Knockdown of lncRNA-NEAT1 expression inhibits hypoxia-induced scar fibroblast proliferation through regulation of the miR-488-3p/COL3A1 axis

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Abstract. Long non-coding (lnc)RNA nuclear-enriched transcripts 1 (NEAT1) has been demonstrated to be involved in the inhibition of hypoxia-induced scar fibroblast proliferation, but the specific mechanism remains undetermined. The present study found that with the decrease of oxygen concentration, lncRNA NEAT1 was upregulated in hypoxia-induced scar fibroblasts, which promoted the mRNA and protein expression levels of collagen (COL)-I, COL-III and α-smooth muscle actin, thereby suppressing hypoxia-induced scar fibroblasts proliferation. In addition, the microRNA (miR)-488-3p/COL3A1 axis was involved in lncRNA NEAT1's regulation of the proliferation of hypoxia-induced scar fibroblasts. In conclusion, the knockdown of lncRNA-NEAT1 expression can inhibit hypoxia-induced scar fibroblasts proliferation through regulation of the miR-488-3p/COL3A1 axis, which will provide a novel therapeutic target for the treatment of hypertrophic scars.

Introduction

Hypertrophic scar is a type of fibrotic skin disease caused by abnormal healing of skin injuries including skin burns (1), which is characterized by excessive proliferation of fibroblasts, epidermal interstitial transformation and collagen deposition (2). Hypertrophic scars commonly occur in injured skin areas and cause pain, itching and other symptoms, which cause great psychological effects to the patients. At present, the main clinical treatment methods for scars include surgical excision and steroid therapy, but the pathological molecular mechanism of scar formation remains to be elucidated. Therefore, the present study on the pathological mechanism of scar formation may reveal a novel therapeutic target for the treatment of hypertrophic scars.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs that contain >200 nucleotides. Studies have demonstrated that lncRNAs significantly affect complicated pathological processes of various diseases, e.g., cardiovascular diseases (3), cerebral ischemic diseases and carcinomas (4). lncRNAs have no protein-coding capability, but they bind to micro (mi)RNA as competitive endogenous RNAs and regulate the expression of downstream target genes, thus serving a critical role in various biological cellular processes and malignant diseases. LncRNA nuclear-enriched transcripts 1 (NEAT1) is a tumor growth regulator that plays an essential role in different types of cancer (5,6) including breast (7), gastric (8) and lung (9) cancer. It has been demonstrated that lncRNA NEAT1 sponges miRNA (miR)-129 to regulate the epithelial-mesenchymal transition (EMT) and inflammatory response of renal fibrosis through regulation of collagen (COL)-I (10). Nonetheless, the molecular regulation mechanism of lncRNA NEAT1 in hypertrophic scar formation remains unclear.

The role of miRNA in the progression of malignant diseases has attracted extensive attention (11). miRNA can competitively block the translation of downstream target genes and negatively regulate the expression level of target genes, thus serving a key part in the treatment of hypertrophic scars (12). Wang *et al* (13) found that miR-31-5p participates in the formation of hypertrophic scars (HSs) by inhibiting FIH and regulating the expression of HIF-1α. Bi *et al* (14) found that miR-98 inhibits the proliferation of hypertrophic scar fibroblasts by targeting COL1A1. In addition, Li *et al* (15) found that lncRNA8975-1 regulates the expression of COL3A1, COL1A1 and α-smooth muscle actin (α-SMA) and inhibits the proliferation of fibroblasts in hypertrophic scars. COL3A1 has been identified as a marker for fibroblast differentiation in hypertrophic scar formation, implying that the expression level of COL3A1 is associated with the pathological process of hypertrophic scars. Given the abnormal proliferation of fibroblasts, it was hypothesized that COL3A1 is involved in the proliferation of fibroblasts during scar formation. The present study aimed to investigate the inhibitive effects of lncRNA NEAT1 on cell proliferation of hypertrophic scars and elucidate the molecular mechanism of the miR-488-3p/COL3A1 axis in regulating scar fibroblast proliferation.

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Materials and methods

Cell culture and treatments. Scar fibroblasts purchased from American Type Culture Collection were incubated in Dulbecco's Modified Eagle's Medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin in 5% CO_2 at 37˚C. The groups of the experiment included the control group (21% O_2), the 10% hypoxia group (10% O_2), the 5% hypoxia group (5% O_2) and the 1% hypoxia group (1% O_2). After 48 h of culture, the experiments were started.

Bioinformatics analysis. Bioinformatics analysis was performed to predict the downstream miRNA and mRNA of lncRNA NEAT1 using Starbase (https://starbase.sysu.edu.cn/).

Cell transfection. Scar fibroblasts underwent transfection with 20 nM miR-488-3p mimic or the corresponding negative control (Shanghai GenePharma Co., Ltd.) by using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h at 37˚C. For COL3A1 overexpression, the recombinant sense expression vector plasmid Cytomegalovirus promoter DNA 3.1 for COL3A1 (1 μg/μl pcDNA3.1-COL3A1; Invitrogen; Thermo Fisher Scientific, Inc.) was constructed by subcloning the cDNA fragment of COL3A1 containing the complete coding sequence between *Kpn*I and *Bam*HI. Short hairpin RNA (shRNA) targeting NEAT1 (sh-NEAT1) and their negative control (sh-NC) were purchased from Shanghai GenePharma Co., Ltd. Sequences were cloned in the pEGFP plasmid. miR-488-3p mimic (miR-488-3p; 5'-UUGAAAGGC UAUUUCUUGGUC-3') and mimic control (miR-NC; 5'-UUC UCCGAACGUGUCACGUTT-3' and 5'-ACGUGACACGUU CGGAGAATT-3'), miR-488-3p inhibitor (anti-miR-488-3p; 5'-CUGUUCCUGCUGAACUGAGCCA-3') and inhibitor control (anti-miR-NC; 5'-CAGUACUUUUGUGUAGUA CAA-3') were also purchased from Shanghai GenePharma Co., Ltd. Scar fibroblasts were seeded into 24-well plates at a density of $2.0x10^4$ cells/well, following which 50 nM synthetic oligonucleotides or 2 μg vectors were transfected into the cells using Lipofectamine® 2000 for 24 h at 37˚C (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Following transfection, cells were incubated in fresh DMEM at 37˚C for 24 h and finally collected for the subsequent experiments.

