Genes & Nutrition

RESEARCH

Open Access

Genistein inhibited endocytosis and fibrogenesis in keloid via CTGF signaling pathways

Chun-Te Lu[1,2], Jiunn-Liang Ko[3,4], Chu-Chyn Ou[5,6], Chih-Ting Hsu[3,7], Yu-Ping Hsiao[3,8] and Sheau-Chung Tang[9*]

Abstract

Background This study aimed to evaluate soy isoflavones' effect and potential use—specifically genistein—in treating human keloid fibroblast cells (KFs) and in a keloid tissue culture model.

Methods To investigate the effects of genistein on keloid, a wound-healing assay was performed to detect cell migration. Flow cytometry was used to measure apoptosis. Western blotting and immunofluorescence staining were performed to detect the expression of target proteins. KF tissues were isolated, cultured, and divided into the control, silenced connective tissue growth factor (CTGF) proteins, and shNC (negative control) groups.

Results Genistein suppressed cell proliferation and migration, triggering the cell cycle at the G2/M phase and increasing the expression of p53 dose-dependent in keloids. Genistein inhibited the expression of COL1A1, FN, and CTGF mRNA and protein. Knockdown CTGF reduced the migrated ability in KFs. Genistein also abated TGF-β1-induced keloid fibrosis through the endocytosis model. Separated and cultured the keloid patient's tissues decreased the cell migration ability by genistein treatment and was time-dose dependent.

Conclusions This study indicated that genistein-induced p53 undergoes cell cycle arrest via the CTGF pathway-inhibited keloid cultured cells, and genistein suppressed the primary keloid cell migration, suggesting that our research provides a new strategy for developing drugs for treating keloids.

Highlights

1. Genistein decreased proliferation and promoted cell cycle arrest at the G2/M phase in keloid cells.
2. Genistein inhibited the expression of COL1A1, FN, and CTGF mRNA and protein expressions.
3. Genistein enhanced endocytosis in keloids and blocked the stimulation of growth factor.
4. Genistein has therapeutic effects in treating keloids and preventing recurrence.

Keywords Genistein, Keloid, Collagen, CTGF, Fibronectin

*Correspondence:
Sheau-Chung Tang
s6160051@nutc.edu.tw; s6160051@gmail.com
Full list of author information is available at the end of the article

Introduction

Keloid disease (KD) is a complex fibroproliferative disorder caused by excessive skin wound healing [5]. Keloids behave like benign tumors due to their uncontrolled growth, exhibiting similar characteristics. The excessive keloid scarring is attributed to heightened proliferation and excess collagen deposition by fibroblasts in the extracellular matrix (ECM) [4]. It is characterized by increased itching and enlargement, along with hypertrophic scars that cause distress to patients. Unlike hypertrophic scars, keloids often grow beyond the boundaries of the original wound. Keloids exhibit significant invasiveness and cellular migration capability, contributing to severe cosmetic disfigurement and limitations in joint mobility for those affected. To this day, treatments for keloid scars include surgical excision and laser; however, these are only partially effective. They can easily recur and continue to increase. Solely using surgical excision for keloid treatment is associated with recurrence rates as high as 45–99% [15]. The development of improved treatments will require a deeper understanding of the molecular mechanisms that cause healing wounds to progress to keloid scars.

Transforming growth factor-β (TGF-β1) is a versatile protein orchestrating various cellular processes, including cell growth, differentiation, motility, and extracellular matrix production during routine wound healing. However, its dysregulation has been associated with excessive scar formation and fibrotic disorders [11, 29, 35]. Elevated levels of TGF-β1 can trigger fibroproliferative disorders by promoting collagen synthesis, thereby exacerbating keloid formation. Additionally, connective tissue growth factor (CTGF or CCN2) serves as a critical mediator of the fibrotic response to TGF-β1, stimulating fibroblast proliferation and synthesizing extracellular matrix (ECM) proteins such as collagen and fibronectin [16, 40]. KFs are implicated as mediators of elevated growth factor, chemokine, or cytokine production and the excessive accumulation of ECM components, such as type I/III collagen 1A1 and fibronectin [3, 34]. TGF-β signaling pathways, intricately woven and multifaceted, hold pivotal significance in the pathogenesis of keloid scars. CTGF, intimately linked to dermal fibrosis, distinctly amplifies TGF-β activity [22]. Inhibiting CTGF expression emerges as a promising avenue in the therapeutic approach to keloids, heralding significant implications for treatment interventions.

Genistein, a natural flavonoid compound predominantly sourced from legumes, has garnered significant attention for its role in tumor prevention and treatment [13]. Genistein exhibits diverse biological actions in vitro depending on the cell type; these include antioxidant, anti-inflammation, anti-angiogenesis, and apoptosis [14, 23, 31]. Previous studies have found that genistein can inhibit the proliferation of fibroblasts and cancer cells [25, 26]. Recognized as a receptor tyrosine kinase inhibitor (TKI), it selectively targets VEGF receptors (VEGFRs), FGF receptors (FGFRs), and PDGF receptors (PDGFRs), exhibiting established effectiveness in antiangiogenesis and the management of diverse cancer types. The ameliorated efficacy of genistein was analyzed in cultured normal human dermal fibroblasts (NHDFs) [16] and culture keloid fibroblasts [6]. Studies indicated that genistein was a biologically active compound and induced G2/M arrest and apoptosis in various cancer cells. These include hepatocellular carcinoma (HCC) [39], renal cell carcinoma (RCC) [27], colorectal cancer (CRC) (X. [10]), prostate cancer, and gynecologic malignancies [28]. Genistein has been implicated in anti-inflammatory and anti-skin aging responses during wound healing [7]. Although some literature discusses whether genistein may increase or inhibit COL1A1 and FN expression, no studies have ever delved into whether genistein effectively treats keloid scars.

This study investigates whether genistein influences CTGF-induced proliferation, migration, and ECM deposition in both KFs and NHDFs cultured in vitro. Furthermore, it seeks to explore the modulation of keloids by genistein, elucidating its effects on critical proteins such as CTGF, COL1A1, and FN.

Materials and methods

Reagents and chemicals

Genistein, phosphate-buffered saline, CCK-8 kit, α-SMA Ab (#A-5228), and anti-β-actin (#A-4700) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The broad-spectrum caspase inhibitor Z-VAD-FMK (#sc-3067), anti-CTGF (#sc-14939), anti-MMP2 (#sc-13595), anti-fibronectin (#sc-88422), anti-Cdk2 (#sc-136191), anti-cyclin A (#sc-53228), anti-p21(#sc-6246), and anti-P53(#sc-126), anti-DNMT-3a(#sc-56656), anti-DNMT-3b(# sc-393845) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, USA). Anti-COL1A1 (#91,144), HRP-linked anti-mouse, anti-FGFR1(#9740), anti-VEGFR1(#64,094), anti-PDGFRα(#3174), anti-PD GFRβ(#3169), anti-PCNA(#13,110), and anti-rabbit IgG antibodies were purchased from Cell Signalling Technology (Beverly, MA, USA). Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin, F-actin Alexa Fluor™ 594 Phalloidin (#A12381), G-actin Deoxyribonuclease I, Alexa Fluor™ 488 Conjugate (#D12371), fetal bovine serum, goat anti-mouse IgG H&L (FITC) (#ab6785), and goat anti-rabbit IgG H&L (FITC) (#ab6717) were purchased from Gibco-Invitrogen™ (Carlsbad, CA, USA). Anti-fibronectin (#610077) was obtained from BD (Becton Dickinson,

NY, USA). Anti-CDK1 (#06–966) was purchased from Millipore (Merck Millipore, Bedford, MA, USA), and anti-Ki-67 (#GTX16667) was purchased from GeneTex (GeneTex Inc, Irvine, CA, USA).

Cell culture and primary keloid spheroid culture

Keloid epidermal fibroblast (KF) cells and Normal human dermal fibroblasts (NHDF) cells were acquired from the Food Industry Research and Development Institute, Taiwan. The cells were cultured with DMEM supplement with 10% FBS, 25 mM HEPES (Hyclone), penicillin (100 IU/mL), streptomycin (50 mg/mL) at 37 ℃ in a humidified atmosphere with 5% CO_2 and sub-cultured every 2–3 days.

Human keloid-derived fibroblast cell cultures

Keloid tissues were obtained from the keloid patients ($n=5$) after acquiring informed consent according to a protocol approved by the Taichung General Hospital of Medicine Institutional Review Board (TCVGH-IRB No. SF23056CSF23056C). All experiments involving humans were performed in adherence to the Helsinki Guidelines. Keloid tissue was washed with PBS, and tissues were homogeneous and cut into 5 mm × 5 mm × 10 mm diameter pieces onto HydroCell 24-well plates (Nunc, Rochester, NY) containing 0.1% collagen 1A1ase DMEM medium. After four days, the dermis and epidermis were separated, and the dividing single KFs were collected for subsequent studies. Cells were grown in DMEM containing 10% fetal bovine serum (FBS; Biowest, France) and 1% A/A in a humidified incubator with 5% CO_2 at 37 °C. Cells at passages 3–6 from three separate cell lines were used in the below experiments. Human keloid-derived fibroblast cell cultures treated with genistein or chemicals were the same with culture cell lines.

RNA extraction and quantitative PCR

The RNA was isolated from KEL and NHDF cells using the rare RNA reagent. RNA isolation, RT-PCR, and quantitative PCR were performed on an Applied Biosystems instrument as described previously [32]. The sequences of the primers for each gene were listed in supplementary Table 1. The quantification of mRNA expression was standardized to the endogenous control GAPDH. The relative gene expression was evaluated using the $2^{-\Delta\Delta Cq}$ method.

