

# LINC00052 promotes breast cancer cell progression and metastasis by sponging miR-145-5p to modulate TGFBR2 expression

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**Abstract.** Long non-coding RNAs (lncRNAs) may participate in biological regulatory mechanisms of tumors. The aim of the present study was to uncover the molecular mechanism of the lncRNA LINC00052 in the tumorigenesis of breast cancer (BC). LINC00052 expression in BC tissues and cell lines was detected by reverse transcription-quantitative PCR analysis. The Cell Counting Kit-8, proliferation, Transwell and wound healing assays were employed to confirm the effect of LINC00052 on cell proliferation, migration and invasion. The cell localization of LINC00052 was estimated by cytoplasmic nuclear separation assay. Finally, the potential regulatory mechanism of LINC00052 in BC was detected by western blot analysis. The expression levels of LINC00052 were found to be significantly higher in BC tissues compared with those in the adjacent normal tissues. Downregulation of LINC00052 expression *in vitro* significantly suppressed the proliferation, migration and invasion of BC cells. LINC00052 was mainly expressed in the cytoplasm and was considered to bind with microRNA (miR)-145-5p based on various databases. Notably, the high expression levels of LINC00052 led to the low expression levels of miR-145-5p and high expression levels of TGF- $\beta$  receptor II (TGFBR2). In conclusion, the findings of the present study demonstrated that LINC00052 may sponge miR-145-5p to upregulate TGFBR2 expression in order to promote the proliferation and metastasis of BC cells. Therefore, LINC00052 may be an effective potential target for the diagnosis and treatment of BC.

## Introduction

According to global cancer statistics in 2018, breast cancer (BC) is the most commonly diagnosed malignancy and the leading cause of cancer-associated mortality among women, with ~17,000,000 new cases occurring annually worldwide in 2018 (1,2), posing a serious threat to the well-being and survival of patients. With advances in cancer research, several signal transduction pathways, including MAPK and PI3K-Akt, and signaling pathway-associated molecules have been found to be abnormally activated in BC, which may serve an important role in the occurrence and development of cancer (3). However, due to the complexity of eukaryotic genome expression, numerous aspects of the molecular regulation of BC remain elusive. Therefore, it is urgent to identify potential biological biomarkers with higher specificity and sensitivity for BC.

Long non-coding RNAs (lncRNAs) are segments of non-coding RNA >200 nucleotides in length (4) that are mostly present in the nucleus and cytoplasm. They are formed by transcription of RNA polymerase II and then matured by splicing and head-to-tail junction (5). In recent years, with the development and maturity of gene microarray and RNA sequencing technology, the research in the field of lncRNA has gradually advanced. It has been demonstrated that, although lncRNAs are a class of non-coding RNAs, they may regulate gene expression by epigenetic, transcriptional and post-transcriptional pathways, thereby regulating cell proliferation and metabolism, and that they participate in the regulation of various pathological processes in organisms (6,7).

As previously reported, LINC00052 is a type of lncRNA that serves an important role in cancer cell migration and invasion (8). For example, Xiong *et al* (8) reported that LINC00052 could sponge microRNA (miRNA/miR)-485-3p and miR-128 to regulate the expression levels of neurotrophic receptor tyrosine kinase 3 (NTRK3) and promote hepatocellular carcinoma (HCC) cell metastasis. In addition to the role of LINC00052 in HCC, it was proved that it could promote cell proliferation and metastasis in gastric cancer (9). Despite these findings, the exact role and function of LINC00052 in BC remains unclear.

miRNAs are a class of small non-coding RNAs that are 18-25 nucleotides in length and are present in plants, fungi,

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animals and viruses (10). miRNAs can bind directly to the 3'-untranslated regions (UTRs) of their target mRNAs; each miRNA can bind and regulate several target genes, and at the same time it can also be regulated by several genes (11). It has been demonstrated that an lncRNA may bind to a miRNA as a competing endogenous RNA (ceRNA) to affect the occurrence and development of tumors (12). However, the mechanism through which LINC00052 binds to miRNAs in BC remains unclear.

The aim of the present study was to investigate LINC00052 expression in BC tissues and compare it with that in adjacent normal tissue. The effects of LINC00052 on the proliferation and metastasis of BC cells were investigated by Cell Counting Kit-8 (CCK-8), wound healing and Transwell assays, and the interactions among LINC00052, miR-145-5p and TGF- $\beta$  receptor II (TGFBR2) were explored by reverse transcription-quantitative PCR (RT-qPCR) and western blot analyses. The aim was to determine whether LINC00052 regulated TGFBR2 expression by sponging miR-145-5p and to elucidate the functional role of LINC00052 in BC, in the hope of providing a new perspective on the diagnosis and treatment of BC.

## Patients and methods

**Human tissue samples.** A total of 45 pairs of BC and adjacent tissues (>1 cm from the tumor) were collected from patients at the Tongji Medical College of Huazhong Science and Technology University (Wuhan, China) between July 2017 and December 2017. The 45 fresh BC samples were acquired from surgery and immediately frozen in liquid nitrogen and kept at -80°C until total RNA was extracted. All patients were female, with a median age of 55 years old (range, 33-67 years old). None of the patients had received preoperative chemotherapy or radiotherapy. The procedures followed the ethical standards of the responsible committees on human experimentation (institutional and national). Written informed consent was obtained from each patient. The present study was approved by the Ethics Committee of Tongji Hospital at Tongji Medical College of Huazhong Science and Technology University.

