LncRNA SNHG14 accelerates breast cancer progression through sponging miR-543 and regulating KLF7 expression

Daoping Zhang[1] · **Xu Ding**[1] · **Meirong Peng**[1]

Abstract

Purpose Dysregulation of long non-coding RNAs (lncRNAs) is being found to have relevance to human cancers, including breast cancer (BC). The aim of this study was to further explore the functional role and molecular mechanisms of small nucleolar RNA host gene 14 (SNHG14) on BC progression.
Methods The expression levels of SNHG14, miR-543, and krüppel-like factor 7 (KLF7) mRNA were determined by quantitative real-time PCR. Western blot analysis was used to evaluate KLF7 protein level. Cell proliferation, apoptosis, and migration and invasion abilities were detected by Cell Counting kit-8 assay, flow cytometry, and transwell assay, respectively. The direct interactions between miR-543 and SNHG14 or KLF7 were confirmed using dual-luciferase reporter assays.
Results Our data indicated that SNHG14 expression was increased in BC tissues and cells, and SNHG14 knockdown mitigated the proliferation, migration, and invasion and facilitated apoptosis of BC cells. SNHG14 directly interacted with miR-543. MiR-543 mediated the regulatory effects of SNHG14 silencing on BC cell behaviors. Moreover, KLF7 was a direct target of miR-543. Overexpressed miR-543-mediated anti-proliferation, anti-migration, anti-invasion, and pro-apoptosis effects were mediated by KLF7. Furthermore, SNHG14 modulated KFL7 expression through acting as a competing endogenous RNA (ceRNA) of miR-543 in BC cells.
Conclusion Our study suggested that SNHG14 knockdown hindered BC progression in vitro at least partly through acting as a ceRNA of miR-543 and modulating KLF7 expression, providing evidence for SNHG14 as a potential target for BC therapy.

Keywords Breast cancer · SNHG14 · miR-543 · Krüppel-like factor 7 (KLF7) · ceRNA

Introduction

Breast cancer (BC) remains the most common malignancy in women, with an estimated 2.1 million new BC cases in 2018 around the world [1]. Due to the westernized lifestyle, especially increases in obesity and physical inactivity, the incidence and mortality of BC are on the rise in China. Although the developments of advanced treatment and earlier detection have improved the overall survival of BC patients, BC is still the leading cause of cancer-related deaths among women in China [2, 3]. Hence, it is very urgent to identify more effective molecular targets for BC treatment.

Long non-coding RNAs (lncRNAs) are a very heterogeneous type of RNA molecules that surpass 200 nucleotides in length, are involved in a wide range of critical biological processes [4]. Increasing lines of evidence have recently shown that deregulated lncRNAs play crucial roles in human cancer, including BC [5, 6]. Small nucleolar RNA host gene 14 (SNHG14) has been established as a potential oncogenic lncRNA in many human cancers, including colorectal cancer, ovarian cancer and cervical cancer [7–9]. SNHG14 was also found to be upregulated in BC tissues, and overexpressed SNHG14 facilitated BC cell growth and invasion [10]. Moreover, a previous document reported that SNHG14 enhanced BC cell chemoresistance to trastuzumab [11]. Therefore, in this study, we aimed to further explore the functional role and underlying mechanisms of SNHG14 on BC progression.

MicroRNAs (miRNAs) are 18–22 nucleotides long, endogenous small non-coding RNA molecules that direct posttranscriptional suppression of target mRNAs [12]. They

✉ Meirong Peng
pengmr86@163.com

1 Department of Laboratory Medicine, Jingmen No. 1 People's Hospital, No.6 Zhongtian Street, Dongbao District, Jingmen 448000, Hubei, China

are widely acknowledged to be associated with a wide range of human disease, including cancer [13]. MiR-543 has been demonstrated to serve as a potential oncomiR or tumor-suppressor in numerous human cancers [14–16]. A previous report uncovered that highly expressed miR-543 hindered BC cell growth, cell cycle progression, and accelerated the apoptosis, illuminating its repressive effect on BC progression in vitro [17]. Competing endogenous RNA (ceRNA) hypothesis proposes that lncRNAs sequester specific miRNAs by acting as molecular sponges of miRNAs to modulate gene expression [18]. SNHG14 had been demonstrated to act as a ceRNA of miR-193-3p and thus enhanced BC progression in vitro [10]. However, the effect of interplay between SNHG14 and miR-543 remains uncovered.

In this study, our data validated that SNHG14 knockdown mitigated the proliferation, migration and invasion, and facilitated the apoptosis of BC cells. Whereafter, we further explored the underlying molecular mechanisms of SNHG14 on BC cell progression in vitro.

Materials and methods

Clinical tissues and cell culture

The study included 40 tissue samples (20 from the malignant site and 20 from adjacent normal breast tissue) from 20 cases BC patients who were diagnosed by Jingmen No. 1 People's Hospital between October 2016 and September 2017. These patients did not receive preoperative chemotherapy or radiotherapy. All fresh samples were immediately stored at − 80 °C until use for RNA isolation. Our study was approved by the Human Research Ethics Committee of Jingmen No. 1 People's Hospital, and written informed consent was signed by each participator before surgery.

MCF-10A human normal mammary epithelial cell line (ATCC®CRL-10317) and two BC cell lines (T-47D, ATCC®HTB-133 and SKBR3, ATCC®HTB-133) were purchased from ATCC (Manassas, VA, USA). BC cells were cultured in DMEM medium (Gibco, Life Technologies GmbH, Darmstadt, Germany), MCF-10A cells were maintained in mammary epithelial basal medium (MEBM, Lonza GmbH, Koln, Germany), containing 10% fetal calf serum (FCS, Bio-One, Frickenhausen, Germany), 1% antibiotics (penicillin/streptomycin, Invitrogen, Blijswijk, the Netherlands) at 37 °C with 5% CO_2 and 95% humidity.

