Long noncoding RNA homeobox A11 antisense promotes transforming growth factor β1-induced fibrogenesis in cardiac fibroblasts

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Abstract. Cardiac fibrosis is closely associated with various heart diseases and is an important pathological feature of cardiac remodeling. However, detailed mechanisms underlying cardiac fibrosis remain largely unknown. Long noncoding RNAs (lncRNAs) are reported to serve significant roles in the development of cardiac fibrosis. The present study aimed to identify the role of a novel lncRNA, homeobox A11 antisense (HOXA11-AS), in cardiac fibrosis. Overexpression of HOXA11-AS in mouse cardiac fibroblasts (CFs) increased the expression of transforming growth factor β1 (TGFβ1) and its downstream molecules, while knockdown of HOXA11-AS inhibited the TGFβ1 signaling pathway. Furthermore, as determined by colony formation and MTT assays, HOXA11-AS overexpression promoted colony formation and viability in mouse CFs, while HOXA11-AS knockdown had the opposite effect. In addition, overexpression of HOXA11-AS increased cell migration and invasion in the Transwell assays, whereas expression knockdown decreased the metastatic ability of cells. In order to explore the detailed mechanism, co-transfection of HOXA11-AS expression plasmid and siTGFβ1 into CFs resulted in increased cell proliferative rate and cell metastasis through the TGFβ1 signaling pathway. Taken together, the present study suggested that the lncRNA HOXA11-AS may be a potential therapeutic target against cardiac fibrosis, and provided a novel insight into the diagnosis and treatment of clinical cardiac fibrosis.

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Introduction

Cardiac fibrosis is among the common pathological features of hypertrophic and dilated cardiomyopathy and may result in ventricular dysfunction, which leads to heart failure (1). Hyperactivity of fibroblasts may result in the accumulation of extracellular matrix proteins with adverse effects on cardiac structure and function, including electrical instability and increased risk of arrhythmogenic cardiac death (2). Upon activation, cardiac fibroblasts (CFs) are transformed into myofibroblasts and thus contribute to the fibrotic response by secreting key fibrogenic mediators. Among these, growth factors are the most widely studied mediators implicated in cardiac fibrosis, including transforming growth factor β (TGFβ) and platelet-derived growth factor (3). The main manifestations of cardiac fibrosis include excessive viability of CFs and the abundant accumulation of extracellular matrix (ECM). Cardiac fibrosis is also characterized by trans-differentiation of CFs into activated myofibroblasts and overexpression of α-smooth muscle actin (α-SMA), as well as ECM components (4). Despite the great efforts that have been made to slow down the progression of cardiac fibrosis during the past decades, it remains a threat to public health.

Long noncoding RNAs (lncRNAs) are a category of RNAs that lack the potential to code proteins and contain >200 nucleotides (5,6). To date, lncRNAs have been demonstrated to serve significant roles in intracellular and extracellular activities, such as transcriptional modification, gene splicing, gene arrangements and tumorigenesis (7-9). It has been reported that lncRNAs are also involved in the progression of cardiac fibrosis. The mechanisms by which lncRNAs regulate cardiac fibrosis remain to be elucidated, although it has been suggested that the underlying mechanisms may involve the regulation of pro-fibrogenic factors by lncRNAs, particularly the regulation of CFs by lncRNAs (10). The roles of lncRNAs in cardiac fibrosis have recently received increasing attention. For instance, Zhang *et al* (11) demonstrated that depletion of interleukin (IL)-17 alleviated cardiac fibrosis and ameliorated cardiac function through inhibiting lncRNA AK081284 in diabetic mice. Furthermore, PFL was identified as a pro-fibrotic lncRNA in respect to cardiac fibrosis in mice (12).

Homeobox A11 antisense (HOXA11-AS) is a recently identified lncRNA, and studies have mainly examined its role in tumorigenesis. For instance, in human non-small cell lung cancer (NSCLC), it was reported to promote cell viability and invasion through regulating the expression of microRNA-124 (13). Upregulation of HOXA11-AS was observed to increase cell viability via the HOXA11-AS-LATS1 axis in human hepatocellular carcinoma (14). However, to the best of our knowledge, the role of HOXA11-AS in cardiac fibrosis has not been identified to date.

The current study aimed to explore the effects of HOXA11-AS on cell viability and metastasis through upregulation and knockdown methods. Colony formation, cell viability and Transwell assays were used to investigate the underlying association of HOXA11-AS and TGFβ1. The results may help identify novel methods to diagnose and treat patients with cardiac fibrosis in clinical practice.

Materials and methods

Mice. The present study was approved by the Ethics Committee of the Department of Cardiology, Zhejiang Hospital (Hangzhou, China). A total of 40 neonate mice (C57BL6/J) were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China) and housed under standard conditions of 21±2˚C, 55% humidity and a 12-h light/dark cycle. The mice were sacrificed before 1 week of age. Briefly, neonate mice were anesthetized by placing them in a sealed isoflurane chamber, at an isoflurane concentration of 2% v/v and a flow rate of 0.6 l/min. After ~30 sec, mice ceased movement and were placed on wet ice to induce hypothermic anesthesia as previously reported (15). The ventricular apex was harvested and subject to isolation of primary cells via trypsinization and differential centrifugation steps, similar to those reported in a previous study (16). Following the isolation of cells, mice were sacrificed by cervical dislocation (16).