CCK-8 assay. Scar fibroblasts transfected with designated vectors for 72 h were seeded into 96-well plates at a density of $1x10^3$ cells/well. Cell viability was detected by Cell Counting Kit-8 assay (CCK-8 assay; Beyotime Institute of Biotechnology) at 48 h following the manufacturer's instructions. The optical density at 450 nm of each well was determined by using a microplate reader.

Luciferase reporter assay. lncRNA NEAT1 with or without miR-488-3p putative target sites were synthesized and obtained from Shanghai GenePharma Co., Ltd. and cloned into the Xho I/Not I sites of a psiCHECK vector (Promega Corporation). For transfection assays, cells were seeded into 24-well plates and transfected with miR-488-3p mimics or miR-488-3p inhibitor using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. After further incubation for 24 h, the transfected cells were harvested, lysed and centrifuged (10,000 x g, 10 min, 4˚C) to obtain the supernatant for the luciferase assay by Dual-Luciferase Reporter Assay kit (Promega Corporation) according to the manufacturer's instructions. Luciferase activity was normalized to *Renilla* luciferase activity.

Immunofluorescence staining. Transfected cells were fixed with 4% paraformaldehyde for 15 min and permeabilized by 0.1% Triton X-100 (Beyotime Institute of Biotechnology) for 30 min at room temperature. After being blocked in normal goat serum (Beijing Solarbio Science & Technology Co., Ltd.) for 15 min at room temperature, the cells were incubated with Ki-67 primary antibody at a 1:200 dilution (Abcam) in a wet box at 4˚C overnight. Then, Cy3-labeled goat anti-rabbit IgG secondary antibody at a 1:200 dilution (Beyotime Institute of Biotechnology) was added to incubate the cells at room temperature for 60 min. Next, the cells were rinsed with PBS and the nucleus counterstained with DAPI for 10 min at room temperature (Beyotime Institute of Biotechnology). Finally, the cells were sealed with the mounting medium (Beijing Solarbio Science & Technology Co., Ltd.) and fluorescence was observed under a fluorescent microscope (Olympus Corporation; magnification, x400) by a pathology experimenter blinded to the experimental or control group.

Reverse transcription-quantitative (RT-q)PCR. A total of 1 μg RNA was extracted from scar fibroblasts by using TRIzol® (Thermo Fisher Scientific, Inc.). Reverse transcription was performed using RT Reagent kit (cat. no. RR037A; Takara, Bio, Inc.). qPCR (cat. no. RR820A; Takara, Bio, Inc.) was performed using SYBR Green mix (Takara Bio, Inc.) with primers specific to miR-488-3p (Guangzhou RiboBio Co., Ltd.). The PCR conditions were as follows: 95˚C for 10 min, followed by 40 cycles of 95˚C for 30 sec, 60˚C for 30 sec and 72˚C for 1 min. Relative quantification of the miRNA expression was calculated through the $2^{-\Delta\Delta Cq}$ method (16). Primers: miR-488-3p forward, 5'-ACACTCCAGCTGGGTTGAAAG GCTATTTC-3' and reverse, 5'-CTCAACTGGTGTCGT GGAGTCGGCAATTCAGTTGAGGACCAAGA-3'; NEAT1 forward, 5'-GGAGAGGGTTGGTTAGAGAT-3' and reverse, 5'-CCTTCAACCTGCATTTCCTA-3'; U6 forward, 5'-CTC GCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGA ATTTGCGT-3'; and GAPDH forward, 5'-GCACCGTCAAGG CTGAGAAC-3' and reverse 5'-GGATCTCGCTCCTGGAAG ATG-3'. U6 and GAPDH were selected as the housekeeping gene to normalize the expression of miRNA and mRNA.

Western blotting. Total protein lysates were generated using RIPA lysis buffer supplemented with protease and phosphatase inhibitor mixtures (cat. no. KC-440; Shanghai KangChen Biological Technology Co. Ltd.). Nuclear proteins were extracted by using the NE-PER nuclear and cytoplasmic extraction reagents (Pierce; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The concentration of the protein in cells lysates was detected using a BCA kit (Beijing Solarbio Science & Technology Co., Ltd.).