Oligonucleotide and plasmid transfection

For the knockdown of SNHG14, cells were introduced with siRNA specifically against SNHG14 (si-SNHG14) or a scrambled oligonucleotide sequence (si-NC) as a negative control. For miR-543 overexpression or silencing, cells were transfected with synthetic miR-543 mimic, the inhibitor of miR-543 (anti-miR-543) or corresponding negative control (miR-NC mimic or anti-miR-NC). KLF7 upregulation in cells was performed using KLF7 overexpression vector (Vector-KLF7), and nontarget vector (Vector-NC) was used as a negative control. All oligonucleotides and plasmids were obtained from Ribobio (Guangzhou, China) and transfected into BC cells using the X-tremeGENE HP transfection reagent (Roche, Mannheim, Germany) following the manufacturer's guidance.

Quantitative real-time PCR

The RiboPure™ RNA Purification kit (Invitrogen) was applied to extract the total RNA from BC tissues and cells, in accordance with the protocols of manufacturers. To quantify the expression of SNHG14 and krüppel-like factor 7 (KLF7) mRNA, cDNA was obtained from total RNA (50 ng) using a High-Capacity RNA-to-cDNA™ kit (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands), and then subjected to quantitative real-time PCR (qRT-PCR) using iQ™ SYBR Green PCR Supermix (Bio-Rad Laboratories, Marnes La Coquette, France) referring to producer's guidance. Level of miR-543 was detected using TaqMan Reverse Transcription kit (Applied Biosystems) and TaqMan MicroRNA assay kit (Applied Biosystems) following the manufacturers' instructions. The Bio-Rad CFX96 Real-Time PCR Detection system (Bio-Rad Laboratories) was used for qRT-PCR, and β-actin and U6 snRNA were used as endogenous controls for normalization. Relative expression levels of SNHG14, KLF7 and miR-543 were determined by the $2^{-\Delta\Delta Ct}$ method. Primers for PCR amplification were listed as follows: SNHG14: 5′-TTTGCTGGTATGGATGGCCC-3′ (sense), and 5′-TCCACACTGACGACACATCA-3′ (antisense); miR-543: 5′-GTATGAAAACATTCGCGGTGC-3′ (sense), and 5′-TATGGTTGTTCACGACTCCTTCAC-3′ (antisense); KLF7: 5′- CGTTGAAACTGGTGGCCAAG-3′ (sense), and 5′-CCTGTGTGAGTCCTCTGGTG-3′ (antisense); β-actin: 5′-CTCGCCTTTGCCGATCC-3′ (sense), and 5′-GGGGTACTTCAGGGTGAGGA-3′ (antisense); U6: 5′-GCTTCGGCAGCACATATACTAAAAT-3′(sense), and 5′-CGCTTCACGAATTTGCGTGTCAT-3′ (antisense).

Cell proliferation assay

Cells were transfected with si-NC, si-SNHG14, miR-NC mimic, miR-543 mimic, si-SNHG14 + anti-miR-NC, si-SNHG14 + anti-miR-543, miR-543 mimic + Vector-NC, or miR-543 mimic + Vector-KLF7. Then, about 3×10^3 transfected cells were seeded in each well of 96-well plates. After 0, 24, 48 and 72 h culture, cell proliferation ability was detected using the Cell Counting kit-8 (CCK-8, US EverBright Inc., Suzhou, China) following the guidance of

manufacturers. The absorbance in each well was determined at the optical density of 450 nm by a SpectraMax Gemini XPS microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Transwell migration assay

A 24-transwell plate containing 8-μm pore insert (Corning® incorporated Life Sciences, Acton, MA, USA) was used for cell migration assay. Briefly, cells were transfected with the indicated oligonucleotide or/and plasmid, and then seeded in the upper compartment of 24-transwell plates in serum-free DMEM media. Grown medium containing 10% FCS was placed into the lower chamber as the chemoattractant. 24 h later, the cells that moved through the underside of the inserts were fixed with 90% ethyl alcohol and stained with 0.2% crystal violet. The number of migrated cells was determined under an Olympus BX53 optical microscope (100× magnification, Olympus, Tokyo, Japan) in 10 randomly selected fields.

Transwell invasion assay

Inserts containing 8-μm pores in 24-transwell plates were precoated with 0.5% Matrigel (Corning® incorporated Life Sciences) for cell invasion assay. To be brief, transfected cells in serum-free medium were seeded into the upper compartment of 24-well plates with Matrigel-coated inserts. DMEM media plus 10% FCS was placed into the lower chamber. After 24 h incubation, the penetrated cells through the pores of inserts were fixed, stained with 0.2% crystal violet and counted using the Olympus BX53 optical microscope at 100× in 10 randomly selected fields.

Flow cytometry for cell apoptosis

Transfected cells were harvested and trypsinized using 0.05% trypsin/EDTA (TaKaRa, Beijing, China). A FITC-Annexin V Apoptosis Detection kit (BD Biosciences, Heidelberg, Germany) was used to determine cell apoptosis capacity, in accordance with the recommendations of manufacturers. In brief, cells were re-suspended in staining buffer, and then double-stained with 5 μL of Annexin-V-FITC and 2 μL of PI at room temperature for 15 min. The apoptotic rate was analyzed by a FACSCanto II flow cytometry (BD Biosciences) using CellQuest software.

Bioinformatics

Bioinformatic analysis for the directly interactional miRNAs of SNHG14 was carried out using starBase v.2.0 software (http://starbase.sysu.edu.cn/starbase2/mirLncRNA.php). TargetScan Human 7.1 software (http://www.targetscan.

org/vert_71/) was used to predict the potential targets of miR-543.

Dual-luciferase reporter assay

SNHG14 luciferase reporter (SNHG14-WT) and KLF7 3′-UTR reporter (KLF7-WT) harboring the putative miR-543-binding sequence, and site-directed mutants in the seeded region (SNHG14-Mut and KLF7-Mut) were constructed by Ribobio. To verify the targeted correlation between SNHG14 and miR-543, SNHG14-WT or SNHG14-Mut was transfected into cells, together with miR-NC mimic or miR-543 mimic. To validate whether KLF7 was a target of miR-543, cells were cotransfected with KLF7-WT or KLF7-Mut and miR-NC mimic or miR-543 mimic. The luciferase activity was assessed 48 h post-transfection using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) following the producer's guidance.

