorylation levels of Smad2 and Smad3 in cells were detected by western blotting (**A**–**C**). (C: Group C; T: Group T; A: Group A; A+T: Group A + T.) RNA was isolated and the expressions of SNAI1 (**D**), SNAI2 (**E**), TWIST1 (**F**), TWIST2 (**G**), and ZEB1 (**H**) were measured by PCR. The relative mRNA expression analysis of each group was performed using GraphPad Prism 5 v5.01 software. Each bar represents the mean ± standard deviation (n=3 in (A–C); n=4 in (D–H)). ns, not significant. **P <0.01, ***P <0.001 and ****P <0.0001. (C: Group C; T: Group T; A: Group A; A+T: Group A + T).

Discussion

The complex pathogenesis and easy-to-relapse characteristics of keloids make it very difficult to treat, and there are only few therapeutic drugs used clinically.[1,2] In 2018, the World Health Organization defined keloids as a member of a group of diseases "characterized by increased fibrous tissue deposition in the skin and subcutaneous tissue",[32] which inspired us to explore the potential of treating keloids through anti-fibrosis agents. Angiotensin-(1-7) is a bioactive peptide with anti-inflammatory, anti-oxidative, and anti-fibrotic effects and has shown efficacy in the treatment of various fibrotic diseases.[27,28] In this study, by exploring the effects of Ang-(1-7) on the morphological changes and migration of HaCaT cells in the EMT model induced by TGF-β1 in vitro, as well as the changes in the levels of EMT-related proteins

and gene expression, we not only explained part of the mechanism of action of Ang-(1-7) but also provided a theoretical basis of Ang-(1-7) as a therapeutic agent for keloids.

It has been previously reported that TGF-β1 was used to induce HaCaT cells to mimic the EMT process of keloid-derived keratinocytes.[33,34] Following treatment with 10 ng/mL TGF-β1 for 72 h (figure), HaCaT cells lost their typical cobblestone-like morphology and developed mesenchymal-like cell morphology. Excessive wound healing is a characteristic of keloids,[9] and the process of EMT is also accompanied by an increase in cell motility. In our experiments, Ang-(1-7) could reverse the increased TGF-β1–induced cell migration ability, which indicates that Ang-(1-7) helps to attenuate excessive wound healing in vitro wound healing models. At the protein level, we selected E-cadherin and claudin-1 as epithelial cell markers, because E-cadherin acts as an epithelial adhesion molecule to maintain epithelial stability and claudin-1 is the main component of tight junction complexes in epithelial cells, maintaining cell polarity and barrier function.[35] α-SMA and vimentin were selected as mesenchymal cell markers, and Western blotting was performed to evaluate their expression levels. Our results showed that epithelial markers were down-regulated and mesenchymal markers were up-regulated in HaCaT cells treated with TGF-β1, and the EMT process was successfully induced and largely inhibited by Ang-(1-7) at the protein level, all of which indicated that Ang-(1-7) reversed EMT to some extent. Although so far, fibroblasts and myofibroblasts have been the focus of most keloid research,[6] recent studies have shown that epithelial cells are also involved in the fibrosis process. Some scholars believe that keratinocytes can be involved in the process of EMT and they directly promote fibrosis.[36,37] Thus, the inhibition of EMT by Ang-(1-7) in keratinocytes can likely contribute to treatment of keloids.

EMT in response to TGF-β1 and fibrosis is mainly through Smad-dependent pathway, upon stimulation by TGF-β1, TGF-β1 receptors trigger intracellular signaling to phosphorylate Smad2 and Smad3, which translocate to the nucleus where they interact with other transcription factors to regulate TGF-β Transcription of responsive genes. To further investigate the mechanism of Ang-(1-7) inhibition of EMT, we analyzed the phosphorylation levels of Smad2 and Smad3 and the expression levels of TGF-β1-Smad downstream target genes.