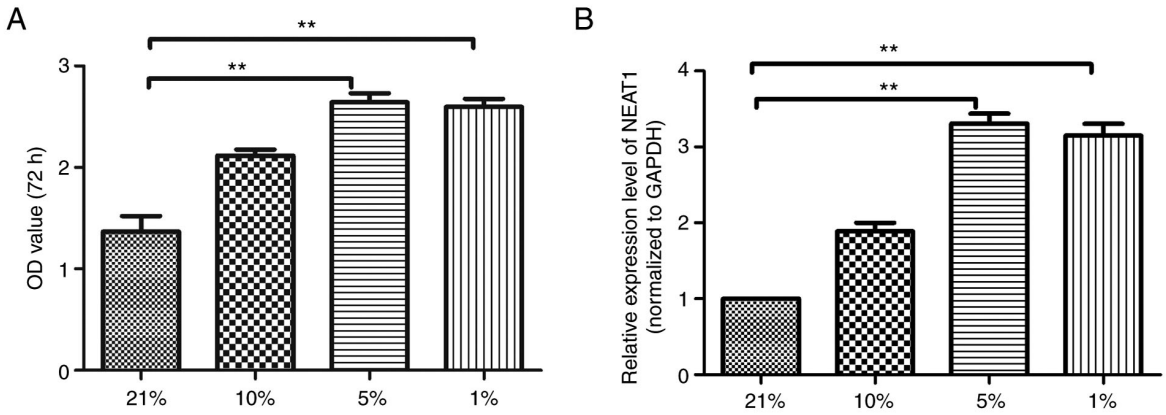

Figure 1. The expression of lncRNA-NEAT1 in scar fibroblasts under different hypoxic conditions. (A) The cell viability under different hypoxic conditions detected by CCK-8 kit. (B) The expression of NEAT1 in scar fibroblasts under different hypoxic conditions detected by reverse transcription-quantitative PCR. The data are presented as mean ± SEM, vs. 21% group, **P<0.01, n=6. lncRNA, long non-coding RNA; NEAT1, nuclear-enriched transcripts 1; OD, optical density.

Proteins (40 μg) were loaded onto a 5-10% polyacrylamide gel, separated by electrophoresis and transferred onto a polyvinylidene difluoride (PVDF) membrane. Then, the PVDF membrane was blocked with a 5% solution of non-fat milk at room temperature for 3 h and incubated with rabbit polyclonal antibodies to COL-I (1:1,000 cat. no. ab260043; Abcam) and COL-III (1:1,000; cat. no. ab7778; Abcam), α-SMA (1:1,000; cat. no. 19245s; Cell Signaling Technology, Inc.) and GAPDH (1:1,000; cat. no. 5174s; Cell Signaling Technology, Inc.) at 4˚C overnight. Horseradish peroxidase-conjugated goat anti-rabbit (cat. no. BA1054) or anti-mouse (cat. no. BA1050) antibody (1:15,000) (Wuhan Boster Biological Technology, Ltd.) was utilized as a secondary antibody, incubated at 37˚C for 1 h. Proteins were visualized by an enhanced chemiluminescence system using the FluorChem FC system (ProteinSimple). ImageJ software V.1.4 (National Institutes of Health) was used to measure the gray values of the bands and analyze the changes in relative protein expression levels.

Statistical analyses. Statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). The data are shown as mean ± standard error of the mean (SEM) from three independent experiments. Statistical analyses were conducted using Student's t-test or ANOVA followed by Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Upregulated lncRNA-NEAT1 expression in scar fibroblasts under different hypoxic conditions. First, the viability of scar fibroblasts under different hypoxic conditions was detected by CCK-8 kit. As shown in Fig. 1A, the CCK-8 results indicated that with the decrease of oxygen concentration, the viability of scar fibroblasts was significantly enhanced; the viability of scar fibroblasts in the 5% group was the highest. The expression of lncRNA NEAT1 in scar fibroblasts under different hypoxic conditions was detected. The RT-qPCR analysis results (Fig. 1B) implied that compared with the 21% group, the expression of lncRNA NEAT1 in scar fibroblasts in the 5% group and the 1% group were markedly increased (P<0.001).

Effects of lncRNA NEAT1 on hypoxia-induced scar fibroblast proliferation. RT-qPCR analysis was performed to detect the expression of lncRNA NEAT1 in different groups. As shown in Fig. 2A, compared with the NC group, the expression of lncRNA NEAT1 in the lncRNA NEAT1 group and the hypoxia (5%) + lncRNA NEAT1 group was significantly reduced, whereas that in the hypoxia (5%) + NC group was increased. To investigate the effect of lncRNA NEAT1, the hypoxia-induced scar fibroblasts proliferation was detected by CCK-8 kit. The results (Fig. 2B) demonstrated that compared with the NC group, the viability of scar fibroblasts in the hypoxia (5%) + NC group was substantially enhanced while that in the hypoxia (5%) + lncRNA NEAT1 group was inhibited. Ki-67 protein can be used as a proliferation marker for hypertrophic scar fibroblasts, hence RT-qPCR analysis and immunofluorescence staining were performed to detect the expression of Ki-67 in scar fibroblasts. The results (Fig. 2C) indicated that compared with the NC group, the expression of Ki-67 in the hypoxia (5%) + NC group was significantly increased while that in the hypoxia (5%) + lncRNA NEAT1 group was markedly reduced, which is consistent with the immunofluorescence staining results (Fig. 2D). Therefore, lncRNA NEAT1 can inhibit the proliferation of hypoxia-induced scar fibroblasts.

Effects of lncRNA NEAT1 on steady-state protein levels of COL-I, COL-III and α-SMA in scar fibroblasts. To investigate the regulatory mechanism of lncRNA NEAT1 in scar fibroblasts, RT-qPCR analysis and western blotting were performed to detect the mRNA and protein expression levels of COL-I, COL-III and α-SMA in scar fibroblasts. The RT-qPCR results (Fig. 3A-C) indicated that compared with the NC group, the mRNA expression of COL-I, COL-III and α-SMA in the Hy + NC group was substantially upregulated while that in the Hy + lncRNA NEAT1 group was downregulated, which is consistent with the western blotting results (Fig. 3D). In summary, the RT-qPCR results and the western blotting results demonstrated that lncRNA NEAT1 can downregulate the expression levels of COL-I, COL-III and α-SMA in hypoxia-induced scar fibroblasts.