Western blot for KLF7 expression

Cells were transfected with the indicated oligonucleotide or/and plasmid for 48 h. Subsequently, cells were harvested and lysed in RIPA lysis buffer (Beyotime, Shanghai, China) referring to the manufacturer's protocols. Total protein (100 μg) was separated by SDS-PAGE on a 10% polyacrylamide gel and blotted onto polyvinylidene fluoride (PVDF) membrane (GE Healthcare, Buckinghamshire, UK). The membranes were probed with anti-KLF7 (1:1000; Abcam, Cambridge, UK) or anti-GAPDH (1:2000; Abcam) antibody, and then incubated with horseradish peroxidase (HRP)-conjugated IgG secondary antibody (1:5000; Abcam). Chemiluminescence (ECL) reagents (GE Healthcare) were used to detect chemiluminescent signals, and the densitometry of protein bands was analyzed by ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

All experiment data were presented as mean ± SD using SPSS software v.21 (SPSS, Chicago, IL, USA). Differences between groups were compared by a Student's t test or one-way ANOVA. P values < 0.05 were considered significant.

Results

SNHG14 expression was increased in BC tissues and cells

For a preliminary observation for the involvement of SNHG14 on BC progression, firstly, we determined the expression of SNHG14 in BC tissues and corresponding

normal breast tissues. As demonstrated by qRT-PCR, SNHG14 expression was elevated in BC tissues compared with normal controls (Fig. 1A). Subsequently, we detected SNHG14 expression in BC cells (T-47D and SKBR3). In line with BC tissues, SNHG14 was significantly upregulated in BC cells, as compared with normal group (Fig. 1B). These data together indicated a significant upregulation of SNHG14 in BC tissues and cells.

Knockdown of SNHG14 mitigated the proliferation, migration and invasion and facilitated apoptosis of BC cells

To validate the function of SNHG14 on BC progression in vitro, we performed "phenocopy" silencing by siRNA against SNHG14 (si-SNHG14). Transfection of si-SNHG14, but not a negative sequence, markedly reduced the expression of SNHG14 in T-47D and SKBR3 cells (Fig. 2A, B). After that, CCK-8 assays showed that in comparison to

Fig. 1 SNHG14 expression was increased in BC tissues and cells. The expression of SNHG14 was determined by qRT-PCR in 20 pairs of BC tissues and adjacent normal tissues (**A**), normal mammary epithelial MCF-10A cells, T-47D and SKBR3 cells (**B**). *$P < 0.05$
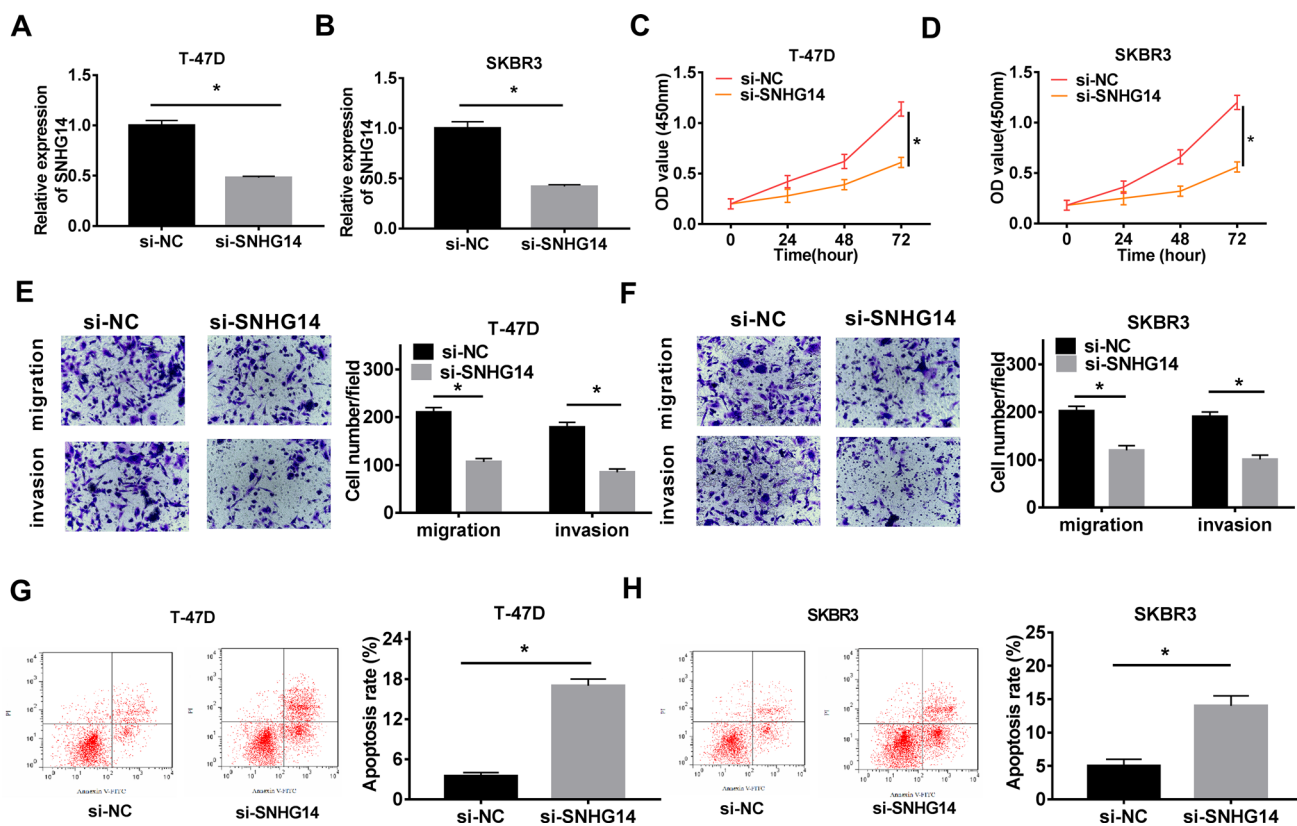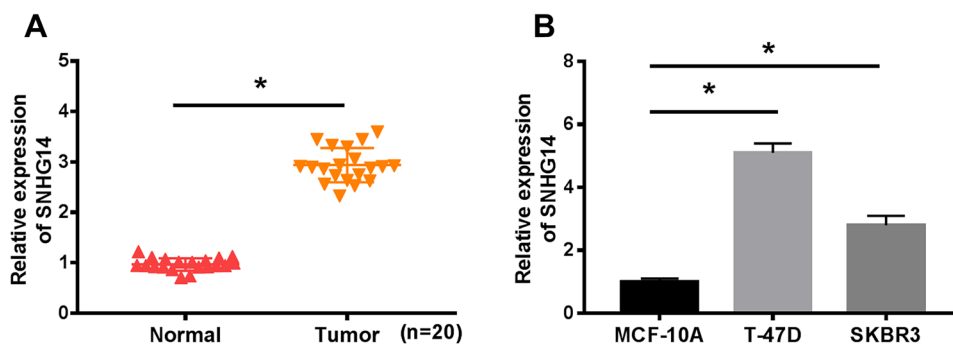

