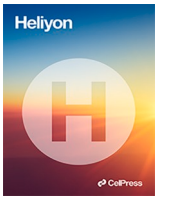
Heliyon 10 (2024) e38821

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Periostin regulates the activity of keloid fibroblasts by activating the JAK/STAT signaling pathway

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ARTICLE INFO

Keywords:
KEL-FIB
JAK/STAT
Migration
POSTN
Proliferation

ABSTRACT

A keloid is secondary to skin trauma or has spontaneously manifested as an overgrowth and occurs when the skin heals abnormally after an injury. The main pathological manifestations are abnormal proliferation of keloid fibroblasts (KEL-FIB). This study researched periostin (POSTN) on keloid fibroblasts (KEL-FIB) and the associated mechanism, aiming to provide a reference for the targeted therapy of keloid. We got tissues from Second People's Hospital of Guangxi Zhuang Autonomous Region between June 2022 and March 2023. POSTN expression was increased in keloid skin tissue and KEL-FIB than normal skin tissue and normal fibroblasts. We collected and inoculated KEL-FIB cells, transfection of si-NC (Silencing of POSTN negative control), si-POSTN (Silencing of POSTN), pcDNA-NC (Overexpression of POSTN negative control), and POSTN (Overexpression of POSTN) (Thermo Fisher Scientific) used Lipofectamine 2000 transfection reagent. Wound closure, cell proliferation viability, migrated cell numbers, and POSTN, p-JAK2, p-STAT3 protein levels were reduced in the si-POSTN group. Wound closure, cell proliferation viability, migrated cell numbers, and POSTN, p-JAK2, p-STAT3 protein levels were elevated in the POSTN group. POSTN protein levels did not changed and wound closure, cell proliferation viability, migrated cell numbers, were reduced in the POSTN + S-Ruxolitinib group. The study results indicated that POSTN promotes cell migration and proliferation by activating the JAK/STAT pathway, promoting KEL-FIB development.

1. Introduction

A keloid is secondary to skin trauma or has spontaneously manifested as an overgrowth [1,2] and occurs when the skin heals abnormally after an injury [3]. The main pathological manifestations are abnormal proliferation of keloid fibroblasts (KEL-FIB), abnormal deposition of extracellular matrix [4]. The clinical manifestations of keloid are crusting and plaques on the skin surface, tingling, and itching [5,6], which has a high relapse rate after treatment [7,8], so studying the mechanism of keloid development is vital to improve patient outcomes.

After skin tissue trauma, fibroblasts are overactive, proliferate, and secrete extracellular matrix, forming pathological scar tissue that is difficult for the body to absorb and remodel [9]. Periostin (POSTN) has an important role in healing trauma and scars [10,11].

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https://doi.org/10.1016/j.heliyon.2024.e38821
Received 5 March 2024; Received in revised form 5 September 2024; Accepted 30 September 2024
Available online 2 October 2024

Table 1
Information on keloid tissue and normal skin tissue specimens taken from the same patients.

Sample		Keloid/Normal skin
Number		33/33
Sex	Female	19 (57.58 %)
	Male	14 (42.42 %)
Age (year)		34.30 ± 9.45
Location		Pre- and postauricular area (6.06 %)
		Left neck (9.09 %)
		Right neck (6.06 %)
		Cheek (18.18 %)
		Eyelid (6.06 %)
		Thigh (12.12 %)
		Prothorax (9.09 %)
		Breast (6.06 %)
		Back (9.09 %)
		Lip (12.12 %)
		Pinna (6.06 %)
Previous treatment		0
Other skin diseases		0

Xu et al. [12] showed that POSTN level is upregulated in keloid tissue and KEL-FIB and that POSTN regulates KEL-FIB migration and collagen synthesis. Numerous studies have confirmed that silencing of POSTN expression inhibits fibroblast proliferation and migration and delays wound healing and remodeling [12–15]. POSTN interacts with the extracellular matrix components to influence cell biological functions, promote cell adhesion, activate gene expression, and promote wound healing [16–19], making it a vital target for keloid intervention therapy.

The Janus tyrosine kinase/signal transducer and activator of transcription (JAK/STAT) pathway is composed of tyrosine kinase–associated receptors JAK and STAT [20]. When cytokines bind to the receptor, molecule undergoes dimerization and a conformational change, then JAK is phosphorylated and forms a docking site with specific amino acids. The phosphorylated STAT protein is activated to form a dimer, and the dimeric STAT binds to specific DNA elements, affect gene expression [21,22]. It could participates cell growth, differentiation, and functional regulation [23] and are elevated in keloids [24]. Xiao et al. [25] demonstrated that IL-6 causes fibrotic injury of the peritoneal membrane by JAK/STAT pathway. Transcriptome analysis showed that STAT3 is vital in keloid fibroblasts [26]. Based on the association of JAK/STAT and POSTN with KEL-FIB, in this study, we hypothesized that POSTN could regulate the progress of keloid fibroblasts through JAK/STAT pathway.

