JOURNAL OF PLASTIC SURGERY AND HAND SURGERY
2023, VOL. 57, NOS. 1–6, 129–136
https://doi.org/10.1080/2000656X.2021.2017944

ARTICLE

ADAM17 regulates the proliferation and extracellular matrix of keloid fibroblasts by mediating the EGFR/ERK signaling pathway

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ABSTRACT

To investigate the role of a disintegrin and metalloprotease protein 17 (ADAM17) in regulating the proliferation and extracellular matrix (ECM) expression of keloid fibroblasts (KFs) via the epidermal growth factor receptor (EGFR)/extracellular signal-regulated kinase (ERK) pathway. ADAM17 expression in keloid tissues was detected by western blotting. KFs were isolated, cultured and divided into the control, shNC (negative control), shADAM17, transforming growth factor-β1 (TGF-β1), TGF-β1 + shNC and TGF-β1 + shADAM17 groups. The expression of ECM was detected by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Western blotting was performed to detect the expression of proteins. Cell proliferation was detected by a 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay, while cell invasion and migration were examined by Transwell and wound healing assays. The expression of ADAM17 was increased in keloid tissues and KFs. Compared with the control group, the expression of p-EGFR and p-ERK/1/2/ERK1/2, as well as the expression of collagen I, collagen III, connective tissue growth factor (CTGF) and α-smooth muscle actin (α-SMA), were decreased in KFs from the shADAM17 group, with decreased cell proliferation, invasion and migration. In contrast, the TGF-β1 group presented the opposite trend in these aspects. In addition, compared with the TGF-β1 group, KFs from the TGF-β1 + shADAM17 group had decreased ECM expression, proliferation, invasion and migration. ADAM17 expression was upregulated in keloid tissues. Silencing ADAM17 may inhibit the activity of the EGFR/ERK pathway to limit the deposition of ECM in KFs with reduced proliferation, invasion and migration.

Abbreviations: ADAM17: a disintegrin and metalloprotease protein 17; ANOVA: one-way analysis of variance; BCA: bicinchoninic acid; CTGF: connective tissue growth factor; DMEM: Dulbecco's modified Eagle's medium; DMSO: dimethylsulfoxide; ECM: extracellular matrix; ECL: enhanced chemiluminescence; EGFR: epidermal growth factor receptor; ERK: extracellular signal-regulated kinase; ELISA: enzyme-linked immunosorbent assay; FBS: fetal bovine serum; HE: Hematoxylin and Eosin; JNK: c-Jun N-terminal kinase; KFs: keloid fibroblasts; MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; NC: negative control; NFs: Normal fibroblasts; PBS: phosphate buffer solution; PBST: phosphate buffer solution with Tween 20; PVDF: polyvinylidene fluoride; qRT-PCR: quantitative reverse transcriptase polymerase chain reaction; SD: standard deviation; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; OD: optical density; TGF-β: transforming growth factor-β

ARTICLE HISTORY
Received 6 August 2021
Revised 23 November 2021
Accepted 8 December 2021

KEYWORDS
ADAM17; EGFR/ERK signaling pathway; keloid; fibroblast; extracellular matrix

Introduction

Pathological scars, including hypertrophic scars and keloids, are the product of wound healing [1], while human skin trauma, inflammation, burns and surgery affect scar formation [2,3]. Keloids have been clinically considered a 'benign skin tumor' with the features of aggressive growth and abnormal accumulation of extracellular matrix (ECM) without any sign of spontaneous recession [4,5], which may result in deformity and dysfunction, thus affecting patients' skin appearance and mental health and reducing the quality of life [6]. Currently, the pathogenesis of keloids remains unclear, and related research is urgently needed [7].

A disintegrin and metalloprotease protein 17 (ADAM17), belonging to the ADAM protein family, is a transmembrane zinc-dependent metalloprotease implicated in diverse biological processes, including cell migration and adhesion [8]. ADAM17 was shown to be crucial for protecting the skin barrier via proteolytic activation of epidermal growth factor receptor (EGFR) ligand, which can bind to the extracellular part of EGFR to activate EGFR tyrosine kinase and the corresponding downstream signaling pathways (such as extracellular signal-regulated kinase (ERK)/c-Jun N-terminal kinase (JNK)/p38), thus playing a role in regulating the pathological process of inflammation and tumors [9,10]. As reported, EGFR is very important for wound healing of the skin, and in particular, it is also the basement substance of ADAM17, which could work jointly to maintain complete skin development [11]. In addition, activation of the EGFR/ERK pathway was observed to take part in transforming growth factor-β (TGF-β)-dependent renal fibrosis [12]. Transforming growth factor-β1 (TGF-β1) has been credited as the most powerful profibrotic cytokine since it can aggravate keloid formation by regulating fibroblast formation and inhibiting the degradation of collagen and other ECM proteins [13]. Evidence has pointed out the role of ADAM17/EGFR-dependent ERK activation in mediating thrombin-stimulated connective tissue growth factor (CTGF) expression in human lung fibroblasts, and consequently, silencing ADAM17 could alleviate the degree of

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lung fibrosis [14]. Hence, we speculated that ADAM17 may play a regulatory role in keloid fibroblasts (KFs) by mediating the EGFR/ERK pathway.