Migration assay and cell proliferation assay

Cells (1×10^4 cells/well) were seeded on the coverslips, and the silicon inserts (ibidi GmbH, Gräfeling, Germany) were used for cells in two individual wells as the wound closure seeding model. After 24 h, the culture insert was removed, and the cells were treated with or without genistein and treated with different concentrations of genistein (40 or 200 μM) for 16, 24, and 48 h. For the cell proliferation assay, cells were treated with genistein at various concentrations (25, 50, 100, 200, and 400 μM) for 1, 3, 5, and 7 days. The cells were washed with PBS and incubated for 2 h with CCK-8 reagent; the OD absorbance was recorded at 450 nm using a microplate reader (Tecan Group Ltd.)

Immunochemistry

Cells were extracted with RIPA buffer (Millipore) to yield cell lysates. The total protein concentration in each sample was assayed using the Bradford method. (Bio-rad). Each sample's aliquots (20 μg total protein) were then loaded on 12.5% SDS–polyacrylamide gels. After electrophoresis, the proteins were transferred onto a nitrocellulose blotting membrane (Amersham Biosciences, GE Healthcare, Chicago, IL) and immunoblotted with specific antibodies—all antibodies at a dilution of 1: 1000 and incubated at 4 ℃ overnight. The complete protocol of the western blot analysis has been described in a previous publication [41]. PVDF membranes were washed three times and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:20,000) for 1 h at room temperature. The ECL Plus Reagent Kit (Roche Applied Science, Indianapolis, IN, USA) was used to detect the protein signals with an X-ray film (Fuji Film).

Immunofluorescence assay

For immunofluorescence assay, under the same conditions, all samples were fixed in 4% paraformaldehyde overnight, permeabilized with 100% methanol for 2 min, and blocked in 5% BSA for 1 h at room temperature. Samples were incubated with primary antibodies overnight in the dark. F-actin Alexa Fluor™ 594 Phalloidin, G-actin Deoxyribonuclease I, Alexa Fluor™ 488 Conjugate, COL1A1 (E6A8E) Rabbit mAb (CST #39952), and Mouse Anti-Fibronectin (BD #610077) diluted 1:1000 was used to detect the target proteins. The samples were then stained with secondary antibodies (Alexa Fluor Registered 594 goat anti-mouse IgG [1:200] and Alexa Fluor Registered 488 goat anti-rabbit IgG [1:200]) for 1 h and mounted with DAPI (Invitrogen). The fluorescent images were obtained with a fluorescence microscope (DP70; Olympus). The intensity value was compared with the control or genistein-treated group, and then the grayscale images were analyzed using Image J software.

Immunohistochemistry assay

The patient's tissues were cut into 5 mm × 5 mm × 10 mm diameter pieces onto HydroCell 24-well plates and cultured within containing genistein (40, 200 μM) DMEM medium for 3, 7, and 14 days. Changed the medium

every day until the tissues were harvested on the specified number of days, then fixed in 4% paraformaldehyde for 24 h at 4 °C, washed with water for at least 4 h, and finally embedded in paraffin. According to the manufacturers ' guidelines, the paraffin Sects. (4 μm thick) were stained with hematoxylin and eosin (H&E, ab245880, Abcam, Cambridge, UK). Sections were incubated overnight at 4 °C with primary antibodies: COL1A1(1:200), FN (1:250), CTGF (1:200) mAb stain. After washing three times with Tris-buffered saline containing 0.025% Triton X-100, the sections were incubated with secondary antibodies for 1 h at room temperature. Images were captured with an Olympus BX53 microscope. The optical density of each image was quantified using ImageJ imaging software.

Flow cytometry assay

Cells were seeded at 1.5×10^5 /well and treated with genistein at 40, 100, and 200 μM for 24 h. Cells were harvested and washed with cold PBS, followed by fixation in 70% ethanol at 4℃ overnight. Fixed cells were treated with RNase (100 μg/ml, 50 μl) and stained with 100 μl Annexin V binding solution containing Annexin V-FITC and propidium iodide (PI). Resuspended cells were incubated for 15 min at room temperature. The apoptosis assays were run and analyzed by fluorescent cell sorting with the BD FACSJazz Cell Sorter (BD Bioscience, San Jose, CA).

Statistical analyses

The quantification of the western blots was performed using ImageJ software. The expression levels of each protein were normalized to those of corresponding β-actin or GAPDH, respectively. Results are reported as means standard deviation. The student's *t*-test was used to compare the differences between the two groups. All experiments were performed in triplicates. The two-tailed *p*-value < 0.05 in statistical analysis was defined as statistically significant.

Results

Genistein inhibited migration proliferation in KFs

To investigate genistein's effect on keloids' migration ability, cells were pre-treated with genistein at 40 μM or 200 μM, and images were captured at 16 and 24 h using a wound healing assay. Compared to the control, genistein significantly decreased the migration of KFs, with the extent of decrease dependent on the concentration of genistein (Fig. 1A). The percentage decrease was normalized to the untreated control, as shown in Fig. 1A (right) ($p < 0.05$). Next, we evaluated the effect of genistein on the proliferation of KFs using the CCK-8 assay. Compared with the control group, various concentrations of genistein (25 μM, 50 μM, 100 μM, and 200 μM) at days 1, 3, 5, and 7 inhibited the proliferation of KFs in a time- and concentration-dependent manner in Fig. 1B ($p < 0.05$). However, genistein (25 μM, 50 μM, 100 μM, and 200 μM) did not affect cell proliferation on the first day but significantly inhibited wound healing. To elucidate the mechanisms by which genistein suppresses the proliferation of KFs, we analyzed cell cycle distribution using flow cytometry. After 24 h of pre-treatment with genistein at concentrations of 40 μM or 200 μM, the proportion of cells markedly increased from approximately 17.8% to 38.2% in the G2/M phase while decreasing from 70.7% to 43.5% in the G0/G1 phase in response to genistein. In contrast, the cell numbers in the S phase remained unaltered upon the application of genistein (Fig. 1C).

To further explore the regulation of apoptosis-related proteins by genistein, KFs were treated with genistein at concentrations of 40 μM or 200 μM for 24 h, and the apoptosis status was determined by flow assay. The proportion of total apoptotic (Annexin V-positive) cells increased concentration-dependent

(See figure on next page.)

Fig. 1 Genistein inhibits migration and proliferation in human keloid fibroblast (KF) cells **A** KFs were treated with 0, 40, and 200 μM genistein, and the migration was by wound healing assays. The wound closure was captured at 0, 16, and 24 h after removing the culture insert. The magnification of 100 × was used. Dotted lines were used to indicate areas of cell migration. The quantification of cell migration using the percentage of Image J software. Significant differences were compared in the genistein group (40, 200 μM) with the control group of the human KFs. All experiments have at least three independent experiments. *$p < 0.05$ vs. control, **$p < 0.01$ vs. control. **B** Proliferation rates of human KFs were detected by CCK-8 assay. Cells were treated with genistein at 25, 50, 100, 200, and 400 μM for 1,3,5 and 7 days, respectively. Cell viability % = [(OD test – OD blank)/(OD control-OD blank)] × 100%. **C** The cell numbers in each cell cycle phase were analyzed by flow cytometry following treatment with genistein for 24 h. The total number in different cell cycle phases was measured and presented as a percentage on the right side. Values represent the means ± SD, $n = 3$/each group. *$p < 0.05$, ** $p < 0.01$ vs. the control. **D** KFs were treated with 40 and 200 μM genistein for 24 h. The apoptosis was detected by Annexin V/PI double staining assay. The ratio of stained cells in each group was determined. *$p < 0.05$ vs. control. DATA were analyzed using FlowJo7.6 software (Tree Star, Inc.). **E** KFs were pretreated with the broad-spectrum caspase inhibitor Z-VAD-FMK (Santa Cruz Biotechnology, Dallas, TX, USA) at 20 μM for 4 h, then treated with or without genistein 200 μM for 24 h. The wound closure was captured at 0, 16, and 24 h after removing the culture insert. The quantification of cell migration using the percentage of Image J software