2. Materials and methods

2.1. Clinical subjects and subgroups

Keloid and normal skin tissue were taken from selected keloid patients (n = 33) (see Table 1) at The Second People's Hospital of Guangxi Zhuang Autonomous Region between June 2022 and March 2023. Keloid and normal tissues were taken from the same selected keloid patients. Tissues were recorded as keloid or normal skin tissue (Trimmed normal skin around keloids). The Hospital approved this study procedure, patients signed informed-consent forms. POSTN mRNA and protein expression were examined using clinical samples.

2.2. Cells

The cells studied were human scar skin fibroblasts (KEL-FIB) (American Type Culture Collection Cell Bank, Manassas, VA, USA) and normal fibroblasts (Mingzhou, Zhejiang, China). The KEL-FIB and normal fibroblasts were cultured in DMEM medium (Thermo Fisher Scientific, Waltham, MA, USA) in 5 % CO2 humidified atmosphere at 37 °C. POSTN mRNA and protein expression, cell biological functions (migration, proliferation) and signal protein expression were examined using cells.

2.3. Cell culture, transfection and grouping

KEL-FIB cells were collected at the logarithmic growth stage and inoculated into 6-well plates (1×10^4 cells/well). Transfection of si-NC (Silencing of POSTN negative control), si-POSTN (Silencing of POSTN), pcDNA-NC (Overexpression of POSTN negative control), and POSTN (Overexpression of POSTN) used Lipofectamine 2000 reagent (Thermo Fisher Scientific, Waltham, Massachusetts, USA) when cell confluence reached 60–70 %. Added plasmid-lipid complex to the culture wells, incubated it at 37 °C in 5 % CO_2. Replaced complete culture medium (10 % FBS RPMI-1640) after 12 h, collected cells after 48 h, and analyzed transfected cells. JAK2 inhibitor S-Ruxolitinib (M3030, concentration at 5 μmol/mL) was added to a part of the POSTN group, designated as the POSTN + S-Ruxolitinib group. POSTN levels was analyzed to evaluate the transfection efficiency. si-NC, si-POSTN were designed, synthesized (GenePharma, Shanghai, China). The full-length POSTN cDNA was amplified by PCR using human cDNA as a template, and then the fragment was

Table 2
si-RNA sequence.

Gene	forward sequence (5′–3′)	reverse sequence (5′–3′)
si-POSTN	GAGAGCAACGTGAATGTTGAA	TTCAACATTCACGTTGCTCTC
si-NC	AACACCGAACGAGACACGATT	ACGAGACACGAACGGAGAATT

Table 3
Primer sequence.

Gene	forward sequence (5′–3′)	reverse sequence (5′–3′)
POSTN	TGCCCAGCAGTTTTGCCCAT	CGTTGCTCTCCAAACCTCTA
GAPDH	CATGTTCGTCATGGGTGTGAACC	GGTCATGAGTCCTTCCACGATACC