In this study, KFs were induced by TGF-β1, and then the role and mechanism of ADAM17 were investigated in regulating the proliferation of KFs and the expression of ECM by mediating the EGFR/ERK pathway, aiming to find a novel method for the prevention and treatment of keloids.

Materials and methods

Ethics statement

This study was conducted after the approval of the Ethics Committee of our hospital. All patients signed the written informed consent form prior to the study.

Study subjects

From January 2019 to December 2020, keloid tissues and normal skin tissues around the scar were obtained from 15 patients who underwent surgical resection in our hospital. The diagnostic criteria of scarring included scar tissue growing aggressively beyond the original damage scope, more than 9 months of disease without any signs of spontaneous recession, and recurrence after surgical resection [15]. Patients who had received hormone, radiation, topical or local injection of any drug treatment or patients with other skin, immune and infectious diseases were excluded.

Hematoxylin and eosin (HE) staining

Paraffin sections were cleared in xylene and rehydrated by conventional gradient alcohol. Next, sections were placed into Harris Hematoxylin Stain for 10 min and washed with tap water for 1 min. Subsequently, the sections were differentiated with 1% hydrochloric ethanol for 30 s, reblued with tap water for 15 min, and stained with 1% eosin ethanol for 3 min. After differentiating with 90% alcohol for 30 s, the sections were washed with 95% alcohol for 1 min, rinsed with xylene carbonate for 1 min, and washed with xylene three times (2 min each time). Finally, sections were mounted with neutral resin and observed with a microscope.

Isolation and culture of keloid fibroblasts

Keloid and normal tissues collected during operations were treated by removing epidermis and subcutaneous tissues under sterile conditions. As previously described, primary KFs and normal human skin fibroblasts (NFs) were isolated from clinical tissues [16]. In short, tissue samples were digested by 0.1% type I collagenase solution at 37 °C for 4–6 h. During the digestion period, the plate was removed every half hour for gentle shaking to guarantee complete and thorough digestion. Next, an equal volume of fresh Dulbecco's modified Eagle's medium (DMEM) was added to neutralize the remaining type I collagenase, followed by 5 min of centrifugation at 2000 rpm at room temperature. The supernatant was discarded. An appropriate volume of fresh culture medium was added to suspend cell precipitation and centrifuged again. Then, the supernatant was discarded. DMEM containing 10% fetal bovine serum (FBS) was added for primary culture with conditions of 5% CO_2 at 37 °C. When cells covered 90% of the plate, 0.25% trypsin was used for digestion and passaging. Fibroblasts in logarithmic growth phase (approximately the 4th generation) were used for subsequent experiments.

Cell grouping and transfection

KFs were divided into six groups: the control, shNC (negative control), shADAM17, TGF-β1, TGF-β1 + shNC and TGF-β1 + shADAM17 groups. The control group was KFs without any treatment. KFs were treated with 10 ng/mL TGF-β1 for 24 h in the TGF-β1 group [17]. KFs in the shNC group and shADAM17 group were transfected with negative control (NC) shRNA and ADAM17 shRNA, respectively. KFs in the TGF-β1 + shNC and TGF-β1 + shADAM17 groups were transfected with both NC shRNA/ADAM17 shRNA and treated with TGF-β1 for 24 h. ADAM17 shRNA (5′-GCGATCACGAGAACAATAAGA-3′) and NC shRNA (5′-GTTCTCCGAACGTGTCACGT-3′) were purchased from Shanghai Genechem Co., Ltd. (Shanghai, China). KFs collected in the logarithmic growth phase were inoculated into six-well plates. When cell confluence reached 70–80%, cell transfection was performed according to the instructions of Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA).

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay

At 48 h after cell transfection, each cell group was adjusted to a density of 1×10^5 cells/mL, and 100 μL cell suspensions were transferred to 96-well plates, which were placed in an incubator for culture at 37 °C with 5% CO_2. After culture for 24, 48 and 72 h, MTT solution (Promega, Madison, WI) was added to each well at 10 μL/well for 4 h of culture at 37 °C. The supernatant was discarded carefully, and 150 μL of dimethylsulfoxide (DMSO) was added to each well for 10 min of oscillation. After the crystals fully dissolved, an enzyme-linked immunosorbent assay (ELISA) reader was used to read the optical density (OD) value of cells in each well at a wavelength of 490 nm. The experiment was independently repeated three times.

Transwell invasion assay

Matrigel was melted at 4 °C overnight and diluted 1:3 with precooled serum-free medium. Next, Matrigel was added to the precooled Transwell chamber, which was incubated for 2 h at 37 °C for solidification of Matrigel. Serum-free DMEM was used for centrifugation and collection of the cell suspension, which was adjusted to a density of 2×10^4 cells/mL. Next, 100 μL of cell suspension was added to the upper Transwell chamber, and 600 μL of complete culture medium was added to the 24-well plate in the lower chamber. Cells were cultured for 24 h at 37 °C with 5% CO_2. The chamber was removed, washed with PBS twice, fixed in paraformaldehyde for 20 min, and washed with phosphate buffer solution (PBS) once again prior to adding 500 μL of 0.1% crystal violet stain to 24-well plates. Then, the chamber was placed in the plate to soak the membrane in culture medium for 5 min, followed by washing with PBS two times and wiping the remaining stain in the upper chamber using cotton swabs. Finally, the plate was placed upside down for air drying. Cells were observed and photographed under a microscope. The experiment was repeated three times independently.

