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RESEARCH ARTICLE

Decorin attenuates hypertrophic scar fibrosis via TGFβ/Smad signalling

Jiangtao Cui[1,2,3,4] | Shiyi Zhang[1,2,3] | Kiran Acharya[1,2,3] | Yan Xu[1,2,3] | Heng Guo[5,4] | Tong Li[1,2,3] | Donghe Fu[1,2,3] | Zizhen Yang[1,2,3] | Lingnan Hou[1,2,3] | Xiaotao Xing[1,2,3] | Xiaoyi Hu[1,2,3]

[1]Key Laboratory of Shaanxi Province for Craniofacial Precision Medicine Research, College of Stomatology, Xi'an Jiaotong University, Xi'an, Shaanxi, China

[2]Laboratory Center of Stomatology, College of Stomatology, Xi'an Jiaotong University, Xi'an, Shaanxi, China

[3]Department of Cranio-Maxillofacial Trauma and Plastic Surgery, College of Stomatology, Xi'an Jiaotong University, Xi'an, Shaanxi, China

[4]Biomedical Experimental Center of Xi'an Jiaotong University, Xi'an, Shaanxi, China

[5]The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, China

Correspondence
Xiaotao Xing and Xiaoyi Hu, Department of Cranio-Maxillofacial Trauma and Plastic Surgery, College of Stomatology, Xi'an Jiaotong University, Xi'an 710004, Shaanxi, China.
Email: xingxiaotao110@126.com and doctorhu@mail.xjtu.edu.cn

Abstract

The management of hypertrophic scars (HSs), characterized by excessive collagen production, involves various nonsurgical and surgical interventions. However, the absence of a well-defined molecular mechanism governing hypertrophic scarring has led to less-than-ideal results in clinical antifibrotic treatments. Therefore, our study focused on the role of decorin (DCN) and its regulatory role in the TGF-β/Smad signalling pathway in the development of HSs. In our research, we observed a decrease in DCN expression within hypertrophic scar tissue and its derived cells (HSFc) compared to that in normal tissue. Then, the inhibitory effect of DCN on collagen synthesis was confirmed in Fc and HSFc via the detection of fibrosis markers such as COL-1 and COL-3 after the overexpression and knockdown of DCN. Moreover, functional assessments revealed that DCN suppresses the proliferation, migration and invasion of HSFc. We discovered that DCN significantly inhibits the TGF-β1/Smad3 pathway by suppressing TGF-β1 expression, as well as the formation and phosphorylation of Smad3. This finding suggested that DCN regulates the synthesis of collagen-based extracellular matrix and fibrosis through the TGF-β1/Smad3 pathway.

KEYWORDS
Decorin, fibroblasts, hypertrophic scar, proliferation, TGF-β1/Smad3

1 | INTRODUCTION

Hypertrophic scarring (HS) is a benign skin fibroproliferative disorder characterized by hyperplasia of collagen formation during skin trauma repair.[1] HS in the maxillofacial region not only hinders oromaxillary functions but also contributes to a decrease in patient aesthetic appearance. These combined effects pose significant challenges to both the physical and mental well-being of affected individuals. Currently, a range of treatment regimens, such as medications, laser therapy, surgical intervention and comprehensive therapies,[2–4] are being utilized to prevent and postpone the occurrence of hypertrophic scars (HSs). Nevertheless, the lack of a comprehensive understanding of the molecular mechanism underlying hypertrophic scar formation has hindered the development of effective treatments aimed at blocking hypertrophic scar formation.[5] Thus, identifying the key genes involved in hypertrophic scar formation and exploring their regulatory mechanisms could provide new insights and propose an ideal strategy for the treatment of HSs.

Abbreviations: DCN, Decorin; Fc, normal skin-derived fibroblasts; HS, hypertrophic scar tissue; HSFc, hypertrophic scar-derived fibroblasts; NS, normal skin tissue; OE, overexpression; siRNA, small interfering RNA.

Transforming growth factor β1 (TGF-β1) is closely associated with the pathogenesis of HSs.[6] Decorin (DCN) is a stromal proteoglycan that binds TGF-β through its core protein, preventing TGF-β from interacting with its receptors.[7] DCN has multifaceted biological functions in immunity, inflammation and tissue fibrosis.[7–9] Moreover, DCN is an oncosuppressive molecule.[10,11] Previous research has indicated that fibroblasts from postburn hypertrophic scar tissue exhibit reduced synthesis of DCN compared to that of normal fibroblasts.[12] Järvinen TA et al. developed a multifunctional therapeutic recombinant DCN protein with the aim of creating a targeted antifibrotic molecule.[13,14] In hypertrophic scar fibroblasts (HSFc), the downregulation of DCN and the differentiation of myofibroblasts induced by TGF-β1 were reversed upon the inhibition of miR-181b.[15] Taken together, these findings indicate that hypertrophic scar formation is closely associated with DCN expression, suggesting that DCN is a key regulator of HS.