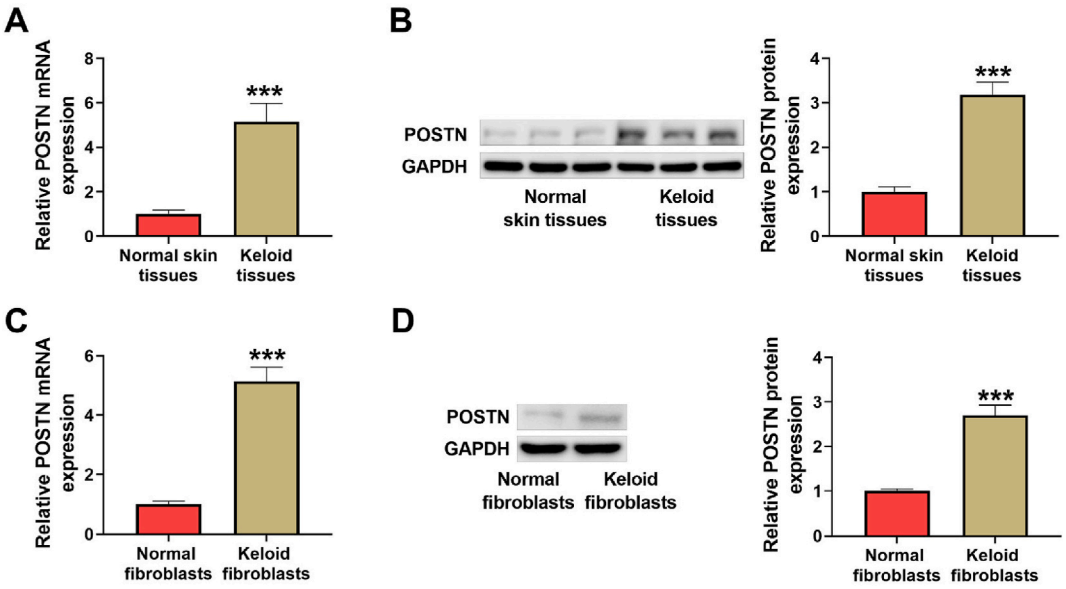

Fig. 1. POSTN was highly expressed in keloid tissue. (A) POSTN mRNA levels in Normal skin (n = 33) and Keloid tissues (n = 33) and **(C)** in Normal fibroblasts and Keloid fibroblasts were determined by qRT-PCR. **(B)** POSTN protein level in Normal skin (n = 33) and Keloid tissues (n = 33) and **(D)** in Normal fibroblasts and Keloid fibroblasts were determined by western blotting. Each group expriments were repeated more than three times. $P < 0.001$.

cloned into pcDNA3.1 plasmid, named POSTN plasmid. The empty pcDNA3.1 plasmid as a negative control, named pcDNA-NC. The sequence see Table 2.

2.4. qRT-PCR

The cells (normal fibroblasts and KEL-FIB) and tissues (normal and keloid) were collected for extracting total RNA by TRIzol RNA Reagent (Thermo Fisher Scientific). cDNA was synthesized by reverse transcription kit (K1691, Thermo Fisher Scientific), qRT-PCR was carried out by T100 thermal cycler (Bio-Rad, Hercules, CA, USA) according to instructions. Synthesized primers by Sangon Biotechnology Inc. (Shanghai, China) sequences (Table 3). POSTN mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method.

2.5. Western blotting

Total protein was obtained by lysing each cell (normal fibroblasts and KEL-FIB) or tissue (normal and keloid) using RIPA lysis solution (Beyotime, Shanghai, China). Using a NanoDrop2000c spectrophotometer (Thermo Fisher Scientific) determined the concentration, using a 15 % polyacrylamide separation gel performed electrophoresis, using wet transfer to the polyvinylidene difluoride membrane (IPVH00010, Merck). Blocked the membrane with 5 % skim milk powder (Beyotime) for 1 h, primary anti-POSTN, JAK2, p-JAK2, STAT3, p-STAT3, and GADPH antibodies diluted 1:1000 were added (Cell Signaling Technology, USA), incubated overnight at 4 °C. next, added the goat anti-rabbit secondary antibody IgG (1:10000, Abcam, Cambridge, UK), reacted for 40 min, Used MF-ChemiBIS 2.0 Image System (Bio-Rad) for imaging. Analyze the protein bands using Image J.