Cell culture and transfection. Mouse CFs were isolated with collagenase type I (40507ES60; Shanghai Shengsheng Biotechnology Co., Ltd., Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) at 37˚C and an atmosphere that contained 5% CO_2, according to a previous study (17). Cell transfections were performed with Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. At 6 h after transfection, the culture medium was replaced with fresh medium. For upregulation, the HOXA11-AS-expressing plasmid was cloned into a pcDNA3.1 vector with *Hind*III and *Xho*I restricted enzyme sites. For knockdown, specific small interfering RNAs (siRNAs) against HOXA11-AS (siHOXA11-AS) and TGFβ1 (siTGFβ1) were used, which were designed and synthesized by GenePharma Co., Ltd. (Shanghai, China). The siHOXA11-AS targeting sequence was 5'-CGG AAUAUCGGAAUAAAGUUU-3', and the siTGFβ1 targeting sequence was 5'-CCAACUAUUGCUUCAGCUC-3'. The siRNAs were dissolved into a concentration of 20 μM using ddH_2O and diluted into 20 nM when used in the cellular experiments. Transfection was performed 48 h prior to the assessments, unless otherwise stated. TGFβ1 recombinant protein (cat. no. 10804-H08H) was purchased from Sino Biological Inc., Wuhan, China.

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA from cultured CFs was isolated by TRIzol reagent (Thermo Fisher Scientific, Inc.) at 1 ml/well in 6-well plates. RNA concentration was quantified with Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.), and a total of 1 μg RNA was reversely transcribed into cDNA with PrimeScript™ RT reagent kit (Takara Biotechnology Co., Ltd., Dalian, China) using the following protocol: 37˚C for 15 min and 85˚C for 5 sec. Next, qPCR assays were performed with SYBRGreen reagent (Takara Biotechnology Co., Ltd.) in a ABI 7900 machine (Thermo Fisher Scientific, Inc.) according to the following procedure: 95˚C for 2 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 30 sec. The primers used in qPCR were as follows: TGFβ1 forward, 5'-CTCCCGTGGCTT CTAGTGC-3', and reverse, 5'-GCCTTAGTTTGGACAGGA TCTG-3'; α-SMA forward, 5'-GTCCCAGACATCAGGGAG TAA-3', and reverse, 5'-TCGGATACTTCAGCGTCAGGA-3'; type I collagen (COL1) forward, 5'-GCTCCTCTTAGGGGC CACT-3', and reverse, 5'-CCACGTCTCACCATTGGGG-3'; fibronectin (FN) forward, 5'-ATGTGGACCCCTCCTGAT AGT-3', and reverse, 5'-GCCCAGTGATTTCAGCAAAGG-3'; GAPDH forward, 5'-AGGTCGGTGTGAACGGATTTG-3', and reverse, 5'-TGTAGACCATGTAGTTGAGGTCA-3'. The results were quantified with the $2^{-\Delta\Delta Cq}$ method (18). Each experiment was repeated three times in triplicate.

Luciferase reporter assay. Luciferase reporter assays were performed with Dual-luciferase Reporter Assay kit (E1910; Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. Briefly, CFs transfected with TGFβ1 luciferase plasmid and *Renilla* plasmid (control plasmid) were treated with siHOXA11-AS or HOXA11-AS-expressing plasmid for 48 h, and then lysed for 20 min at room temperature using a shaker at 150 rpm. Subsequently, the lysate was incubated with luciferase assay reagent II, and the absorbance of all wavelengths was collected immediately. The Stop & Glo reagent (Thermo Fisher Scientific, Inc.) was then added, and the absorbance of the plate re-read; the first reading was the TGFβ1 luciferase and the second one was the *Renilla* luciferase (the control luciferase). The luciferase activity was calculated based on the ratio of two readings.

Colony formation assay. A total of 100 CFs were seeded into 12-well plates, and transfected with siHOXA11-AS or with HOXA11-AS-expressing plasmid in the presence or absence of TGFβ1 recombinant protein (10 ng/ml) or siTGFβ1 (20 ng/ml) in triplicate. Following incubation for 14 days, the colonies were fixed with ice-cold methanol for 10 min at room temperature and stained with crystal violet for 15 min (1%). Subsequently, the number of colonies was assessed under a light microscope at a magnification of x200 (Nikon Corporation, Tokyo, Japan). Only colonies containing >50 cells were considered.

Cell viability assay. Cell viability was assessed by MTT assays. Briefly, a total of $1x10^3$ CFs/well were seeded in 96-well plates and treated with siRNA against HOXA11-AS (siHOXA11-AS)