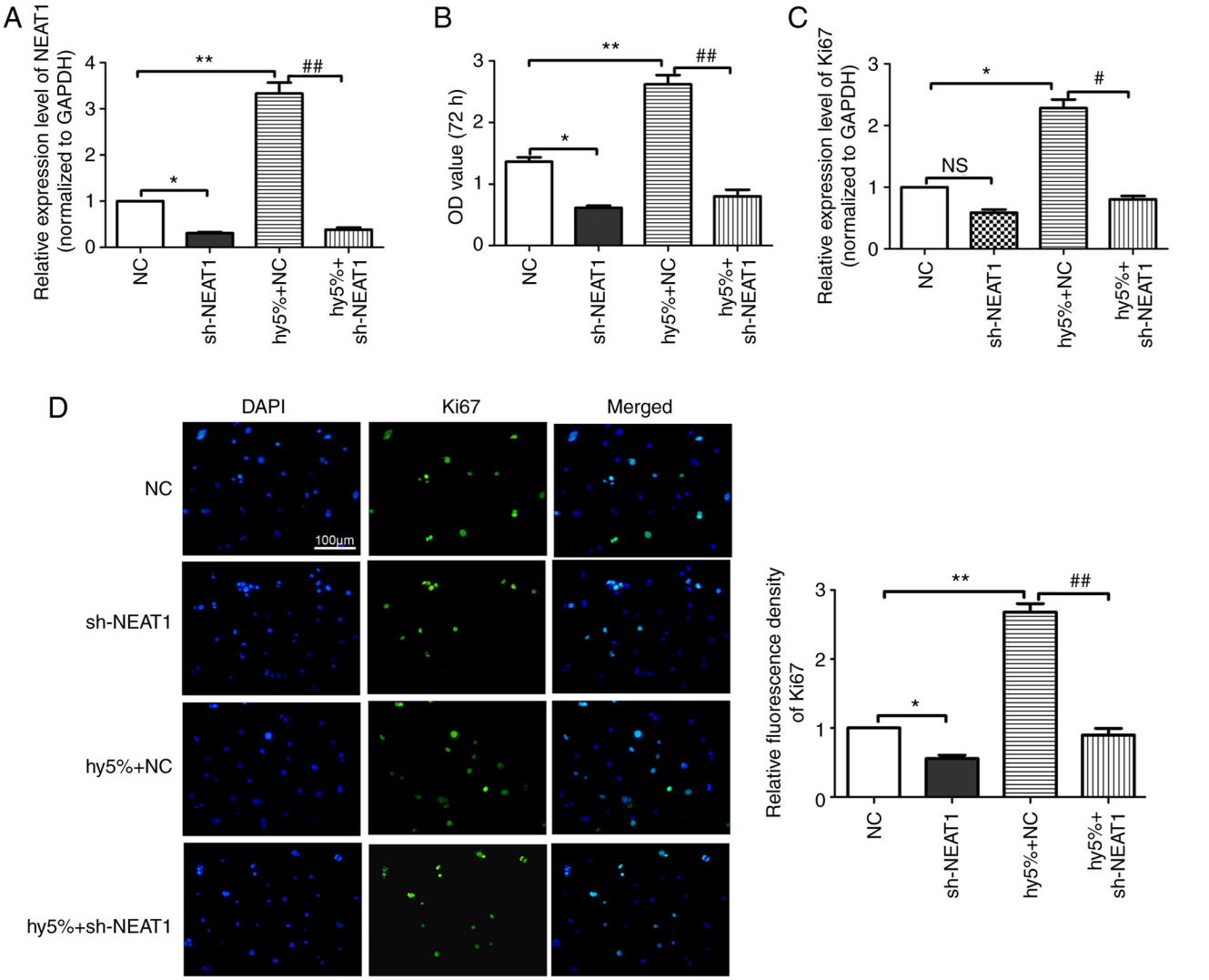

Figure 2. Effects of lncRNA NEAT1 on hypoxia-induced scar fibroblast proliferation. (A) The mRNA expression level of NEAT1 in different groups detected by RT-qPCR analysis. (B) The ability of proliferation in hypoxia-induced scar fibroblasts measured by CCK-8 kit. (C) The mRNA expression of Ki-67 in hypoxia-induced scar fibroblasts assessed by RT-qPCR. (D) The positive rate of Ki-67 in fibroblasts detected by immunofluorescence staining: the representative staining results (left) and the statistical results (right). The data are presented as mean ± standard error of the mean, vs. NC group, *P<0.05, **P<0.01; vs. hy 5% + lncRNA NEAT1 group, $^{\#}$P<0.05, $^{\#\#}$P<0.01, n=6. lncRNA, long non-coding RNA; NEAT1, nuclear-enriched transcripts 1; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; sh, short hairpin; hy, hypoxia.

lncRNA NEAT1 directly targeted miR-488-3p. Luciferase activity of scar fibroblasts (Fig. 4A and B) transfected with miR-488-3p mimics was markedly reduced compared with the mimics control group (P<0.01). In addition, the RT-qPCR results (Fig. 4C) demonstrated that the expression of miR-488-3p in the lncRNA NEAT1 group was significantly increased compared with the NC group. Therefore, it was confirmed that lncRNA NEAT1 directly targets miR-488-3p.

miR-488-3p directly targets COL3A1. As indicated by the results of the luciferase activity assay (Fig. 5A and B), luciferase activity of scar fibroblasts transfected with miR-488-3p mimics was substantially reduced compared with the mimics control group (P<0.01). In addition, compared with the mimics control group, the expression level of miR-488-3p (Fig. 5C) in the miR-488-3p mimics group was significantly increased. Compared with the inhibitor control group, the expression level of miR-488-3p was significantly reduced in the miR-488-3p inhibitor group. Furthermore, the RT-qPCR analysis results (Fig. 5D) indicated that the mRNA expression level of COL3A1 in the miR-488-3p mimics group was substantially downregulated compared with the mimics control group, whereas that in the miR-488-3p inhibitor group was increased compared with the inhibitor control group. The western blotting results (Fig. 5E) were consistent with the RT-qPCR results. Given the above results, it was concluded that miR-488-3p directly targets COL3A1 in scar fibroblasts.