The TGF-β/Smad signalling pathway serves as the primary regulator of collagen formation in fibroblasts and myofibroblasts.[16] Prolonged activation of this pathway stimulates excessive proliferation of fibroblasts and myofibroblasts, leading to excessive collagen formation and deposition.[17] However, the relationship between DCN and the TGF-β/Smad signalling pathway in HSs is unclear. Further exploration of the relationship between them is essential to unveil the molecular mechanism underlying HS.

In light of the challenges associated with HS, our research focused on DCN and explored its regulatory mechanism within the TGF-β1/Smad3 signalling pathway to elucidate its antifibrotic properties. Our findings demonstrated that DCN can reduce collagen deposition and suppress the proliferation, invasion and migration of HSFc in vitro. Moreover, DCN plays a crucial role in mitigating hypertrophic scar formation by inhibiting the TGF-β1/Smad3 signalling pathway. Overall, our study suggested that DCN is a promising potential therapeutic target for treating HSs.

2 | MATERIALS AND METHODOLOGY

2.1 | Cell culture

Human HSFc and normal fibroblasts (Fc) were obtained from CTCC (Meisen, Zhejiang, China). Upon reaching 80% confluence, the cells were passaged. The cells were then digested using 0.25% trypsin (Gibco, USA) and centrifuged at 800 rpm for 5 min. The cell pellet was resuspended in DMEM supplemented with 10% FBS (Meisen, Zhejiang, China), counted and passaged at a ratio of 1:3. HSFc and Fc were cultured in a 5% CO2 humidified atmosphere at 37°C. Cells from passages 4 to 7 were utilized for this study.

2.2 | Transfection group

DCN knockdown siRNA and DCN-overexpressing lentiviruses were purchased from Gene Pharma Company (Shanghai, China) and HanBI Company (Shanghai, China), respectively. The human DCN siRNA target sequences used are shown in Supplementary Table S2. HSFc and Fc cells were plated into 6-well plates until they reached 50% confluence before transfection. siRNAs were transfected into HSFc and Fc with a jet PRIME Reagent (Polyplus, Shanghai, China). The appropriate DCN-overexpressing lentivirus and vector (multiplicity of infection = 20) were separately added to the wells. The fibroblasts were then divided into a control group (blank control group), siDCN-1 group, siDCN-2 group (DCN knockdown group), vector group (DCN-overexpressing lentivirus-negative control group) and OE-DCN group (DCN-overexpressing lentivirus group).

2.3 | Cell invasion assay

The upper chamber of a 24-well transwell plate with an 8-μm aperture of the filter membrane (Corning, NY) was filled with 200 μL of serum-free medium, and a cell suspension of HSFc was seeded at a density of 5×10^4/well. In total, 500 μL of complete medium supplemented with FBS was added to the lower chamber and incubated for 24 h. Then, the HSFc was fixed with 4% paraformaldehyde for 30 min and washed with PBS three times. HSFs were dyed with 0.5% crystal violet staining solution (Booster, Wuhan, China) and incubated for 30 min at room temperature. After washing with PBS, the number of migrated cells in three random fields was observed and counted via microscopy.

2.4 | Western blotting

Total protein was extracted from cultured fibroblasts and homogenized in RIPA lysis buffer (Booster, Wuhan, China). Total protein was quantified using the BCA method (Boster, Wuhan, China). Protein samples (25 μg) were then separated by 8% SDS–PAGE and transferred onto PVDF membranes (Millipore, MA, USA). The membranes were first blocked with 5% nonfat milk for 2 h and then washed with TBST three times at room temperature. The membranes were incubated with primary antibodies specific for DCN (1:1000, Santa Cruz, USA), GAPDH (1:1000, Santa Cruz, USA), Col1 (1:2000, Abcam, Cambridge, UK), COL-3 (1:1000, Abmart, Shanghai, China), Smad3 (1:1000, Abcam, Cambridge, UK), p-Smad3 (1:1000, Abcam, Cambridge, UK) and TGF-β1 (1:1000, Abcam, Cambridge, UK) at 4 °C overnight. The next day, the membranes were incubated with an HRP-conjugated anti-rabbit or mouse antibody (Boster, Wuhan, China) at room temperature for 1 h and then washed with TBST three times at room temperature. The immunoreactive signals on the membrane for chemiluminescence detection of proteins were visualized using an ECL Kit (Bioscience, Shanghai, China) on a ChemiDoc XRS Imaging System (Bio-Rad), and the protein expression intensity was analysed using ImageJ software.