Fig. 2 SNHG14 knockdown mitigated the proliferation, migration and invasion and facilitated apoptosis of T-47D and SKBR3 cells. T-47D and SKBR3 cells were transfected with si-SNHG14 or negative control si-NC for the indicated time point. (**A** and **B**) 48 h after transfection, SNHG14 expression was assessed by qRT-PCR in trans- fected cells. (**C** and **D**) After 0, 24, 48 and 72 h transfection, cell proliferation ability was detected using CCK-8 assay. (**E** and **F**) 24 h after transfection, cell migration and invasion capacities were determined by transwell assay. (**G** and **H**) After 48 h transfection, cell apoptosis was evaluated by flow cytometry. *$P < 0.05$

negative control, SNHG14 silencing significantly weakened cell proliferation ability in the two cells (Fig. 2C, D). Transwell results revealed that SNHG14 knockdown strikingly hampered cell migration and invasion capacities compared with negative control (Fig. 2E, F). Additionally, flow cytometry data demonstrated that SNHG14 depletion highly promoted cell apoptosis (Fig. 2G, H). All these results established a notion that SNHG14 knockdown hindered BC cell proliferation, migration, and invasion and enhanced cell apoptosis.

SNHG14 directly interacted with miR-543

To further investigate the molecular mechanisms by which SNHG14 regulated BC cell progression in vitro, we implemented a detailed analysis for its target miRNAs using online software starBase v.2.0. Of interest, these predicted data showed a putative binding site for miR-543 in SNHG14 (Fig. 3A). To confirm this, we carried out a dual-luciferase reporter assay using SNHG14 luciferase reporter (SNHG14-WT). In contrast to a scrambled negative sequence, transfection of miR-543 mimic significantly decreased the luciferase activity of SNHG14-WT (Fig. 3B, C). Whereas, site-directed mutant of the miR-543-binding sequence remarkably abolished the effect of miR-543 on reporter gene expression under the same conditions (Fig. 3B, C). Subsequently, we confirmed whether the miR-543-binding site was functional. As expected, in comparison to negative control, miR-543 expression was prominently promoted by SNHG14 knockdown in the two BC cells (Fig. 3D, E). In addition, qRT-PCR results showed that miR-543 level was significantly downregulated in the two BC cells, as compared with MCF-10A cells (Fig. 3F). These data together strongly pointed to a role of SNHG14 as a molecular sponge of miR-543.

Si-SNHG14-mediated anti-proliferation, anti-migration, anti-invasion and pro-apoptosis effects were abated by restored expression of miR-543

A previous report had demonstrated that miR-543 weakened BC progression in vitro through repression of cancer cell proliferation and cell cycle, as well as enhancement of cell apoptosis [17]. Given our data that SNHG14 directly interacted with miR-543, we further explored whether miR-543 mediated the regulatory effect of SNHG14 knockdown on BC cell progression in vitro. To validate this, T-47D and SKBR3 cells were cotransfected with si-SNHG14 and miR-543 inhibitor (anti-miR-543). As demonstrated by qRT-PCR, cotransfection of anti-miR-543 substantially reversed the promotional effect of si-SNHG14 on miR-543 expression in the two cells compared with negative control (Fig. 4A,
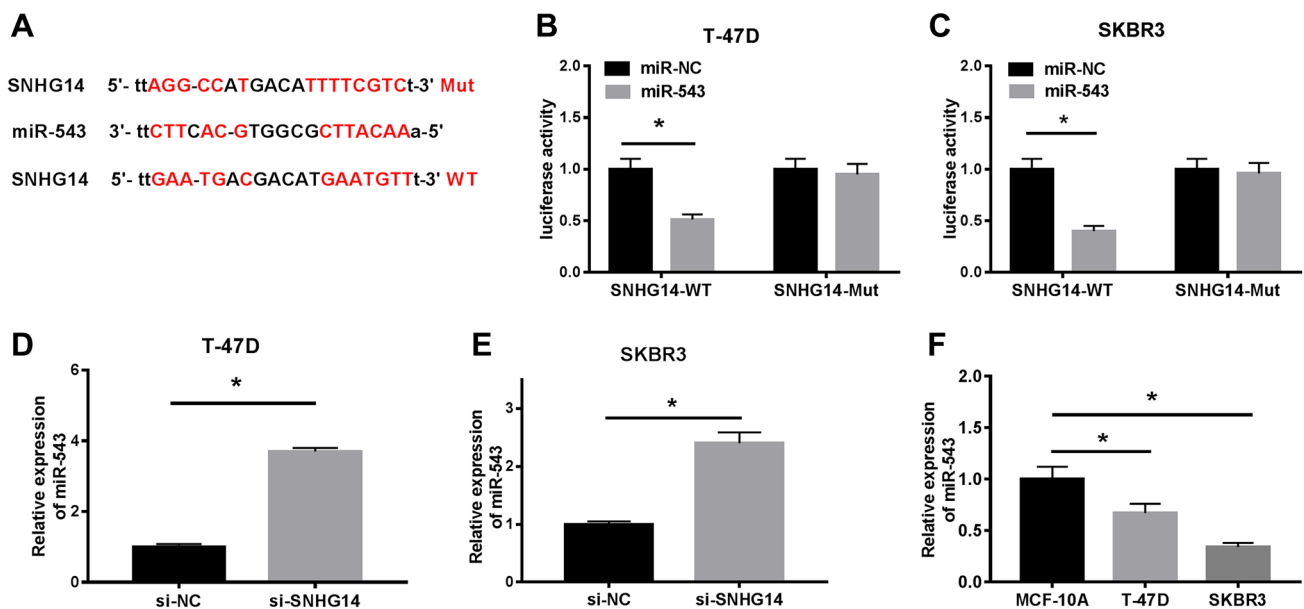