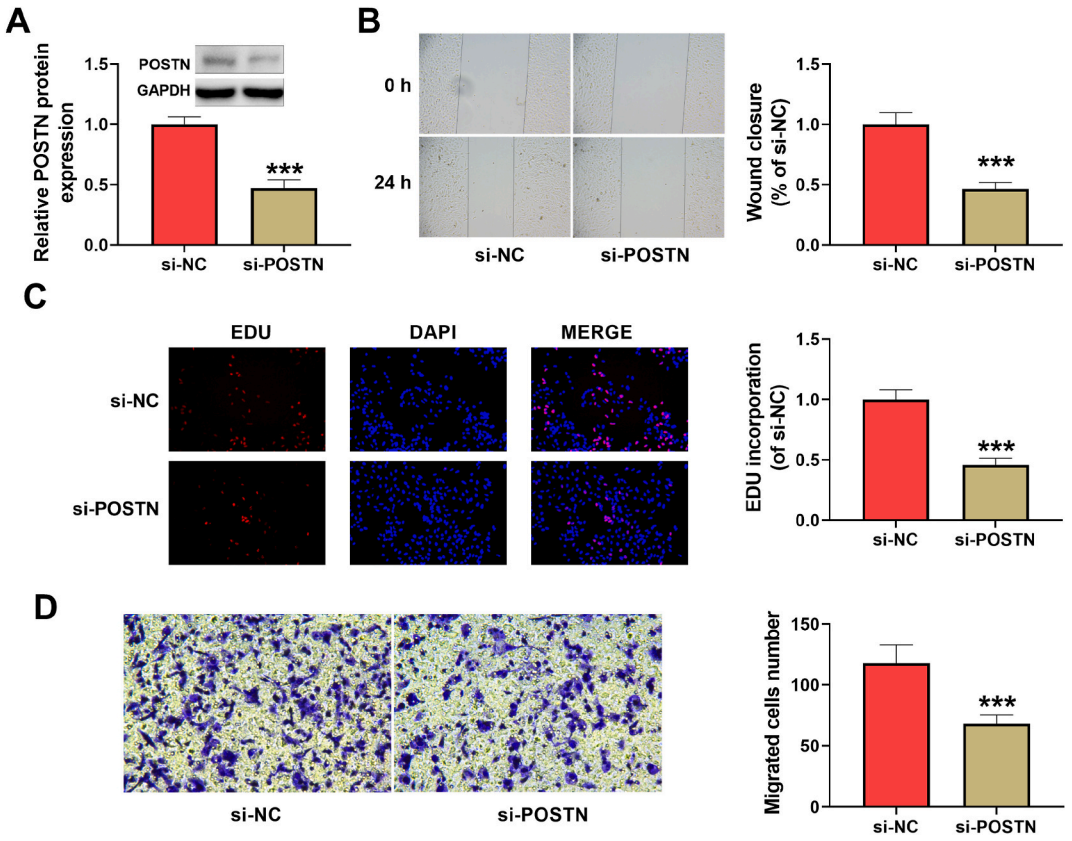

Fig. 2. The effect of silencing of POSTN on KEL-FIB. (A) POSTN protein level in si-NC and si-POSTN were determined by western blotting. **(B)** The relative wound closure in si-NC and si-POSTN were determined by scratch assay. **(C)** The cell proliferation viability in si-NC and si-POSTN were determined by EDU/DAPI assay. **(D)** The migrated cells number in si-NC and si-POSTN were determined by transwell assay. Each group expriments were repeated more than three times. $P < 0.001$.

2.6. Scratch test

Inoculated the KEL-FIB transfected cells in six-well plates, and confluence was close to 100 %. The medium was discarded, then cell layer was scratched with pipette tip, Rinse off free cells with PBS (Beyotime, Guangzhou, China). Next, added 2 mL of serum-free medium (Thermo Fisher Scientific, Waltham, Massachusetts, USA) for assessment at 0 h and 24 h. Each scratch was photographed under an inverted phase contrast microscope (XD-RFL, Sunny Optical, China), and the relative wound closure was analyzed using ImageJ software.

2.7. Transwell assay

To test the migration capability, each group of KEL-FIB of transfected cells was resuspended in 200 μL serum-free culture medium, inoculated into the upper chambers (8.0 μm pores) which inserted into 24-well plates. 600 μL essential medium supplemented with 15 % fetal bovine serum was added to space between chamber and well. After incubation for 24 h at 37 °C, removed non-migrating cells by using a swab, fixed migrating cells with 4 % paraformaldehyde (Beyotime, Guangzhou, China) on the lower surface for 20 min, stained with 0.1 % crystal violet (Merck) for about 15 min. Photographs were obtained under phase-contrast microscopy (Olympus, Tokyo, Japan).

2.8. 5 - Ethynyl - 2′- deoxyuridine/4′, 6-diamidino-2-phenylindole dihydrochloride staining (EDU/DAPI)

Inoculated the KEL-FIB transfected cells in 48-well plates at 1×10^4 cells/mL, 150 μL 50 μmol/L EDU medium (APEIBIO, USA) was added. Cultured the cells for 2 h (37 °C), washed three times with 200 μL PBS, then added 4 % paraformaldehyde, reacted for 40 min. Next, 200 μL 2 g/L glycine (Merck) was added and reacted for 5 min, then the solution was discarded, washed the cells three times with 200 μL PBS containing 0.5 % Triton X-100 (Merck). Finally, Apollo staining solution (RiboBio, China) was added, incubated the cells for 20 min in a dark room, added DAPI to each well at a concentration of 1 mg/L for 20 min in dark room. Observed, photographed using a fluorescent inverted microscope.