The EMT process is coordinated by transcription factors (TFs) called EMT-TFs, and the core EMT-TF is composed of Snail (encoded by SNAI1), Slug (encoded by SNAI2), ZEB1, ZEB2, TWIST1, and TWIST2.[38] The SNAI and ZEB families are highly conserved zinc finger transcription factors that are considered to be key EMT regulators,[39] and both SNAI1 and SNAI2 have been shown to inhibit E-cadherin expression and induce EMT.[40] TWIST, a helix-loop-helix transcription factor similar to the ZEB and SNAI families, is a potent inducer of EMT and a marker of stem cell state. SMADs activated by TGF-β1 can interact with EMT-TFs in subsequent transcriptional regulation, eg, TGF-β–activated SMADs and Snail synergistically down-regulate E-cadherin, tight junction proteins, and other epithelial proteins in mouse mammary epithelial cells Expression of markers.[41] In a lung fibrosis study, copper sulfate activated the TGF-β1/Smad pathway to trigger lung fibrosis by increasing the protein and mRNA expression levels of TGF-β1, p-Smad2, and p-Smad3.[42]

Our experimental results showed that elevated levels of p-Smad2/Smad2 and p-Smad3/Smad3 induced by TGF-β1 were inhibited by Ang-(1-7), and the mRNA levels of SNAI1, SNAI2, TWIST1, TWIST2 and ZEB1 increased significantly. However, these changes were inhibited by Ang-(1-7), indicating that Ang-(1-7) inhibited the TGF-β1-Smad signaling pathway in the in vitro EMT model of HaCaT cells. This re-emphasizes that the regulation between TGF-β1/Smad signaling pathway and EMT-related transcriptional regulators plays an important role in the occurrence and development of fibrotic diseases.

Several previous studies have demonstrated the beneficial effects of Ang-(1-7) in various physiological and pathological conditions. In the cardiovascular system, Ang-(1-7) has been shown to improve endothelial function, lower blood pressure, and inhibit the development of cardiac hypertrophy and fibrosis.[43] In the respiratory system, Ang-(1-7) has been shown to reduce pulmonary hypertension and improve lung function in animal models of lung injury.[44] In the renal system, Ang-(1-7) has been shown to protect against renal injury and fibrosis.[45] Despite encouraging results from preclinical studies, the clinical application of Ang-(1-7) remains limited. The short half-life and rapid degradation of Ang-(1-7) in vivo make it difficult in clinical application. Several approaches have been proposed to overcome these limitations, including the development of stable analogues and the use of gene therapy to increase endogenous production of Ang-(1-7),[46] Ang-(1-7) has emerged as an important peptide with beneficial effects in various physiological and

pathological conditions. Further studies are needed to better understand its mechanism of action and develop effective therapeutic strategies.

In conclusion, to our knowledge, this is the first study to show that Ang-(1-7) inhibits TGF-β1–induced EMT in HaCaT cells by inhibiting the TGF-β1-Smad signaling pathway. Our experimental results show that Ang-(1-7) can effectively inhibit the EMT process in HaCaT cells, but whether A directly inhibits the fibrosis process promoted by EMT is still unclear, the therapeutic potential and clinical application of Ang-(1-7) in keloids merits further investigation.

Acknowledgments

We are grateful to the Southwest Medical University for its support of our research.

Funding

This research was funded by Joint Project of Southwest Medical University and Luzhou city, the project number: 02×00150027. This research was also funded by the (Luzhou-Medical University) Cooperation Project (No. 2019LZXNYDZ08).