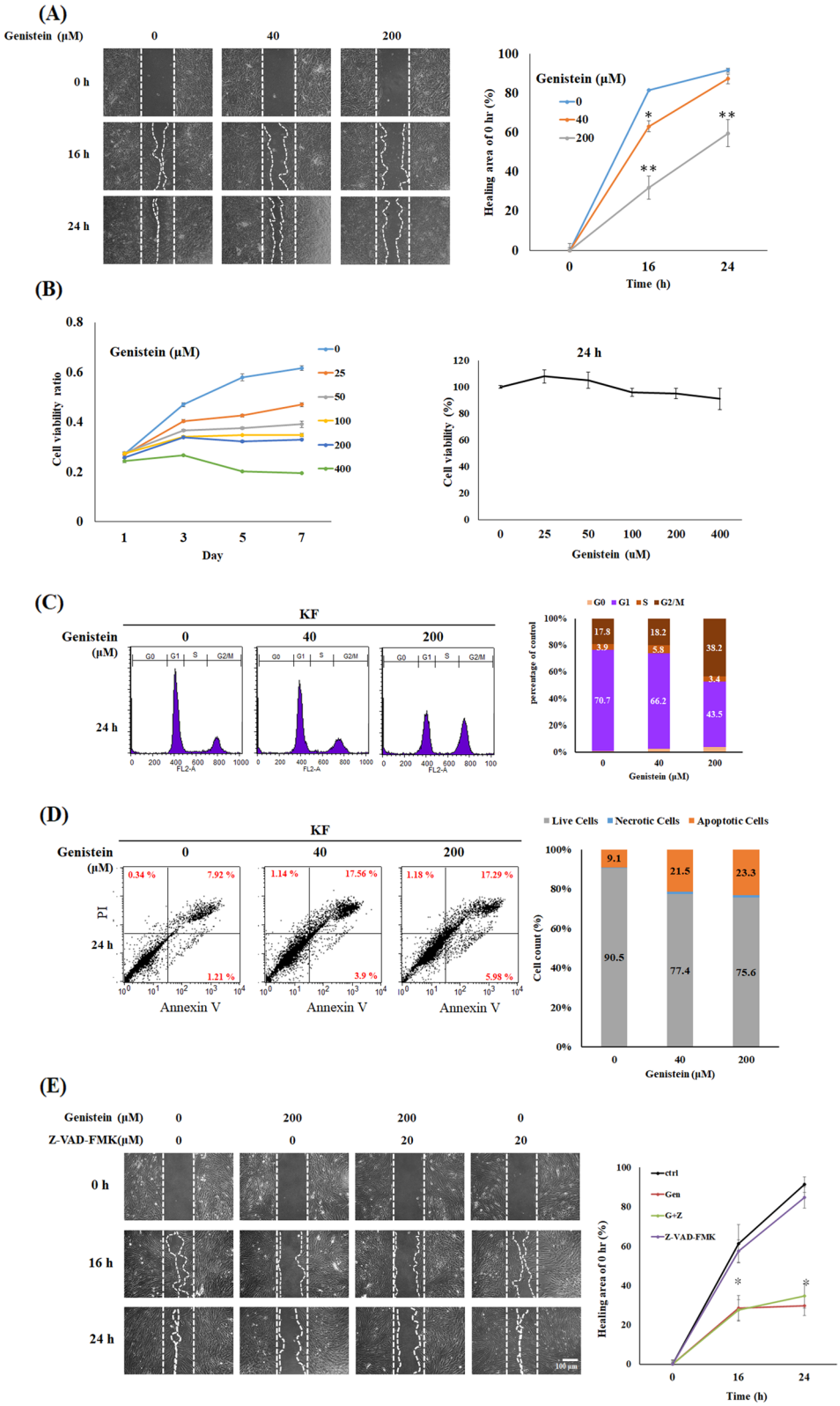

Fig. 1 (See legend on previous page.)

with genistein treatment. The percentage of apoptotic cells increased after treatment with genistein from 7.92% to 17.29%. The genistein slightly increased the apoptotic cell percentage (increased by 9.37%). The proportions of KFs at the late stage of apoptosis were not significant in response to genistein (from 1.21% to 5.98%) (Fig. 1D). KFs were pretreated with the broad-spectrum caspase inhibitor Z-VAD-FMK (20 μM) with or without genistein (200 μM) for 16, 24 h and determined by wound healing assay. Genistein significantly inhibited the KFs migration ability (Fig. 1E). Destroy apoptosis did not affect the ability of genistein to inhibit the wound healing of KFs. The quantification curve was presented on the right. Based on the above results, genistein reduced the migration and proliferation of keloid cells.

Genistein triggered the cell cycle arrest of KFs

To further analyze whether genistein regulates the expression of proteins related to the cell cycle, KF cells were treated with genistein at 40 μM or 200 μM for 24 h, and the total proteins were determined by western blot assay. FN and COL1A1 protein levels inhibited expression by genistein treatment, and the results were consistent with Fig. 1. Moreover, genistein inhibited CTGF protein levels in a dose-dependent manner. Interestingly, genistein significantly enhanced the level of p53 and inhibited Cdk1 and Cdk2 expression in KFs. Those results demonstrated that genistein likely destroys keloids by increasing the p53 protein and reducing Cdk1, 2 protein expression. We showed that genistein enhanced cell cycle arrest at the G2/M phase in Fig. 1. To assess further the cell cycle and proliferation levels, cell proliferation factor (PCNA, proliferating cell nuclear antigen) and Ki67 were analyzed by western blot assay. As in Fig. 2, PCNA and Ki67 protein expression were decreased by genistein treatment and in a dose-dependent manner (Fig. 2A). The protein quantification plot was presented in Fig. 2B ($p < 0.05$).

To investigate the effect of CTGF on the genistein of keloid cells, cells were stimulated with CTGF at various concentrations (50, 100, and 200 ng/ml), and the protein levels were determined by western blot assay. COL1A1 protein level was increased by CTGF treatment and in a dose-dependent manner (Fig. 2C). While pre-treated with genistein 1 h before CTGF co-cultured 24 h, COL1A1 significantly decreased the protein expression in a dose dependent. In addition to inhibiting collagen and FN, CTGF inhibits the expression of its upstream CTGF protein. Those data implied that genistein inhibited CTGF and keloid cells' expression of the downstream protein (COL1A1 and FN).

Genistein inhibits CTGF expression and affects the migration of KFs

To investigate the biological function of CTGF in KFs, CTGF was knocked down by transfection with silenced CTGF. Then, we explored the specific mechanism of CTGF in alleviating genistein-induced migration. We used a western blot to evaluate the protein levels of CTGF knockdown (#951 and #952) in human keloid tissues. The detection of CTGF gene expression also has a consistent effect (Fig. 3A). Compared with parental cell, knockdown of CTGF (especially in #952) group reduced the migrated ability in KFs (Fig. 3B). Interfering with CTGF expression minimizes the ability of keloids to crawl. Genistein inhibits the expression of CTGF and reduces the migration ability of keloid cells. Such a result is like that in Fig. 1A.

Genistein suppressed TGF-β1-induced KFs collagen 1A1, fibronectin, and CTGF

To explore the effects of several stimulants on keloid cells, NHDF and KF cells were treated with chemicals (TSLP, SDF, or TGF-β1), respectively. Compared with other chemicals, COL1A1 and FN significantly increased their protein expressions stimulated by TGF-β1. The relative folds up to 5 and 3 compared to the control group. ($p < 0.05$) (Fig. 4A). We also observed that TGF-β1 treatment increased COL1A1 and FN mRNA expression (Fig. 4B). The results were consistent with the protein expression. Those results indicated that TGF-β1 could promote the expression of COL1A1 and FN in KFs. Subsequent studies used TGF-β1 as a stimulator. To investigate how genistein affects the COLA1A or FN in TGF-β1 treatment cells, NHDF and KF cells were treated with genistein at 40 or 200 μM for 48 h after TGF-β1 (20 ng/ml) pre-treated, and western blot assay determined the FN and COLA1A protein expressions. The relative proteins (CTGF, α-SMA, and MMP-2) were also detected in their expression in this assay. As shown in Fig. 4C, TGF-β1 has significantly increased the protein expression, and genistein (40 and 200 μM) dramatically decreased the FN, COL1A1, and CTGF protein expression and exhibited a dose-dependent manner ($^{*}p < 0.05$). The fold expression of the proteins was quantified on the right side of Fig. 4C. However, there was no effect on the α-SMA and MMP-2 proteins. To clarify how the genistein affects the mRNA expression of FN, COL1A1, CTGF, and other relative genes (COL1A2, COL3A1, DNMT3a, 3b, LARP6, and FOXO2), KF cells were treated alone with genistein at 40 or 200 μM for 24 h and determined those genes by real-time PCR assay. Genistein decreased all relative gene expression (DNMT3a, 3b, LARP6, and FOXO2), among which FN, COL1A1, and CTGF have a concentration-dependent inhibition phenomenon on genistein (40 or