**Cell lines and culturing.** The human BC cell lines (MDA-MB-231, MDA-MB-468, T47D, SKBR3 and MCF-7) and the normal breast MCF10A cell line were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. The MDA-MB-231 cells were cultured in L15 medium, while the MDA-MB-468 and T47D cell lines were cultured in RPMI-1640 medium, and the MCF-7 and SKBR3 cell lines were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Sigma-Aldrich; Merck KGaA) at 37°C with 5% CO<sub>2</sub>.

**Database analysis.** The present study included 1,222 samples obtained from The Cancer Genome Atlas (TCGA; <https://portal.gdc.cancer.gov>) database, consisting of 1,109 tumor samples and 113 normal breast tissue samples, and 113 pairs of cancer and adjacent normal tissues. The BC RNA expression data (level 3) of the corresponding patients were downloaded from TCGA Data Portal (November 2017). The RNA sequencing raw reads (lncRNA and mRNA) were

post-processed and normalized using TCGA RNASeqv2 system (TCGA; <https://portal.gdc.cancer.gov>). The normalized miRNA expression data were downloaded from TCGA Data Portal and quantile normalized before performing the analysis. TargetScan Human v7.2 ([http://www.targetscan.org/mamm\\_31/](http://www.targetscan.org/mamm_31/)) was used to predict the TGFBR2 gene targeted by miR-145-5p. DIANA-LncBase (<http://www.microrna.gr/LncBase>) was used to predict the LINC00052/miR-145-5p interactions.

**Cell transfection.** Small interfering (si)RNAs against LINC00052 and a scrambled control siRNA used as a negative control (si-NC) were purchased from Guangzhou RiboBio Co., Ltd. miR-145-5p mimics (miR-145-5p-mimics), inhibitors (miR-145-5p-inhibitor), or corresponding scrambled controls (miR-NC) were synthesized by Guangzhou RiboBio Co., Ltd. siRNAs, miR mimics, miR inhibitors and their NC oligonucleotides (50 nM) were transfected using Lipofectamine™ 3000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) into the MDA-MB-231 and MCF-7 cells separately in 6-well plates of cells at 50-60 or 70-80% confluency, respectively, according to the manufacturer's protocol. Each well was transfected with 50 nM siRNAs. After 48 h of transfection at 37°C, the cells were collected for subsequent experiments. The sequences of siRNAs used are listed in Table I.

**Cell proliferation assay.** MDA-MB-231 and MCF-7 cells (5x10<sup>3</sup> cells/well) were treated with si-NC, si-1 and si-2 separately (50 nM) for 8 h at 37°C. After transfection, cells were digested with trypsin, and 2x10<sup>3</sup> cells/well were seeded in a 96-well culture plate. Cell proliferation was determined using a CCK-8 assay for 10 min (Dojindo Molecular Technologies, Inc.) and the absorbance was measured at 450 nm (Molecular Devices, LLC).

**Wound healing assay.** The MDA-MB-231-NC, MDA-MB-231-siLINC00052-1, MDA-MB-231-siLINC00052-2, MCF-7-NC, MCF-7-siLINC00052-1 and MCF-7-siLINC00052-2 cells were seeded in 6-well plates at 2x10<sup>5</sup> cells/well. When the confluence had reached 90%, the scratch wounds were created across each well using a 200- $\mu$ l sterile micropipette plastic tip. Subsequently, the cells were cultured for 24 h at 37°C with serum-free medium (L15 medium for MDA-MB-231 cells or DMEM for MCF-7 cells; Thermo Fisher Scientific, Inc.). Images of each scratch were captured for 5 fields of view in three triplicates at 0 and 24 h using a light microscope (Carl Zeiss AG; magnification, x200). Cells in the marked area between the lines and the edges of the wound were counted using Image-Pro Plus 6.0 (Media Cybernetics, Inc.). The cell migration rate was calculated using GraphPad Prism 5.0 (GraphPad Software, Inc.).

**Cell migration and invasion assay.** Following MCF-7 cell transfection with NC, si-1 and si-2, Transwell migration and Matrigel invasion assays, using a 24-well chamber with an 8- $\mu$ m-pore filter (Corning, Inc.), were used to investigate the *in vitro* effects of LINC00052 on cell migration and invasion. For the Transwell migration assay, the MDA-MB-231 cells, transfected with siRNA, were trypsinized and 200  $\mu$ l cell suspension (2x10<sup>4</sup> cells/ml) was added to the upper chamber of

Table I. Sequences of siRNA, mimics, inhibitors and the NC used for transfection.

Name	Sequences
siRNA-NC	5'-TTCTCCGAACGTGTCACGTdTdT-3'
siLINC00052-1	5'-UUAUUCACAUCACUGCAU GTT-3'
siLINC00052-2	5'-UUUCAGAU AUGCCAAGCUC TT-3'
NC mimic sense	5'-UUUGUACUACACAAAAGUACUG-3'
NC mimic anti-sense	3'-AAACAUGAUGUGUUUUAUGAC-5'
miR-145-5p mimic sense	5'-GUCCAGUUUUCCCAGGAAUCCCU-3'
miR-145-5p mimic anti-sense	3'-AGGGAUUCUGGGAAAACUGGAC-5'
NC inhibitor	5'-CAGUACUUUUGUGUAGUACAAA-3'
miR-145-5p inhibitor	5'-AGGGAUUCUGGGAAAACUGGAC-3'

si, small interfering; NC, negative control; miR, microRNA.

Table II. Sequences of RT-qPCR primers.