Wound healing assay

KFs in each group were inoculated into six-well plates at a density of 2×10^5 cells/mL and placed in an incubator for culture at 37 °C with 5% CO_2. When cells adhered to the plate wall, a sterile pipette tip was used to gently scratch lines, with even strength, and the pipette was vertical to the plate. PBS was used to wash away detached cells. DMEM containing 1% FBS was added to culture cells at 37 °C with 5% CO_2. At 0 and 24 h after scratching, cells were observed with an inverted microscope, and pictures were taken. ImageJ software (Bethesda, MD) was used to analyze the scratching area for quantitative analysis. The migration rate of cells was calculated with the formula: migration rate=(0 h scratching area – 24 h scratching area)/0 h scratching area. The experiment was independently repeated three times.

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

Total RNA of cells was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA), and ultraviolet spectrophotometry was used to determine the RNA concentration. Based on the manufacturer's instructions for PrimeScript™ RT Master Mix (Takara, Otsu, Japan), RNA was reverse transcribed into cDNA. An appropriate volume of cDNA was used as a template for PCR with a SYBR® Premix Ex Taq™ fluorescent quantitative kit (Takara, Otsu, Japan). Reaction conditions included 95 °C for 10 min, 40 cycles of 95 °C for 30 s, and annealing temperature for 30 s, followed by incubation at 72 °C for 5 min.

Table 1. Primer sequences for qRT-PCR in this study.

Gene	Primer sequences (5′–3′)	Annealing temperature (°C)
Collagen I	Forward: GGCGGCCAGGGCTCCGACCC Reverse: AATTCCTGGTCTGGGGCACC	60
Collagen III	Forward: TGGTGTTGGAGCCGCTGCCA Reverse: TGGTGTTGGAGCCGCTGCCA	60
CTGF	Forward: ACAAGGGCCTCTTCTGTGACTT Reverse: GGTACACCGTACCACCGAAGAT	60
α-SMA	Forward: CATCATGCGTCTGGATCTGG Reverse: GGACAATCTCACGCTCAGCA	60
GAPDH	Forward: TCACCATCTTCCAGGAGCG Reverse: TCACCATCTTCCAGGAGCG	60

Primer sequences were designed with Primer 5.0 software (Table 1), which were synthesized by GenScript (Nanjing) Co., Ltd. (Nanjing, China). Relative expression of target genes was normalized to GAPDH (Shanghai GenePharma Co., Ltd., Shanghai, China) and calculated using the $2^{-\triangle\triangle Ct}$ method, with $\Delta Ct = Ct_{target\ gene} - Ct_{GAPDH}$; $\Delta\Delta Ct = \Delta Ct_{Experimental\ groups} - \Delta Ct_{Control\ group}$. Relative transcription levels of target mRNA $= 2^{-\triangle\triangle Ct}$. The experiment was independently repeated three times.

Western blotting

Protein lysate was used to extract total proteins according to the manufacturer's instructions. Protein concentration was detected using a bicinchoninic acid (BCA) kit. To perform 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis, 40 μL of protein was loaded into each lane. Proteins were transferred to polyvinylidene fluoride (PVDF) membranes at a voltage of 100 V (2 h), which were blocked in phosphate buffer solution with Tween 20 (PBST) buffer (containing 5% defatted milk powder) for 1 h. Next, primary antibodies, including antibodies against ADAM17, collagen I, collagen I, CTGF, α-smooth muscle actin (α-SMA), p-EGFR, ERK1/2, p-ERK1/2 and GAPDH monoclonal antibodies (all from Abcam, Cambridge, UK), were added and incubated overnight at 4 °C. The membrane was washed with PBST buffer again (5 times × 3 min) prior to development and visualization by enhanced chemiluminescence (ECL) solution. A gel imaging system (Bio-Rad, Hercules, CA) was used for image collection and gray value analysis. With GAPDH as the loading control, the relative expression of target proteins was expressed as the gray value ratio of target protein to GAPDH.

Statistical analysis

All data were analyzed using SPSS 21.0 (SPSS, Inc., Chicago, IL). Measurement data are expressed as the mean ± standard deviation (SD) and were tested by Student's *t*-test. Comparisons among multiple groups were analyzed by one-way analysis of variance (ANOVA) with Tukey's *post hoc* test. A value of $p < .05$ was considered to be statistically significant.