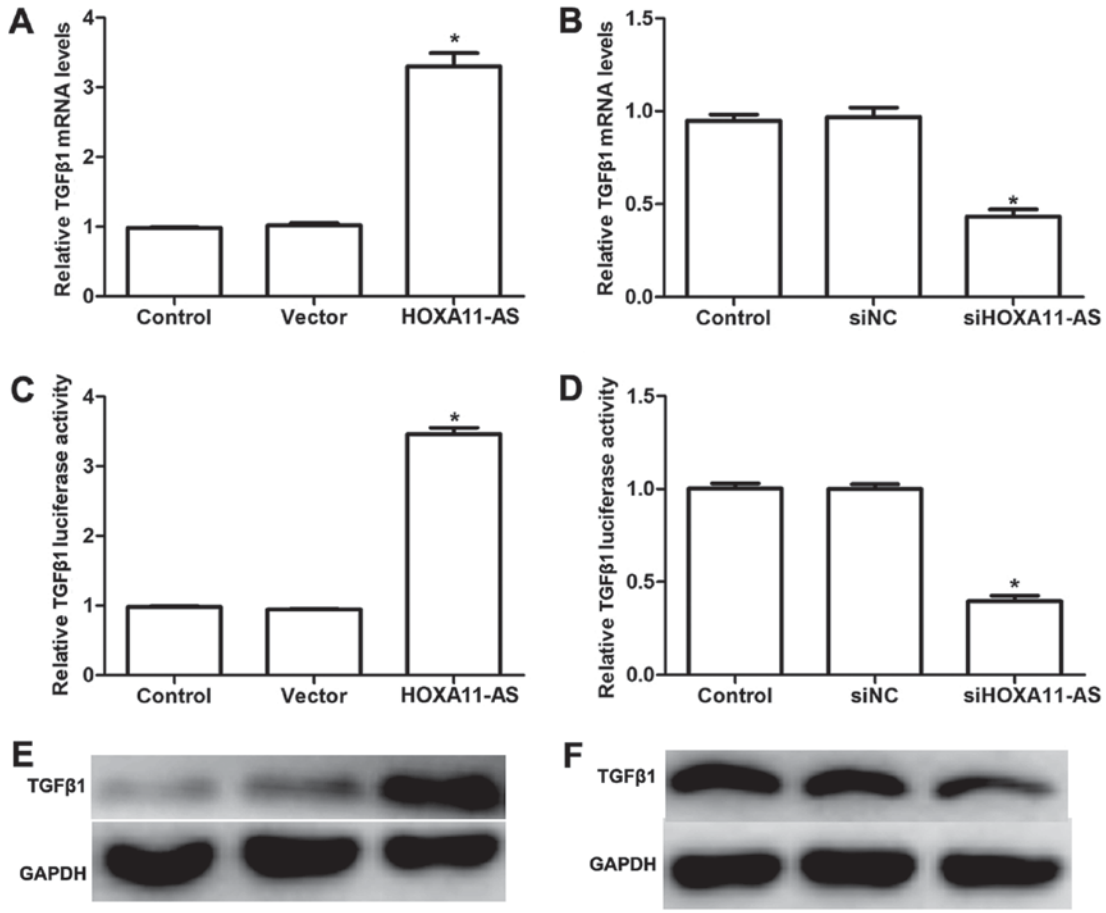

Figure 1. Long noncoding RNA HOXA11-AS positively regulated the expression of TGFβ1 in mouse cardiac fibroblasts. mRNA levels of TGFβ1 were detected by reverse transcription-quantitative polymerase chain reaction in CFs transfected with (A) HOXA11-AS-expressing plasmid and (B) siHOXA11-AS. TGFβ1 luciferase activity of CFs transfected with (C) HOXA11-AS-expressing plasmid and (D) siHOXA11-AS. TGFβ1 protein levels detected by western blot analysis in CFs where HOXA11-AS was (E) overexpressed or (F) downregulated. GAPDH was used as a control. *P<0.05 vs. control group. CFs, cardiac fibroblasts; HOXA11-AS, homeobox A11 antisense; TGFβ1, transforming growth factor β1; si-, small interfering RNA; NC, negative control.

or negative control siRNA (siNC) for 48 h. Next, 10 μl MTT (5 μg/ml) was added into each well, and cells were cultured for an additional 3 h at 37˚C in darkness. Next, the formazan crystals were dissolved in 100 μl dimethyl sulfoxide, and the absorbance of the 96-well plate was read at 570 nM with a plate reader (Thermo Fisher Scientific, Inc.).

Transwell assay. A total of 5,000 CFs/well were seeded into 12-well plates and transfected with siHOXA11-AS or HOXA11-AS-expressing plasmid for 48 h. Next, cells were collected and diluted into a concentration of $5x10^5$ cells/ml with culture medium without FBS. For the migration assay, a 150 μl of cells was added into the upper Transwell chambers (Corning, Inc., Corning, NY, USA) and 600 μl medium with 10% FBS was added into the lower chamber. After 8 h of incubation in a 37˚C incubator, the chambers were washed three times with warm PBS, fixed with methanol at room temperature for 5 min and stained with crystal violet (1%) for 5 min. For the invasion assay, the upper chambers were first coated with Matrigel (Corning, Inc.) for 6 h at 37˚C. Following staining, images of the cells on the lower surface of the chamber were obtained with a Nikon light microscope, and the number of cells in six random fields-of-view was counted. All experiments were repeated in triplicate.