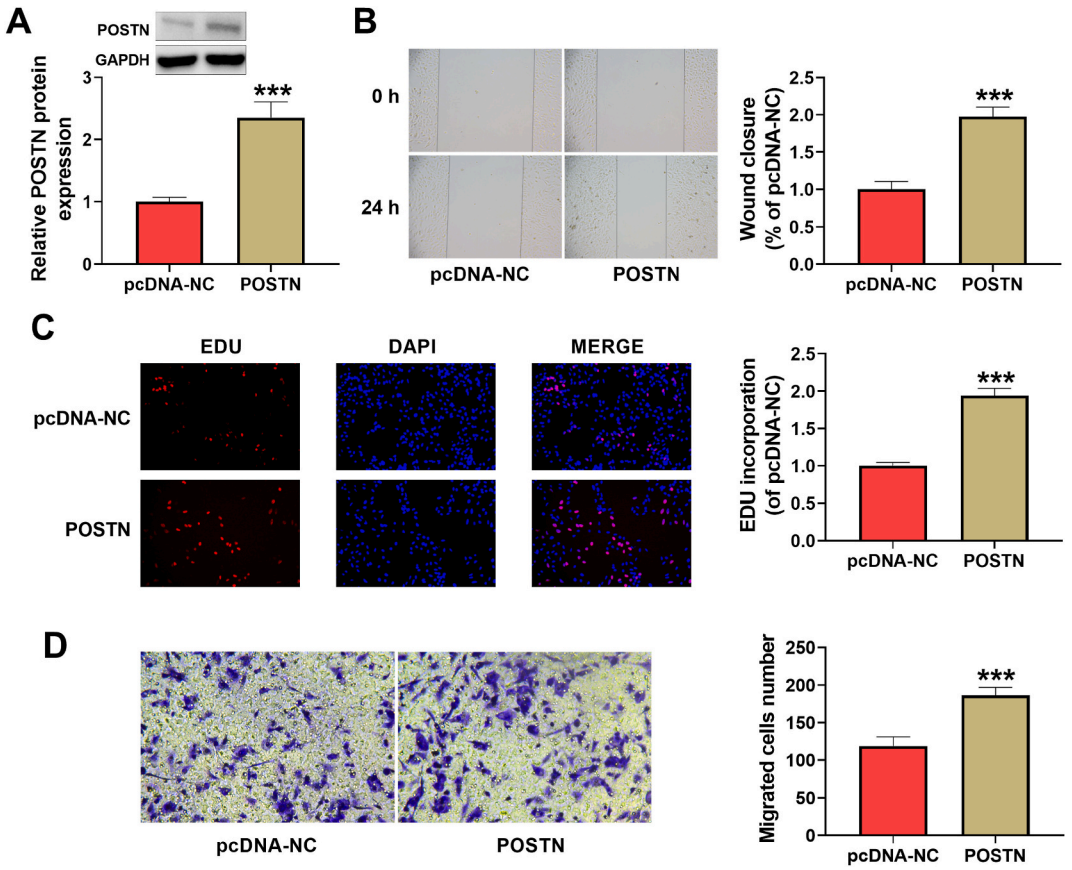

Fig. 3. The effect of overexpression of POSTN on KEL-FIB. (A) POSTN protein level in pcDNA-NC and POSTN were determined by western blotting. **(B)** The relative wound closure in pcDNA-NC and POSTN were determined by scratch assay. **(C)** The cell proliferation viability in pcDNA-NC and POSTN were determined by EDU/DAPI assay. **(D)** The migrated cells number in pcDNA-NC and POSTN were determined by transwell assay. Each group expriments were repeated more than three times. $P < 0.001$.

2.9. Statistical analysis

The data are given as mean ± SD. Statistical analysis used SPSS Statistics 22.0 (IBM, Armonk, NY, USA). Investigations were repeated more than three times. Student's *t*-test or ANOVA was employed for the analysis of differences. $P < 0.05$ was taken to indicate statistical significance.

3. Results

3.1. The expression of POSTN in keloid tissue

To investigate the expression POSTN in cell and tissue, qRT-PCR and Western blot assay were utilized. Results indicated that POSTN levels in keloid tissue and KEL-FIB were elavated than in normal skin tissue and fibroblasts (see Fig. 1A–D), confirming that POSTN was highly expressed in keloid tissue and KEL-FIB.

3.2. The impact of silencing of POSTN on KEL-FIB

In this study, KEL-FIB cells were transfected with POSTN small interfering RNA, and POSTN protein expression decreased (see Fig. 2A), confirming successful silencing. Scratch test and transwell assay indicated that silencing of POSTN significantly repressed the migration rate in KEL-FIB (see Fig. 2B and D). In addition, EDU staining suggested that cell proliferation viability were inhibited by silencing POSTN (Fig. 2C). According to the above results, we found that POSTN could regulate KEL-FIB progression and silencing POSTN expression inhibited the migration and proliferation of KEL-FIB, consistent with previous studies [27].