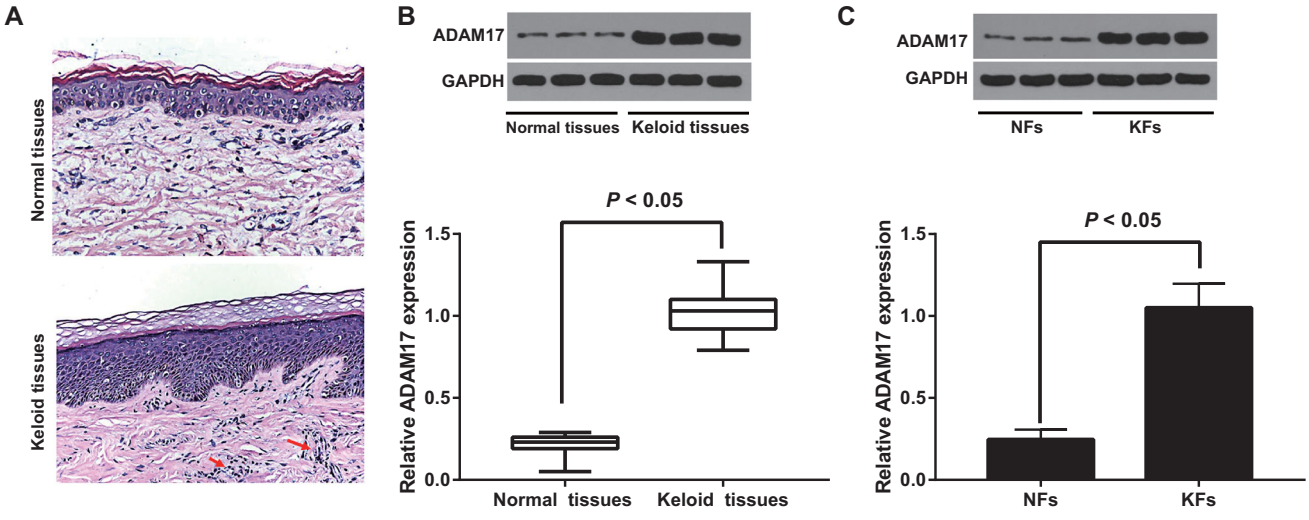

Figure 1. ADAM17 expression in keloid tissues and fibroblasts. (A) Normal skin tissues and keloid tissues were observed after HE staining; the red arrow indicates inflammatory cell infiltration; (B) ADAM17 protein expression in human keloid tissues and normal skin tissues was detected by western blotting; (C) ADAM17 protein expression in NFs and KFs was detected by western blotting. NFs: normal fibroblasts; KFs: keloid fibroblasts.