Western blot analysis. Western blot analysis was performed in CFs transfected with siHOXA11-AS or HOXA11-AS-expressing plasmid for 48 h. Briefly, total proteins from cultured cells were extracted by radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Nanjing, China). Protein concentration was monitored with bicinchoninic acid methods (Thermo Fisher Scientific, Inc.). Next, a total of 30 μg protein was loaded onto a 12% SDS-PAGE gel and transferred to a polyvinylidene difluoride membrane. Subsequent to blocking with 5% milk at room temperature for 1 h, the membranes were incubated with primary antibodies against TGFβ1 (ab64715; Abcam, Cambridge, MA, USA), α-SMA (ab5694; Abcam), COL1 (ab6308; Abcam) and FN (ab18265; Abcam), as well as GAPDH (sc-47724; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at 4˚C overnight. The dilution was 1:1,000 for all primary antibodies. Samples were then incubated with horseradish peroxidase-conjugated IgG secondary antibodies, which were purchased from Santa Cruz Biotechnology, Inc. for 1 h at room temperature (sc-2004 and sc-2005; dilution, 1:2,000). Immunoreactivities were determined by enhanced chemiluminescent autoradiography (Thermo Fisher Scientific, Inc.) with the ImageQuant LAS 4000 device (GE Healthcare Bio-Sciences, Uppsala, Sweden).

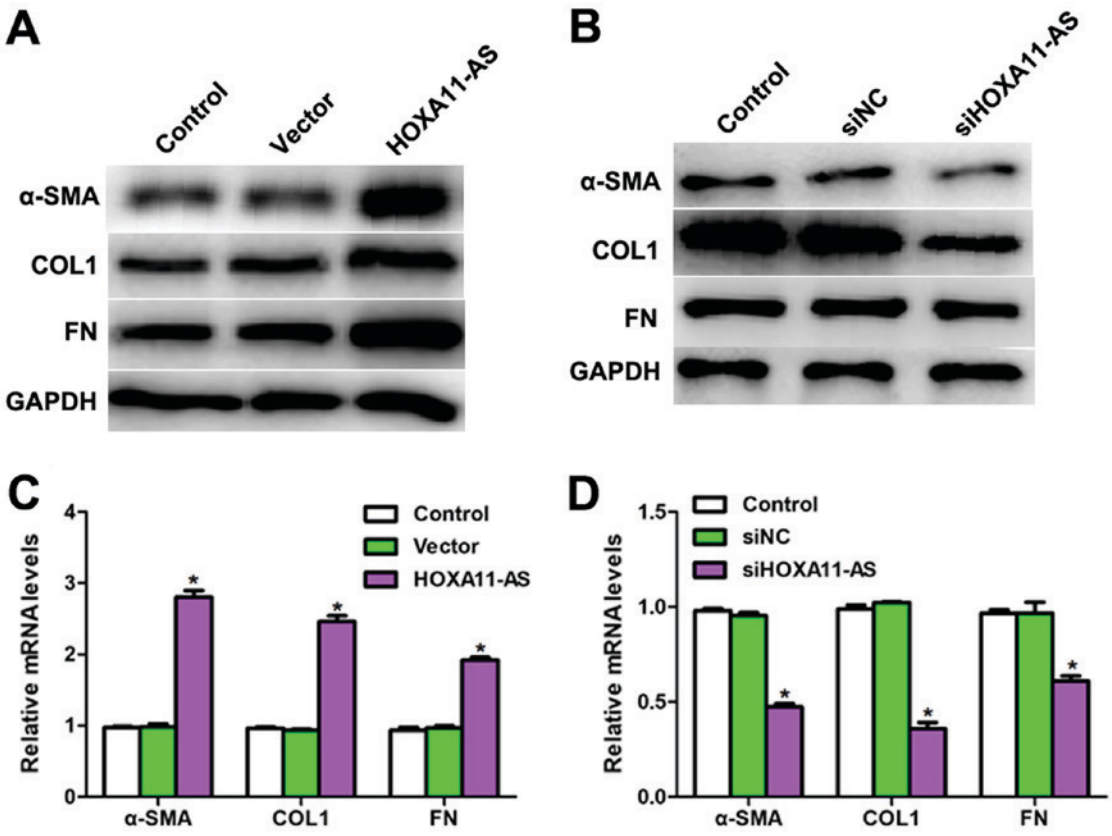

Figure 2. Long noncoding RNA HOXA11-AS regulated the levels of proteins and mRNAs associated with the TGFβ1 signaling pathway. Western blot analysis examined the protein levels of α-SMA, COL1, FN and GAPDH in CFs exhibiting (A) HOXA11-AS overexpression by plasmid transfection, and (B) HOXA11-AS knockdown by siRNA transfection. mRNA levels of α-SMA, COL1 and FN were detected in CFs transfected with (C) HOXA11-AS-expressing plasmid or (D) siRNA. *P<0.05 vs. control group. CFs, cardiac fibroblasts; HOXA11-AS, homeobox A11 antisense; TGFβ1, transforming growth factor β1; siRNA, small interfering RNA; α-SMA, α-smooth muscle actin; COL1, type I collagen; FN, fibronectin; NC, negative control.

Statistical analysis. All data are presented as the mean ± standard deviation. A two-tailed Student's t-test was used to compare the means of two groups, whereas one-way analysis of variance was used for comparisons among multiple groups, followed by the least-significant-difference post hoc test. P<0.05 was considered to denote a statistically significant difference.