Sequences	RT-qPCR primers
TGFBR2F	5'-TGTGATGTGAGATTTCCACCTGT-3'
TGFBR2R	5'-TGTTCTCGTCATTCTTCTCCATAC-3'
LINC00052F	5'-GTGAACCTTCGACCTTGGACTT-3'
LINC00052R	5'-AGAGAGGGAGGGAGACTGAGATT-3'
miR-145-5pF	5'-CCTTGTCCTCACGGTCCAGT-3'
miR-145-5pR	5'-AACCATGACCTCAAGAACAGTATTT-3'
GAPDHF	5'-GGAAGCTTGTCAATGGAAATC-3'
GAPDHR	5'-TGATGACCTTTTGGCTCCC-3'
U6F	5'-GCTTCGGCAGCACATATACTAAAAT-3'
U6R	5'-CGCTTCACGAATTTGCGTGTCAT-3'

F, forward; R, reverse; TGFBR2, TGF- $\beta$  receptor II; RT-qPCR, reverse transcription-quantitative PCR; miR, microRNA; si, small interfering.

each insert (Corning, Inc.) containing the uncoated membrane and resuspended in L15 medium without serum (200  $\mu$ l). The lower chambers were supplemented with 30-40% FBS (500  $\mu$ l). After incubation for 24 h at 37°C, the upper surface of the membrane was removed with a cotton tip, while the cells on the lower surface were stained for 10-15 min with 0.1% crystal violet at room temperature. For the invasion assay, Matrigel chambers (BD Biosciences) were performed according to the manufacturer's instructions. Briefly, the transfected MDA-MB-231 cells (200  $\mu$ l; 5,000 cells per well) were collected, resuspended in DMEM without serum, and then added to the upper chamber containing Matrigel (50  $\mu$ l). DMEM medium (500  $\mu$ l) supplemented with 10% FBS was added to the lower chambers and incubated overnight at 37°C. The cells on the upper surface were scraped, whereas the invasive cells on the lower surface were fixed for 20 min at room temperature, then stained with 0.1% crystal violet for 10-15 min at room temperature. The migratory and invasive abilities were determined by counting the cells that migrated to the lower surface of the membrane. All cells were counted

under a light microscope (Carl Zeiss AG) at a magnification of x200 in  $\geq 5$  randomly selected fields in triplicate.

**Cytoplasmic and nuclear fractionation.** Cytoplasmic and nuclear fractionation was performed using a PARIS™ kit (Ambion; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. MDA-MB-231 or MCF-7 cells ( $1 \times 10^7$  cells/well) were collected and resuspended in cell fractionation buffer for cytoplasmic and nuclear fractionation, and then placed on ice for 10 min. After centrifugation with 5,000  $\times$  g for 30 sec at 4°C according to the manufacturer's instructions, the supernatant and nuclear pellet were separated using a cell disruption buffer to be preserved for RNA extraction.

**RT-qPCR analysis.** Total RNA from the human tissue samples or the cell lines was extracted using TRIzol® reagent (Takara Biotechnology Co., Ltd.) according to the manufacturer's protocol. Total RNA was reverse transcribed using HiScript® II RT SuperMix (Vazyme Biotech Co., Ltd.) according to the manufacturer's protocol. The sequences of primers used are listed in Table II. U6 and GAPDH were used as the control for miR-145-5p and TGFBR2, respectively. qPCR was performed in a Roche LightCycle480 II Real-Time PCR Detection System using SYBR Premix Ex Taq (cat. no. RR420A; Takara Bio, Inc.). OneStep PCR parameters for lncRNA and TGFBR2 quantification were as follows: 37°C for 60 min for RT, 10 min at 95°C and then 45 cycles of 20 sec at 95°C and 5 min at 72°C. All the measurements were conducted in triplicates. Quantitative mRNA data were normalized and presented as a ratio to GAPDH calculated using the  $2^{-\Delta\Delta C_q}$  method (13).

**Western blot analysis.** Since the premise of the lncRNA-miRNA-mRNA ceRNA regulatory network occurrence was in the cytoplasm, the separation of cytoplasmic and nuclear fractions was first performed to confirm that LINC00052 was expressed in the cytoplasm. Subsequently, the cytoplasm and nuclear fractions were isolated, and the proteins were extracted from the cytoplasm for further experimental verification of the ceRNA regulatory network. Briefly, cells transfected with miR-145-5p inhibitor and LINC00052 siRNAs, or transfected with miR-145-5p mimic were lysed in RIPA buffer [50 mM Tris (pH 7.4), 150 mM