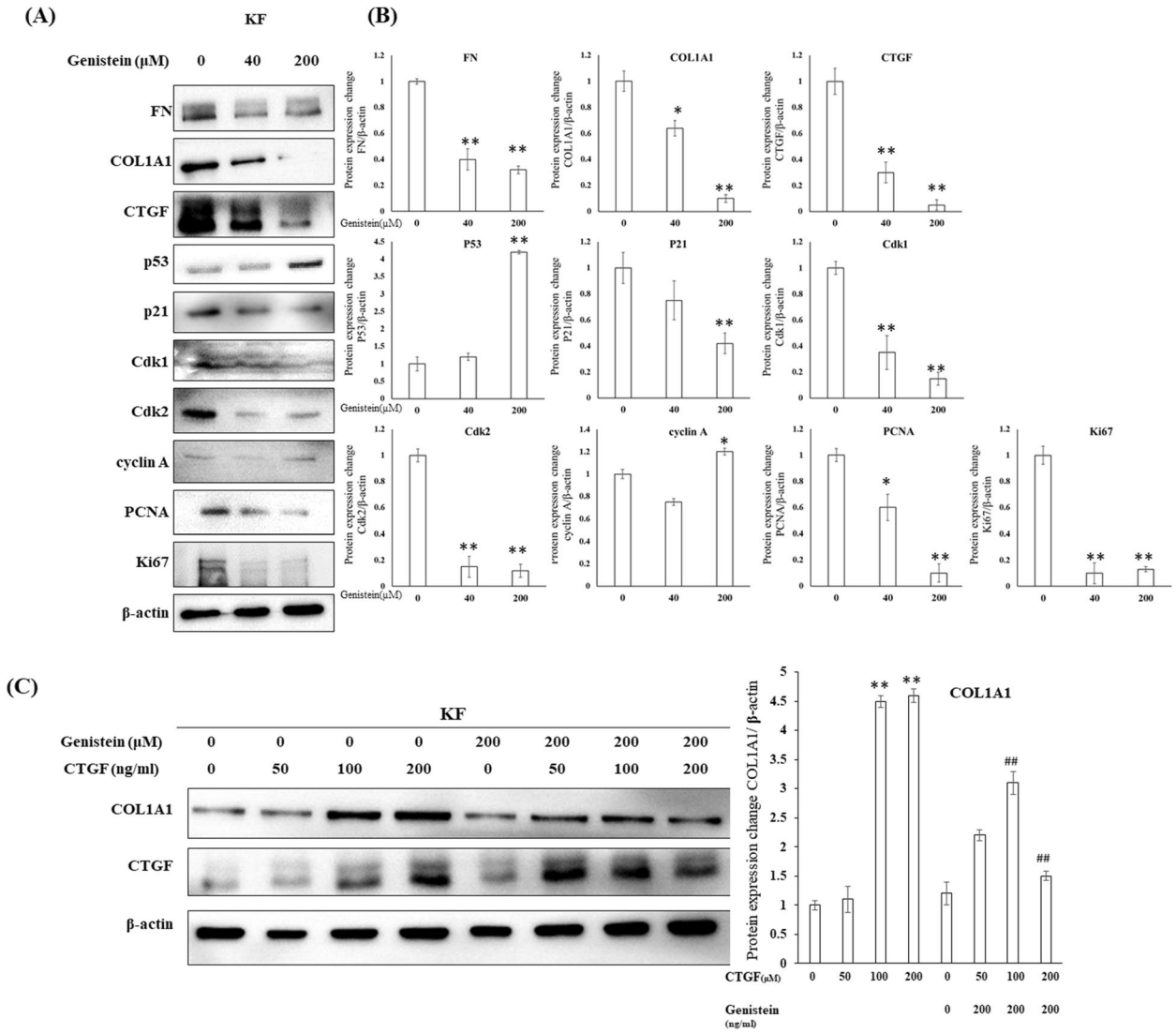

Fig. 2 Genistein affects the cell cycle of KFs **A** KFs were treated with 40 and 200 μM genistein for 24 h. Protein expression levels of fibronectin (FN), collagen IA1, cyclin A, Cdk2, p21, and p53 in KFs based on western blot analysis. **B** The quantification of protein levels using the percentage of Image J software. Significant differences were compared in the genistein group (40, 200 μM) with the control group of the KFs. $^{*}p < 0.05$ vs. control, $^{**}p < 0.01$ vs. control. **C** Protein expression levels of collagen and CTGF were analyzed by western blot in KFs pre-treated with CTGF for 1 h and co-treated with genistein for 24 h. Beta-actin was the internal control and was normalized by the loading protein. All experiments have at least three independent experiments

200 μM) treated cells ($^{*}p < 0.05$). This result was consistent with the protein assay (Fig. 4D). The above results indicate that the expression of FN, COL1A1, and CTGF genes and proteins are highly sensitive to genistein and significantly reduced in genistein-treated keloid cells.

Disruption of cholesterol decreased the inhibitory effect of genistein on KFs

Cholesterol is the major structural component of lipid rafts/caveolae. It is reported that the cholesterol-depleting agent methyl-β-cyclodextrin (MβCD) inhibits keloid endocytosis [42]. To determine whether lipid rafts/caveolae are related to the inhibitory effect of genistein on KFs, MβCD was used to investigate whether lipid rafts/caveolae mediate cellular migration in response to genistein. The results indicated that treatment with MβCD alone had a minor inhibitory effect on cell migration ability. Interestingly, compared to treatment with genistein alone, MβCD significantly mitigated the inhibitory effect of genistein on KF migration (Fig. 5A). Genistein may affect the functions of growth factor receptors by enhancing their

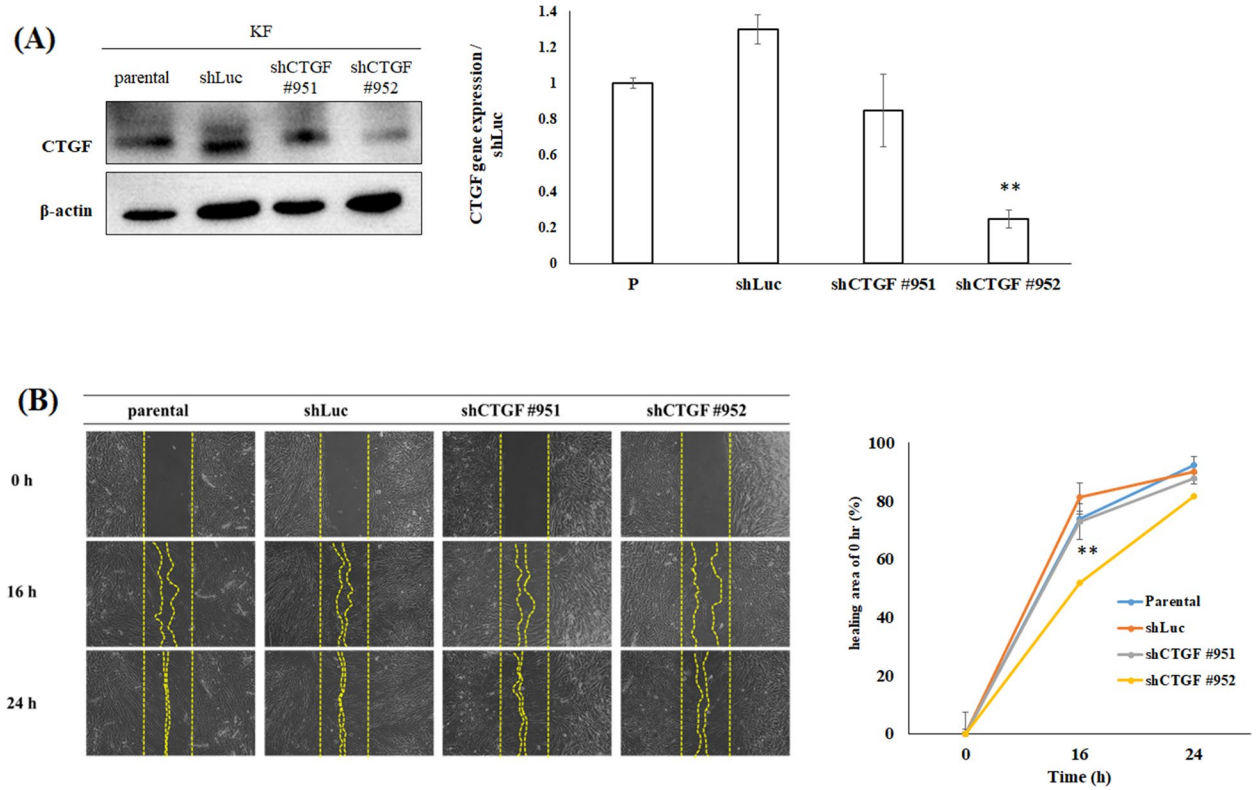

Fig. 3 Effect of CTGF silence on keloids **A** Protein expressions of CTGF and β-actin in KFs were determined by Western blot. ImageJ software was used to quantify the band intensities of CTGF and β-actin. Data showed the relative expression of CTGF standardized by the β-actin protein level. **B** KFs shLuc and KF-shCTGF cells were determined for migration by wound healing assays. The wound closure was captured at 0, 16, and 24 h after removing the culture insert. The magnification of 100 × was used. Dotted lines were used to indicate areas of cell migration

endocytosis. This process can be disrupted by removing cellular cholesterol in the lipid raft domain.

G-actin molecules polymerize into actin filaments (F-actin), which influence cellular dynamic behavior like migration and play pivotal roles in cell polarity during migration. To explore whether genistein affects the expression of actin proteins and inhibits the migration behavior of keloid cells, cells were treated with genistein (40, 200 μM) with or without MβCD. The fluorescence microscope detected the cell appearance and fluorescence intensity of G-actin and F-actin. The fluorescence intensity was quantified and shown on the right (Fig. 5B). Compared to the control group, genistein has inhibited the fluorescence signal of G-actin (*$p < 0.05$), and MβCD significantly mitigated the inhibitory effect of genistein on KF actin expression.

Additionally, genistein significantly reduced the fluorescent signal of COL 1A1 and FN proteins. Still, this

(See figure on next page.)

Fig. 4 Genistein abated TGF-β1-induced expression of FN, COL1A1, and CTGF in NHDF and KF NHDF and KFs have seeded 1×10^5 cells/well in a culture medium for 24 h before treatment. **A** Cells treated with different chemicals (TGF-β1: 20 ng/ml; TSLP: 100 ng/ml; or SDF: 20 ng/ml) for 24 h. The total protein levels of FN and COL1A1 were represented by western blots assay. Protein levels were quantified relative to actin expression to correct for loading differences and normalized to control levels. The quantified fold of protein expression was shown on the right side. (*$p < 0.05$ vs. control, **$p < 0.01$ vs. control.) **B** Cells treated with TGF-β1 (20 ng/ml) for 24 h. mRNA expression levels of FN and CCOL1A1 in NHDFs were examined by qPCR. GAPDH was used as an internal control. Values are means ± SD. All experiments have at least three independent experiments. *$p < 0.05$ vs. control, **$p < 0.01$ vs. control. **C** Cells were pre-treated with genistein (40 or 200 μM) for 1 h and treated with TGF-β1 (20 ng/ml) for 24 h. The total protein levels of FN, COL1A1, CTGF, α-SMA, and MMP-2 were detected by western blot in NHDF and KF cell lines. Actin was used as an internal control in each lane. The quantified fold of protein expression was shown on the right side. (*$p < 0.05$ vs. control, **$p < 0.01$ vs. control.) **D** KF cells were treated with genistein (40, 200 M) for 24 h, and the genes (FN, CTGF, COL1A1, COL 1A2, COL 3A1, DNMT1, DNMT3a, DNMT3b, LARP6, and FOXO2) expression were determined by qPCR. GAPDH was the internal control, and the associated statistical analysis was meant ± SD. All experiments of at least three independent experiments. *$p < 0.05$ vs. control, **$p < 0.01$ vs. control