2.5 | Real-time quantitative PCR

Total RNA from the different groups was extracted using TRIzol reagent (Sparkjade, Shandong, China). A total of 0.5 μg of RNA was

reverse transcribed using 2×SPARKscript II RT Plus Master Mix (Sparkjade, Shandong, China). RT–qPCR was conducted with SYBR Green qPCR Mix (SparkJade, Shandong, China) on a real-time polymerase chain reaction system (Bio-Rad, Hercules, California). The reaction conditions were as follows: predenaturation at 94°C for 2 min, followed by 40 cycles of polymerase chain reaction denaturation at 94°C for 5 s and annealing at 60°C for 30 s. The Ct (threshold cycle) value of each sample was calculated, and the relative mRNA expression was normalized to the β-actin value using the relative quantification ($2^{-\Delta\Delta Ct}$) method. The primer sequences are listed in Supplementary Table S1.

2.6 | Statistical analysis

All experiments were performed in triplicate, and the data are presented as the mean ± SD. Student's test was used to compare data between two different groups. One-way ANOVA was applied to compare data among multiple groups. Statistical analysis was performed, and graphs were generated using Prism software. $p < 0.05$ ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) indicated statistical significance.

3 | RESULTS

3.1 | Hypertrophic scarring characterized by decreased DCN and increased collagen

Hypertrophic scarring is a fibroproliferative condition primarily originating from the reticular dermis and characterized by excess collagen deposition. Bioinformatics analyses confirmed the decreased expression of DCN in keloid tissue compared to normal skin tissue (Figure 1A). We aimed to determine whether DCN play the same role in HS as keloid tissue. We performed RT–qPCR, immunohistochemistry and immunofluorescence assays to investigate the expression of DCN in HSs and hypertrophic scar-derived fibroblasts. The results from the RT–qPCR assay revealed a decrease in DCN gene expression and an increase in COL-1 and COL-3 gene expression in hypertrophic scar tissues (Figure 1B) and hypertrophic scar-derived fibroblasts (HSFc) (Figure 1C). Immunohistochemical staining and immunofluorescence staining revealed significantly lower DCN protein expression in HSs than in normal skin (NS) (Figure 1D, E). Further immunofluorescence analyses confirmed a marked decrease in DCN expression in cultured HSFc compared to that in fibroblasts (Fc) (Figure 1F). These findings collectively suggest that DCN is downregulated in HSs and