Results

LncRNA HOXA11-AS positively regulates the expression of TGFβ1 in mouse CFs. Firstly, the present study examined the effects of HOXA11-AS on the expression of TGFβ1. As shown in Fig. 1A, when CFs were transfected with HOXA11-AS-expressing plasmid, the mRNA level of TGFβ1 was significantly increased, while TGFβ1 mRNA was significantly decreased in cells transfected with siHOXA11-AS, as compared with the levels of the control groups (Fig. 1B). Dual-luciferase reporter assays also demonstrated that overexpression of HOXA11-AS in CFs markedly upregulated the luciferase activity of TGFβ1, whereas depletion of HOXA11-AS with specific siRNA knocked down the luciferase signal of TGFβ1 (Fig. 1C and D). Subsequently, western blot analysis was also performed to determine the regulatory effect of HOXA11-AS on the pro-inflammatory cytokine TGFβ1. As shown in Fig. 1E and F, transfection with HOXA11-AS-expressing plasmid elevated the protein levels of TGFβ1, while knockdown of HOXA11-AS in CFs suppressed this protein. All of these results suggested that the lncRNA HOXA11-AS positively regulated the expression of the key profibrotic factor TGFβ1.

LncRNA HOXA11-AS regulates the protein and mRNA levels of TGFβ1 signaling pathway. Based on the results displayed in Fig. 1, the role of HOXA11-AS in the TGFβ1 downstream signaling pathway was further examined. As shown in Fig. 2A, the protein levels of α-SMA, COL1 and FN were all upregulated by transfection of HOXA11-AS-expressing plasmid in CFs, while the inner control GAPDH level remained stable. Similarly, the expression levels of these downstream genes of TGFβ1 were inhibited when CFs were treated with siHOXA11-AS (Fig. 2B). The mRNA levels were also detected with RT-qPCR analysis, and it was revealed that the mRNA levels of α-SMA, COL1 and FN were markedly increased when HOXA11-AS was overexpressed, while these levels significantly decreased when HOXA11-AS was downregulated in mouse CFs (Fig. 2C and D). These results, along with the findings displayed in Fig. 1, suggested that HOXA11-AS regulated the TGFβ1 signaling pathway in mouse CFs.

Transcript levels of HOXA11-AS are associated with the viability of mouse CFs. Next, the role of HOXA11-AS on the viability of mouse CFs was examined by colony formation and MTT assays. It was observed that overexpression of HOXA11-AS in CFs increased the colony number by >2-fold

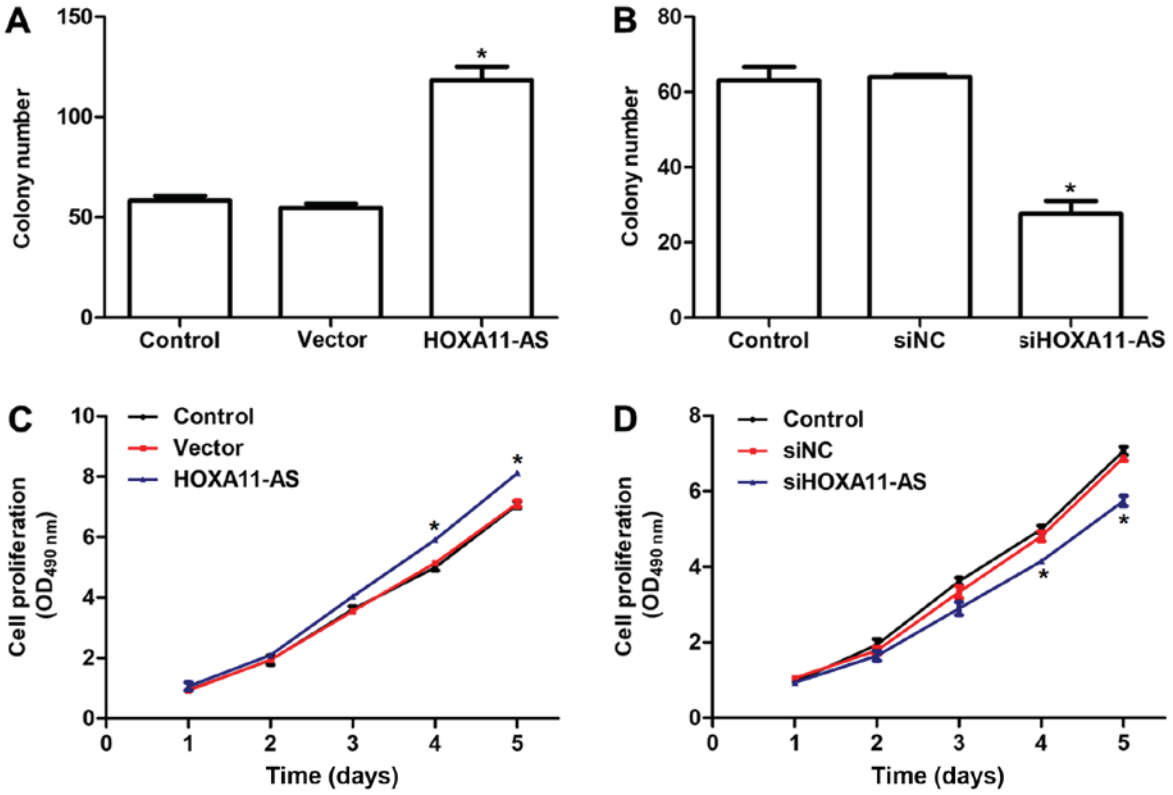

Figure 3. Transcript levels of HOXA11-AS were associated with cell viability in mouse CFs. Colony formation assays were performed in mouse CFs transfected with (A) HOXA11-AS-expressing plasmid and (B) siHOXA11-AS. MTT assays were conducted to examine the proliferation of mouse CFs in 5 consecutive days following treatment with (C) HOXA11-AS-expressing plasmid and (D) siHOXA11-AS. *P<0.05 vs. control group. CFs, cardiac fibroblasts; HOXA11-AS, homeobox A11 antisense; TGFβ1, transforming growth factor β1; siRNA, small interfering RNA; NC, negative control.