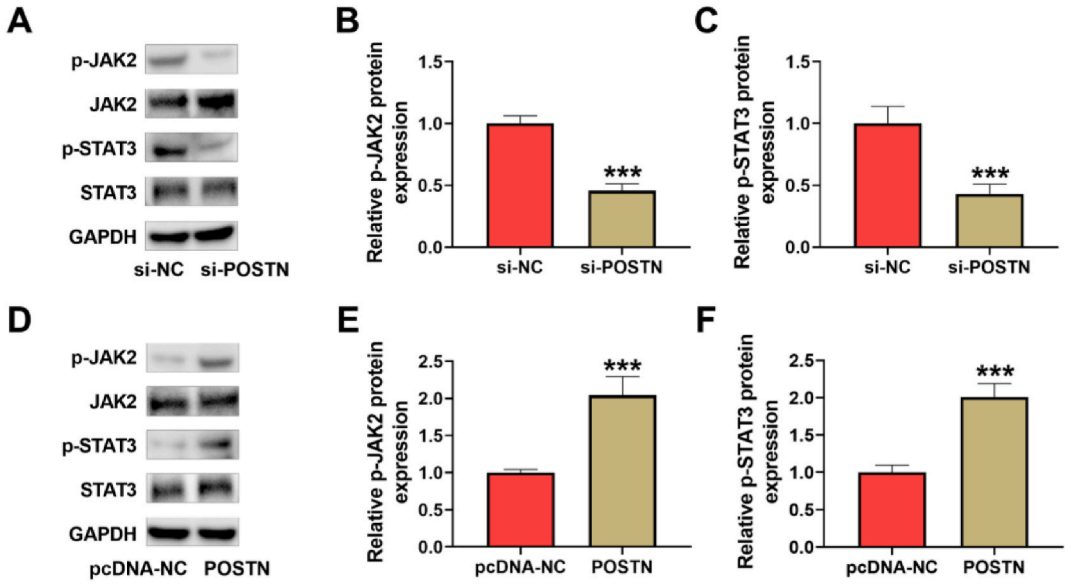

Fig. 4. The effect of POSTN on JAK/STAT pathway–related factors. (A–C) The p-JAK2 and p-STAT3 protein levels in si-NC and si-POSTN were determined by western blotting. **(D–F)** The p-JAK2 and p-STAT3 protein levels in pcDNA-NC and POSTN were determined by western blotting. Each group expriments were repeated more than three times. $P < 0.001$.

3.3. The impact of overexpression of POSTN on KEL-FIB

To further confirm the role of POSTN in KEL-FIB, transfected cells with a POSTN overexpression plasmid. The results revealed that POSTN protein levels increased (see Fig. 3A), confirming successful overexpression. Next, the relative wound closure, cell proliferation viability, and migrated cells numbers were examined, and the results showed that all three increased (see Fig. 3B–D), indicating that overexpression POSTN promoted the migration and proliferation of KEL-FIB.

3.4. The effect of POSTN on JAK/STAT pathway–related factors

This study has revealed that silencing of POSTN expression inhibits KEL-FIB proliferation and migration and that overexpression of POSTN promotes KEL-FIB proliferation and migration. Some studies have confirmed that JAK/STAT involves cell growth, migration, death, and the immune response [28–30]. Based on this, in this study, we determined phosphorylated JAK2 and STAT3 protein expression to address the relationship of the POSTN and JAK/STAT in process of KEL-FIB. The results showed that p-JAK2 and p-STAT3 protein levels decreased after silencing of POSTN expression (see Fig. 4A–C), and p-JAK2 and p-STAT3 protein levels increased after overexpression of POSTN (see Fig. 4D–F), indicating that POSTN promotes JAK/STAT pathway–related factors expression, activates the JAK/STAT pathway, promotes the development of KEL-FIB.

3.5. S-Ruxolitinib partially reversed the effect of POSTN overexpression

In this study, S-Ruxolitinib was selected as a JAK2 inhibitor. The results showed that overexpression of POSTN improve the POSTN protein expression level, S-Ruxolitinib did not affect POSTN protein levels (see Fig. 5A), the relative wound closure (see Fig. 5B), cell proliferation viability (see Fig. 5C), and migrated cells numbers (see Fig. 5D) decreased in the POSTN + S-Ruxolitinib group. This suggests that S-Ruxolitinib can partially reverse the phenomenon that overexpression POSTN promotes cell migration and proliferation.