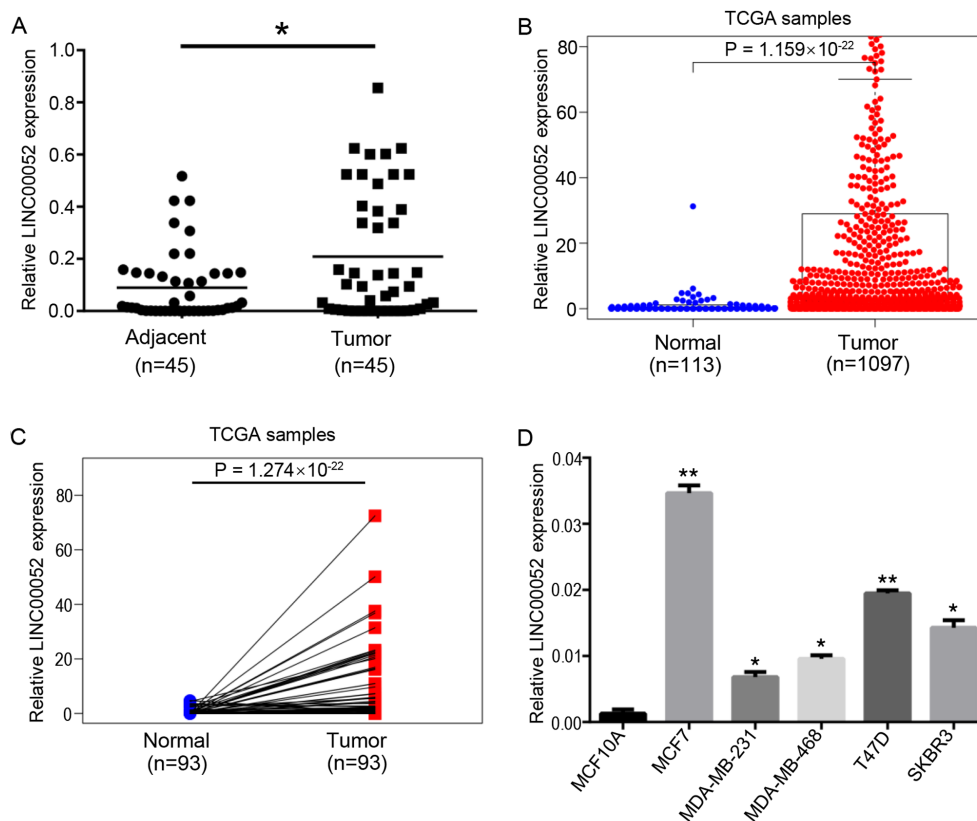


Figure 1. LINC00052 expression in breast cancer. (A) LINC00052 expression in clinical breast cancer tissues compared with in matched adjacent normal tissues (n=45). (B) LINC00052 expression in breast cancer and normal breast tissues was analyzed using TCGA database. (C) LINC00052 expression in 113 paired breast cancer samples and adjacent tissues from TCGA. (D) LINC00052 expression in breast cancer and normal breast cell lines was determined by reverse transcription-quantitative PCR analysis. \*P<0.05. \*\*P<0.01. TCGA, The Cancer Genome Atlas.

NaCl, 1 NP-40, 0.5% sodium deoxycholate; Beyotime Institute of Biotechnology] containing protease inhibitor cocktail (Abcam), followed by protein concentration detection using the Bradford protein assay. Next, 20  $\mu$ g proteins/lane were subjected to 10% SDS-PAGE and transferred onto nitrocellulose membranes (Merck KGaA). The membranes were blocked at 26°C in TBS-Tween-20 (0.1%) with 5% skimmed milk for 1 h, then incubated overnight with the TGFBR2 primary antibody (1:200; cat. no. ab184948) at 4°C. Blots were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:3,000; cat. no. ab150077; Abcam) and goat anti-mouse IgG (1:3,000; cat. no. ab150113; Abcam) secondary antibodies for 1 h at 26°C, then detected using an enhanced chemiluminescence kit (Thermo Fisher Scientific, Inc.), imaged using a Gel Doc XR+ imaging system (Bio-Rad Laboratories, Inc.) and analyzed using Image Lab software v3.0 (Bio-Rad Laboratories, Inc.). GAPDH was used as an internal reference. Mouse anti-GAPDH (1:1,000; cat. no. ab8245) and rabbit anti-TGFBR2 were purchased from Abcam.

**Statistical analysis.** Statistical analysis was performed using SPSS 17.0 (SPSS, Inc.). and the results are presented as the mean  $\pm$  SEM. The Kolmogorov-Smirnov test was used to assess the normality assumption of data distribution. A paired t-test (for adjacent vs. normal tissues) or the Mann-Whitney U test were used to compare the difference between two groups when applicable. Expression differences among three groups were analyzed by ANOVA followed by Tukey's post-hoc test.

P<0.05 was considered to indicate a statistically significant difference.

## Results

**LINC00052 expression is upregulated in BC.** In the present study, RT-qPCR was used to detect LINC00052 expression in 45 pairs of BC and adjacent normal breast tissues. Compared with in normal breast tissues, LINC00052 expression in BC tissues was significantly upregulated (Fig. 1A). Subsequently, LINC00052 expression in BC was analyzed using TCGA database. The expression levels of LINC00052 in BC tissues were significantly higher compared with those in normal tissues (Fig. 1B). In addition, the results from 113 pairs of cancer samples and adjacent tissues from TCGA demonstrated that LINC00052 expression in tumor tissues was significantly higher compared with that in paracancerous tissues (Fig. 1C). Similarly, higher expression levels of LINC00052 were observed in BC cell lines compared with that in the normal breast MCF10A cells (Fig. 1D).

**LINC00052 silencing inhibits BC cell proliferation, migration and invasion.** To further explore the role of LINC00052 in BC development, two LINC00052 siRNAs, named si-1 and si-2, were used. In the transfection experiment, si-1 and si-2 achieved good gene interference effects in MDA-MB-231 and MCF-7 cells (Fig. 2A). The CCK-8 assay demonstrated that, once LINC00052 was silenced, the proliferative ability of