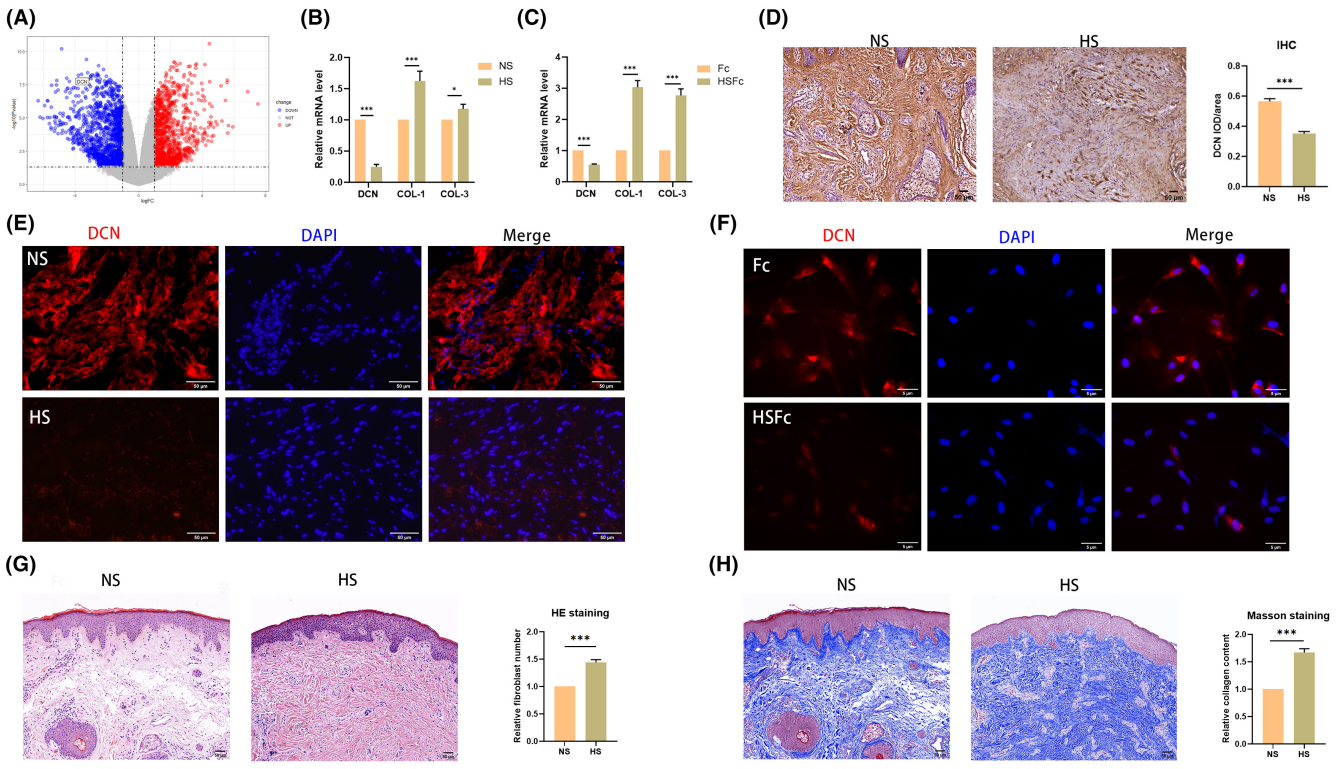

FIGURE 1 The expression of DCN and collagens in hypertrophic scars. (A) Bioinformatics search of the GEO database (GSE117887) to compare the DEGs between normal skin tissues and keloid scars. (B) RT–qPCR analysis showing DCN, COL-1 and COL-3 mRNA expression in HS and NS. (C) RT-qPCR analysis showing DCN, COL-1 and COL-3 mRNA expression in HSFc and Fc. (D) Immunohistochemical staining of DCN in the NS and HS groups. (E) Immunofluorescence staining for DCN in the NS and HS groups. (F) Immunofluorescence staining for DCN in Fc and HSFc. (G) HE staining analysis. (H) Masson staining analysis. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; unpaired *t* test. Fc, normal skin-derived fibroblasts; HS, hypertrophic scar tissue; HSFc, hypertrophic scar-derived fibroblasts; NS, normal skin tissue.

their derived fibroblasts. To investigate the characteristics of collagen deposition, histological detection techniques were employed. Compared with those in normal skin, Masson staining and HE staining revealed a disordered distribution of collagen fibres, increased collagen content, microvessel formation and thickening of the epithelium in hypertrophic scar tissue (Figure 1G, H). These findings indicate that DCN may play a role in regulating the expression, deposition and distribution of collagen in HSs.

3.2 | DCN inhibits collagen deposition

To investigate the role of DCN in HS, we utilized two small interfering RNAs (siRNAs) to knock down DCN and a lentivirus to overexpress DCN. The effectiveness of DCN knockdown and overexpression (OE) was evaluated through RT–qPCR and Western blotting (Supplementary Figure S1). Fluorescence microscopy confirmed successful transfection of the overexpressing lentiviruses containing enhanced green fluorescent protein into Fc (Supplementary Figure S1) and HSFc (Figure 2A). We investigated the impact of DCN on collagen formation. RT–qPCR and Western blotting analyses were subsequently conducted to assess the influence of DCN on collagen formation. In HSFc, DCN knockdown significantly enhanced both the mRNA (Figure 2B) and protein (Figure 2D) expression of COL-I and COL-III, whereas DCN overexpression notably suppressed the expression of collagens (Figure 2C, E). Similarly, in Fc under the same conditions, both the gene and protein levels of COL-I and COL-III followed the same

