LINC00514 upregulates CCDC71L to Promote Cellular Process in Triple-Negative Breast Cancer by Sponging miR-6504-5p and miR-3139

Xiao Luo
China-Japan Union Hospital of Jilin University
Hui Wang (whui66@jlu.edu.cn)
China-Japan Union Hospital of Jilin University https://orcid.org/0000-0002-1608-3782

Primary research

Keywords: LINC00514, miR-6504-5p, miR-3139, CCDC71L, TNBC

Posted Date: December 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-120260/v1

Version of Record: A version of this preprint was published on March 23rd, 2021. See the published version at https://doi.org/10.1186/s12935-021-01875-2.

Abstract

Background: Long noncoding RNAs (lncRNAs) have recently identified as essential gene modulators in numerous cancers. Previous studies have confirmed the oncogenic role of long intergenic nonprotein-coding RNA 00514 (*LINC00514*) in some cancers. Nevertheless, its biological function and mechanism remain elusive in triple-negative breast cancer (TNBC).

Methods*:* Herein, we detected *LINC00514 expression level in TNBC tissues and cells via RT-qPCR. The function of LINC00514 in TNBC cellular activities was assessed via colony formation, EdU, wound healing, transwell assays and flow cytometry analysis.*

Results: *The binding between* miR-6504-5p/miR-3139 and LINC00514/CCDC71L was validated by luciferase reporter assay. The data indicated that *LINC00514 expression was* increased in TNBC tissues and cells. Furthermore, it was manifested that silenced *LINC00514 restrained cell proliferative, migratory and invasive abilities and accelerated cell apoptosis.* In mechanism, *LINC00514 was revealed to sequester* miR-6504-5p and miR-3139 in TNBC cells. Furthermore, the low level of miR-6504-5p and miR-3139 was discovered in TNBC tissues and cells. Accordingly, we discovered overexpression of miR-6504-5p or miR-3139 retarded cell growth and migration in TNBC. Later, CCDC71L was recognized as a common downstream gene of miR-6504-5p and miR-3139. Rescue assay verified that overexpressed CCDC71L countervailed the inhibitive influence of *LINC00514 knockdown in TNBC cellular process.*

Conclusion: MiR-6504-5p and miR-3139 were involved in *LINC00514*-mediated cellular activities through regulating CCDC71L expression, which provided a novel *LINC00514*/miR-6504-5p/miR-3139/CCDC71L axis in TNBC.

Introduction

Breast cancer (BC) has been commonly acknowledged as a predominant type of cancer among women globally (1). It has various subtypes with multiple clinical outcomes and diverse biological behaviors because of the heterogeneous property (2). Among which, triple-negative breast cancer (TNBC) was an aggressive subtype losing Her2 amplification, progesterone receptor and estrogen receptor, and accounted for about 10–25% of total BC cases (3, 4). Additionally, TNBC patients presented worse clinical outcome, higher incidence and higher risk of distant metastasis (5, 6). Thus, it was quite urgent to probe the novel biomarkers and potential molecular mechanisms underlying TNBC progression.

As a group of RNAs, long noncoding RNA (lncRNA) is lack of protein translation abilities and comprises over 200 nucleotides (7). Abnormally expressed lncRNAs were commonly discovered in multiple cancer types and their dysregulation were widely reported in numerous cellular activities, like cancer initiation, apoptosis, migration and metastasis (8). Formerly, lncRNA SNHG1 was reported to highly express in non-small-cell lung cancer and aggravated proliferative and invasive capacities of cells (9). Downregulation of BLACAT1 suppressed cell proliferation and arrested cell cycle at G0/G1 phase in cervical cancer (10). Besides, some lncRNAs have been recognized to play critical roles in TNBC (11, 12). For example, lncRNA

MIR100HG was identified as oncogenic gene by promoting cell proliferation in TNBC (13). LncRNA linc-ZNF469-3 was upregulated and enhanced lung metastasis in TNBC (14). Therefore, looking for the new biomarkers from lncRNAs could be a prospective treatment choice for the therapy of TNBC.