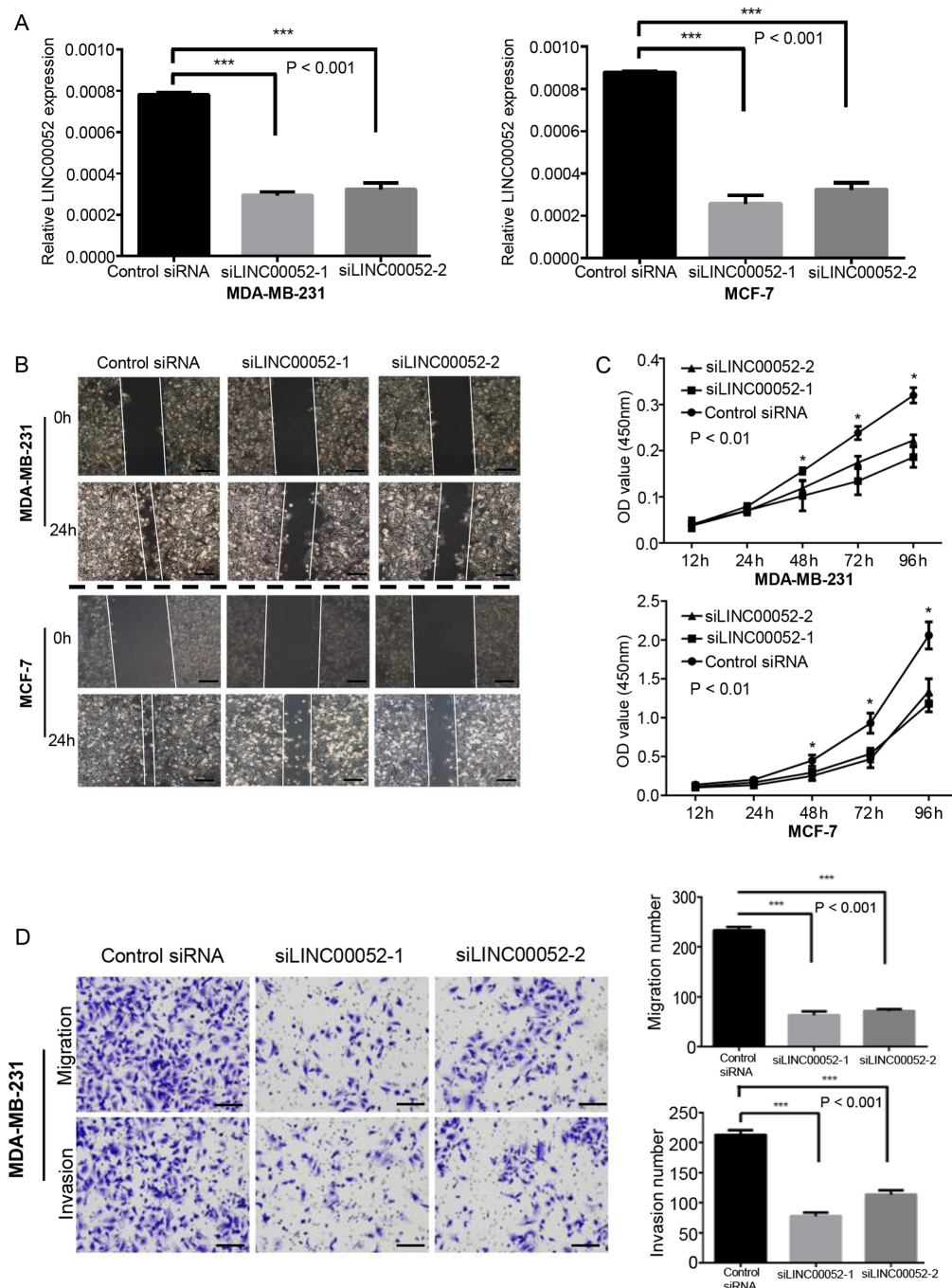


Figure 2. LINC00052-knockdown significantly inhibits cell proliferation, migration and invasion in breast cancer cells *in vitro*. (A) LINC00052 expression in MDA-MB-231 and MCF-7 cells transfected with control siRNA or siLINC00052-1/2 was determined by reverse transcription-quantitative PCR analysis. \*\*\*P<0.001. (B) Wound healing assays were used to evaluate the effects of LINC00052-knockdown on MDA-MB-231 and MCF-7 cell migration. Scale bar, 100  $\mu$ m. (C) The effect of LINC00052-knockdown on the proliferation of MDA-MB-231 and MCF-7 cells was examined using Cell Counting Kit-8 assays. \*P<0.05 vs. siLINC00052-1/2. (D) Transwell migration and invasion assays revealed that LINC00052-knockdown inhibited the migration and invasion of MDA-MB-231 cells. Scale bar, 100  $\mu$ m. \*\*\*P<0.001. siRNA, small interfering RNA; OD, optical density.

MDA-MB-231 and MCF-7 cells was significantly decreased, indicating that LINC00052 may serve an important role in promoting the proliferation of BC cells (Fig. 2B). The wound healing assay demonstrated that the migratory ability of MDA-MB-231 and MCF-7 cells was markedly lower compared with that in the control group following LINC00052 knockdown (Fig. 2C). The Transwell assay results revealed that the migration and invasion of MDA-MB-231 cells transfected with LINC00052 siRNA were significantly inhibited

(Fig. 2D). MCF-7 cells were also used for the Transwell assay, but after 48 h of observation, no cells had migrated to the lower chamber (data not shown). Overall, the present results suggested that LINC00052 may promote the proliferation, migration and invasion of BC cells.

**Cellular localization and target prediction of LINC00052.** According to previous studies, lncRNAs in the nucleus can function as scaffolds for proteins to form ribonucleoprotein