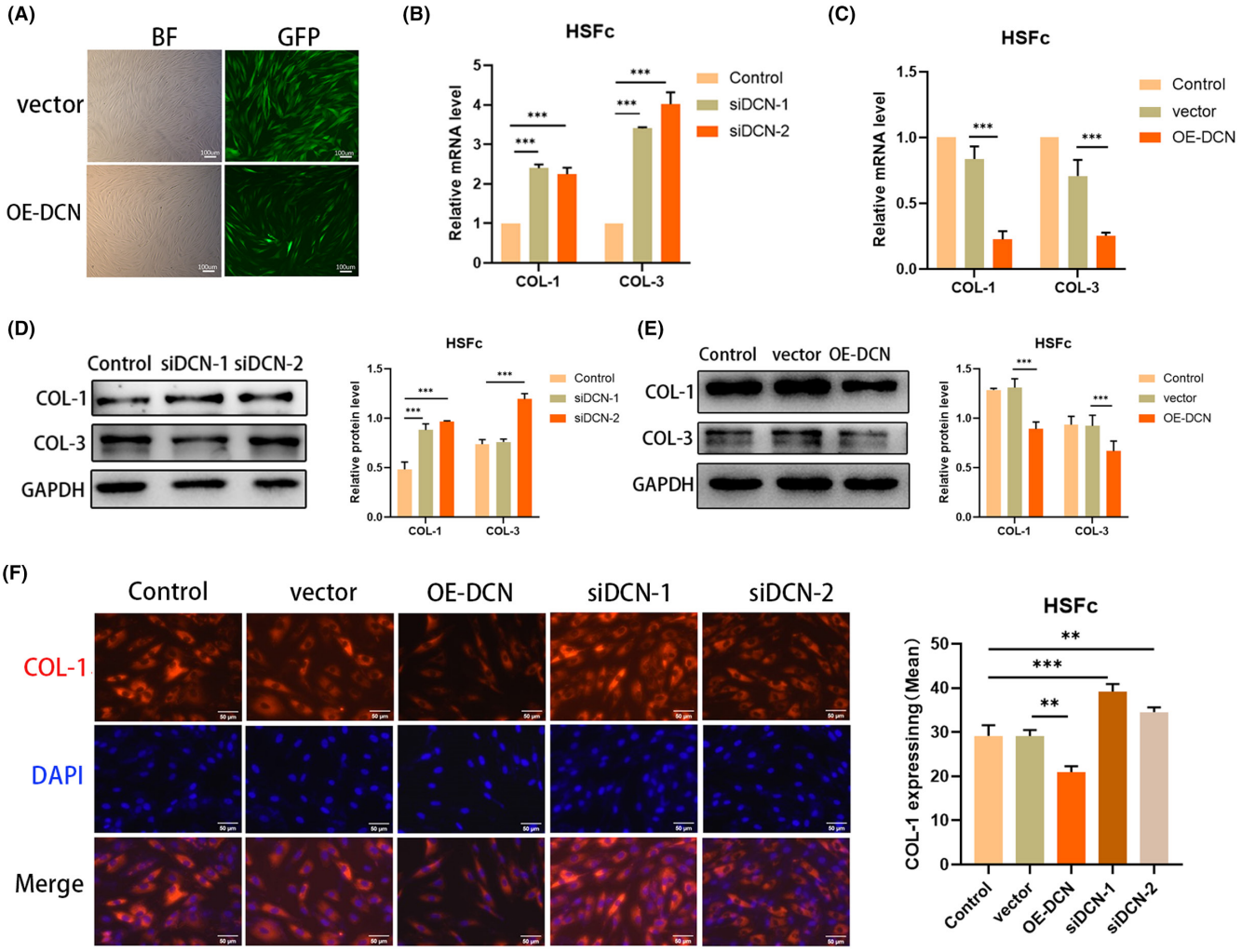

FIGURE 2 DCN inhibits collagen formation. (A) Fluorescence microscopy confirmed successful transfection of the overexpressing lentiviruses in HSFc; (B) RT-qPCR analysis showing the expression of COL-1 and COL-3 mRNA after DCN knockdown in HSFc; (C) RT-qPCR analysis showing the expression of COL-1 and COL-3 mRNA after DCN overexpression in HSFc; (D) Western blotting analysis showing the expression of COL-1 and COL-3 proteins after DCN knockdown in HSFc and quantitative statistics; (E) Western blotting analysis showing the expression of COL-1 and COL-3 proteins after DCN overexpression in HSFc and quantitative statistics; (F) Immunofluorescence analysis showing the expression of COL-1 proteins after DCN knockdown and overexpression in HSFc and quantitative statistics. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; unpaired t test. Fc, normal skin-derived fibroblasts; HSFc, hypertrophic scar-derived fibroblasts; OE, overexpression; siRNA, small interfering RNA.

trend (Supplementary Figure S3). Furthermore, immunofluorescence analysis revealed that DCN knockdown resulted in increased fluorescence intensity of COL-1 compared to that of the control, while the DCN overexpression group exhibited lower fluorescence intensity in HSFc (Figure 3F). These findings strongly suggest that DCN plays a crucial role in inhibiting collagen formation.

3.3 | DCN represses cytological function

Scar formation is closely linked to the excessive proliferation of scar fibroblasts.[18] Therefore, we investigated the effects of DCN on regulating the proliferation, invasion and migration of HSFc. Given that the overproliferation of fibroblasts is a critical aspect of scar formation, we conducted a CCK-8 assay to assess the impact of DCN knockdown and overexpression on HSFc proliferation. Our results demonstrated that DCN knockdown promoted HSFc proliferation, whereas DCN overexpression inhibited it (Figure 3A, C). To further elucidate the influence of DCN on the functional behaviour of HSFc, we examined its effects on cell invasion and migration. Cell invasion assay revealed that DCN knockdown increased the invasion of HSFc (Figure 3B), while DCN overexpression decreased invasion (Figure 3D). Consistent results were obtained from the scratch assay (Figure 3E–G). Overall, our findings suggest that DCN overexpression suppresses the proliferation, invasion and migration properties of HSFc, whereas DCN knockdown has the opposite effect.