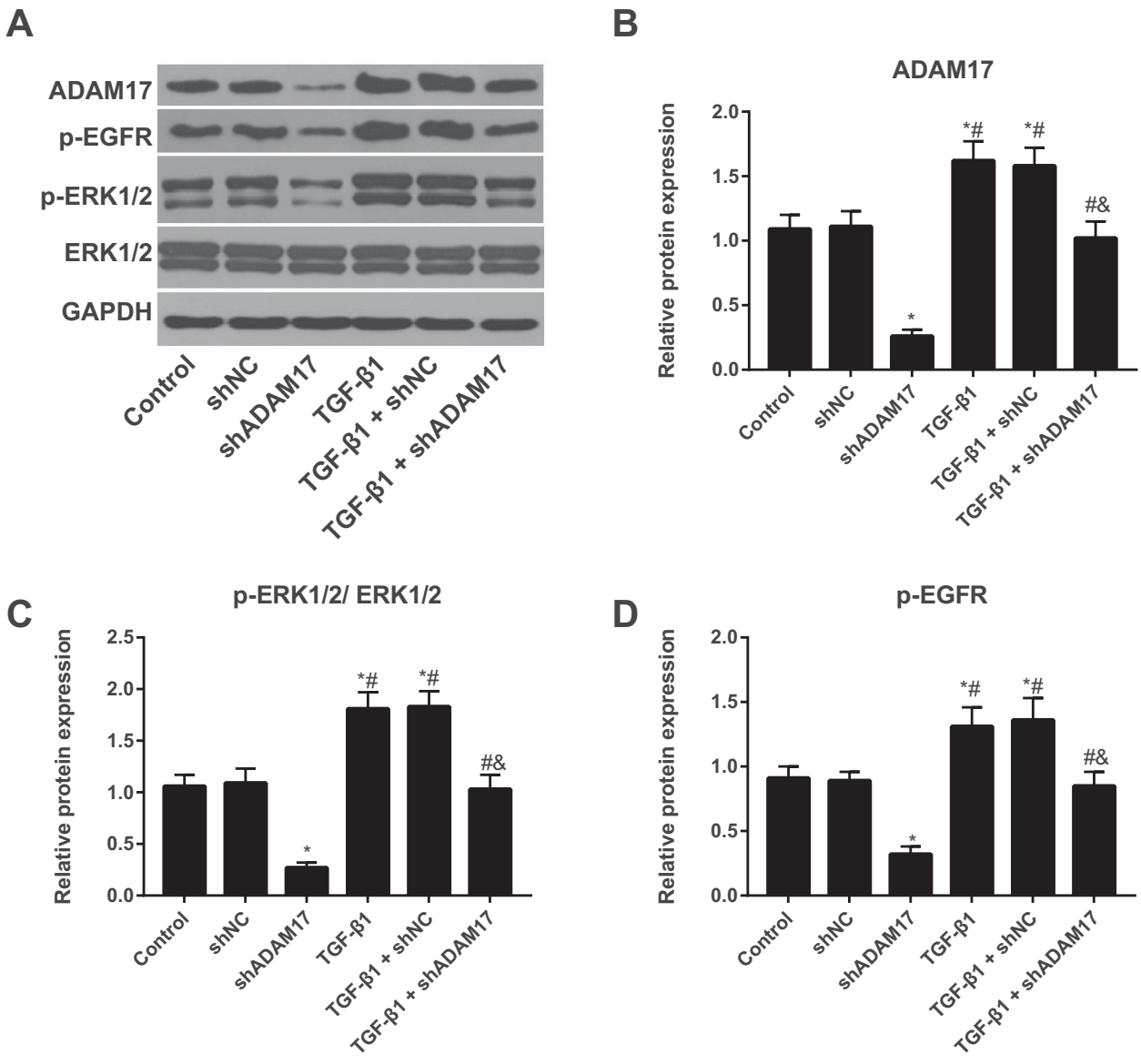

Figure 2. Expression of ADAM17 and the EGFR/ERK pathway in keloid fibroblasts (KFs). (A) Expression of ADAM17 and EGFR/ERK pathway-related proteins in KFs was determined by western blotting; (B–D) comparison of the expression of ADAM17 (B), p-EGFR (C) and p-ERK1/2/ERK1/2 (D) in KFs among different groups; $^{*}p<.05$ vs. the control group and shNC group; $^{\#}p<.05$ vs. the shADAM17 group; $^{\&}p<.05$ vs. the TGF-β1 group and TGF-β1 + shNC group.

Results

ADAM17 expression in keloid tissues and fibroblasts

The age of the 15 patients was 39.2 ± 5.6 years old, with nine males and six females. HE staining (Figure 1(A)) was performed to observe normal skin tissues and keloid tissues. Compared with normal tissues, keloid tissues showed inflammatory cell infiltration (red arrow), massive fibroblast proliferation and collagen deposition. The protein expression of ADAM17 in keloid tissues and isolated KFs was evaluated by western blotting (Figure 1(B,C)). Based on the results, ADAM17 protein expression was higher in keloid tissues and KFs than in normal tissues and NFs (all $p< .05$).

Expression of ADAM17 and EGFR/ERK pathway in KFs

The expression of ADAM17 and EGFR/ERK pathway-related proteins was determined by western blotting (Figure 2). Compared with the control and shNC groups, the expression of ADAM17, p-EGFR and p-ERK1/2/ERK1/2 in KFs from the shADAM17 group was decreased but increased in the TGF-β1 group (all $p< .05$). In addition, compared with the TGF-β1 group, these proteins were decreased in KFs from the TGF-β1 + shADAM17 group (all $p< .05$). The above data indicate that ADAM17 could inhibit the activity of the EGFR/ERK pathway in KFs.

ADAM17 knockout affects ECM expression by mediating the EGFR/ERK pathway

The expression of ECM-related genes and proteins in KFs among different groups was measured by qRT-PCR and western blotting, respectively (Figure 3). Compared with the control and shNC groups, the expression of collagen I, collagen III, CTGF and α-SMA was downregulated in KFs from the shADAM17 group and upregulated in KFs from the TGF-β1 group (all $p< .05$). In comparison with the TGF-β1 group, the above factors were reduced in KFs from the TGF-β1 + shADAM17 group (all $p< .05$), showing that ADAM17 knockout may reduce TGF-β1-induced ECM deposition of KFs by inhibiting the EGFR/ERK pathway.