lncRNA NEAT1 inhibited hypoxia-induced scar fibroblasts proliferation through regulation of miR-488-3p/COL3A1 axis. To detect the mRNA expression level of COL3A1 in scar fibroblasts after transfection, RT-qPCR analysis was performed. The results (Fig. 6A) demonstrated that compared with the pcDNA3.1 group, the mRNA expression level of COL3A1 in the pcDNA3.1-COL3A1 group was significantly increased. The western blotting results (Fig. 6B) indicated that

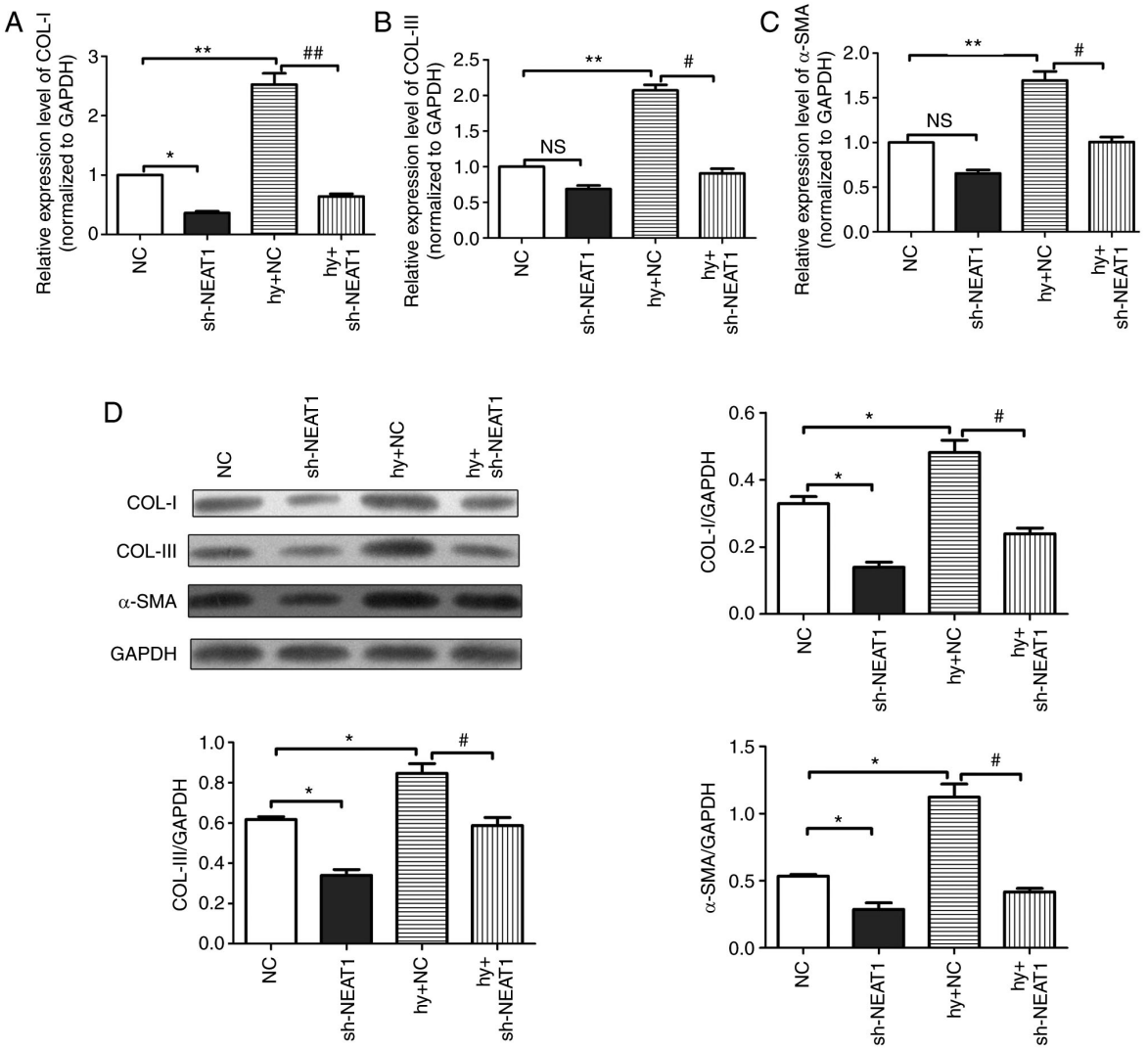

Figure 3. Effects of lncRNA NEAT1 on the steady-state protein levels of COL-I, COL-III and α-SMA in scar fibroblasts. The mRNA expression levels of (A) COL-I, (B) COL-III and (C) α-SMA in scar fibroblasts detected by reverse transcription-quantitative PCR. (D) The protein expression levels of COL-I, COL-III and α-SMA in scar fibroblasts detected by the western blotting assay. All protein expression levels were normalized to GAPDH. The data are presented as mean ± standard error of the mean, vs. NC group, *P<0.05, **P<0.01; vs. Hy + NC group, $^{\#}$P<0.05, $^{\#\#}$P<0.01, n=6. long non-coding RNA; NEAT1, nuclear-enriched transcripts 1; COL, collagen; α-SMA, α-smooth muscle actin; sh, short hairpin; Hy, hypoxia; NC, negative control; NS, not significant.