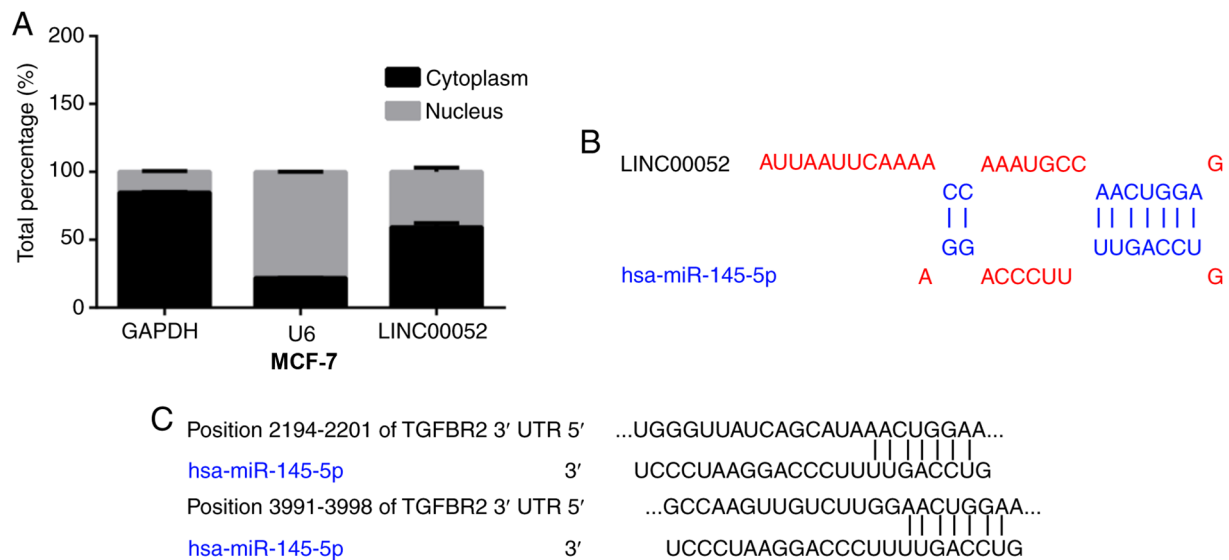


Figure 3. LINC00052 expression in both the cytoplasm and nucleus, the prediction of the binding sites between LINC00052 and miR-145-5p, and miR-145-5p and TGFBR2 (A) The subcellular cytoplasmic/nuclear location of LINC00052 varied, as demonstrated by reverse transcription-quantitative PCR analysis. (B) The binding sites between LINC00052 and miR-145-5p were predicted using the DIANA-LncBase database. (C) The binding sites between miR-145-5p and TGFBR2 were predicted using TargetScan bioinformatics database. TGFBR2, TGF- $\beta$  receptor II; miR, microRNA; UTR, untranslated region.

complexes and as guides to recruit chromatin-modifying complexes to target genes (14-16). In addition, lncRNAs in the cytoplasm may function as ceRNAs by competitively binding to miRNAs (17,18). Thus, the intracellular distribution of LINC00052 was investigated by cytoplasmic/nuclear localization experiments. GAPDH has always been considered as an enzyme that only distributes in the cytoplasm (19-21). Therefore, GAPDH was used as the internal reference for cytoplasmic and nuclear fractionation. The cytoplasmic and nuclear fractions were first isolated, and then the mRNA expression levels were detected by RT-qPCR. The results indicated that GAPDH RNA was relatively stable in the cytoplasm and U6 was relatively stable in the nucleus, while LINC00052 was expressed in both the cytoplasm and nucleus (Fig. 3A). In the present study, the bioinformatics prediction software TargetScan, was used to predict the target gene of miR-145-5p, and the binding sites between miR-145-5p and TGFBR2 were successfully predicted, revealing that there was >1 site of TGFBR2 binding to miR-145-5p (Fig. 3C). Furthermore, the binding site between LINC00052 and miR-145-5p was obtained using the DIANA-LncBase database (Fig. 3B). The aforementioned results provide theoretical evidence for the regulatory mechanism of action of LINC00052.

*miR-145-5p is involved in LINC00052 upregulating TGFBR2 expression in BC.* In order to elucidate whether miR-145-5p was involved in the regulation of TGFBR2 expression mediated by LINC00052, the mRNA and protein expression levels of TGFBR2 were detected by RT-qPCR and western blot analysis, respectively, after transfection. In the transfection experiment, miR-145-5p mimic and miR-145-5p inhibitor achieved good gene interference effects in MDA-MB-231 and MCF-7 cells (Fig. 4C and D). The results demonstrated that knockdown of LINC00052 in BC cells significantly decreased the mRNA (Fig. 4E and F) and protein (Fig. 4A and B) expression levels of TGFBR2, and significantly increased

the expression levels of miR-145-5p (Fig. 4E and F). However, knockdown of LINC00052 combined with knockdown of miR-145-5p in BC cells was associated with lower protein expression levels of TGFBR2 compared with those with miR-145-5p-knockdown alone (Fig. 4G and H). In addition, overexpression of miR-145-5p significantly decreased the protein expression levels of TGFBR2, and knockdown of miR-145-5p significantly increased the protein expression levels of TGFBR2 (Fig. 4G and H). These results suggested that LINC00052 may upregulate the expression levels of TGFBR2 by interacting with miR-145-5p.

## Discussion

Previous studies have established that lncRNAs serve a key role in tumorigenesis and tumor development (22-25). However, the potential functions and underlying mechanisms of these lncRNAs in BC remain unclear. A previous study reported low expression levels of LINC00052 in HCC, and that LINC00052 inhibited the migration and invasion of HCC cells by targeting NTRK3 and erythrocyte membrane protein band 4.1 like 3 (8). By contrast, Salameh *et al* (26) reported that LINC00052 expression was upregulated in BC and promoted tumor growth through HER3 signaling. The different mechanisms of action of LINC00052 in BC progression warrant further investigation. It has been reported that LINC00052 has an oncogenic function in gastric cancer, suggesting that LINC00052 may act as either an oncogene or a tumor suppressor, with its different functions depending on the different biological processes (9).