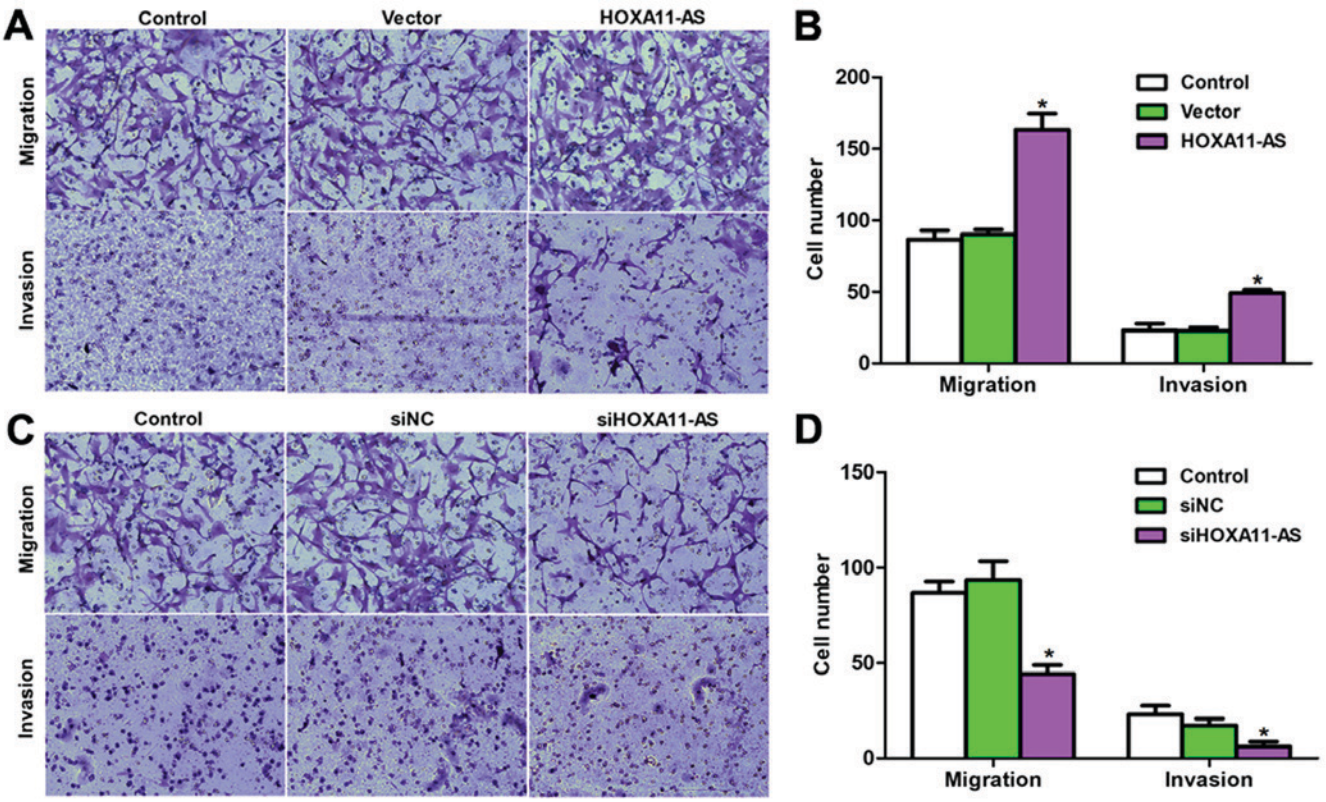

Figure 4. HOXA11-AS expression was positively correlated with the migration and invasion of mouse cardiac fibroblasts. (A) Representative images, and (B) quantification of the migration and invasion abilities of cells transfected with HOXA11-AS-expressing plasmid in five random fields. (C) Representative images, and (D) quantification of the migration and invasion abilities of cells transfected with siHOXA11-AS in five random fields. *P<0.05 vs. control group. HOXA11-AS, homeobox A11 antisense; TGFβ1, transforming growth factor β1; siRNA, small interfering RNA; NC, negative control.