4. Discussion

The main effector cells of keloids are fibroblasts, and proliferation, migration, and invasion are important indicators of keloid progression [31,32]. Studies have shown that keloid fibroblasts have the ability to overproliferate, resulting in incomplete apoptosis and abnormal synthesis of collagen and inducing continuous proliferation of keloid tissue [33–35].

POSTN is an important factor for promoting angiogenesis, inhibiting apoptosis of vascular endothelial cells in scar tissue, promoting vascular endothelial cell function, allowing scar fibroblasts to tolerate a hypoxic and hypotrophic state in which normal fibroblasts have difficulty surviving, and promoting scar formation and development [36]. Tischler et al. [37] demonstrated that POSTN is involved in keloid formation, and several other studies [38–43] confirmed that POSTN expression in keloids is higher, which promotes invasion and migration of MSC cells. Crawford et al. [44] and Zhang et al. [27] confirmed that silencing of POSTN expression

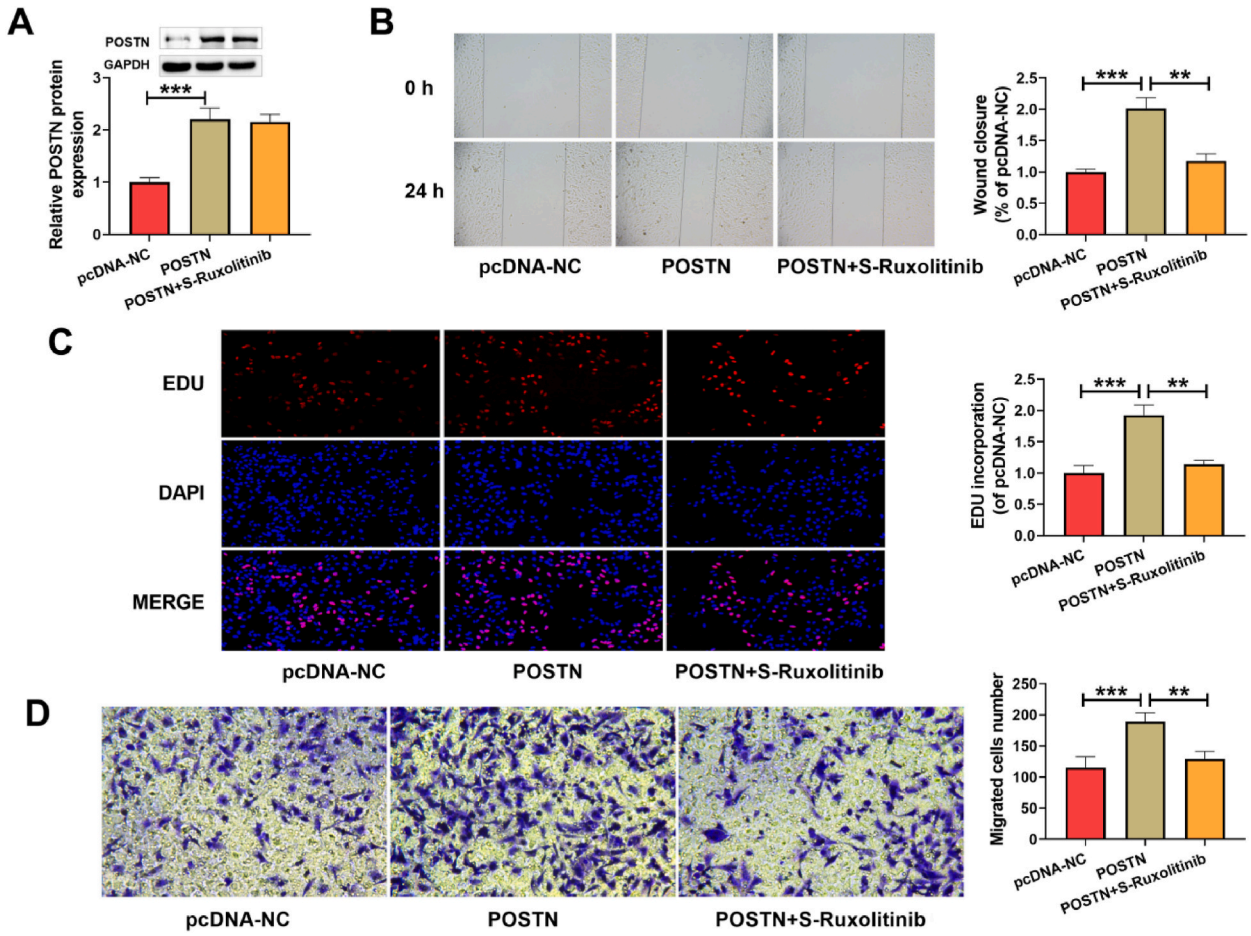

Fig. 5. S-Ruxolitinib partially reversed the effect of POSTN overexpression. (A) The POSTN protein levels in pcDNA-NC, POSTN, POSTN + S-Ruxolitinib were determined by western bloting. **(B)** The relative wound closure in pcDNA-NC, POSTN, POSTN + S-Ruxolitinib were determined by scratch assay. **(C)** The cell proliferation viability in pcDNA-NC, POSTN, POSTN + S-Ruxolitinib were determined by EDU/DAPI assay. **(D)** The migrated cells number in pcDNA-NC, POSTN, POSTN + S-Ruxolitinib were determined by transwell assay. Each group expriments were repeated more than three times. $P < 0.05$.

inhibited KEL-FIB proliferation and migration. Elliott et al. [45] showed that silencing of POSTN expression decreased the number of traumatic fibroblasts in mice, decreased the ability to synthesize collagen matrix, and slowed wound contraction and healing and that overexpression of POSTN reversed this phenomenon. This study showed that POSTN was upregulated in keloid tissue and KEL-FIB.

Numerous reports have indicated that signal pathways are vital in the development of KEL-FIB. Hu et al. [46] and Li et al. [47] reported that TIEG1 and DKK3 attenuate the progress of KEL-FIB by inhibiting TGF-β/Smad pathway, regulating fibroblast migration, proliferation. Further, Euler et al. [48] demonstrated that IFN-γ reduces KEL-FIB proliferative viability by activating the JAK1/STAT1 pathway, and Lv et al. [49] demonstrated that silencing of circCOL5A1 expression reduced KEL-FIB migratory and proliferation capacity by inhibitig PI3K/AKT pathway. Additional studies have shown that POSTN is associated with the JAK/STAT pathway: Amara et al. [50] demonstrated that POSTN promotes liver fibrosis and cirrhosis progression through the JAK/STAT pathway, and Dai et al. [51] demonstrated that POSTN mediates peritoneal inflammation and injury through JAK/STAT. Based on association between POSTN, JAK/STAT, and KEL-FIB, this study hypothesized that POSTN regulates KEL-FIB development through the JAK/STAT pathway, results confirmed that POSTN promotes KEL-FIB development by activating JAK/STAT pathway.