It was well known that lncRNAs acted as biological regulators through a variety of mechanisms (15), such as regulating alternative splicing (16), modifying chromosome (17), and sponging microRNA (miRNA) (18). Long intergenic nonprotein-coding RNA 00514 (LINC00514) was a newly identified lncRNA, which could exert functions in cancers. Nowadays, great attention has been attracted on LINC00514 due to its critical role in the malignancy of papillary thyroid cancer (19) and the progression of osteosarcoma (20). Nevertheless, the detail function and regulatory mechanism of LINC00514 in TNBC were highly unclear. In this study, we high expression of LINC00514 was found in TNBC tissues and cells, and LINC00514 played oncogenic role in TNBC via miR-6504-5p/miR-3139/CCDC71L axis.

Materials And Methods

Tissue samples

TNBC samples and corresponding adjacent samples were collected from Fifty-two TNBC patients at China-Japan Union Hospital of Jilin University（Jilin, China). Informed consents were signed by each patient. Prior to operation, no patients suffered radiotherapy or chemotherapy. After collecting, each sample was instantly preserved at -80°C in liquid nitrogen for further use. The use of tissues was obtained the approval from the Ethical Review Board of China-Japan Union Hospital of Jilin University （Jilin, China).

Cell lines

Human normal breast epithelial cell (MCF-10A), human TNBC cell lines (MDA-MB-468, HCC1937, MDA-MB-436, MDA-MB-231) and HEK293T cell line were all commercially provided by ATCC, and maintained in an incubator containing 5% CO_2 at 37°C. For cell culture, DMEM medium (Thermo Fisher Scientific, CA, USA) supplementing 10% FBS and 1% Pen/Strep solution was applied.

Cell transfection

The specific shRNAs targeting LINC00514 (sh-LINC00514#1/2) were obtained from GenePharma Company to silence LINC00514 via utilizing Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) with sh-NC as control. The pcDNA3.1/LINC00514, pcDNA3.1/CCDC71L and empty NC vector, and miR-6504-5p/miR-3139 mimics/inhibitor and NC mimics/inhibitor, were all provided by GenePharma Company. Cell samples were reaped after 48 h.

RT-qPCR

The extraction of total RNAs from tissues and cells was conducted by TRIzol reagent (Invitrogen) with a RecoverAll™ Total Nucleic Acid Isolation kit (Ambion). Utilizing the Prime Script™ RT reagent kit (Takara,

Dalian, China), reverse transcription reactions were performed. Later, the employment of SYBR Premix Ex Taq (Takara Bio, Shiga, Japan) was for RT-qPCR on StepOnePlus System (Applied Biosystems). Based on $2^{-\Delta\Delta CT}$ method, gene expressions were calculated with normalization to GAPDH or U6.

Colony formation assay

Transfected TNBC cells were planted to 6-well plates, and each well was filled with 5×10^3 cells. After two weeks, cells were fixed by 5% paraformaldehyde, and then 0.1% crystal violet solution was supplemented for staining. Finally, colonies (more than 50 cells) were counted and recorded.

EdU assay

For the measurement of cell proliferation, Edu assay kit (RiboBio, China) was used. In brief, TNBC cells in each group with treatment of EdU were stained by DAPI. Under fluorescence microscope (Nikon, Japan), visualized images of EdU-positive cells were obtained.

Flow cytometry analysis

TNBC cells were planted in 6-well plates and then rinsed in PBS. Later, 1 μL of PI (Invitrogen) and 2.5 μL Annexin V conjugated with FITC were added into binding buffer. Subsequently, the binding buffer was used to resuspend the cells after trypsinization. After 15 minutes, apoptotic TNBC cells were identified by flow cytometry (BD Biosciences).

Western blot analysis

By RIPA buffer (Thermo Fisher Scientific), proteins of TNBC cells were lysed, and then protein concentration was confirmed by BCA-kit (Beyotime, Shanghai, China). Separated by SDS-PAGE, proteins were transferred to PVDF membranes. Then, the membranes were blocked in 5% skim milk and incubated with primary antibodies (Abcam, Cambridge, MA) overnight at 4 °C. Washed by 0.1% TBST in triple, the membranes were incubated with secondary antibody at 37 °C for an hour. The loading control was GAPDH. The results were analyzed and visualized by ECL detection reagent (GE Healthcare, Chicago, IL).