Disclosure

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

References

1. Marneros AG, Krieg T. Keloids – clinical diagnosis, pathogenesis, and treatment options. *J Dtsch Dermatol Ges*. 2004;2(11):905–913. doi:10.1046/j.1439-0353.2004.04077.x
2. Atiyeh BS, Costagliola M, Hayek SN. Keloid or hypertrophic scar: the controversy: review of the literature. *Ann Plast Surg*. 2005;54(6):676–680. doi:10.1097/01.sap.0000164538.72375.93
3. Ding J, Tredget EE. The Role of Chemokines in Fibrotic Wound Healing. *Adv Wound Care*. 2015;4(11):673–686. doi:10.1089/wound.2014.0550
4. Satish L, Evdokiou A, Geletu E, Hahn JM, Supp DM. Pirfenidone inhibits epithelial–mesenchymal transition in keloid keratinocytes. *Burns Trauma*. 2020;8. doi:10.1093/burnst/tkz007
5. Bock (Seifert) O, Yu H, Zitron S, Bayat A, Ferguson MW, Mrowietz U. Studies of Transforming Growth Factors Beta 1–3 and their Receptors I and II in Fibroblast of Keloids and Hypertrophic Scars. *Acta Dermato-Venereologica*. 2005;85(3):216–220. doi:10.1080/00015550410025453
6. Tamaoki M, Imanaka-Yoshida K, Yokoyama K, et al. Tenascin-C regulates recruitment of myofibroblasts during tissue repair after myocardial injury. *Am J Pathol*. 2005;167(1):71–80. doi:10.1016/S0002-9440(10)62954-9
7. Nangole FW, Agak GW. Keloid pathophysiology: fibroblast or inflammatory disorders? *JPRAS Open*. 2019;22:44–54. doi:10.1016/j.jpra.2019.09.004
8. Kuwahara H, Tosa M, Egawa S, Murakami M, Mohammad G, Ogawa R. Examination of Epithelial Mesenchymal Transition in Keloid Tissues and Possibility of Keloid Therapy Target. *Plast Reconstr Surg Glob Open*. 2016;4(11):e1138. doi:10.1097/GOX.0000000000001138
9. Hahn JM, McFarland KL, Combs KA, Supp DM. Partial epithelial-mesenchymal transition in keloid scars: regulation of keloid keratinocyte gene expression by transforming growth factor-β1. *Burns Trauma*. 2016;4:30. doi:10.1186/s41038-016-0055-7
10. Yang C-E, Moon SJ, Kim SJ, et al. Epithelial-mesenchymal transition in keloid tissue. *Arch Plast Surg*. 2018;45(06):600–601. doi:10.5999/aps.2017.01214
11. Kong D, Li Y, Wang Z, Sarkar FH. Cancer Stem Cells and Epithelial-to-Mesenchymal Transition (EMT)-Phenotypic Cells: are They Cousins or Twins? *Cancers*. 2011;3(1):716–729. doi:10.3390/cancers30100716
12. Wendt MK, Schiemann WP. Therapeutic targeting of the focal adhesion complex prevents oncogenic TGF-beta signaling and metastasis. *Breast Cancer Res*. 2009;11(5):R68. doi:10.1186/bcr2360
13. Ma X, Chen J, Xu B, et al. Keloid-derived keratinocytes acquire a fibroblast-like appearance and an enhanced invasive capacity in a hypoxic microenvironment in vitro. *Int J Mol Med*. 2015;35(5):1246–1256. doi:10.3892/ijmm.2015.2135
14. Hahn JM, McFarland KL, Combs KA, Supp DM. Partial epithelial-mesenchymal transition in keloid scars: regulation of keloid keratinocyte gene expression by transforming growth factor-β1. *Burns Trauma*. 2016;4:s41038-016-0055–7.
15. Zhang M, Liu S, Guan E, et al. Hyperbaric oxygen therapy can ameliorate the EMT phenomenon in keloid tissue. *Medicine*. 2018;97(29):e11529. doi:10.1097/MD.0000000000011529
16. Do DV, Ong CT, Khoo YT, et al. Interleukin-18 system plays an important role in keloid pathogenesis via epithelial-mesenchymal interactions. *Br J Dermatol*. 2012;166(6):1275–1288. doi:10.1111/j.1365-2133.2011.10721.x
17. Jiang X, Zhang Z, Song C, et al. Glaucocalyxin A reverses EMT and TGF-β1-induced EMT by inhibiting TGF-β1/Smad2/3 signaling pathway in osteosarcoma. *Chem Biol Interact*. 2019;307:158–166. doi:10.1016/j.cbi.2019.05.005
18. Xu J, Lamouille S, Derynck R. TGF-β-induced epithelial to mesenchymal transition. *Cell Res*. 2009;19(2):156–172. doi:10.1038/cr.2009.5
19. Rygiel KA, Robertson H, Marshall HL, et al. Epithelial–mesenchymal transition contributes to portal tract fibrogenesis during human chronic liver disease. *Lab Invest*. 2008;88(2):112–123. doi:10.1038/labinvest.3700704
20. Lee JH, Massagué J. TGF-β in developmental and fibrogenic EMTs. *Semin Cancer Biol*. 2022;86:136–145. doi:10.1016/j.semcancer.2022.09.004
21. U T, S A, N U, C P, Y N, P V. Molecular signalings in keloid disease and current therapeutic approaches from natural based compounds. *Pharm Biol*. 2015;53.