Fig. 3 SNHG14 directly interacted with miR-543 and regulated miR-543 expression. (**A**) Nucleotide resolution of the predicted miR-543 binding site in SNHG14 and mutated miR-543 binding sequence. (**B** and **C**) SNHG14 wild-type luciferase reporter plasmid (SNHG14-WT) harboring the putative miR-543-binding site and site-directed mutant in the seeded region (SNHG14-Mut) were constructed and transfected into T-47D and SKER3 cells, respectively, together with miR-543 mimic or miR-NC mimic. Then, the luciferase activity was detected using a luciferase reporter assay system. (**D** and **E**) T-47D and SKER3 cells were transfected with miR-543 mimic or miR-NC mimic, and then miR-543 expression was assessed by qRT-PCR. (**F**) MiR-543 level was determined by qRT-PCR in MCF-10A, T-47D and SKBR3 cells. *$P < 0.05$
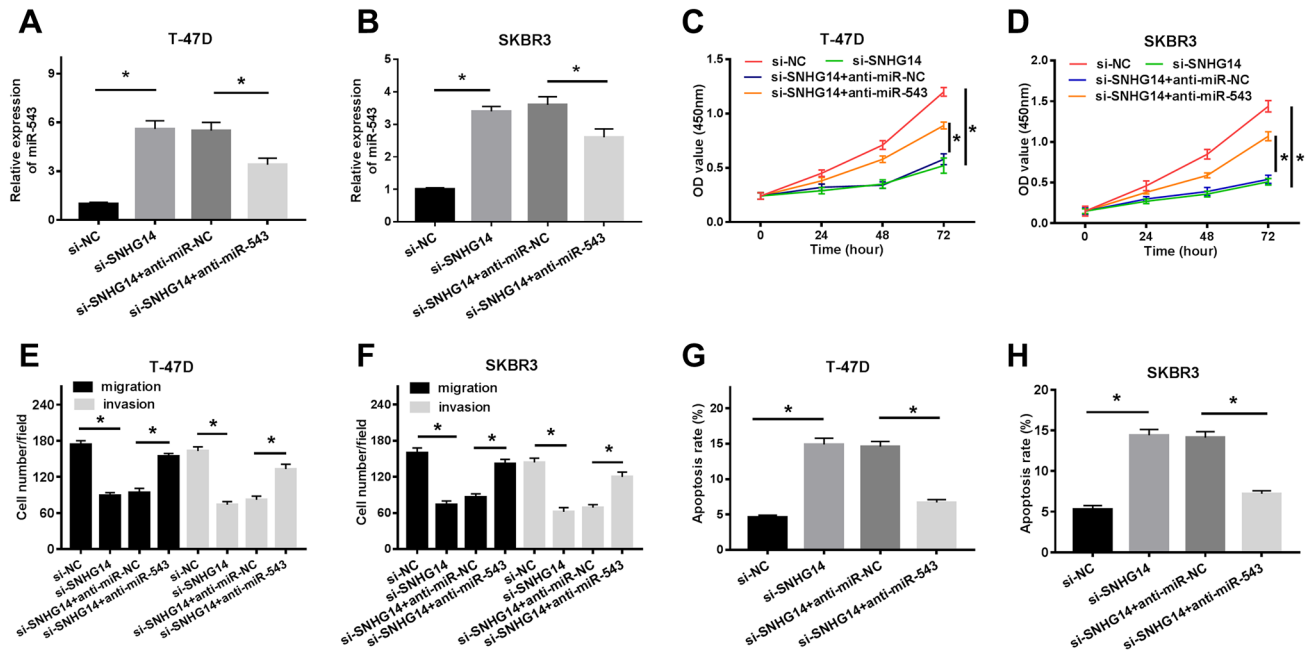
Fig. 4 SNHG14 knockdown regulated BC cell progression in vitro by miR-543. T-47D and SKBR3 cells were transfected with si-SNHG14 alone, or together with anti-miR-543 or negative control anti-miR-NC. (**A** and **B**) After 48 h transfection, miR-543 expression was detected by qRT-PCR in transfected cells. (**C** and **D**) 0, 24, 48 and 72 h after transfection, cell proliferation capacity was monitored by CCK-8 assay. (**E** and **F**) 24 h after transfection, cell migration and invasion abilities were determined using transwell assay. (**G** and **H**) After 48 h transfection, cell apoptosis was assessed by flow cytometry. *$P < 0.05$

B). Subsequent experiments results revealed that in contrast to negative control, si-SNHG14-mediated anti-proliferation, anti-migration, anti-invasion and pro-apoptosis effects were manifestly abrogated by the restoration of miR-543 expression (Fig. 4C–H). Together, these data suggested that miR-543 mediated the regulatory effects of SNHG14 silencing on BC cell proliferation, migration, invasion, and apoptosis.