Wound healing assay

To make cells adhere, TNBC cells were cultured in 96-well plates all night with each well filled by 5×10^4 cells. Later, sterile pipette tip was used to scratch the wounds. After 24 h, wounds were imaged following washing in PBS.

Transwell invasion assay

Transfected TNBC cells were reaped and placed into the upper transwell chamber (8 μm pores) coated with Matrigel (BD Biosciences). The serum-free medium was placed in the upper chambers, while the lower chamber was supplemented with medium containing 10% FBS. Incubated for 24 h, cells were fixed and stained. Then, an inverted microscope was used for the counting of invasive cells.

Subcellular fractionation assay

PARIS Kit (Invitrogen) was used to isolated nuclear and cytoplasmic RNA in TNBC cells. Then, the extraction of subcellular fractions was carried out. Later, the fractions were subjected to RT-qPCR with normalization to GAPDH (cytoplasm control) or U6 (nucleus control).

RNA pull down assay

LINC00514 and NC-lncRNA labeled with biotin were transfected into MDA-MB-468 and HCC1937 cells. The lysates of TNBC cells were used for conducting an incubation with streptavidin magnetic beads for 4 h at 4 °C. Subsequently, precooled lysis buffer and salt buffer was applied to rinse the beads. With the extraction of pull-down RNAs, the levels of miRNAs binding to LINC00514 were detected.

Luciferase reporter assay

LINC00514-WT/Mut and CCDC71L-WT/Mut vectors were separately constructed by cloning wild type (WT) and mutant (Mut) miR-6504-5p or miR-3139 binding site in LINC00514 sequence or CCDC71L 3'-UTR to pmirGLO (Promega) vectors. Then, above luciferase vectors were transfected with miR-6504-5p mimics, miR-3139 mimics or NC mimics into TNBC cells for 48 h. Finally, luciferase activity was examined with Dual Luciferase Assay System (Promega).

Statistical analysis

Through using GraphPad Prism 6 (GraphPad), data comparison between or over two groups were statistically analyzed by Student's t test or one-way ANOVA. Three biological repeats were included in all experimental procedures with results presenting as mean ± SD. The analysis of correlation between genes was conducted by Spearman's correlation analysis. P less than 0.05 was considered as cut-off value.

Results

LINC00514 was an upregulated lncRNA in TNBC

Firstly, we analyzed LINC00514 expression profile in TNBC tissues and cell lines by RT-qPCR. Compared with corresponding adjacent tissues, LINC00514 expression was significantly increased in TNBC tissues (Fig. 1A). Importantly, LINC00514 expression was higher in patients at advanced stage (III-IV stage) than that in early stage (I-II stage) (Fig. 1B). In addition, upregulation of LINC00514 was found in TNBC cell lines with comparison of MCF-10A cell line (Fig. 1C). Taken together, LINC00514 was highly expressed in TNBC tissues and cell lines.

LINC00514 expedited TNBC cellular process

Considering aberrant LINC00514 expression in TNBC, we then explored its biological function in TNBC via conducting loss-of-function assays. MDA-MB-468 and HCC1937 cells, which presenting higher

LINC00514 expression, was used for the investigation. At first, LINC00514 expression was stably silenced by transfecting sh-LINC00514#1/2 in MDA-MB-468 and HCC1937 cells (Fig. 2A). Then, colony formation assay showed that cell proliferation was repressed by LINC00514 downregulation (Fig. 2B). Consistently, the same result was observed in EdU assay (Fig. 2C). However, the apoptosis rate was promoted in LINC00514-silenced TNBC cells through flow cytometry analysis (Fig. 2D). Western blot manifested the upregulation of Bax protein level and the downregulation of Bcl-2 protein level in sh-LINC00514 group (Fig. 2E). According to wound healing assay, cell migration was suppressed by silencing LINC00514 (Fig. 2F). Likewise, LINC00514 knockdown inhibited cell invasion via the result of transwell assay (Fig. 2G). Overall, LINC00514 accelerated cell proliferation, migration and invasion, and retarded cell apoptosis in TNBC.