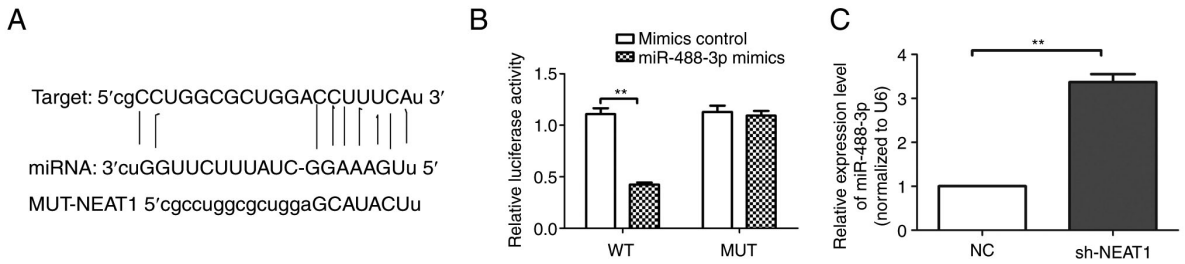

Figure 4. LncRNA NEAT1 directly targets miR-488-3p. (A) The miR-488-3p target sites in lncRNA NEAT1 revealed by sequence prediction via Starbase. (B) The interaction between lncRNA NEAT1 and miR-488-3p and luciferase activity in scar fibroblasts determined by the dual luciferase assay. (C) The mRNA expressions of miR-488-3p in fibroblasts detected by reverse transcription-quantitative PCR. The data are presented as mean ± standard error of the mean, vs. mimics control and NC group, **P<0.01, n=6. LncRNA; long non-coding RNA; NEAT1, nuclear-enriched transcripts 1; miR, microRNA; NC, negative control; MUT, mutant; WT, wild type.

compared with the Hy + NC group, the protein expression level of COL-III in the Hy + lncRNA NEAT1 group was substantially reduced; compared with the Hy + lncRNA NEAT1 + inhibitor control group, the protein expression level of COL-III in the Hy + lncRNA NEAT1 + miR-488-3p inhibitor group was substantially increased; compared with the Hy + lncRNA