22. Hong Y-K, Chang Y-H, Lin Y-C, Chen B, Guevara BEK, Hsu C-K. Inflammation in Wound Healing and Pathological Scarring. *Adv Wound Care*. 2023;12(5):288–300. doi:10.1089/wound.2021.0161
23. Yan L, Cao R, Wang L, et al. Epithelial–mesenchymal transition in keloid tissues and TGF-β1 –induced hair follicle outer root sheath keratinocytes. *Wound Repair Regener*. 2015;23(4):601–610. doi:10.1111/wrr.12320
24. Grande MT, Sánchez-Laorden B, López-Blau C, et al. Snail1-induced partial epithelial-to-mesenchymal transition drives renal fibrosis in mice and can be targeted to reverse established disease. *Nature Med*. 2015;21(9):989–997. doi:10.1038/nm.3901
25. A M, A G-P, M N, P A, C E. The expression of the renin-angiotensin-aldosterone system in the skin and its effects on skin physiology and pathophysiology. *J Physiol Pharmacol*. 2019;70.
26. Ghatage T, Goyal SG, Dhar A, Bhat A. Novel therapeutics for the treatment of hypertension and its associated complications: peptide- and nonpeptide-based strategies. *Hypertens Res*. 2021;44(7):740–755. doi:10.1038/s41440-021-00643-z
27. Sumners C, Peluso AA, Haugaard AH, Bertelsen JB, Steckelings UM. Anti-fibrotic mechanisms of angiotensin AT 2 -receptor stimulation. *Acta Physiol (Oxf)*. 2019;227(1):e13280. doi:10.1111/apha.13280
28. Simões e Silva A, Silveira K, Ferreira A, Teixeira M. ACE2, angiotensin-(1-7) and M as receptor axis in inflammation and fibrosis. *Br J Pharmacol*. 2013;169(3):477–492. doi:10.1111/bph.12159
29. Bernasconi R, Thriene K, Romero-Fernández E, et al. Pro-inflammatory immunity supports fibrosis advancement in epidermolysis bullosa: intervention with Ang-(1-7). *EMBO Mol Med*. 2021;13(10). doi:10.15252/emmm.202114392
30. Okamura K. Angiotensin(1–7) attenuated Angiotensin II-induced hepatocyte EMT by inhibiting NOX-derived H2O2-activated NLRP3 inflammasome/IL-1β/Smad circuit. *Free Radic Biol Med*. 2016;97:531–543. doi:10.1016/j.freeradbiomed.2016.07.014.
31. Zhou JP, Tang W, Feng Y, et al. Angiotensin-(1–7) decreases the expression of collagen I via TGF-β1/Smad2/3 and subsequently inhibits fibroblast–myofibroblast transition. *Clin Sci*. 2016;130(21):1983–1991. doi:10.1042/CS20160193
32. Marty P, Chatelain B, Lihoreau T, et al. Halofuginone regulates keloid fibroblast fibrotic response to TGF-β induction. *Biomed. Pharmacother.* 2021;135:111182. doi:10.1016/j.biopha.2020.111182
33. Miyake Y, Nagaoka Y, Okamura K, Takeishi Y, Tamaoki S, Hatta M. SNAI2 is induced by transforming growth factor-β1, but is not essential for epithelial-mesenchymal transition in human keratinocyte HaCaT cells. *Exp Ther Med*. 2021;22(4):1–10. doi:10.3892/etm.2021.10558