KLF7 was a direct target of miR-543

MiRNAs are widely accepted to exert biological function through posttranscriptional suppression of target mRNAs. Hence, to further understand the role of miR-543 in BC progression, we used TargetScan Human 7.1 software to help identify the targets of miR-543. In silico prediction of miR-543 targets showed that KLF7 contained a putative target sequence for miR-543 in its 3′-UTR (Fig. 5A). Transfection of miR-543 mimic, but not miR-NC control, significantly reduced the activity of a luciferase reporter gene fused to the wild-type KLF7 3′-UTR (Fig. 5B, C). However, the activity of a mutant KLF7 3′-UTR luciferase reporter was not influenced by miR-543 overexpression (Fig. 5B, C), suggesting that the action of miR-543 on KLF7 depended on the presence of miR-543-binding site with 3′-UTR. Moreover, the protein level of KLF7 was highly decreased by miR-543 overexpression in the two BC cells (Fig. 5D, E). All these

data strongly implied that KLF7 was directly targeted and repressed by miR-543.

KLF7 mediated the regulatory effects of miR-543 on BC cell proliferation, migration, invasion, and apoptosis

To provide further mechanistic insight into the link between miR-543 and KLF7 on BC cell progression in vitro, T-47D and SKBR3 cells were transfected with miR-543 mimic alone, or together with KLF7 overexpression plasmid (Vector-KLF7). In contrast to negative plasmid, transient introduction of Vector-KLF7 strongly alleviated the inhibition of miR-543 overexpression on KLF7 level in the two cells (Fig. 6A, B). Subsequent experimental data revealed that compared with miR-NC group, miR-543 upregulation resulted in an obvious repression on cell proliferation and a distinct suppression of cell migration and invasion, as well as a clear enhancement of cell apoptosis (Fig. 6C–H). Nevertheless, these effects were manifestly reversed by restored expression of KLF7 in the two cells (Fig. 6C–H). Together, these results pointed a notion that overexpressed miR-543-mediated anti-proliferation, anti-migration, anti-invasion and pro-apoptosis effects were mediated by KLF7 in BC cells.
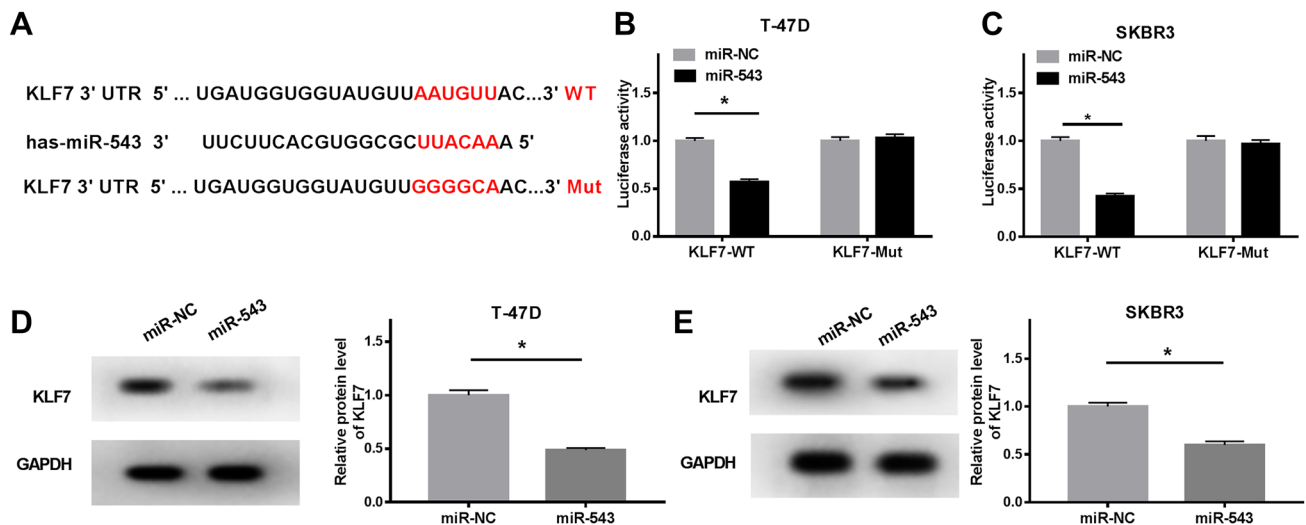
Fig. 5 KLF7 was a direct target of miR-543. (**A**) Schematic of the putative miR-543 binding site in the 3′-UTR of KLF7 and the mutant in the seeded sequence. (**B** and **C**) KLF7 3′-UTR wild-type luciferase reporter plasmid (KLF7-WT) harboring miR-543 binding site and its mutant of the seeded region (KLF7-Mut) were transfected into T-47D and SKBR3 cells, respectively, together with miR-543 mimic or miR-NC mimic, followed by the measurement of the luciferase activity. (**D** and **E**) KLF7 protein expression was determined by western blot in T-47D and SKBR3 cells transfected with miR-543 mimic or miR-NC mimic. *$P < 0.05$

SNHG14 modulated KFL7 expression through acting as a ceRNA of miR-543

Next, we explored whether, if so, how SNHG14 modulated KLF7 expression in BC cells. As expected, in comparison to negative control, the mRNA and protein levels of KLF7 were strikingly reduced by SNHG14 knockdown in both T-47D and SKBR3 cells (Fig. 7A–D), indicating a positive regulation of SNHG14 on KLF7 expression. Intriguingly, cotransfection of anti-miR-543, but not a scrambled negative sequence, significantly abolished si-SNHG14-mediated decreased KLF7 expression in the two cells (Fig. 7A–D). These data together strongly suggested that SNHG14 acted as a ceRNA of miR-543 to modulate KLF7 expression.

Discussion

In recent years, lncRNAs have been postulated to function as essential regulators in tumorigenesis and progression of BC. For example, the high level of lnc00511 was associated with the poor prognosis of BC patients, and lnc00511 over-expression accelerated the proliferation and sphere-formation of BC cells in vitro through sponging miR-185-3p [19]. RUSC1-AS-N expression was manifested to be increased in BC tissues and cells, and its depletion hindered the proliferation and metastasis of BC cells by Wnt/β-catenin signaling pathway [20]. Moreover, a recent study reported that the carcinogenic role of HOX transcript antisense RNA (HOTAIR) could be attributed to its regulatory effect on

BC cell invasion, metastasis, and autophagy [21]. These researches highlighted that some lncRNAs might serve as promising therapeutic targets for improving BC treatment. In this study, our results suggested that SNHG14 knockdown mitigated the proliferation, migration and invasion, and promoted apoptosis of BC cells via miR-543/KLF7 axis.