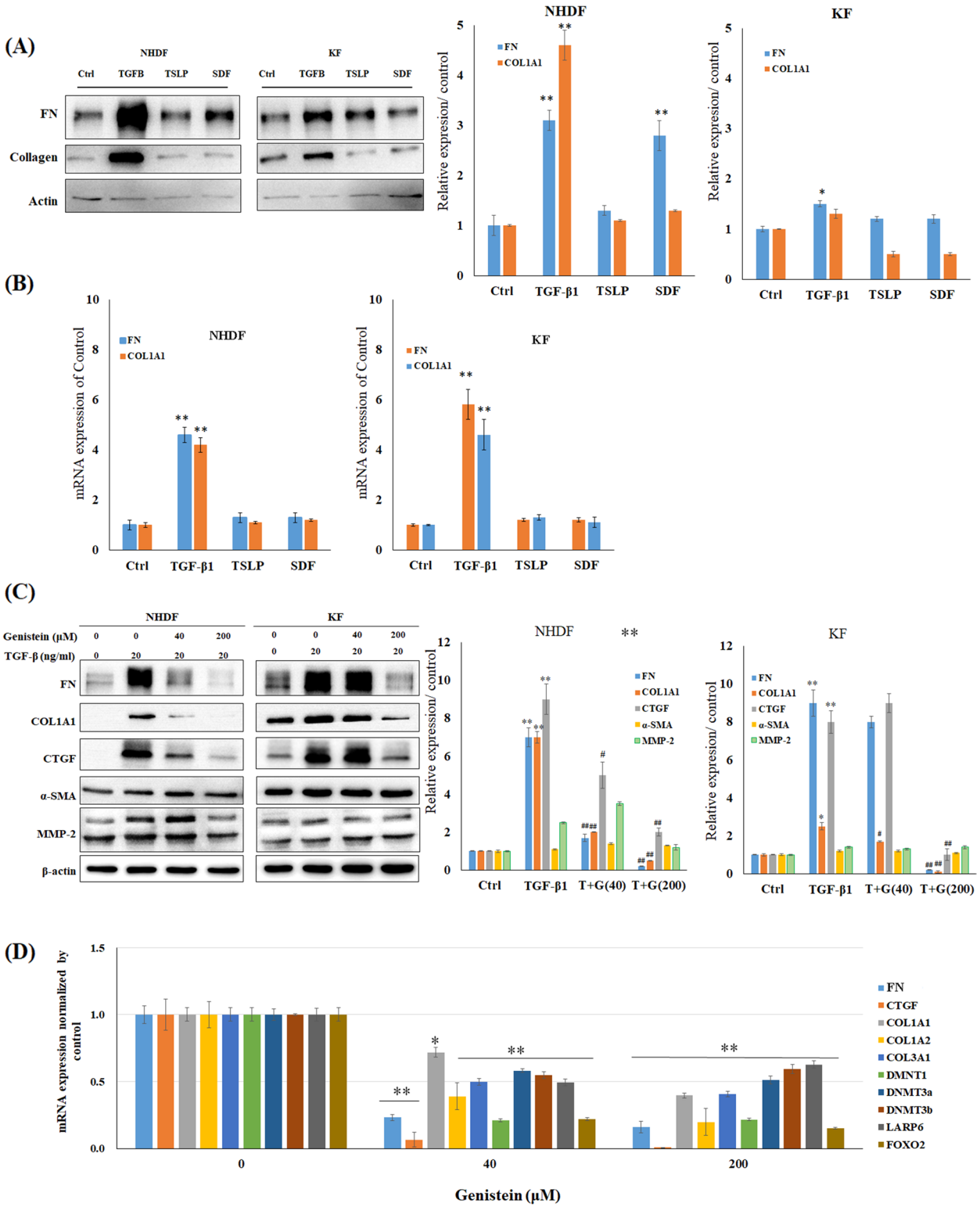

Fig. 4 (See legend on previous page.)

signal was rescued by treatment with genistein combined with MβCD (Fig. 5C). Furthermore, keloids are mediated by growth factors, including FGFR1, VEGFR1, PDGFRα, and PDGFRβ. Genistein treatments are likely to inhibit the functions of these factors. Consistent with the results above, coculture with genistein and the MβCD group recovered the inhibition in keloids (Fig. 5D). The above research results demonstrate that genistein may enhance endocytosis, thereby affecting the functions of growth factor receptors in keloids. MβCD disrupts and removes cellular cholesterol in the lipid raft domain, providing supporting evidence that genistein may control the expansion and crawling of keloids.

Genistein suppressed keloid patients' primary culture cell migration and proliferation

We utilized intralesional keloid excision samples harvested from patients to confirm whether genistein reduces fibroblast migration in human keloid-derived fibroblasts. As shown in Fig. 6A, through wound healing experiments, genistein reduced the migration ability of KFs from patients. These results were consistent with those presented in Fig. 1, particularly evident at 16 h after treatment with a concentration of 200 μM genistein, where a significant decreasing trend was observed (* $p < 0.05$; ** $p < 0.01$). To further confirm whether genistein can affect the expression of COL1A1 or FN proteins in patients' keloids, we employed the western blot method to analyze the protein expression levels. As shown in Fig. 6B, COL1A1 protein expression was significantly decreased in genistein (200 μM)-treated primary culture cells. The quantified graphs are depicted on the right side. Particularly noteworthy is the more significant inhibitory effect of genistein on COL1A1, especially evident under conditions of high-concentration treatment (200 μM) of keloid specimens (* $p < 0.05$; ** $p < 0.01$). Next, to observe the effects of keloid tissues treated with genistein, the finely minced primary tissue was cultured in medium containing genistein (40 or 200 μM) for 3, 7, or 14 days and determined by H&E staining. In Fig. 6C, the keloid dermis was characterized by excessive accumulation of ECM with abnormally thick, tight, and compact collagen fibers in the control group. After being treated with genistein, Keloid cells appear finer collagen, loosely, and have more space (arrow), especially at genistein 200 μM treatment for 14 days. Next, the tissues specifically detected the FN, CTGF, and COL1A1 proteins in the dermal layer of keloids by Immunohistochemistry staining. We observed that COL1A1 protein was inhibited by genistein in both normal and keloid cells. IHC staining revealed that FN and COL1A1 proteins were distributed in the dermal aspect of keloid samples' primary cell culture (Fig. 6C).

All the conclusions mentioned above indicate that genistein inhibited protein expression in keloid patients and reduced the migration ability of primary cultured cells, consistent with the findings from the cell culture model. These results collectively confirm that genistein has therapeutic effects in treating keloids and preventing recurrence.

Discussion

This study demonstrated that genistein significantly inhibited the protein expression of collagen 1A1, fibronectin, and CTGF (Figs. 2 and 4). Genistein could inhibit various pathological phenotypes, including inhibition of keloid cell proliferation, cell cycle arrest at the G2/M phase in vitro (Fig. 1), and inhibition of KF migration in vitro and primary cell culture (Figs. 1 and 6). In addition, using siRNA to silence CTGF expression significantly reduces the migration ability of keloid cells, indicating that CTGF plays a crucial role in inhibiting cell migration by genistein in keloid cells (Fig. 3). MβCD disrupts cell membrane structures was confirmed that genistein blocks various growth factor signaling pathways in keloid cells, thereby achieving the goal of inhibiting keloid cell differentiation (Fig. 5).

Keloids and hypertrophic scars resulting from excessive healing of skin wounds are characterized as pathological scars. Compared with hypertrophic scars, keloids exhibit more severe itching, swelling, and significant invasion of surrounding normal tissue. Therefore, the

(See figure on next page.)

Fig. 5 Genistein enhanced endocytosis step to disrupt the growth factors affecting KFs KFs were co-treated with genistein (40 or 200 μM) and MβCD (1 mM) for 24 h, respectively. **A** Cells were determined by migration using wound healing assays. The wound closure was captured at 0, 18, 24, 42, and 48 h after removing the culture insert. The magnification of 100 × was used. The migrated areas were quantified relative to the control group (*$p < 0.05$; (**$p < 0.01$). **B** The cellular cytoskeletal proteins were labeled using F-actin Red Fluorescent or G-actin Green Fluorescent Cell Linker Kit. The nuclear DNA was stained with a DAPI agent. The fluorescent images were obtained with a fluorescence microscope (DP70; Olympus, JAPAN). Untreated cells were used as control. Data are presented as mean ± SD, and mean intracellular fluorescence intensity of G-actin or F-actin was quantitated using ImageJ software (right side). (*$p < 0.05$ vs. control; # $p < 0.05$ vs. genistein 40 μM group). **C** Immunofluorescence detection of COL1A1 (green): Rabbit mAb (CST #39,952, Cell Signaling Technology), or FN (green): Mouse Anti-Fibronectin (BD #610,077, MilliporeSigma) expression (× 200, bar = 100 μm). Nuclei were stained with DAPI (blue). **D** Protein expression levels of FGFR1, VEGFR1, PDGFRα, and PDGFRβ in KFs based on western blot analysis. β-actin was used as a loading control. (*$p < 0.05$)