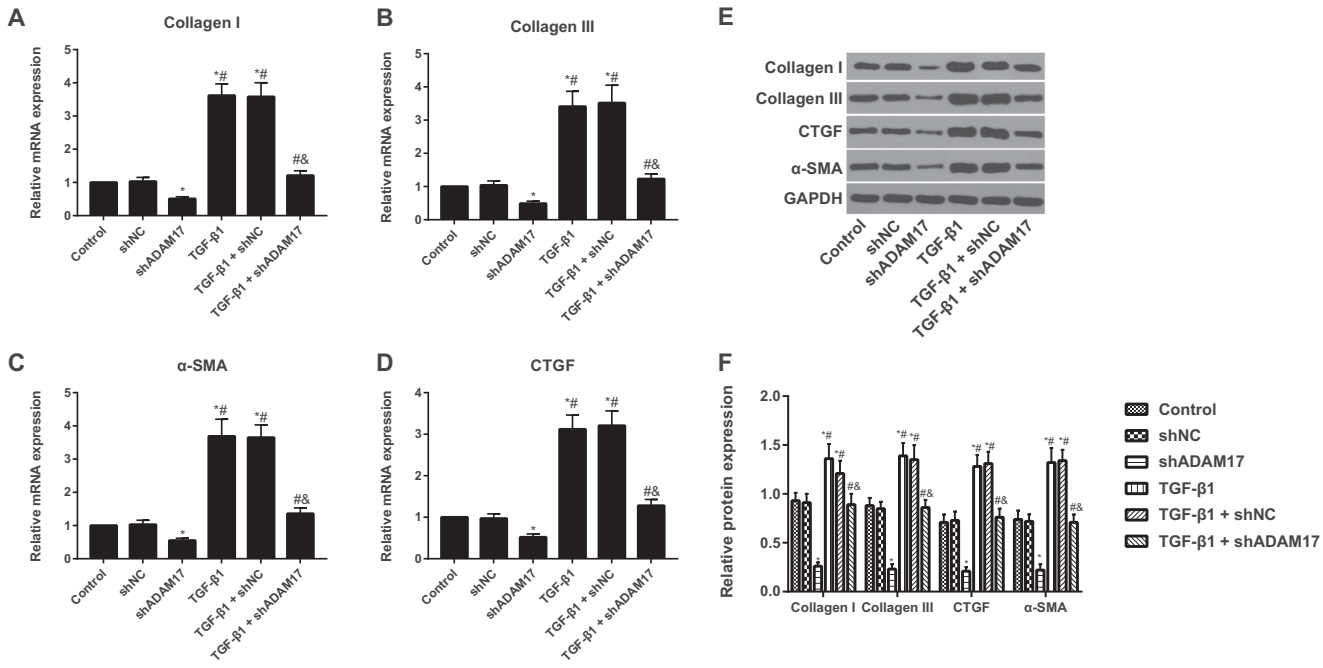

Figure 3. Expression of extracellular matrix (ECM)-related genes and proteins in keloid fibroblasts (KFs). (A–D) Expression of ECM-related genes collagen I (A), collagen III (B), α-SMA, (C) and CTGF (D) in KFs was quantified by qRT-PCR; (E, F) expression of ECM-related proteins was determined by western blotting. $^{*}p<.05$ vs. the control group and shNC group; $^{\#}p<.05$ vs. the shADAM17 group; $^{\&}p<.05$ vs. the TGF-β1 group and TGF-β1 + shNC group.

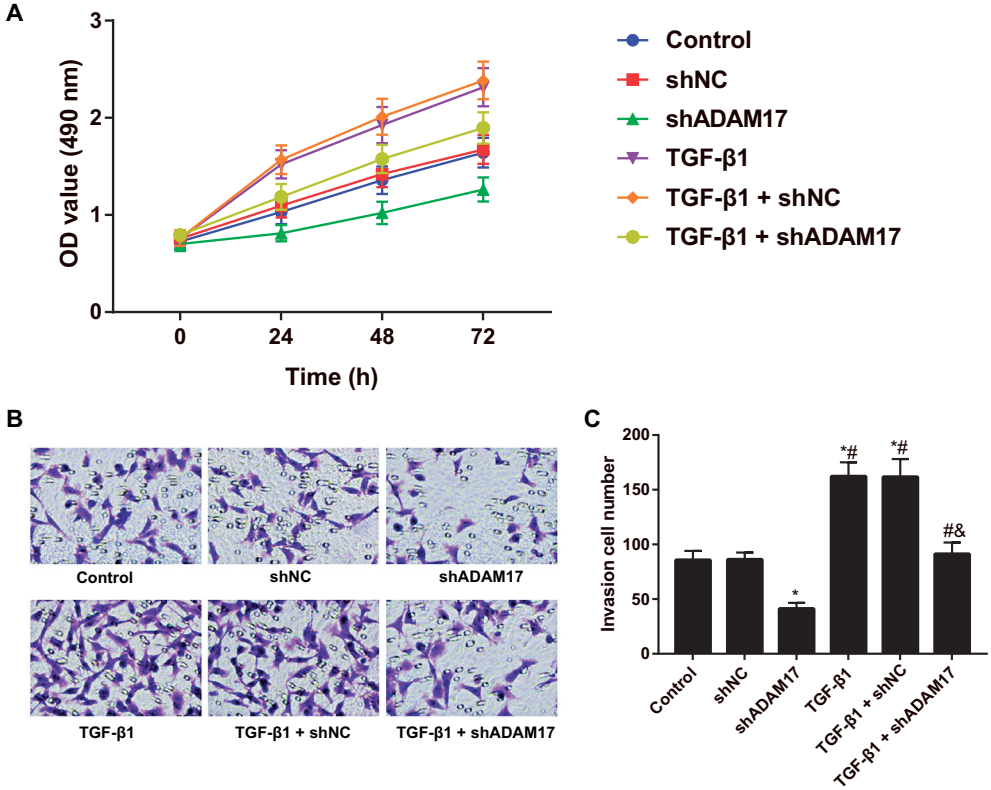

Figure 4. Comparison of the proliferative and invasive abilities of keloid fibroblasts (KFs). (A) The proliferative ability of KFs assessed by MTT assay; (B) the invasive ability of KFs examined by Transwell assay; (C) comparison of invasion cell number of KFs. $^{*}p<.05$ vs. the control group and shNC group; $^{\#}p<.05$ vs. the shADAM17 group; $^{\&}p<.05$ vs. the TGF-β1 group and TGF-β1 + shNC group.