3.4 | DCN affects collagen formation through the TGF-β1/Smad3 pathway

To investigate the impact of DCN on the TGF-β/Smad signalling pathway, which is crucial for collagen formation in fibroblasts and myofibroblasts,[19] Western blotting analysis was conducted to assess p-Smad3 and Smad3 expression. Following DCN knockdown (Figure 4A), increased expression of p-Smad3 and Smad3

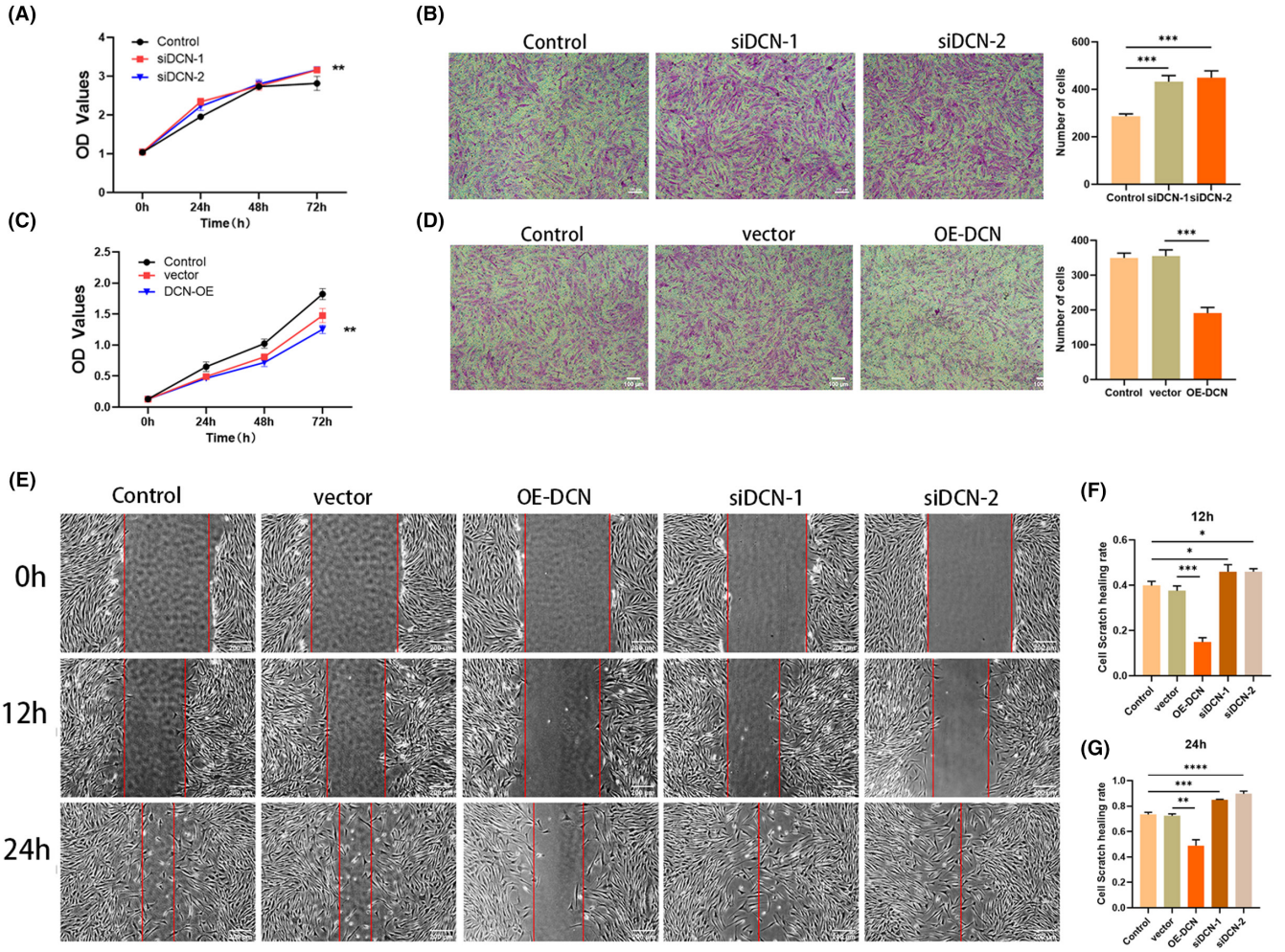

FIGURE 3 DCN is important for HSFc proliferation, invasion and migration. (A, C) CCK-8 assay analysis showing proliferation after DCN knockdown and overexpression in HSFc and quantitative statistics. (B, D) Cell invasion assay analysis showing invasion after DCN knockdown and overexpression in HSFc and quantitative statistics. (E–G) Scratch assay analysis showing migration after DCN knockdown and overexpression in HSFc and quantitative statistics. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; unpaired t test. Fc, normal skin-derived fibroblasts; HSFc, hypertrophic scar-derived fibroblasts; OE, overexpression; siRNA, small interfering RNA.

was observed, whereas the opposite trend was observed upon DCN overexpression (Figure 4B). Furthermore, Western blotting results suggested that DCN overexpression attenuated COL-1, COL-3, p-Smad3 and Smad3 levels in TGF-β1-treated HSFc cells, which exhibited upregulated TGF-β1, p-Smad3 and collagen expression. This finding suggested that DCN influenced the expression of TGF-β1 or inhibited the activity of TGF-β1 simultaneously. These findings further revealed that the antifibrotic effect of DCN is mediated through the TGF-β1/Smad3 pathway in vitro. Understanding the mechanism of action of DCN will facilitate the development of new approaches for its therapeutic use in hypertrophic scar treatment.