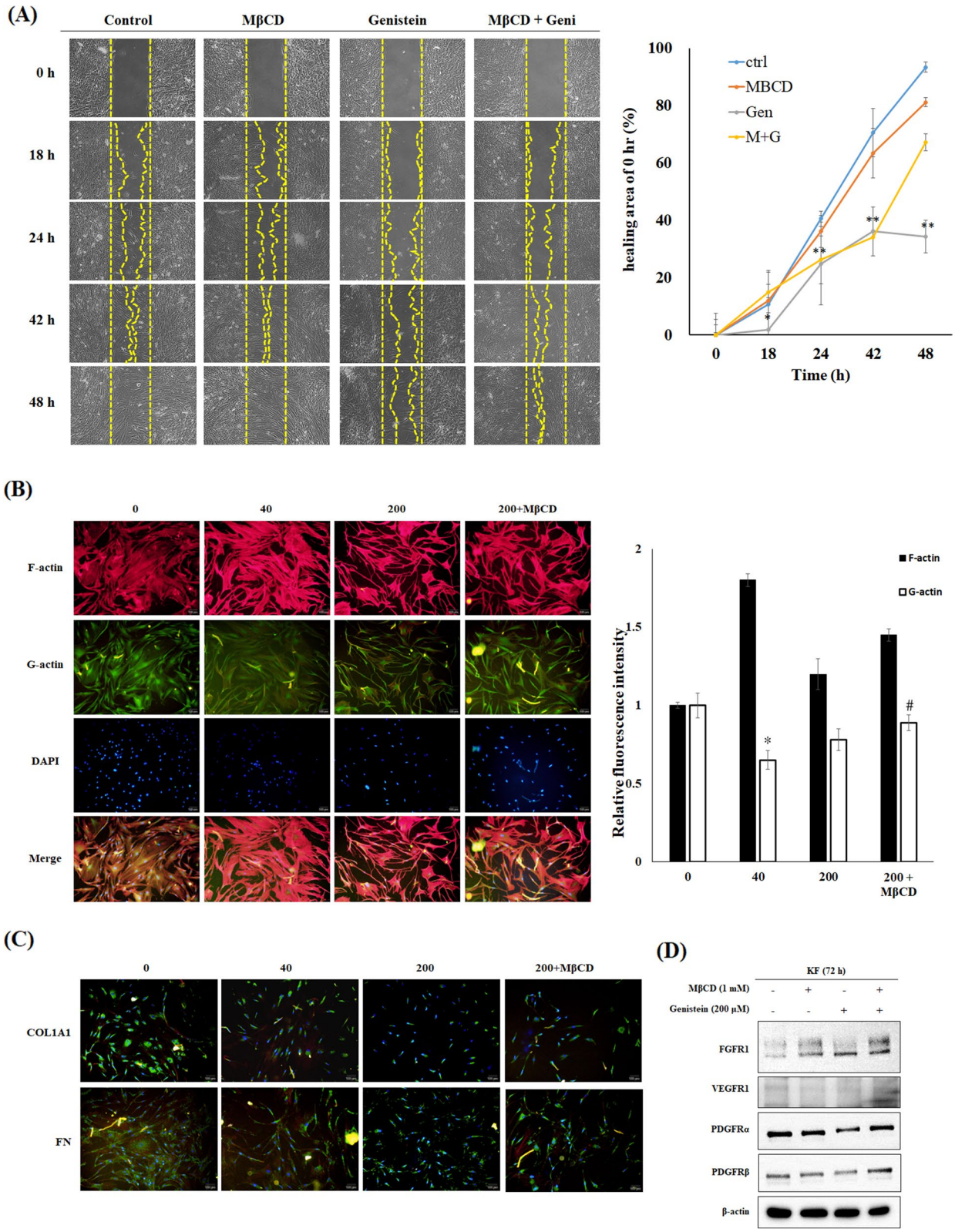

Fig. 5 (See legend on previous page.)

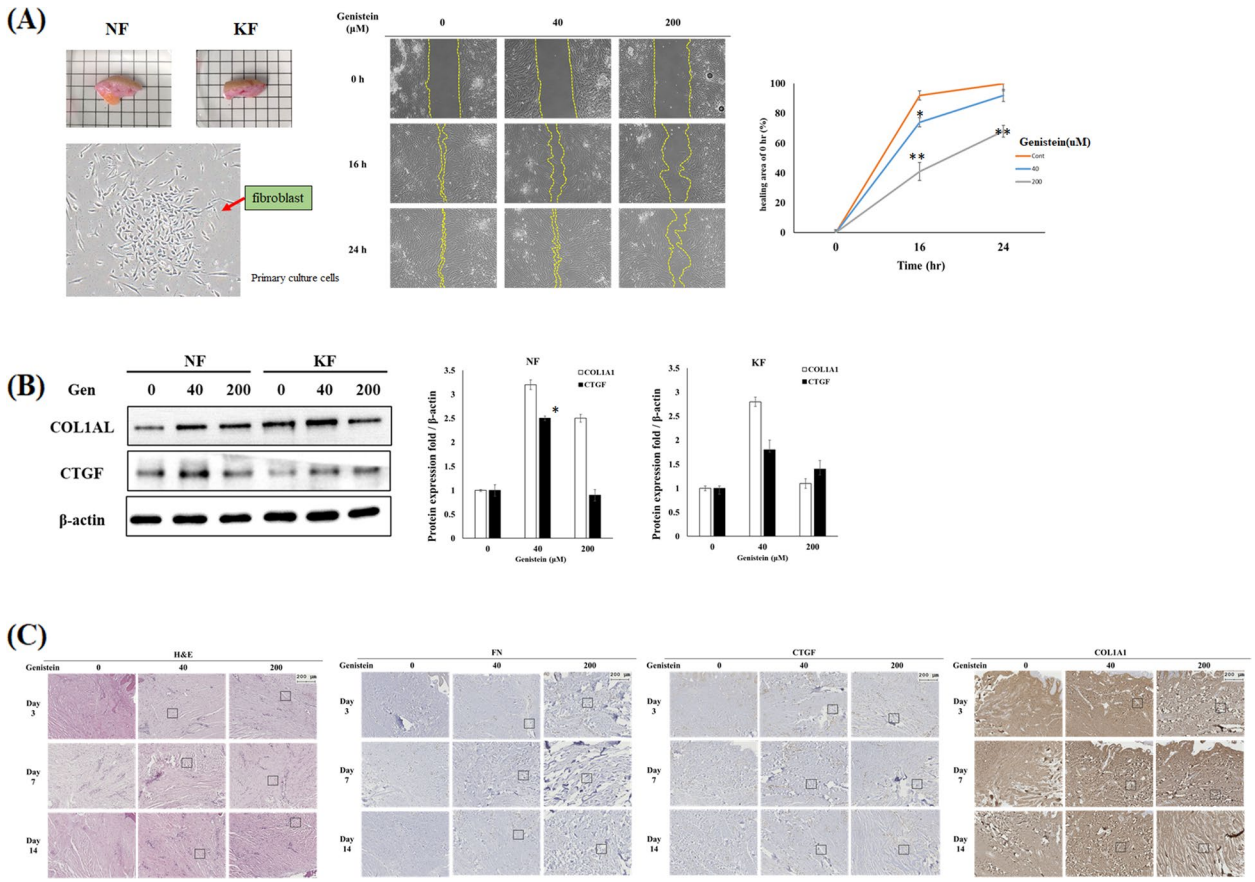

Fig. 6 Genistein repressed cell migration ability and collagen protein expression Cells from keloid patient specimens were cultured with genistein (40 or 200 μM), respectively. **A** Migration assay determined migration ability, and the quantitative graph was displayed on the right. Migrated areas were quantified relative to the control group. (*$p < 0.05$; **$p < 0.01$). **B** Comparing the COL1A1 and CTGF proteins expression of normal fibroblast (NF) and KFs. β-actin was used as the internal loading control. Protein levels were quantified relative to the control group. (*$p < 0.05$; **$p < 0.01$). **C** Hematoxylin and eosin stain represent genistein-treated morphology and epithelial thickness, and the scale bar = 200 μm. Immunohistochemically staining for keloid tissues and detected with COL1A1, FN and CTGF

primary goal in controlling keloid scars is to modulate the migratory ability of scar cells. This study confirms that genistein significantly inhibits the migration ability of keloid cells, as observed in cultured and keloid cells isolated from patients (Figs. 2, 3, and 5). Genistein was also to influence collagen production in human dermal fibroblasts under oxidative stress conditions. However, many works of literature still have different opinions on the speed of wound healing or collagen production and repair of genistein. Genistein exhibits multidirectional biological action [18]. This may be related to the concentration used. Such as, a low concentration of genistein (1 μM) restores collagen expression reduced by t-BHP reagent induction, whereas a high concentration (100 μM) of genistein enhances the inhibitory effect of t-BHP [30]. Other studies have also shown that the concentration of genistein has different results in regulating collagen [25, 26]. Despite genistein's specific effects on anti-inflammation and peroxide reduction, it exerts a biphasic effect on collagen expression. The genistein used in this study is between 40–200 μM, which is a high concentration compared to other literature and inhibits collagen production. The results are the same [19, 37, 38].