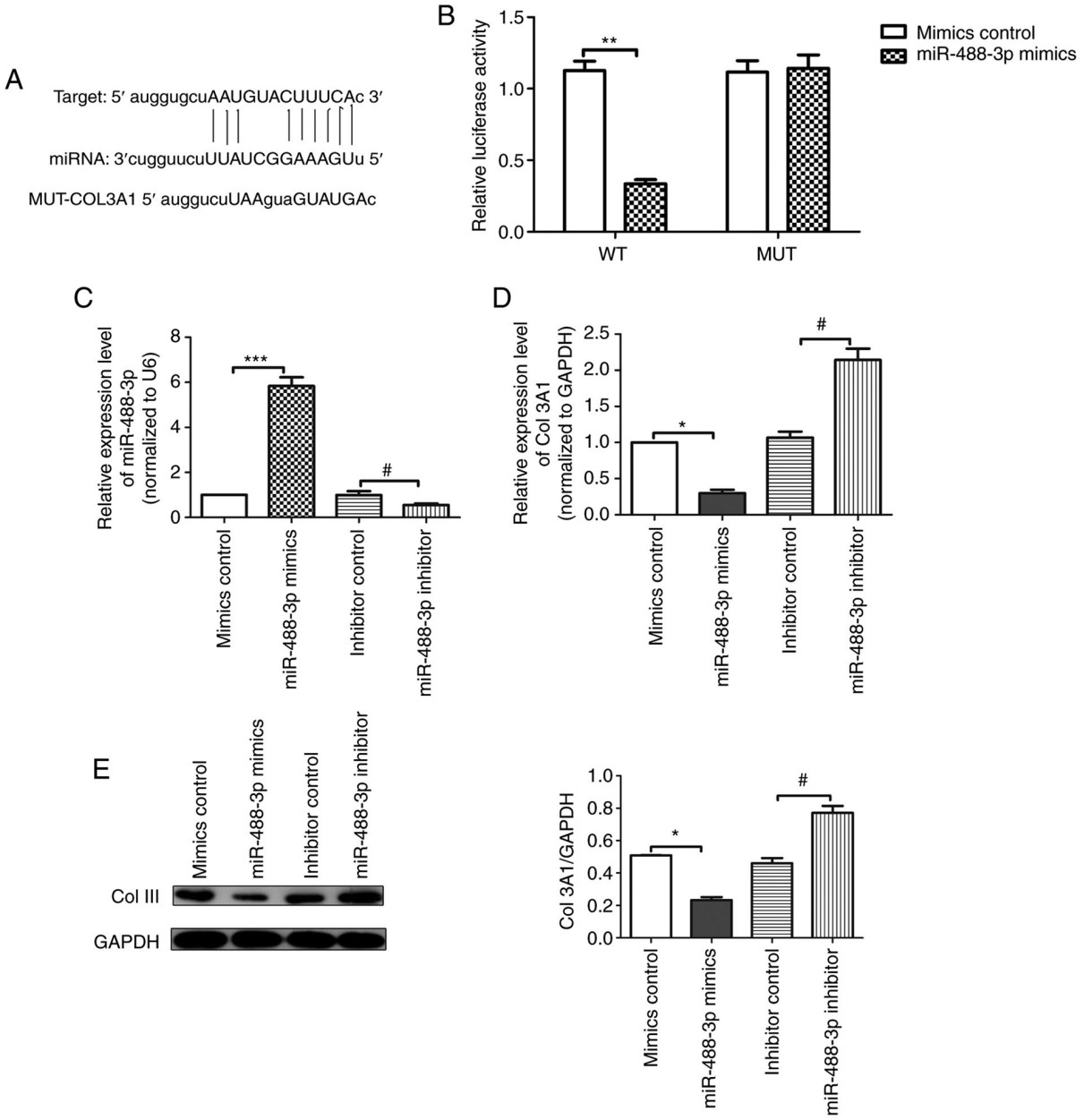

Figure 5. miR-488-3p directly targets COL3A1. (A) The miR-488-3p target sites on COL3A1 revealed by sequence prediction via Starbase. (B) The interaction between miR-488-3p and COL3A1 and luciferase activity in scar fibroblasts verified by the dual-luciferase assay. (C) The mRNA expression of miR-488-3p in fibroblasts detected by RT-qPCR analysis. (D) The mRNA expression of COL3A1 in fibroblasts detected by RT-qPCR analysis. (E) The protein expression of COL-III in scar fibroblasts detected by the western blotting assay. The data are presented as mean ± standard error of the mean, vs. mimics control and NC group, *P<0.05, **P<0.01 and ***P<0.001; $^{\#}$P<0.05. n=3. miR/miRNA, microRNA; COL, collagen; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; MUT, mutant; WT, wild type.

NEAT1 + pcDNA3.1 group, the protein expression level of COL-III in the Hy + lncRNA NEAT1 + pcDNA3.1-COL3A1 group was markedly increased. The proliferation of scar fibroblasts was detected by CCK-8 kit. The results (Fig. 6C) suggested that compared with the Hy + NC group, the viability of scar fibroblasts in the Hy + lncRNA NEAT1 inhibitor group was substantially inhibited; compared with the Hy + lncRNA NEAT1 + inhibitor control group, the viability of scar fibroblasts in the Hy + lncRNA NEAT1 + miR-488-3p inhibitor group was significantly enhanced; compared with the Hy + lncRNA NEAT1 + pcDNA3.1 group, the viability in the Hy + lncRNA NEAT1 + pcDNA3.1-COL3A1 group was increased with statistic difference (P<0.05). Meanwhile, the expression of Ki-67 protein in scar fibroblasts was detected through RT-qPCR analysis and the results (Fig. 6D) were consistent with the CCK-8 assay results. Given the above, lncRNA NEAT1 inhibited hypoxia-induced scar fibroblast proliferation through regulation of miR-488-3p/COL3A1 axis.

Discussion

Hypertrophic scar is a common proliferative disease associated with abnormal wound healing responses (17), so clarifying its pathological mechanism is conducive to determining appropriate treatment strategies. Abnormal proliferation and apoptosis of scar fibroblasts directly or indirectly affect