In the present study, it was observed that LINC00052 expression was upregulated in BC through TCGA database analysis and RT-qPCR analysis of 45 pairs of BC samples and normal tissues. In addition, RT-qPCR and western blot assays were performed to confirm the effects of LINC00052 on the mRNA and protein expression levels of TGFBR2. The present results revealed that LINC00052 regulated TGFBR2

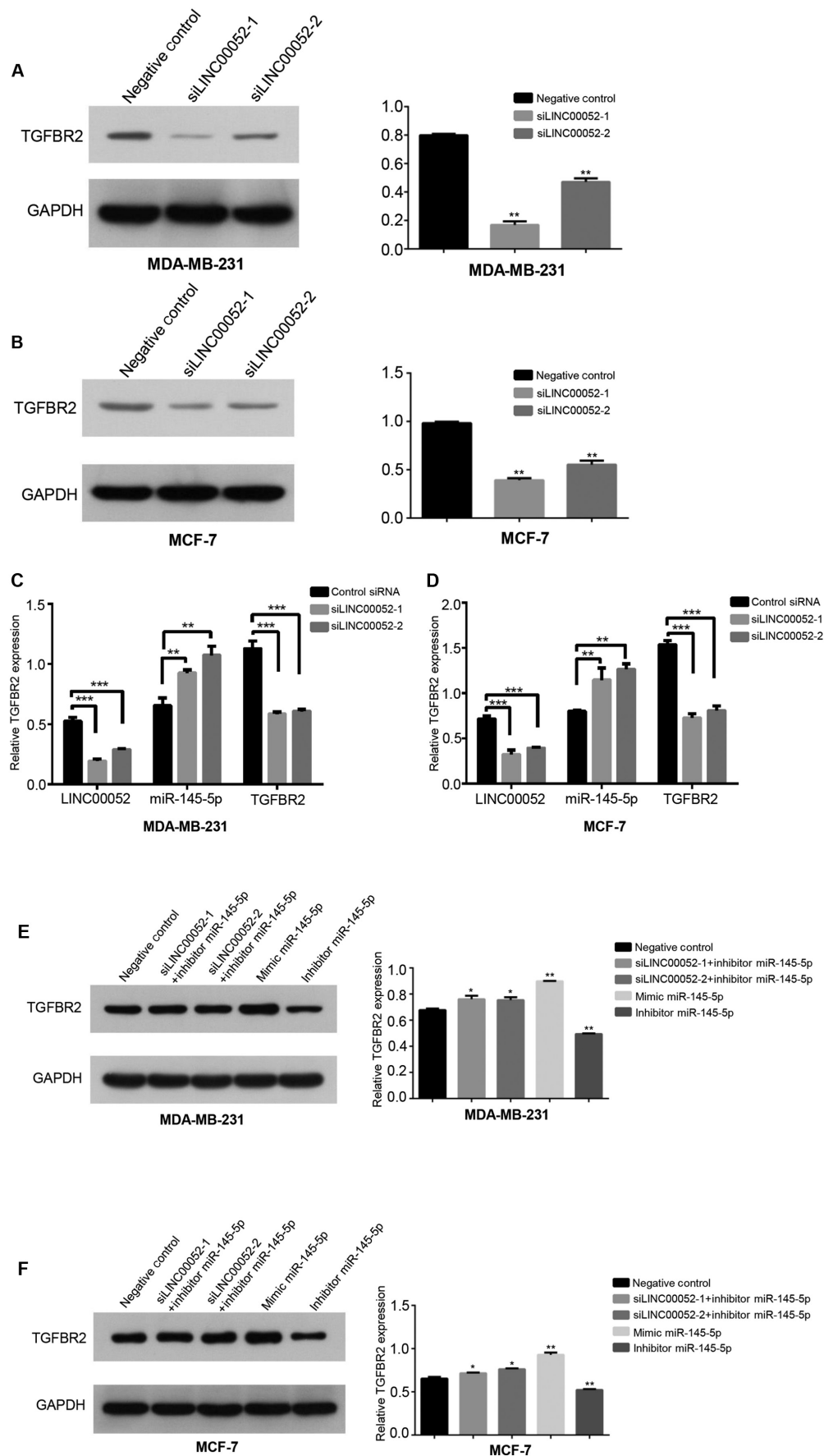


Figure 4. miR-145-5p is involved in the LINC00052-mediated regulation of TGFBR2 expression. TGFBR2 protein expression in (A) MDA-MB-231 and (B) MCF-7 cells was decreased following LINC00052 silencing. miR-145-5p expression in (C) MDA-MB-231 and (D) MCF-7 cells transfected with miR-145-5p mimic and miR-145-5p inhibitor was determined by reverse transcription-quantitative PCR analysis. mRNA expression levels of LINC00052, TGFBR2 and miR-145-5p in (E) MDA-MB-231 and (F) MCF-7 cells following LINC00052 silencing. TGFBR2 protein expression after LINC00052 and miR-145-5p silencing or miR-145-5p overexpression in (E) MDA-MB-231 and (F) MCF-7 cells. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . TGFBR2, TGF- $\beta$  receptor II; miR, microRNA.

expression. These results suggested that LINC00052 may be a factor promoting cell proliferation and metastasis by regulating TGFBR2 in BC. Additionally, the present study revealed that LINC00052 was differentially expressed in BC samples. Unlike previous studies (27-29), LINC00052 expression was upregulated in BC tissue samples compared with in normal breast tissues, possibly acting as a tumor promoter by enhancing cell proliferation and metastasis (25). The underlying reason may be that the overexpression or downregulation of lncRNAs differ among different types of tumor (30). In addition, a novel LINC00052/miR-145-5p/TGFBR2 regulatory axis in BC was identified, resulting in accelerated tumor progression.