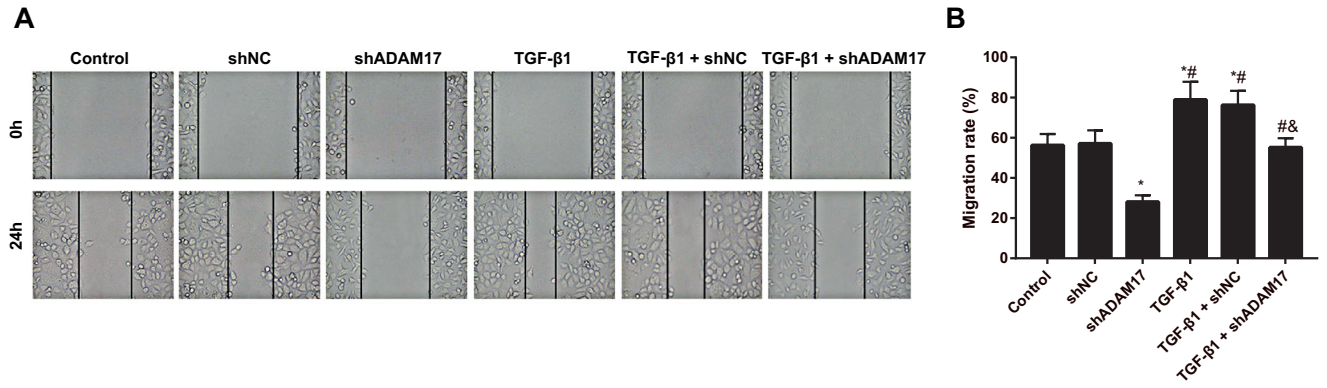

Figure 5. The migration ability of keloid fibroblasts (KFs) measured by wound healing assay. (A) The migration ability of KFs was evaluated by wound healing assay; (B) comparison of the migration rate of KFs. $^{*}p<.05$ vs. the control group and shNC group; $^{\#}p<.05$ vs. the shADAM17 group; $^{\&}p<.05$ vs. the TGF-β1 group and TGF-β1 + shNC group.

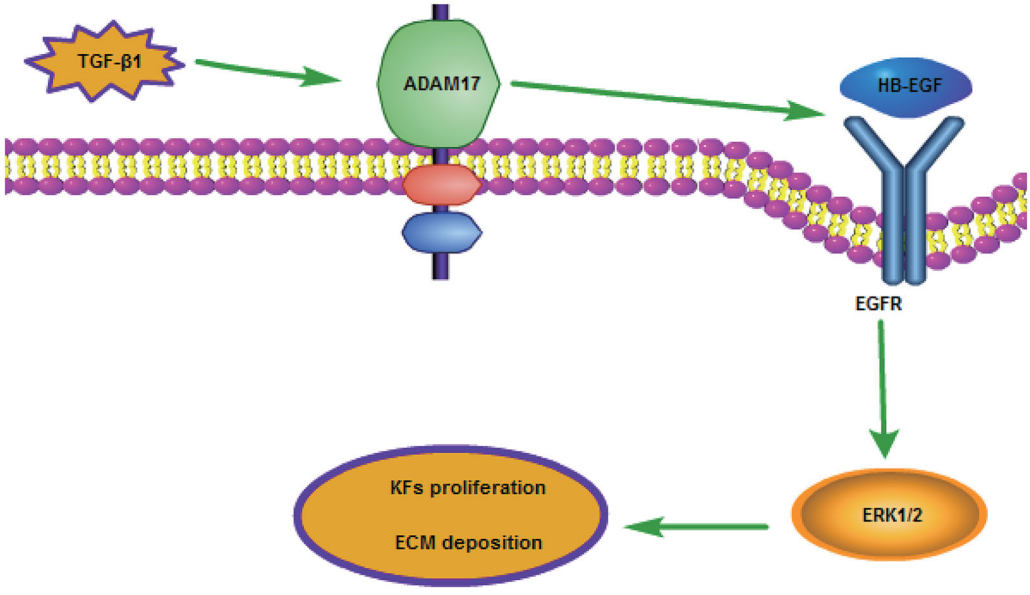

Figure 6. Mechanism of ADAM17 in KFs by mediating the EGFR/ERK signaling pathway. KFs: keloid fibroblasts; ECM: extracellular matrix.

ADAM17 knockout inhibits the proliferation and invasion of KFs by mediating the EGFR/ERK pathway

MTT and Transwell assays were used to detect the proliferation and invasion abilities of KFs, respectively (Figure 4). Compared with the control group and shNC group, KFs in the shADAM17 group declined appreciably in proliferative and invasive abilities, while KFs in the TGF-β1 group were improved in these abilities (all $p<.05$). In comparison with the TGF-β1 group and TGF-β1 + shNC group, KFs in TGF-β1 + shADAM17 decreased regarding proliferative ability and invasive cell number (all $p<.05$). All these findings demonstrate that silencing ADAM17 may inhibit the EGFR/ERK pathway to reduce the proliferation and invasion induced by TGF-β1.

ADAM17 knockout inhibits the migration of KFs by mediating the EGFR/ERK pathway

The migration ability of KFs in each group was measured with a wound healing assay (Figure 5). Compared with the control and shNC groups, KFs in the shADAM17 group had a decreased migration rate, while the TGF-β1 group had an increased migration rate (both $p<.05$). In comparison with the TGF-β1 and TGF-β1 + shNC groups, KFs in the TGF-β1 + shADAM17 group were unregulated in the migration rate (both $p<.05$). These results indicate that silencing ADAM17 may inhibit the EGFR/ERK pathway to reduce TGF-β1-induced migration of KFs (Figure 6).