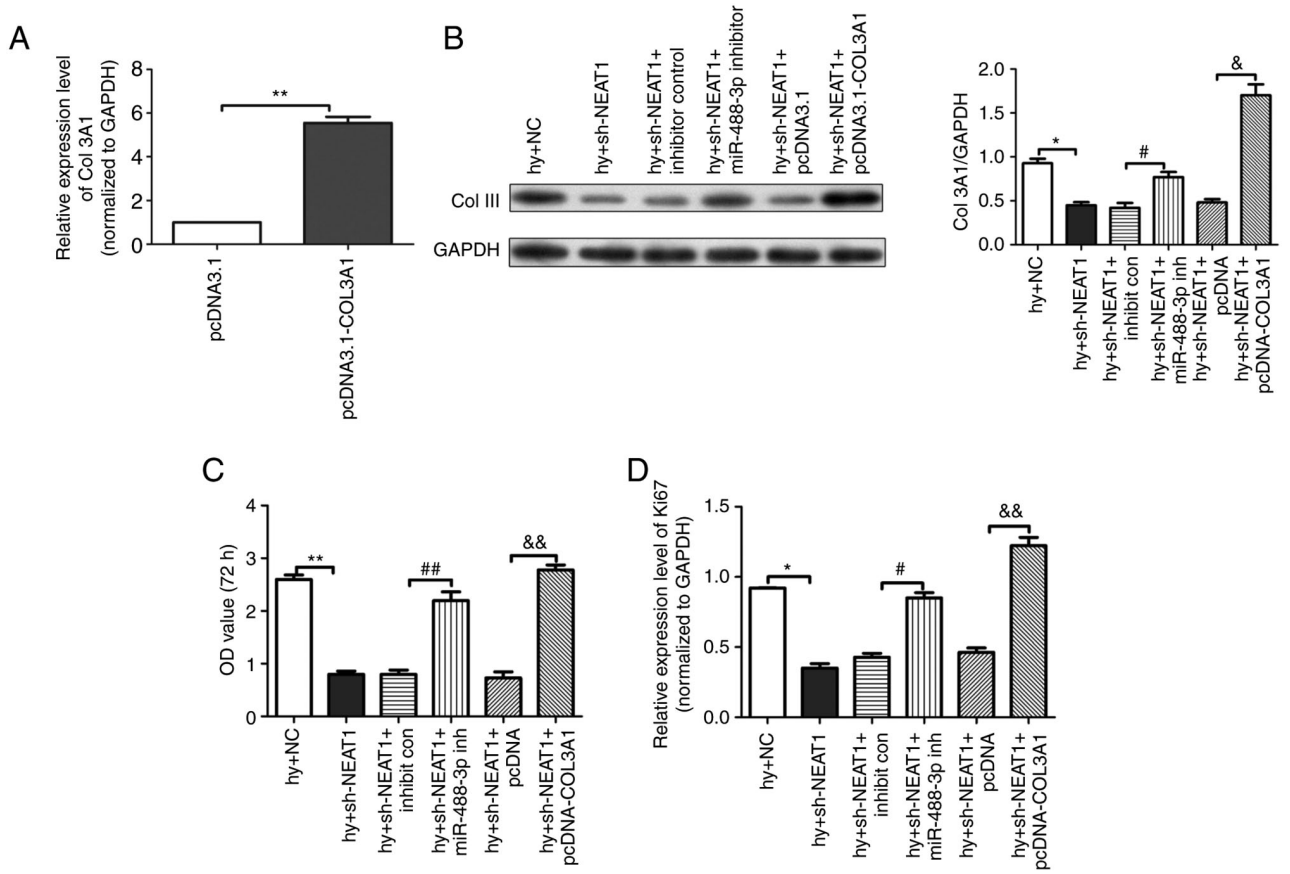

Figure 6. lncRNA NEAT1 inhibited hypoxia-induced scar fibroblasts proliferation through regulation of miR-488-3p/COL3A1 axis. (A) The mRNA expression level of COL3A1 in scar fibroblasts detected by RT-qPCR analysis. (B) The protein expression of COL-III in fibroblasts measured by the western blotting assay. (C) The ability of cell proliferation assessed by CCK-8 kit. (D) The mRNA expression of Ki-67 detected by RT-qPCR analysis. The data are presented as mean ± standard error of the mean, vs. pcDNA3.1 & Hy + NC group, *P<0.05, **P<0.01; vs. Hy + lncRNA NEAT1 + inhibitor control group, $^{\#}$P<0.05, $^{\#\#}$P<0.01; vs. Hy + lncRNA NEAT1 + pcDNA3.1 group, $^{\&}$P<0.05 and $^{\&\&}$P<0.01. n=3. LncRNA; long non-coding RNA; NEAT1, nuclear-enriched transcripts 1; miR/miRNA, microRNA; COL, collagen; RT-qPCR, reverse transcription-quantitative PCR; Hy, hypoxia; NC, negative control; NS, not significant; sh, short hairpin.

their collagen deposition and scar formation (18). With the development of biomedical science, increasing evidences have demonstrated that lncRNAs play a regulatory role in the pathogenesis of various diseases including cancer, myocardial infarction, pulmonary fibrosis and hypertrophic scars by regulating key proteins with competing endogenous (ce) RNAs (19-21). LncRNA NEAT1 regulates FRS2 by targeting miR-29-3p in hypertrophic scar fibroblasts, thereby exacerbating the pathological process of scar formation (22). The results of the present study indicated that lncRNA NEAT1 serves an important role in hypertrophic scars by mediating miR-488-3p/COL3A1 axis. Therefore, it was hypothesized that lncRNA NEAT1 acts as a mediator in the progression of hypertrophic scars.

Hypertrophic scar formation is a complicated pathological process characterized by inflammation, collagen deposition and fibroblast dysfunction. Activated fibroblasts are the main effector cells in this fibrosis process (23). The abnormal proliferation of scar fibroblasts and the inflammation-mediated fibrosis directly affect scar formation. Bai *et al* (24) found that loureirin B suppresses scar fibroblasts proliferation and fibrosis induced by TGF-β1 by downregulating the expression of fibrosis-related molecules by regulating MMPs. Liu *et al* (25) found that miR-6836-3p promotes the proliferation of scar fibroblasts by upregulating the expression of connective tissue growth factor, hence miR-6836-3p may be a potential target in the treatment of hypertrophic scars. The present study investigated the effects of lncRNA NEAT1 on the function of hypoxia-induced scar fibroblasts. The results demonstrated that there was a significant decrease in cell viability and the expression level of Ki-67 protein in the lncRNA NEAT1 group compared with the sh-NC group; silencing lncRNA NEAT1 inhibited the proliferation of fibroblasts and the pathological progression of hypertrophic scars. Nonetheless, the underlying molecular mechanism of the effects of lncRNA NEAT1 on proliferation in hypertrophic scar formation remains to be investigated in future research.

As the main participants of scar formation in wound healing, scar fibroblasts are involved in biological processes including collagen synthesis, extracellular matrix (ECM) formation and deposition (26) and skin fibrosis. TGF-β1 recruits macrophages to release inflammatory factors, promotes the chemotaxis of fibroblasts and smooth muscle and regulates the collagen gene expression in fibrosis (27). Collagens are known to regulate the migration, proliferation and gene expression of cells (28). In hypertrophic scars, fibroblasts synthesize excessive ECM proteins, among which the deposition of collagens, especially COL-I and COL-III, is significantly increased (29).

Consistent with previous studies, the results of the present study confirmed that collagen deposition is significantly increased in hypoxia-induced hypertrophic scars. The present study found that silencing lncRNA NEAT1 targeted miR-488-3p to downregulate the expression levels of COL-I, COL-III and α-SMA in hypoxia-induced fibroblasts under hypoxic pathological conditions. Zhang *et al* (30) concluded that Ft1 activates the PI3K/Akt/mTOR signaling pathway and promotes the expression levels of COL1A1 and COL3A1, thereby stimulating fibroblast proliferation and myofibroblast differentiation and accelerating wound healing. The present study found that silencing lncRNA NEAT1 mediated miR-488-3p/COL3A1 axis, downregulated collagen expression levels and attenuated the process of hypertrophic scarring.

In summary, the knockdown of lncRNA NEAT1 expression inhibited scar fibroblast proliferation through regulation of the miR-488-3p axis and regulated a series of collagens including COL3A1 to serve protective roles in hypertrophic scar formation. The results demonstrated that lncRNA NEAT1 may be a novel therapeutic target for the treatment of hypertrophic scars. Nonetheless, the limitation of this study lies in the fact that the underlying regulatory mechanism of lncRNA NEAT1 in hypertrophic scar formation remains unconfirmed *in vivo* experiments.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HX wrote the manuscript, designed experiments and analyzed the data. XG and YT participated in experiments and data analysis. JW participated in experiments and literature review. HX and XG confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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