Genistein has varying regulatory effects on wound healing in different tissues. Literatures confirm that oral or topical use of genistein can increase collagen deposition, improve wound stiffness, and increase skin wound healing. For example, genistein can promote wound healing in mice dorsal skin [17]; genistein with keratin hydrogel could promising therapeutic molecule for the management of wound repair. keratin-genistein hydrogel is a promising therapeutic molecule for the management of wound repair [20]. Wound healing is related to inflammation and oxidation and is also closely related to the speed of collagen production (J. [9, 24]). Oxidative stress impacts collagen production in human dermal fibroblasts

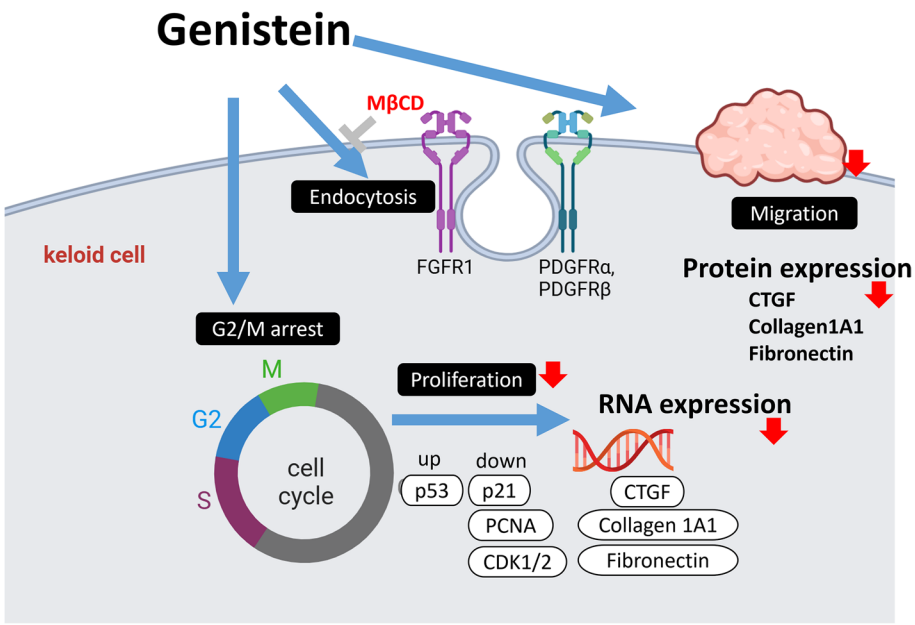

Fig. 7 A schematic representation of the molecular mechanisms of genistein-inhibited endocytosis and fibrogenesis in keloid via CTGF signaling pathways Genistein promoted cell cycle arrest at the G2/M phase and inhibited the expression of COL1A1, FN, and CTGF mRNA and protein expressions. Genistein enhanced endocytosis in keloids and blocked the stimulation of growth factors (PDGF, FGF). This process may depend on the regulation of CTGF. Genistein may attenuate the activity of keloid fibroblasts and reduce keloid formation

[12]. This article is the first to prove that genistein significantly inhibits collagen in keloids with uncontrolled wound healing, and proposes effective biological concentration verification in cell culture models and human keloid specimen models.

Keloid tissues resemble benign tumors, and literature has demonstrated that genistein inhibits the migration ability of cancer cells [2, 8]. This study also confirms that genistein inhibits cell migration in KF cell lines and primary tissue cultures from keloid patients (Figs. 1 and 6). Stress fibers are generally defined as bundles of 10–30 thin filaments, composed of F-actin crosslinked by actin-binding proteins such as α-actinin, fascin and filamin. Well-organized F-actin can form tress fiber. In addition, myofibroblasts activated from fibroblasts usually display strong stress fiber. Hence, cells with a robust actin stress fiber system (contractile phenotype) were observed to be less migratory. Additionally, genistein decreased the expression levels of migration-related proteins such as G-actin and F-actin in KF cells (Fig. 5B). These results indicate that the effective biological concentration of genistein ranges from 40 to 200 μM. The future direction of this study will focus on using genistein gel as a dressing for wounds post-resection of keloid patients.

Treatment for keloids is usually surgery or combined with steroid injections. However, these treatments aim to destroy the bulk of the extracellular matrix that has already been formed and do not target the early stages of wound healing. As a tyrosine kinase blocker, genistein may significantly suppress KF growth by exerting combined inhibitory effects on EGFR. The reduction of EGFR activation can also suppress the transcription of the downstream genes. In the present study, decreasing the expression of cyclin d1 resulted in cell cycle arrest in the G1 phase and the promotion of cell apoptosis. The downregulation of CTGF inhibited the proliferation and differentiation of HKFs and the fibrotic effects of fibroblasts. Genistein substantially accelerated wound repair, which is associated with CTGF regulations. In addition, genistein also reduced the keloid's relative marker expression, such as LARP6, CTGF, Foxf2, and S6RP6 in Fig. 2. Therefore, we think genistein has the potential to abate the over-proliferation of collagen and fibroblast cell synthesis in the keloid's early phase.

We previously explored epigenetic mechanisms in genistein-treated keloid cells, co-treating the demethylating agent 5-aza-dC with genistein for 72 h and measuring protein levels. Treatment with 5-aza-dC restored fibronectin expression but not COL1A1 expression. Our laboratory has also used Western blotting to analyze the protein expression of DNA methyltransferase isoforms (DNMT3a and DNMT3b). Genistein did not increase DNMT3a and DNMT3b in either cell line (data not shown). We confirmed that genistein does not affect keloid scars through the phosphorylation mechanism of the smad2/3 pathway. Taken together, the

above results demonstrate that genistein inhibits the migration of keloids but does not activate the MMP2 and MMP9 pathways that may be involved.

Genistein, a naturally occurring phytoestrogen (commonly found in soy products, including soybeans), is a non-steroidal compound of plant origin whose structure and function are essentially like human estrogen. Often used to improve symptoms of menopause [33]. Genistein has been shown to expedite cutaneous wound healing primarily via mechanisms independent of estrogen receptors [1, 21]. Most of the literature explores the biological activity of genistein through oral ingestion, and some articles explore genistein through topical application [31, 36]. Genistein enhances the healing of diabetic wounds by reducing oxidative stress and suppressing the activity of inducible nitric oxide synthase (iNOS). Our laboratory has previously utilized a gel mixed with genistein as a suitable adjuvant, which could then be commercialized into a gel formulation and tested in animal models. Some studies have similar approaches [36]. Introducing genistein to treatment requires further research into patch or dressing models, which will allow for clarifying the curative effects.

In conclusion, this study indicated that genistein-induced cell cycle arrest, reduced proliferation, migration, and COL1A1, FN target protein expression in KFs and the patient's primary culture. The presence of MβCD significantly alleviates the inhibitory effect of genistein on KFs migration. As shown in Fig. 7, overall, genistein-abated endocytosis and fibrogenesis via CTGF signaling pathways. The present study demonstrated that genistein can be a therapeutic target for keloid. This approach expects genistein gel during the early stages of wound healing, thereby controlling the proliferation of collagen at a specific rate during the wound healing period.

Abbreviations

α-SMA	α-Smooth muscle actin
CTGF	Connective tissue growth factor
FBS	Fetal bovine serum
GAP DH	Glyceraldehyde-3-phosphate dehydrogenase
KF	Keloid fibroblasts
NK	Normal keratinocytes
Real-time PCR	Real-time polymerase chain reaction
ROS	Reactive oxygen species
SDF-1	Stromal cell-derived factor-1
TGF-β1	Transforming growth factor-β
TSLP	Thymic stromal lymphopoietin
VEGF	Vascular endothelial growth factor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12263-024-00758-1.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements
The authors thank all co-workers who contributed to this study. They also sincerely thank the participants for their participation and cooperation.

Authors' contributions
Yu-Ping Hsiao conceived and designed the experiments. Chun-Te Lu contributed to the data statistics. Chih-Ting Hsu and Chu-Chyn Ou performed the experiments. Jiunn-Liang Ko and Sheau-Chung Tang discussed the results and prepared the manuscript. Sheau-Chung Tang revised the manuscript and response letter. All authors read and approved the final manuscript.

Funding
This work was supported by grants from the Ministry of Science and Technology (MOST 111–2320-B-040–018-MY3), National Taichung University of Science and Technology (NTCUST113-028), and Chung Shan Medical University Hospital (CSH-2021-C-028).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The Research Ethics Committee consented to use human material, Institutional Review Board of Taichung Veterans General Hospital: TCVGH-IRB No.: SF23056C).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Division of Plastic and Reconstructive Surgery, Department of Surgery, Taichung Veterans General Hospital, Taichung 407, Taiwan. [2]Institute of Medicine, School of Medicine, College of Medicine, National Yang-Ming Chiao Tung University, Taipei 112, Taiwan. [3]Institute of Medicine, School of Medicine, Chung Shan Medical University, Taichung 402, Taiwan. [4]Department of Medical Oncology and Chest Medicine, Chung Shan Medical University Hospital, Taichung 402, Taiwan. [5]Department of Nutrition, Chung Shan Medical University, Taichung 402, Taiwan. [6]Department of Nutrition, Chung Shan Medical University Hospital, Taichung 402, Taiwan. [7]Manufacturing Class, Puli Brewery, Taiwan Tobacco & Liquor Corporation, Nantou 545, Taiwan. [8]Department of Dermatology, Chung Shan Medical University Hospital, Taichung 402, Taiwan. [9]Department of Nursing, National Taichung University of Science and Technology, Taichung 406, Taiwan.

Received: 28 May 2024 Accepted: 15 October 2024
Published online: 28 October 2024

References

1. Ahn S, Chantre CO, Ardona HAM, Gonzalez GM, Campbell PH, Parker KK. Biomimetic and estrogenic fibers promote tissue repair in mice and human skin via estrogen receptor beta. Biomaterials. 2020;255: 120149. https://doi.org/10.1016/j.biomaterials.2020.120149.
2. Alatawi FS, Faridi U. Anticancer and anti-metastasis activity of 1,25 dihydroxycholecalciferols and genistein in MCF-7 and MDA-MB-231 breast cancer cell lines. Heliyon. 2023;9(11): e21975. https://doi.org/10.1016/j.heliyon.2023.e21975.
3. Babu M, Diegelmann R, Oliver N. Keloid fibroblasts exhibit an altered response to TGF-beta. J Invest Dermatol. 1992;99(5):650–5. https://doi.org/10.1111/1523-1747.ep12668146.
4. Berman B, Maderal A, Raphael B. Keloids and hypertrophic scars: pathophysiology, classification, and treatment. Dermatol Surg. 2017;43(Suppl 1):S3–18. https://doi.org/10.1097/DSS.0000000000000819.
5. Betarbet U, Blalock TW. Keloids: A Review of Etiology, Prevention, and Treatment. J Clin Aesthet Dermatol. 2020;13(2):33–43.