LINC00514 sponged miR-6504-5p and miR-3139 in TNBC

Thereafter, we interrogated the molecular mechanism of LINC00514 that regulated TNBC cellular process. Firstly, we conducted subcellular fractionation assay, discovering that LINC00514 was chiefly distributed in the cytoplasm (Fig. 3A). Extensive documents suggested cytoplasmic lncRNA as a competing endogenous RNA (ceRNA) in cancers (21, 22). Therefore, we hypothesized that LINC00514 might act as a ceRNA through sequestering some specific miRNAs to mediate TNBC progression. Then, we searched the potential miRNAs for LINC00514 by starBase (http://starbase.sysu.edu.cn/). As a result, 8 miRNAs were screened out (Fig. 3B). By performing RNA pull-down assay, miR-6504-5p and miR-3139 were observed to be pulled down by LINC00514 biotin probe compared with Bio-NC group (Fig. 3C). Thus, we speculated miR-6504-5p and miR-3139 as potential miRNAs for LINC00514 in TNBC. As shown in Fig. 3D, binding site of miR-6504-5p or miR-3139 in LINC00514 sequence (LINC00514-WT) and the mutant site (LINC00514-Mut) were presented. Through RT-qPCR, we found miR-6504-5p or miR-3139 expression was significantly increased by respectively transfecting miR-6504-5p mimics or miR-3139 mimics (Fig. 3E). Luciferase reporter assay demonstrated that the luciferase activity of LINC00514-WT reporter was decreased by the overexpression of miR-6504-5p or miR-3139, while no significant change was found in that of LINC00514-Mut reporter (Fig. 3F). This revealed that both miR-6504-5p and miR-3139 could interact with LINC00514. Moreover, levels of miR-6504-5p and miR-3139 were validated to be low in TNBC tissues (Fig. 3G). Importantly, the expression of LINC00514 was negatively associated with that of miR-6504-5p or miR-3139 (Fig. 3H). Data above confirmed that LINC00514 served as sponge of miR-6504-5p and miR-3139 in TNBC.

MiR-6504-5p and miR-3139 were lowly expressed in TNBC and inhibited cellular process

Subsequently, we explored the expression pattern and biological functions of miR-6504-5p and miR-3139 in TNBC cells. Results of RT-qPCR depicted that miR-6504-5p and miR-3139 both expressed at a low level in TNBC cell lines (Fig. 4A). Subsequently, some functional assays were carried out to identify functional role of miR-6504-5p and miR-3139 in TNBC cell growth and migration. Based on results of colony formation and EdU assays, we found miR-6504-5p mimics and miR-3139 mimics inhibited cell proliferation (Fig. 4B-C). As shown in Fig. 4D, the apoptosis of TNBC cells was enhanced via

overexpressed miR-6504-5p or miR-3139. In addition, levels of apoptosis-relevant proteins (Bax and Bl-2) in cells with transfection of miR-6504-5p mimics or miR-3139 mimics were tested. The results indicated that upregulation of miR-6504-5p or miR-3139 increased Bax protein level and reduced Bcl-2 protein level (Fig. 4E). Through wound healing assay, overexpression of miR-6504-5p or miR-3139 remarkably suppressed cell migratory capability (Fig. 4F). Furthermore, transwell assay confirmed the inhibitive role of miR-6504-5p or miR-3139 overexpression in TNBC cell invasion (Fig. 4G). Conclusively, miR-6504-5p and miR-3139 retarded cell growth and migration in TNBC.