TGF- $\beta$  is a secreted ligand that exerts its effects through a transmembrane heteromeric receptor complex, which consists of TGFBR1 and TGFBR2 (31). Aberrant TGF- $\beta$  signaling contributes to tumor metastasis and is a common finding in human cancer (32). Additionally, it has been reported that myeloid cells are recruited into mammary carcinomas with TGFBR2 deletion and directly promote tumor metastasis (33). It has been reported that the combination of TGFBR2 and TGF- $\beta$  can activate the downstream TGF- $\beta$ /Smad signaling pathway in lung cancer, inhibit c-myc expression, promote the expression levels of p21, p15 and other cell cycle inhibitors, block the cell cycle and promote the antitumor effect of the TGF- $\beta$ /Smad signaling pathway (34). A previous study revealed that TGFBR2 could activate the MAPK signaling pathway to promote the proliferation of tumor cells by binding to growth factor receptor-bound protein 2 (Grb2). However, when the binding site of TGFBR2 and Grb2 mutates, TGFBR2 and Grb2 cannot bind, leading to the failure to activate the MAPK signaling pathway, which makes TGFBR2 lose its role in promoting tumor cell proliferation (35). The present study further investigated whether LINC00052 could alter miRNA expression and affect TGFBR2 expression. Using online softwares and RT-qPCR analysis, miR-145-5p was identified as a potential regulatory gene of TGFBR2. miR-145-5p, a putative tumor suppressor, is downregulated in a variety of tumors, including endometrial cancer, laryngeal carcinoma, gastric cancer, pancreatic adenocarcinoma and ovarian cancer (36,37). A recent study has demonstrated that decreased miR-145-5p expression caused by promoter methylation is a prognostic factor for endometrial carcinoma (36). Restoration of miR-145-5p expression results in decreased cell proliferation by targeting fascin actin-bundling protein 1 in laryngeal carcinoma (38). Additionally, miR-145-5p delivered from exosomes has been found to repress the growth and metastasis of pancreatic adenocarcinoma and ovarian cancer cells (36,39). Furthermore, miR-145-5p was found to act as a tumor suppressor by targeting ZEB2 and N-cadherin in gastric cancer (40). The aforementioned studies suggest that miR-145-5p may be a potential therapeutic target in cancer.

Although miR-145-5p has been proven to target a number of protein-coding genes (41,42), the present study demonstrated that miR-145-5p targeted TGFBR2 and LINC00052. First, the DIANA-LncBase database was used to predict the interaction between miRNAs and LINC00052, and the binding site between LINC00052 and miR-145-5p was found; similarly, the binding sites for miR-145-5p and TGFBR2 were also successfully predicted by TargetScan. In addition, inhibition of LINC00052 expression induced upregulation

of miR-145-5p expression and downregulation of TGFBR2 expression, indicating that LINC00052 may exert a negative regulatory effect on miR-145-5p and a positive regulatory effect on TGFBR2. miRNAs have been found to be involved in the regulation of gene expression and serve an important role in a series of basic cellular processes, such as differentiation, invasion, migration, proliferation and apoptosis (43,44), relying on the complementarities between the limited region of sequence at the 5'-end of the miRNA (seed) and the 3'-UTR of specific target mRNAs (44). Bioinformatics analysis revealed that TGFBR2 was targeted by miR-145-5p. Overexpression of miR-145-5p decreased TGFBR2 expression, whereas the inhibition of miR-145-5p increased TGFBR2 expression. Furthermore, whether LINC00052 can promote the proliferation and metastasis of BC cells was investigated. The present results indicated that LINC00052-knockdown significantly inhibited the proliferation and migration of BC cells. Therefore, the results of the present study highlight the importance of the interaction between miRNAs and lncRNAs in BC. In order to elucidate whether miR-145-5p was involved in LINC00052-mediated regulation of TGFBR2 expression, transfection combinations were performed. The results revealed that LINC00052-knockdown or overexpression of miR-145-5p significantly decreased the expression levels of TGFBR2. However, when LINC00052-knockdown was combined with miR-145-5p inhibition, the decrease of TGFBR2 expression caused by LINC00052-knockdown was rescued, suggesting that miR-145-5p may be involved in LINC00052-mediated TGFBR2 expression regulation. The present results may provide new insights into the molecular mechanisms interconnecting LINC00052, TGFBR2 and miR-145-5p.

The present study suggested that LINC00052 may promote cancer progression in BC by regulating miR-145-5p and TGFBR2. To the best of our knowledge, this regulatory pathway has not been reported in BC, which provides a new direction to find novel therapeutic targets for BC. However, the present study presents some limitations. The study was not able to verify the effect of LINC00052 inhibition on the migration and invasion of MCF-7 cells using a Transwell assay, as the MCF-7 cells did not have invasion ability, and the study also lacked *in vivo* xenograft tumor experiments and dual-luciferase reporter assays, which are required in future experiments.

In conclusion, the preliminary results of the present study confirmed that LINC00052 expression was upregulated in BC and that it may competitively bind to miR-145-5p to upregulate TGFBR2 expression, thereby serving a key role in promoting BC.

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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

MD conceived the study. MD and HL designed the study. MD and TX performed the experiments. MD analyzed the data and wrote the manuscript. HL and TX confirm the authenticity of all the raw data. XL made substantial contributions to the conception of the study, secured funding and supervised the study. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

All procedures involving human participants in the present study were approved by the Ethics Committee of Tongji Hospital at Tongji Medical College of Huazhong Science and Technology University (Wuhan, China), and written informed consent from each participant was obtained. The procedures followed the ethical standards of the responsible committee on human experimentation (institutional and national).

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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