6. Cao C, Li S, Dai X, Chen Y, Feng Z, Zhao Y, Wu J. Genistein inhibits proliferation and functions of hypertrophic scar fibroblasts. Burns. 2009;35(1):89–97.
7. Carvalho MT, Araújo-Filho HG, Barreto AS, Quintans-Júnior LJ, Quintans JS, Barreto RS. Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. Phytomedicine. 2021;90: 153636.
8. Chaichian S, Nikfar B, Bidgoli SA, Moazzami B. The Role of Genistein and its Derivatives in Ovarian Cancer: New Perspectives for Molecular Mechanisms and Clinical Applications. Curr Med Chem. 2023. https://doi.org/10.2174/0109298673251713231019091910.
9. Chen, J., Qin, S., Liu, S., Zhong, K., Jing, Y., Wu, X., . . . Peng, C. (2023). Targeting matrix metalloproteases in diabetic wound healing. Front Immunol, 14, 1089001. https://doi.org/10.3389/fimmu.2023.1089001.
10. Chen X, Wu Y, Gu J, Liang P, Shen M, Xi J, Qin J. Anti-invasive effect and pharmacological mechanism of genistein against colorectal cancer. BioFactors. 2020;46(4):620–8.
11. Christmann RB. Another Piece in the Fibrotic Puzzle: TSLP as a Novel Ligand for Fibrocyte Activation. J Investig Dermatol. 2016;136(2):360–2.
12. Darawsha A, Trachtenberg A, Levy J, Sharoni Y. The protective effect of carotenoids, polyphenols, and estradiol on dermal fibroblasts under oxidative stress. Antioxidants. 2021;10(12): 2023.
13. Ge J, Zhou Q, Cheng X, Qian J, Yan Q, Wu C, Zou J. The protein tyrosine kinase inhibitor, Genistein, delays intervertebral disc degeneration in rats by inhibiting the p38 pathway-mediated inflammatory response. Aging (Albany NY). 2020;12(3):2246.
14. Goh YX, Jalil J, Lam KW, Husain K, Premakumar CM. Genistein: a review on its anti-inflammatory properties. Front Pharmacol. 2022;13: 820969.
15. Gold, M. H., Nestor, M. S., Berman, B., & Goldberg, D. (2020). Assessing keloid recurrence following surgical excision and radiation. Burns & Trauma, 8. https://doi.org/10.1093/burnst/tkaa031.
16. Hillege MM, Galli Caro RA, Offringa C, de Wit GM, Jaspers RT, Hoogaars WM. TGF-β regulates collagen type I expression in myoblasts and myotubes via transient Ctgf and Fgf-2 expression. Cells. 2020;9(2):375.
17. Hu X, Zhang H, Chen Z. Effects of four polyphenols on mouse wound healing and the gene expression profile of resveratrol action. Histol Histopathol. 2024;39(1):105–16. https://doi.org/10.14670/HH-18-616.
18. Jurzak M, Adamczyk K, Antonczak P, Garncarczyk A, Kusmierz D, Latocha M. Evaluation of genistein ability to modulate CTGF mRNA/protein expression, genes expression of TGFbeta isoforms and expression of selected genes regulating cell cycle in keloid fibroblasts in vitro. Acta Pol Pharm. 2014;71(6):972–86.
19. Kwon S-H, Chung H, Seo J-W, Kim HS. Genistein alleviates pulmonary fibrosis by inactivating lung fibroblasts. BMB Rep. 2024;57(3):143.
20. Mahajan, N. M., Wanaskar, K., Ali, N., Mahapatra, D. K., Iqbal, M., Bhat, A. R., and Kaleem, M. Innovative wound healing hydrogel containing chicken feather keratin and soy isoflavone genistein. In vivo studies. Gels. 2023;9(6). https://doi.org/10.3390/gels9060462
21. Mitrengova P, Mucaji P, Perzelova V, Dosedla E, Gal P. Genistein: a promising molecule modulating tumour growth and wound healing? Ceska Slov Farm. 2018;67(1):3–13.
22. Naik P. Novel targets and therapies for keloid. Clin Exp Dermatol. 2022;47(3):507–15.
23. Nazari-Khanamiri F, Ghasemnejad-Berenji M. Cellular and molecular mechanisms of genistein in prevention and treatment of diseases: an overview. J Food Biochem. 2021;45(11):e13972.
24. Nguyen AV, Soulika AM. The dynamics of the skin's immune system. Int J Mol Sci. 2019;20(8): 1811.
25. Pawlicka, M. A., Zmorzynski, S., Popek-Marciniec, S., & Filip, A. A. (2022). The Effects of Genistein at Different Concentrations on MCF-7 Breast Cancer Cells and BJ Dermal Fibroblasts. Int J Mol Sci, 23(20). https://doi.org/10.3390/ijms232012360.
26. Pawlicka MA, Zmorzyński S, Popek-Marciniec S, Filip AA. The effects of genistein at different concentrations on MCF-7 breast cancer cells and BJ dermal fibroblasts. Int J Mol Sci. 2022;23(20): 12360.
27. Shafiee G, Saidijam M, Tayebinia H, Khodadadi I. Beneficial effects of genistein in suppression of proliferation, inhibition of metastasis, and induction of apoptosis in PC3 prostate cancer cells. Arch Physiol Biochem. 2022;128(3):694–702.
28. Sharifi-Rad, J., Quispe, C., Imran, M., Rauf, A., Nadeem, M., Gondal, T. A., . . . Sytar, O. (2021). Genistein: an integrative overview of its mode of action, pharmacological properties, and health benefits. Oxidative medicine and cellular longevity, 2021.
29. Shin, J. U., Kim, S. H., Kim, H., Noh, J. Y., Jin, S., Park, C. O., . . . Lee, K. H. (2016). TSLP is a potential initiator of collagen synthesis and an activator of CXCR4/SDF-1 axis in keloid pathogenesis. Journal of Investigative Dermatology, 136(2), 507–515.
30. Sienkiewicz P, Surazynski A, Palka J, Miltyk W. Nutritional concentration of genistein protects human dermal fibroblasts from oxidative stress-induced collagen biosynthesis inhibition through IGF-I receptor-mediated signaling. Acta Pol Pharm. 2008;65(2):203–11.
31. Tang S-C, Hsiao Y-P, Ko J-L. Genistein protects against ultraviolet B–induced wrinkling and photoinflammation in in vitro and in vivo models. Genes Nutr. 2022;17(1):1–11.
32. Tang S-C, Tang L-C, Liu C-H, Liao P-Y, Lai J-C, Yang J-H. Glycolic acid attenuates UVB-induced aquaporin-3, matrix metalloproteinase-9 expression, and collagen degradation in keratinocytes and mouse skin. Biochemical Journal. 2019;476(10):1387–400.
33. Thangavel P, Puga-Olguín A, Rodríguez-Landa JF, Zepeda RC. Genistein as potential therapeutic candidate for menopausal symptoms and other related diseases. Molecules. 2019;24(21):3892.
34. Uitto J, Perejda AJ, Abergel RP, Chu ML, Ramirez F. Altered steady-state ratio of type I/III procollagen mRNAs correlates with selectively increased type I procollagen biosynthesis in cultured keloid fibroblasts. Proc Natl Acad Sci U S A. 1985;82(17):5935–9. https://doi.org/10.1073/pnas.82.17.5935.
35. Um J-Y, Kang SY, Kim HJ, Chung BY, Park CW, Kim HO. Transient receptor potential vanilloid-3 (TRPV3) channel induces dermal fibrosis via the TRPV3/TSLP/Smad2/3 pathways in dermal fibroblasts. J Dermatol Sci. 2020;97(2):117–24.
36. Vu QL, Fang C-W, Suhail M, Wu P-C. Enhancement of the topical bioavailability and skin whitening effect of genistein by using microemulsions as drug delivery carriers. Pharmaceuticals. 2021;14(12): 1233.
37. Wasilewicz R, Wasilewicz J, Pruszyńska-Oszmałek E, Stuper-Szablewska K, Leciejewska N, Kołodziejski PA. Genistein stimulates the viability and prevents myofibroblastic transformation in human trabecular meshwork cells stimulated by TGF-β. Exp Eye Res. 2024;240:109806.
38. Xu Y, Zhang D, Yang H, Liu Y, Zhang L, Zhang C, Zhang H. Hepatoprotective effect of genistein against dimethylnitrosamine-induced liver fibrosis in rats by regulating macrophage functional properties and inhibiting the JAK2/STAT3/SOCS3 signaling pathway. Front Biosci-Landmark. 2021;26(12):1572–84.
39. Yan H, Jiang J, Du A, Gao J, Zhang D, Song L. Genistein enhances radiosensitivity of human hepatocellular carcinoma cells by inducing G2/M arrest and apoptosis. Radiat Res. 2020;193(3):286–300.
40. Zhang T, Wang X-F, Wang Z-C, Lou D, Fang Q-Q, Hu Y-Y, Tan W-Q. Current potential therapeutic strategies targeting the TGF-β/Smad signaling pathway to attenuate keloid and hypertrophic scar formation. Biomed Pharmacother. 2020;129: 110287.
41. Zheng J-Y, Wang S-C, Tang S-C, Hsin I-L, Kang Y-T, Hsu C-T, Ko J-L. Sodium acetate ameliorates cisplatin-induced kidney injury in vitro and in vivo. Chem-Biological Interactions. 2023;369: 110258.
42. Zhou B-Y, Wang W-B, Wu X-L, Zhang W-J, Zhou G-D, Gao Z, Liu W. Nintedanib inhibits keloid fibroblast functions by blocking the phosphorylation of multiple kinases and enhancing receptor internalization. Acta Pharmacol Sin. 2020;41(9):1234–45.

Publisher's Note