4 | DISCUSSION

Hypertrophic scarring is characterized by excessive deposition of collagen and other extracellular matrix, leading to continuous scar growth without a self-healing tendency.[20] This condition not only affects aesthetics but also causes pain, itching and other accompanying symptoms, which can negatively affect the psychological health of patients.[21,22] The pathogenesis of HSs is still unclear. However, the current understanding suggests that the excessive proliferation and apoptosis of fibroblast components are uncontrolled, and the excessive deposition and degradation of extracellular components are imbalanced.[23,24] This study provides a groundbreaking understanding of the mechanism of DCN in hypertrophic scar development. Our research revealed that DCN not only reduces collagen accumulation within HSs but also hinders the proliferation, invasion and migration of HSFc. Moreover, DCN reduced fibrosis by inhibiting the TGF-β1/Smad3 signalling pathway. These findings will provide new ideas for the treatment of HSs.

Regulating intracellular catabolism is an emerging theme among secreted matrix constituents. DCN is a widely present component in the dermis and matrix interstitium and has a strong affinity for collagen fibres. As the first small proteoglycan identified as leucine-rich,[25] it plays a crucial role in regulating cell growth and the synthesis and composition of the extracellular matrix.[26] DCN can bind collagen fibres and control cell-matrix interactions. This study demonstrated decreased DCN expression in HSs and their derived fibroblasts, along with the ability of DCN to alleviate hypertrophic scar formation. These findings align with prior research, providing further support for the role of DCN in scar mitigation.[27]

Hypertrophic scar tissues are characterized by increased collagen levels and disorganized arrangement. Given that fibroblasts play a central role in hypertrophic scar formation, we examined the biological effects of DCN on both normal skin fibroblasts and fibroblasts derived from HSs. Our investigation focused on the impact of DCN on collagen synthesis, proliferation, migration and invasion in fibroblasts. We found that DCN knockdown led to enhanced collagen synthesis and secretion, as well as increased proliferation, migration and invasion of HSFc, while DCN overexpression had the opposite effects. Additionally, previous studies have reported that DCN inhibits corneal fibroblast migration.[28]