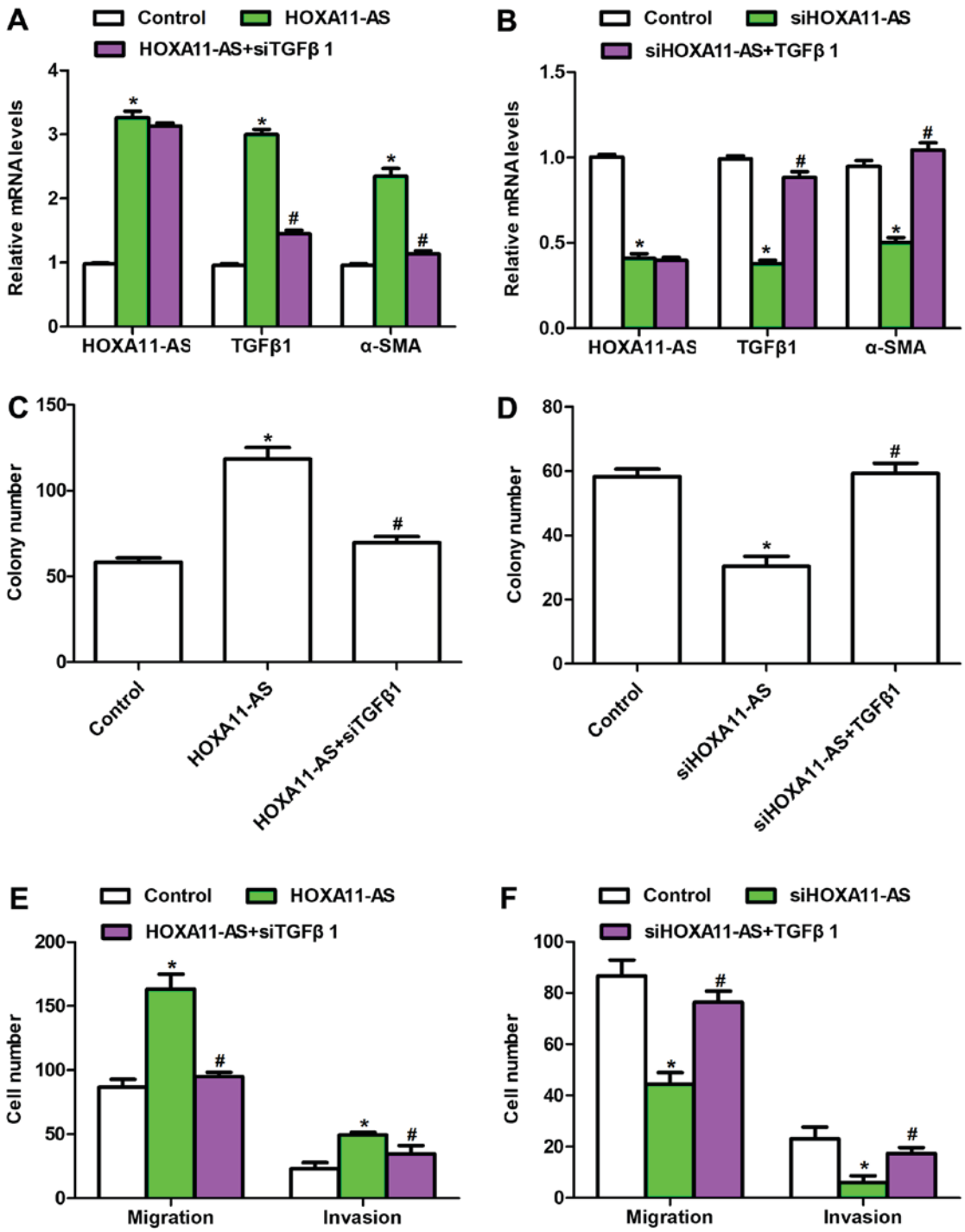

Figure 5. HOXA11-AS regulated the viability and metastasis of mouse CFs through the TGFβ1 signaling pathway. Relative mRNA levels of HOXA11-AS, TGFβ1 and α-SMA were examined by RT-qPCR in cells with (A) HOXA11-AS overexpression in the presence or absence of TGFβ1 knockdown, or (B) HOXA11-AS knockdown with or without TGFβ1 recombinant protein treatment. Colony formation assays were performed in cells with (C) HOXA11-AS overexpression with or without siTGFβ1, or (D) HOXA11-AS knockdown with or without TGFβ1 recombinant protein treatment. Transwell assays were also performed in CFs with (E) HOXA11-AS overexpression in the presence or absence siTGFβ1 transfection, or (F) HOXA11-AS depletion in the presence or absence of TGFβ1 recombinant protein treatment. *P<0.05 vs. control group; #P<0.05 vs. HOXA11-AS or siHOXA11-AS group. CFs, cardiac fibroblasts; HOXA11-AS, homeobox A11 antisense; TGFβ1, transforming growth factor β1; α-SMA, α-smooth muscle actin; siRNA, small interfering RNA; NC, negative control; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

(Fig. 3A), whilst knockdown of HOXA11-AS with specific siRNA suppressed the ability of cells to form colonies, with only ~30 colonies observed in the siHOXA11-AS-treated group (Fig. 3B). Furthermore, there were no notable differences between three groups in the first 3 days of the cell viability assays (Fig. 3C and D). However, overexpression of HOXA11-AS promoted the proliferative rate by 21% on day 4 and 27% on day 5 in mouse CFs (Fig. 3C). Similarly, knockdown of HOXA11-AS in CFs significantly inhibited the cell proliferative rate on days 4 and 5 (Fig. 3D). The aforementioned observations indicated that the transcript levels of HOXA11-AS positively regulated the viability of mouse CFs.

HOXA11-AS expression is positively correlated with metastasis in mouse CFs. Cell viability and metastasis are two main manifestations of cardiac injuries. Therefore, the study next detected the effects of HOXA11-AS on cell metastasis with a Transwell assay. As shown in Fig. 4A and B, approximately 85 and 15 migrating and invading cells, respectively, were observed on the lower surface of the chamber in the control groups. However, more than 160 and 50 cells that migrated and invaded, respectively, through the membrane of the 8-μm pores were counted following HOXA11-AS overexpression in CFs. Similarly, transfection of mouse CFs with specific siRNA against HOXA11-AS inhibited the cell migration and invasion by approximate 50%, as compared with the control cells (Fig. 4C and D). These data suggested that the lncRNA HOXA11-AS positively regulated the migration and invasion of mouse CFs.