STAT3 is highly expressed in keloid tissue and in cultured KEL-FIB, with tyrosine phosphorylation occurring at position 705. Aberrantly activated STAT3 induces abnormal expression of B lymphoma-2 and cyclin D1, and promotes keloid growth [52–54]. Studies has demonstrated that inhibition of JAK2 kinase blocks the STAT3 pathway [53,55]. S-Ruxolitinib is a selective kinase inhibitor that inhibits JAK phosphorylation, causes a blockade of downstream STAT3 phosphorylation, and blocks multiple cytokine signaling pathways [56]. Ladislau et al. [57] revealed that blocking the IFN-induced JAK/STAT pathway inhibited inflammatory diseases and exerted targeted therapeutic effects on dermatomyositis. Alkhalifah et al. [58] showed that S-Ruxolitinib prevented pemphigus vulgaris. The results of this study confirmed that S-Ruxolitinib could partially reverse the promotion of KEL-FIB proliferation and migration after overexpression of POSTN by blocking the POSTN-induced JAK/STAT pathway. Therefore, S-Ruxolitinib intervention in the JAK/STAT pathway might be used clinically to interfere with POSTN expression, inhibit KEL-FIB proliferation and

migration, and regulate KEL-FIB development, providing a new target for keloid-related disease treatment.

In summary, this study suggested that POSTN expression was upregulated in keloid tissue and KEL-FIB, POSTN can activate the JAK/STAT pathway, promote cell proliferation, migration, promote the development of KEL-FIB.

Ethics declarations

The Second People's Hospital of Guangxi Zhuang Autonomous Region approved this study procedure and patients signed informed-consent forms.

Funding

This work was supported by funds from Zunyi Science and Technology and the Big-Data Bureau and Zunyi Medical and Pharmaceutical College (HZ, 2021-222).

Consent to participate

We secured a signed informed consent form from every participant.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

CRediT authorship contribution statement

Jiao Tian: Validation, Supervision. **Xin Liu:** Methodology, Investigation. **Dawei Zhu:** Software, Resources. **Jianyi Li:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We express thanks to Zunyi Medical College, The Second People's Hospital of Guangxi Zhuang Autonomous Region, and The Affiliated Hospital of Zunyi Medical University for their support of this study.

Appendix BSupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38821.

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