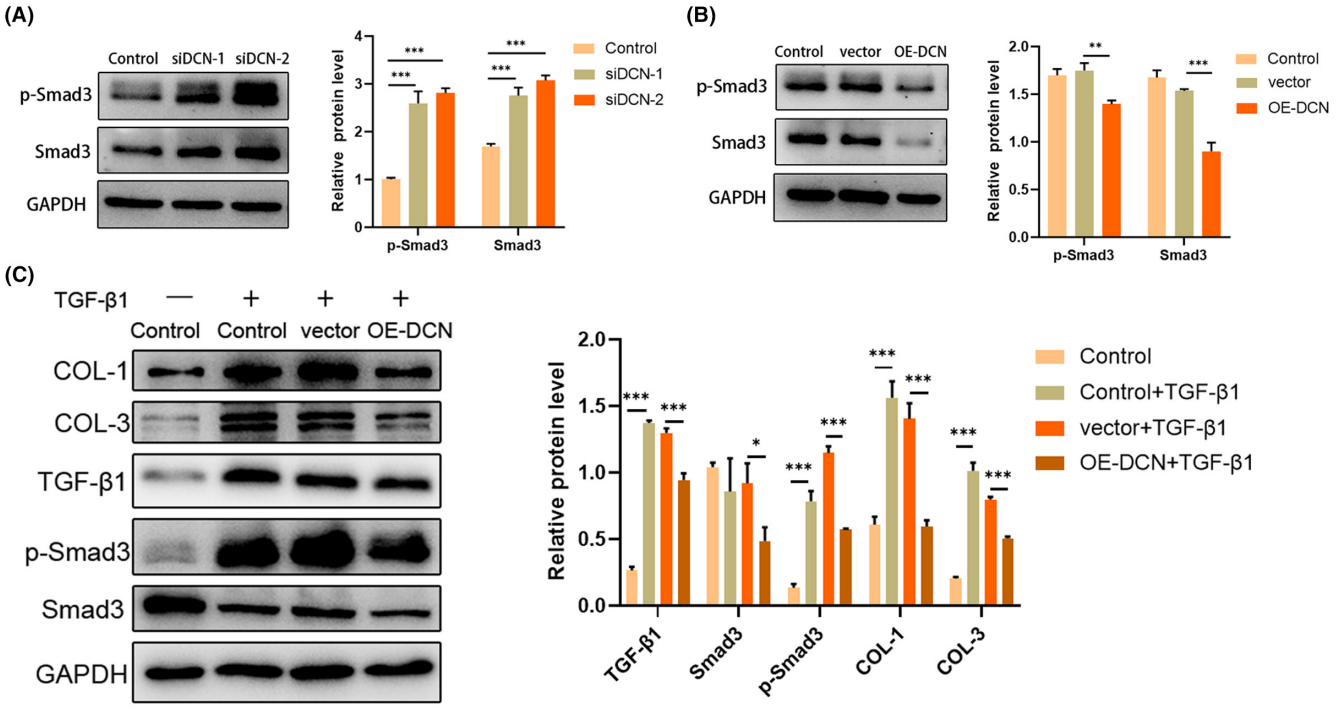

FIGURE 4 DCN regulates the TGF-β1/Smad3 signalling pathway. (A) (B) Western blotting analysis showing the protein expression of Smad3 and p-Smad3 after DCN knockdown and overexpression in HSFc cells and quantitative statistics. (C) Western blotting analysis showing the protein expression of Smad3, p-Smad3, TGFβ1, COL-1 and COL-3 after DCN overexpression in HSFc with/without TGF-β1 (5 ng/mL) and quantitative statistics. ***$p < 0.001$; unpaired t test. OE, overexpression; siRNA, small interfering RNA.

Furthermore, we investigated whether DCN influences the TGFβ/Smad signalling pathway. DCN knockdown was found to enhance p-Smad3 and Smad3 expression, while DCN overexpression had the opposite effect. Smad3 is a key activator of the TGFβ1/Smad signalling pathway, and inhibition of Smad3 phosphorylation has been shown to reduce fibrosis.[29] Our study revealed that DCN overexpression, in conjunction with TGF-β1 treatment, decreased TGF-β1 expression and regulated the expression of Smad3, p-Smad3, COL-1 and COL-3. This finding suggested that DCN may affect TGF-β1 expression or inhibit its activity concurrently. These findings indicate that DCN suppresses TGFβ1/Smad3 signalling to inhibit HSFc expression of COL-1, consistent with observations in cardiac fibrosis.[30]

There are several limitations to our study. First, our investigation was confined to the role of DCN in HSFc in vitro, leaving its in vivo relevance unexplored, although DCN-loaded nanofibers have shown efficacy in reducing ECM-related proteins in vitro.[31] Further studies using animal models of HS are necessary to validate these findings. Additionally, the impact of DCN on collagen fibre assembly, the mechanical properties of the skin, and cellular processes such as autophagy and mitophagy suggest a broader scope for exploration.[32–34] Its multifaceted bioactivity warrants comprehensive investigation in future research to better understand its potential in hypertrophic scar therapy.

AUTHOR CONTRIBUTIONS

Xiaotao Xing and Xiaoyi Hu conceived, designed and supervised the study. Jiangtao Cui and Shiyi Zhang designed and performed the experiments. Yan Xu, Heng Guo, Tong Li, Donghe Fu, Ziyang Yang and Lingnan Hou assisted in collecting, analysing the data and revising the manuscript. Kiran Acharya revised into the language of the manuscript. Jiangtao Cui and Xiaotao Xing wrote and revised the manuscript. All authors reviewed and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors state no conflict of interest.

DATA AVAILABILITY STATEMENT

Datasets related to this article can be found at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE117887, hosted at Gene Expression omnibus.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information.

Figure S1. Validation of DCN knockdown and overexpression efficiency. (A) Fluorescence microscopy confirmed successful transfection of the overexpressing lentiviruses in Fc. (B) The mRNA levels of DCN were detected by RT–qPCR after Fc and HSFc were transfected with siRNA to knock down DCN. (C) The mRNA levels of DCN were detected by RT–qPCR after Fc and HSFc were transfected with DCN-overexpressing lentivirus to overexpress DCN. (D) (E) The protein levels of DCN were detected by Western blotting after Fc and HSFc were transfected with siRNA to knock down DCN. (F) (G) The protein levels of DCN were detected by Western blotting after Fc and HSFc were transfected with DCN-overexpressing lentivirus to overexpress DCN. $^{**}p<0.01$, $^{***}p<0.001$; unpaired *t* test. Fc, normal skin-derived fibroblasts; HSFc, hypertrophic scar-derived fibroblasts. OE, overexpression; siRNA, small interfering RNA.

Figure S2. Lentivirus overexpression vector mapping.

Figure S3. DCN inhibits collagen formation in Fc. (A) RT–qPCR was used to detect the expression of COL-1 and COL-3 mRNA after DCN knockdown in Fc. (B) Western blot ting analysis of COL-1 and COL-3 mRNA expression after DCN knockdown in Fc. (C) RT–qPCR was used to detect the expression of COL-1 and COL-3 mRNA after DCN overexpression in Fc. (D) Western blotting analysis of COL-1 and COL-3 protein expression after DCN overexpression in Fc. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; unpaired *t* test. Fc, normal skin-derived fibroblasts; OE, overexpression; siRNA, small interfering RNA.

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