34. Wang T, Zhang L, Shi C, et al. TGF-β-induced miR-21 negatively regulates the antiproliferative activity but has no effect on EMT of TGF-β in HaCaT cells. *Int J Biochem Cell Biol*. 2012;44(2):366–376. doi:10.1016/j.biocel.2011.11.012
35. Krause G, Winkler L, Mueller SL, Haseloff RF, Piontek J, Blasig IE. Structure and function of claudins. *Biochimica et Biophysica Acta*. 2008;1778(3):631–645. doi:10.1016/j.bbamem.2007.10.018
36. Rybinski B, Franco-Barraza J, Cukierman E. The wound healing, chronic fibrosis, and cancer progression triad. *Physiol Genom*. 2014;46(7):223–244. doi:10.1152/physiolgenomics.00158.2013
37. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009;119(6):1420–1428. doi:10.1172/JCI39104
38. Liu F, Gu L-N, Shan B-E, Geng C-Z, Sang M-X. Biomarkers for EMT and MET in breast cancer: an update. *Oncol Lett*. 2016;12(6):4869–4876. doi:10.3892/ol.2016.5369
39. Reinke LM, Xu Y, Cheng C. Snail represses the splicing regulator epithelial splicing regulatory protein 1 to promote epithelial-mesenchymal transition. *J Biol Chem*. 2012;287(43):36435–36442. doi:10.1074/jbc.M112.397125
40. Conacci-Sorrell M, Simcha I, Ben-Yedidia T, Blechman J, Savagner P, Ben-Ze'ev A. Autoregulation of E-cadherin expression by cadherin–cadherin interactions. *J Cell Biol*. 2003;163(4):847–857. doi:10.1083/jcb.200308162
41. Vincent T, Neve EPA, Johnson JR, et al. A SNAIL1-SMAD3/4 transcriptional repressor complex promotes TGF-β mediated epithelial-mesenchymal transition. *Nat Cell Biol*. 2009;11(8):943–950. doi:10.1038/ncb1905
42. Guo H, Jian Z, Liu H, et al. TGF-β1-induced EMT activation via both Smad-dependent and MAPK signaling pathways in Cu-induced pulmonary fibrosis. *Toxicol Appl Pharmacol*. 2021;418:115500. doi:10.1016/j.taap.2021.115500
43. Chen Y, Fan J, Cao L, et al. Unique mechanistic insights into the beneficial effects of angiotensin-(1-7) on the prevention of cardiac fibrosis: a metabolomic analysis of primary cardiac fibroblasts. *Exp Cell Res*. 2019;378(2):158–170. doi:10.1016/j.yexcr.2019.03.006
44. Liu M-L, Xing S-J, Liang X-Q, et al. Reversal of Hypoxic Pulmonary Hypertension by Hypoxia-Inducible Overexpression of Angiotensin-(1-7) in Pulmonary Endothelial Cells. *Mol Ther Methods Clin Dev*. 2020;17:975–985. doi:10.1016/j.omtm.2020.04.008
45. Choi HS, Kim IJ, Kim CS, et al. Angiotensin-[1–7] attenuates kidney injury in experimental Alport syndrome. *Sci Rep*. 2020;10(1):4225. doi:10.1038/s41598-020-61250-5
46. Touyz RM, Montezano AC. Angiotensin-(1–7) and Vascular Function. *Hypertension*. 2018;71(1):68–69. doi:10.1161/HYPERTENSIONAHA.117.10406