SNHG14 has been demonstrated to function as a potential oncogenic lncRNA in a range of human cancers, such as non-small cell lung cancer, ovarian cancer and cervical cancer [8, 9, 22]. Liu et al. manifested that overexpressed SNHG14 enhanced the progression of gastric cancer by regulation of cancer cell viability, metastasis, and apoptosis via targeting miR-145/SOX9 axis [23]. Additionally, Liu et al. uncovered that SNHG14 upregulation acted as a ceRNA of miR-203 to facilitate destructive behaviors in clear cell renal cell carcinoma cells [24]. In this study, our results indicated that SNHG14 level was significantly increased in BC tissues and cells, and SNHG14 silencing hampered BC cell proliferation, migration and invasion, in line with previous works [10, 11]. Besides, we first manifested that SNHG14 knockdown facilitated the apoptosis of BC cells. In a word, SNHG14 played an oncogenic role in BC, consistent with earlier studies [10, 11, 25].

LncRNAs are widely accepted to act as ceRNAs of specific miRNAs to modulate gene expression, and thus play crucial roles during BC tumorigenesis. Therefore, the starBase v.2.0. software was used to search for the directly interactional miRNAs of SNHG14. Among these candidates, miR-543 was of particular interest in this research, because of its role as important regulators in human cancers,
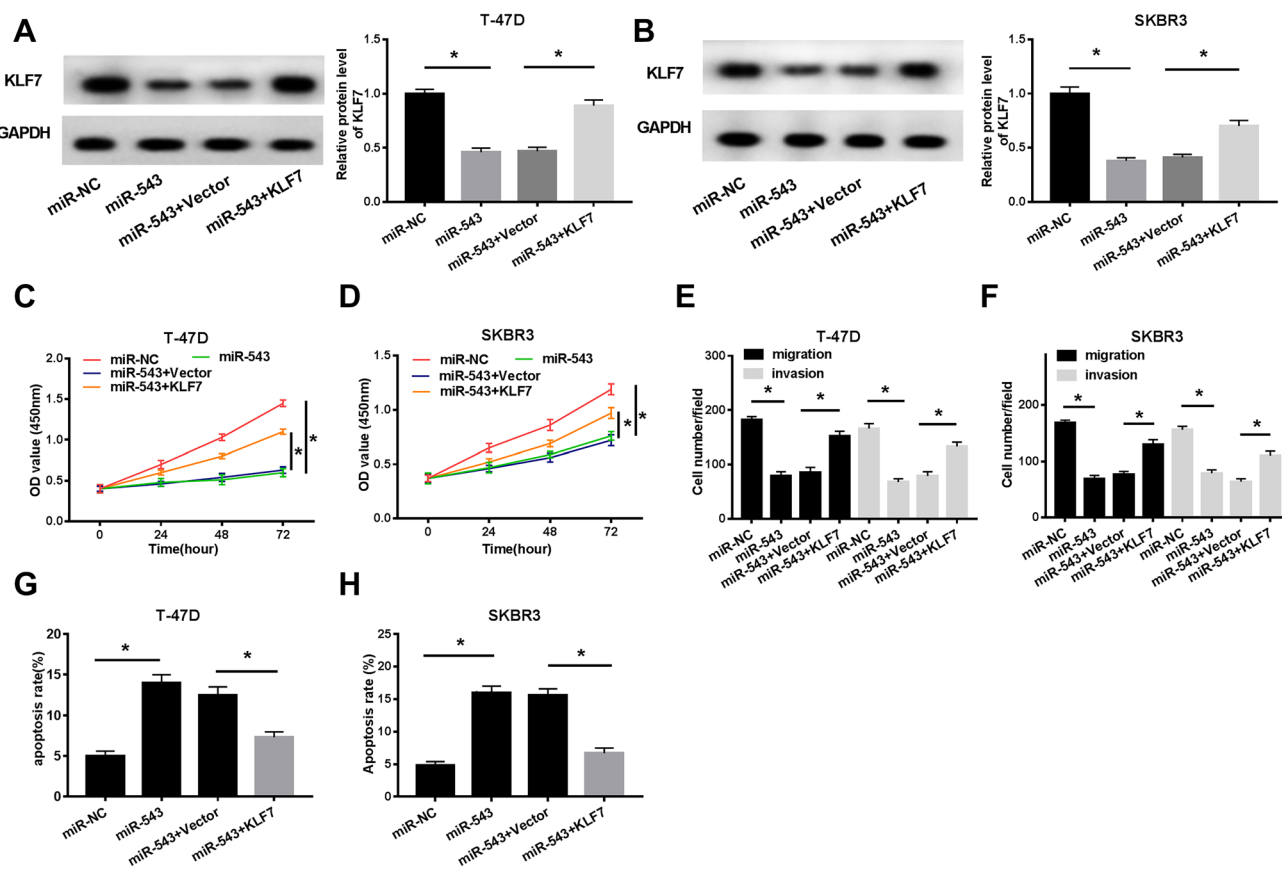
Fig. 6 MiR-543 overexpression regulated BC cell progression in vitro through KLF7. T-47D and SKBR3 cells were transfected with miR-NC mimic, miR-543 mimic, miR-543 mimic + Vector-NC or miR-543 mimic + Vector-KLF7. (**A** and **B**) KLF7 protein level was assessed by western blot 48 h post-transfection. (**C** and **D**) 0, 24, 48 and 72 h after transfection, cell proliferation ability was monitored by CCK-8 assay. (**E** and **F**) Cell migration and invasion capacities were detected by transwell assay. (**G** and **H**) Cell apoptosis was evaluated by flow cytometry. *$P < 0.05$

including cervical cancer, prostate cancer and esophageal cancer [14, 26, 27]. Moreover, a previous document uncovered that high level of miR-543 weakened the proliferation and cell cycle progression, and enhanced cell apoptosis in BC cells by targeting mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase-2 (ERK2) [17]. The regulatory effect of miR-543 was very similar to our finding of SNHG14 silencing on BC cell behaviors. This striking resemblance prompted us to explore miR-543 as a potential molecular mediator of SNHG14 knockdown-induced BC cell behaviors. Intriguingly, we firstly manifested that SNHG14 directly interacted with miR-543 and modulated miR-543 expression in BC cells. Moreover, our data underscored that miR-543 mediated the regulatory effects of SNHG14 silencing on BC cell proliferation, migration, invasion and apoptosis. In short, SNHG14 knockdown alleviated BC cell progression in vitro by miR-543.