Discussion

In the current study, ADAM17 expression was upregulated in keloid tissues and KFs. Similar to our finding, Gao et al. also discovered higher ADAM17 protein expression in cancer-associated fibroblasts extracted from human breast cancer than in normal fibroblasts [18]. Using gene chip analysis, Kawasaki et al. observed obvious ADAM17 upregulation in surgical scar tissues compared with leiomyoma tissues [19]. Hypoxia was revealed by Chen et al. to be able to induce the upregulation of ADAM17 in lung fibroblasts [20]. Charbonneau et al. also found upregulation of ADAM17 induced by hypoxia in fibroblasts and macrophage-like synoviocytes [21]. When skin is damaged, the local tissues may become hypoxic [22]. As reported by Masakazu Kawaguchi and

Suzuki, ADAM17 expression was elevated during the wound healing process, and cytokines, such as tumor necrosis factor-a (TNF-α), TGF-β and 12-O-tetra-decanoylphorbol-13-acetate, activated the expression of ADAM17 [23]. Thus, we speculated that the upregulation of ADAM17 in keloid tissues might be caused by the hypoxic environment, as well as the stimulation of cytokines, such as TGF-β, during the wound healing process. Accordingly, the KFs in our study were treated with TGF-β1, and we observed significantly increased ADAM17 expression in KFs, further suggesting that ADAM17 upregulation in KFs might be partially stimulated by TGF-β1. However, whether other factors are also involved in the upregulation of ADAM17 remains to be investigated.

Another important finding was pointed out in this study: silencing ADAM17 inhibited the protein expression of p-EGFR and p-ERK1/2/ERK1/2, while KFs treated with TGF-β1 presented the opposite trend. In addition to the activation of the Smad signaling pathway, TGF-β can also transactivate the EGFR/ERK1/2 pathway to promote invasive cell migration [24]. Importantly, the activation of ADAM17 leads to the release of the active EGFR ligand HB-EGF, which can activate EGFR autocrine or paracrine signaling [25]. Activated EGFR tyrosine kinase can initiate MAPK signaling pathways, such as the ERK, JNK and p38 MAPK pathways [26]. Evidence suggests that the ERK1/2 pathway is an important molecular pathway associated with cell proliferation, differentiation, migration, aging and apoptosis [27,28]. In the study of Zeng et al., ADAM17 knockout suppressed AngII-induced inflammation of vascular smooth muscle cells by inhibiting the EGFR-ERK1/2 pathway [29]. Taken together, silencing ADAM17 can inhibit the EGFR/ERK pathway in KFs, thereby blocking the downstream pathogenic effect of TGF-β1 on KFs.

In addition, the expression of ECM-related genes and proteins was examined, and as a result, TGF-β1 induced the upregulation of collagen I, collagen III, CTGF and α-SMA in KFs. By binding to TGF-βI and TGF-βII receptors on the cell membrane, TGF-β1 can initiate the phosphorylation of Smad2 and Smad3, and the activated Smad2/Smad3 complex binds to Smad4 to enter the nucleus [30,31]. Next, the Smad complex in the nucleus jointly improves the activity of gene transcription factors, thereby causing the excessive production of α-SMA, collagen I, collagen III, fibronectin (FN) and other cytokines [32,33]. Additionally, CTGF was shown to promote the proliferative division, collagen production and ECM deposition of fibroblasts [34]. In this study, silencing ADAM17 inhibited the expression of the above ECM-related factors. Ou et al. also reported the role of TGF-β in initiating CTGF expression in fibroblasts, but this regulatory effect can be reversed by ADAM17 siRNA [35]. Chen et al. found that inhibiting ADAM17 can mediate the ERK pathway to regulate CTGF expression in lung fibroblasts [14]. Given the above, silencing ADAM17 can mitigate TGF-β1-induced ECM deposition in KFs by suppressing the EGFR/ERK pathway.

Increasing evidence has demonstrated that TGF-β1 promotes the proliferation of KFs during the formation of keloids, while the proliferation of numerous fibroblasts promotes the secretion and accumulation of collagen, thus resulting in an imbalance between the synthesis and degradation of ECM [15,36–38]. In our study, TGF-β1 treatment enhanced the proliferation, invasion and migration of KFs, while silencing ADAM17 contributed to the opposite trend. Consistent with our results, Cui et al. also reported that EDB knockout inhibited the ERK pathway to suppress TGFβ1-induced proliferation and collagen deposition in KFs [17]. In a study by Xiao et al., ADAM17 knockout also reduced the invasion and migration of glioma cells by inhibiting the EGFR-MEK-ERK pathway [39]. All these findings suggest that silencing ADAM17 may inhibit the EGFR/ERK pathway to limit KF proliferation and ECM deposition, thus reducing the invasion and migration abilities of KFs.

To conclude, ADAM17 expression was upregulated in keloids, but silencing ADAM17 could reduce the deposition of ECM and the proliferation, invasion and migration of KFs via inhibition of the EGFR/ERK pathway, which provides a new theoretical basis for the targeted therapy of keloids.

Acknowledgements

Thank you for all the reviewers in this study.

Disclosure statement

The authors declare that they have no competing interests.

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