HOXA11-AS regulates cell viability and metastasis through the TGFβ1 signaling pathway in mouse CFs. To explore the detailed mechanisms underlying the effect of HOXA11-AS in mouse CFs, further investigations were conducted with TGFβ1 recombinant protein treatment or transfection with siTGFβ1. As shown in Fig. 5A, the mRNA levels of HOXA11-AS, TGFβ1 and α-SMA were upregulated when CFs were transfected with HOXA11-AS-expressing plasmid; however, when cells were co-transfected with siTGFβ1 and HOXA11-AS-expressing plasmid, the HOXA11-AS level remained stable, while TGFβ1 and α-SMA mRNA levels were significantly downregulated. The opposite results were observed when HOXA11-AS was depleted in the presence or absence of TGFβ1 recombinant protein treatment (Fig. 5B). In addition, the colony formation assay indicated that overexpression of HOXA11-AS promoted the number of colonies by up to 2-fold compared with the control group, whereas co-treatment with siTGFβ1 reversed the effects of HOXA11-AS on colony formation (Fig. 5C). It was also demonstrated that knockdown of HOXA11-AS in mouse CFs inhibited colony formation, which was consistent with the earlier observations (Fig. 3); however, the colony number was markedly increased upon co-stimulation with TGFβ1 recombinant protein (Fig. 5D). Furthermore, the regulatory effects of HOXA11-AS on cell migration and invasion were examined by co-treatment with siTGFβ1 or TGFβ1 recombinant protein (Fig. 5E and F); the results demonstrated that HOXA11-AS promoted cell metastasis and that the effects were reversed by TGFβ1 knockdown. Taken together, these data indicated that HOXA11-AS regulated cell functions through the TGFβ1 signaling pathway in mouse CFs.

Discussion

CFs are considered to be a uniform cell type, widely distributed in connective tissues, and can be referred to as mesenchymal original cells that secret multiple ECM components, such as collagens and FN (19). CFs are the most common cell type in the heart and serve a key role in the development of cardiac fibrosis (20). The present study explored the role of HOXA11-AS in cardiac fibrosis using CFs, and examined the proliferative rate and metastasis of these cells. The results indicated that HOXA11-AS may serve as a potential therapeutic target of cardiac fibrosis.

The TGFβ1 signaling pathway is a classic and powerful fibrogenic pathway that increases the accumulation of ECM components, leading to cardiac fibrosis (21,22). It has also been reported to be involved in various cellular processes, including cell growth, apoptosis, differentiation and homeostasis (21,23). TGFβ superfamily ligands bind to the type II receptor and recruit the phosphorylated type I receptor, which then phosphorylates receptor-regulated SMADs, finally forming a complex that accumulates in the nucleus. These ligands then act as transcription factors and participate in the regulation of the expression of target genes, including α-SMA, collagen I and FN (24). In the present study, it was first observed that overexpression of HOXA11-AS upregulated the mRNA and protein levels of TGFβ1, whereas knockdown of HOXA11-AS decreased these levels. The luciferase activity of TGFβ1, which reflects its transcriptional activity, was also detected. The luciferase reporter assay results further confirmed that HOXA11-AS positively regulated the transcription of TGFβ1. Subsequently, the protein and mRNA expression levels of α-SMA, collagen I and FN were observed to be increased when CFs were transfected with HOXA11-AS-expressing plasmid. Thus, the data suggested that HOXA11-AS directly upregulated the TGFβ1 signaling pathway.

Cell viability and metastasis are two manifestations of malignancy (25-27), as well as cardiac fibrosis. Therefore, cell viability and metastasis were examined in cells transfected with HOXA11-AS-expressing plasmid or siRNA targeting HOXA11-AS. It was demonstrated that HOXA11-AS promoted the cell proliferative rate, and increased the potential of cells to migrate and invade through the Transwell membrane. Taken together with the former findings that HOXA11-AS upregulated TGFβ1 signaling, it can be hypothesized that HOXA11-AS served its role through regulating the TGFβ1 pathway. To this end, TGFβ1 siRNA or recombinant protein co-treatment with HOXA11-AS-expressing plasmid or siHOXA11-AS, respectively, in CFs was conducted, and the results revealed that HOXA11-AS promoted cell viability and metastasis, which was reversed by knockdown of TGFβ1. These observations directly suggested that HOXA11-AS promoted cardiac fibrosis progression through upregulating the TGFβ1 signaling pathway. As for the detailed mechanism of the regulatory effects of HOXA11-AS on TGFβ1, further investigation is required in future studies.

In conclusion, the current study demonstrated that HOXA11-AS promoted cell proliferation and metastasis by increasing the activity of TGFβ1 in CFs, which further identified the specific role of HOXA11-AS in cardiac fibrosis. The present study attempted to explain the detailed mechanism, which may provide novel evidence for the clinical diagnosis and treatment of cardiac fibrosis in the near future.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JW and XL performed the experiments with assistance from QZ, RP, LZ and ZC. LT provided the funding and designed the project.

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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