Next, we used TargetScan Human 7.1 software to help identify the molecular targets of miR-543. Among the approximately 758 targets, KLF7 was selected for further

research in the study, considering the key function of KLF7 in many cancers, such as glioma, non-small cell lung cancer and cervical cancer [28–30]. Moreover, highly expressed KLF7 was found to be closely correlated with poor prognosis of GC patients, and KLF7 depletion resulted in reduced migration of cancer cells [31]. Besides, a previous report demonstrated that KLF7 was associated with BC progression [32]. These findings from our study described above prompted us to examine whether KLF7 was involved in the regulatory mechanism of SNHG14/miR-543 axis on BC cell behaviors. As expected, we firstly uncovered that KLF7 was directly targeted and repressed by miR-543 in BC cells. Moreover, our data validated that overexpressed miR-543-mediated anti-proliferation, anti-migration, anti-invasion and pro-apoptosis effects were mediated by KLF7. Similar with our findings, Zhao et al. reported that miR-185 targeted KLF7 to hamper the proliferation and invasion in non-small cell lung cancer cells [29]. More importantly, our data firstly substantiated that SNHG14 modulated KFL7 expression through acting as a ceRNA of miR-543 in BC

Fig. 7 KLF7 acted as a ceRNA of miR-543 to modulate KLF7 expression. T-47D and SKBR3 cells were transfected with si-NC, si-SNHG14, si-SNHG14 + anti-miR-NC or si-SNHG14 + anti-miR-543 for 48 h. (**A** and **B**) KLF7 mRNA level was detected by qRT-PCR in transfected cells. (**C** and **D**) KLF7 protein expression was determined by western blot in transfected cells. $*P < 0.05$
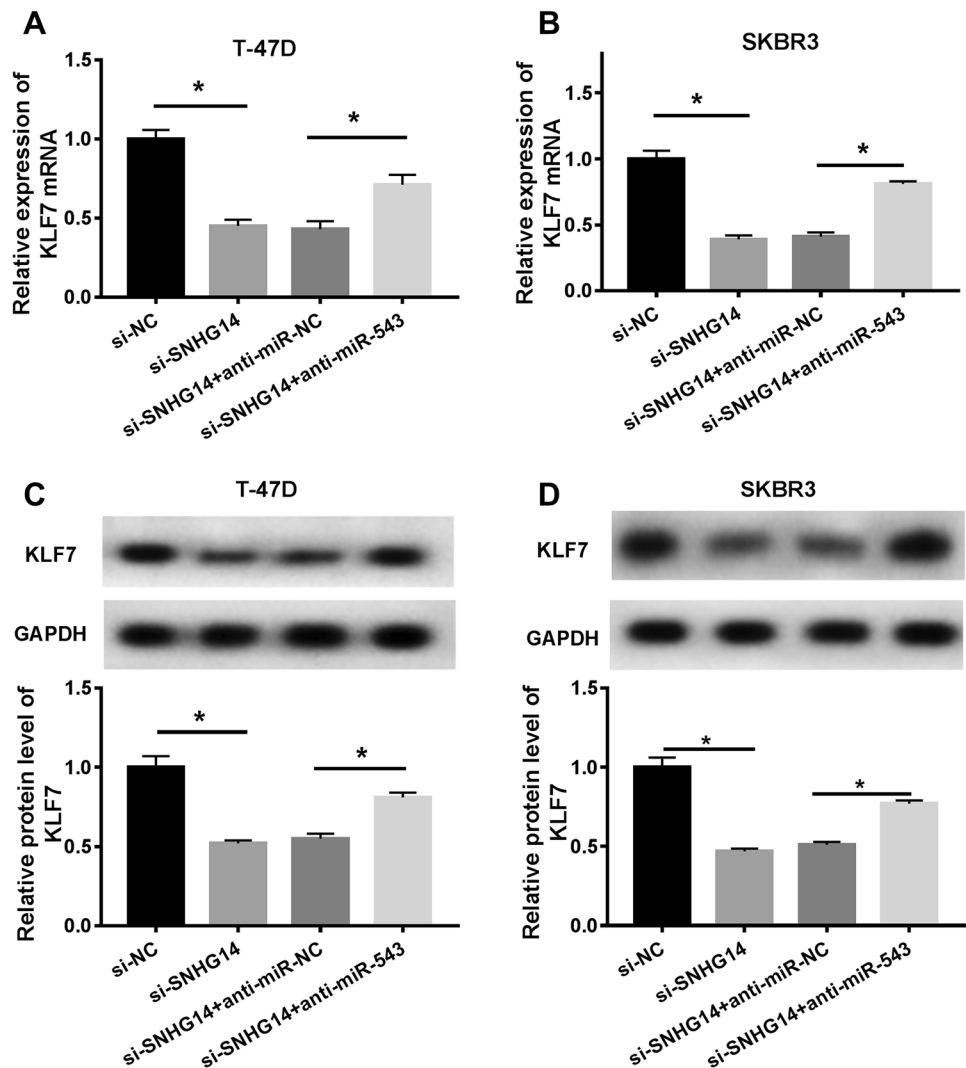
cells. This study is limited in vitro investigation, and more researches in vivo using xenograft model or BC mice model will be carried out in further work.

In conclusion, our study suggested that the knockdown of SNHG14 hampered the proliferation, migration and invasion, and accelerated the apoptosis of BC cells at least in part by acting as a ceRNA of miR-543 and regulating KLF7 expression. Targeting SNHG14 might be a promising therapeutic strategy.

Declarations

Conflict of interest The authors have no interests to disclose.

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