CCDC71L was a common target of miR-6504-5p and miR-3139

To further support ceRNA hypothesis, the downstream genes of miR-6504-5p and miR-3139 were explored. By using starBase, two potential mRNAs were found (Fig. 5A). Then, the expression of these two genes in TNBC cells were testified. As observed, CCDC71L was highly expressed in TNBC cells, while that of AGO1 did not exhibit expression difference (Fig. 5B). Subsequently, we found the binding site between CCDC71L and miR-6504-5p or miR-3139 to construct CCDC71L-WT, and mutated the sites to construct CCDC71L-Mut (Fig. 5C). After miR-6504-5p mimics or miR-3139 mimics transfection, the luciferase activity of CCDC71L-WT reporter was considerably weakened, while that of CCDC71L-Mut reporter remain unchanged (Fig. 5D). Additionally, LINC00514 was verified to overexpress in TNBC cells by pcDNA3.1/LINC00514 (Fig. 5E). Characterized by easy transfection, HEK293T cells were used for further conducting luciferase reporter assay. Data indicated that overexpressed LINC00514 counteracted luciferase activity of CCDC71L-WT that was inhibited by overexpressing miR-6504-5p or miR-3139 while CCDC71L-Mut luciferase activity was not affected (Fig. 5F). Then, we confirmed that miR-6504-5p inhibitor and miR-3139 inhibitor apparently decreased miR-6504-5p and miR-3139 expression, separately, in TNBC cells (Fig. 5G). At last, we found that CCDC71L mRNA and protein levels decreased by silenced LINC00514 were partially restored by inhibiting miR-6504-5p, and the co-transfection of miR-6504-5p inhibitor and miR-3139 inhibitor nearly fully reserved the function of LINC00514 knockdown (Fig. 5H-I). Namely, LINC00514 increased CCDC71L expression via sponging miR-6504-5p and miR-3139 in TNBC.

LINC00514 boosted TNBC cellular activities by upregulating CCDC71L

For further analyzing whether LINC00514 played oncogenic role in TNBC by mediating CCDC71L, some restoration experiments were conducted in MDA-MB-468 and HCC1937 cells. CCDC71L expression was upregulated in TNBC cells (Fig. 6A). As demonstrated, proliferative ability of TNBC cells inhibited by LINC00514 silencing was reversed via overexpressing CCDC71L (Fig. 6B-C). Besides, the elevated cell apoptosis caused by LINC00514 knockdown was counteracted by CCDC71L overexpression (Fig. 6D). Consistently, overexpressed CCDC71L countervailed the effect of LINC00514 deficiency on apoptosis-related proteins levels (Fig. 6E). Meanwhile, LINC00514 knockdown-mediated inhibition on the migratory ability of TNBC cell was recovered by upregulating CCDC71L (Fig. 6F). At last, upregulated CCDC71L offset the suppressive role of sh-LINC00514 transfection in cell invasion (Fig. 6G). Hence, we validated that LINC00514 expedited cell proliferation, suppressed cell apoptosis, and accelerated cell migration and invasion via increasing CCDC71L expression in TNBC.

Discussion

In recent decades, extensive documents have strongly supported the participation of lncRNAs in the pathogenesis and development of human cancers (23–25). As an aggressive subtype of BC, the progression of TNBC was attributed to numerous oncogenes and anti-oncogenes (26, 27). To promote TNBC therapy efficacies and develop novel TNBC treatments, it was critical to fully understand the molecular events, especially lncRNA (14, 28). Previous studies demonstrated that LINC00514 accelerated cell proliferation and invasion in vitro and aggravated tumor growth by targeting miR-204-3p/CDC23 axis in papillary thyroid cancer (19). In osteosarcoma, LINC00514 increased URGCP expression to promote cell cycle and suppress cell apoptosis via sponging miR-708 (20). However, the biological role of LINC00514 was still poorly understood in TNBC. Herein, this study was the first to reveal that LINC00514 exhibited a high level in TNBC tissues and cells, especially in the tissues of patients at advanced stage. Loss-of-function assay manifested that LINC00514 knockdown restrained cell proliferation, induced cell apoptosis, weakened cell migration and invasion. These findings suggested LINC00514 as an oncogenic lncRNA in TNBC.

The mechanisms of lncRNAs have not been fully explored; nevertheless, emerging reports indicated that lncRNA served as ceRNA to release miRNA targets by sequestering miRNA (29). To investigate the underlying regulatory mechanism of LINC00514-mediated cancer development, we conducted in silico studies to research putative miRNA for LINC00514. Through bioinformatics analysis and RNA pull down assay, miR-6504-5p and miR-3139 were predicted to be potential miRNAs. And it was supposed that miR-6504-5p and miR-3139 had the binding sites on LINC00514 sequence. Further, luciferase reporter assay confirmed the binding of LINC00514 to miR-6504-5p and miR-3139. In addition, we found that miR-6504-5p and miR-3139 were both lowly expressed in TNBC tissues and their expressions were negatively associated with that of LINC00514. Therefore, we supposed that LINC00514 interacted with miR-6504-5p and miR-3139 in TNBC.

As another class of noncoding RNA, miRNAs only contained 20–24 nucleotides and exerted critical effects on tumorigenesis and progression (30, 31). MiR-6504-5p and miR-3139 are novel miRNAs whose functions and mechanism have not been depicted in cancers, especially in TNBC. In this study, miR-6504-5p and miR-3139 were both found to be downregulated in TNBC cell lines, and overexpression of miR-6504-5p or miR-3139 significantly inhibited cell proliferation, migration, invasion and contributed to cell apoptosis. This confirmed the anti-oncogenic property of miR-6504-5p and miR-3139 in TNBC. Previously, increasing researched validated that miRNAs could directly bind to mRNA 3'-UTR to post-transcriptionally regulate mRNA translation or degradation (32). Our present study identified CCDC71L, a novel mRNA which has not been explored in cancers, as the common target of miR-6504-5p and miR-3139. Importantly, we discovered that the inhibitive effect of silenced LINC00514 on CCDC71L mRNA and protein levels was partially restored by miR-6504-5p inhibition, but fully rescued by co-inhibition of miR-6504-5p and miR-3139. Rescue assays demonstrated that CCDC71L overexpression counteracted LINC00514 silencing-mediated suppression on TNBC cellular process. Therefore, it was suggested that LINC00514 regulate CCDC71L expression via sponging miR-6504-5p and miR-3139.

In conclusion, our study for the first time uncovered the functional role and molecular mechanism of LINC00514 in TNCB, and discovered that LINC00514 acted as sponge of miR-6504-5p and miR-3139 to increase CCDC71L expression, thereby promoting TNBC cellular process. This finding might be helpful for the further exploration of new TNBC therapy.

Declarations

Funding

Not applicable.

Authors' contributions

Both two co-authors participated in the literature search, analysis and interpretation of the data, and the writing of the manuscript. All authors saw and approved the final manuscript.

Acknowledgments

Not applicable.

Competing Interests

The authors have declared that no competing interest exists.

Ethics approval and consent to participate

The use of tissues was obtained the approval from the Ethical Review Board of China-Japan Union Hospital of Jilin University（Jilin, China).

Consent for publication

Not applicable.

Availability of data and materials

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

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, et al. Global cancer statistics, 2012. Cancer J Clin. 2015;65(2):87–108.
2. Kennecke H, Yerushalmi R, Woods R, Cheang MC, Voduc D, et al. Metastatic behavior of breast cancer subtypes. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2010;28(20):3271–7.

3. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. N Engl J Med. 2010;363(20):1938–48.
4. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. Jama. 2006;295(21):2492–502.
5. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clinical cancer research: an official journal of the American Association for Cancer Research. 2007;13(15 Pt 1):4429–34.
6. Yin WJ, Lu JS, Di GH, Lin YP, Zhou LH, et al. Clinicopathological features of the triple-negative tumors in Chinese breast cancer patients. Breast cancer research treatment. 2009;115(2):325–33.
7. Camacho CV, Choudhari R, Gadad SS. Long noncoding RNAs and cancer, an overview. Steroids. 2018;133:93–5.
8. Lin C, Yang L. Long Noncoding RNA in Cancer: Wiring Signaling Circuitry. Trends in cell biology. 2018;28(4):287–301.
9. Lu Q, Shan S, Li Y, Zhu D, Jin W, et al. Long noncoding RNA SNHG1 promotes non-small cell lung cancer progression by up-regulating MTDH via sponging miR-145-5p. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2018;32(7):3957–67.
10. Shan D, Shang Y, Hu T. Long noncoding RNA BLACAT1 promotes cell proliferation and invasion in human cervical cancer. Oncology letters. 2018;15(3):3490–5.
11. Liu L, Yu D, Shi H, Li J, Meng L. Reduced lncRNA Aim enhances the malignant invasion of triple-negative breast cancer cells mainly by activating Wnt/β-catenin/mTOR/PI3K signaling. Pharmazie. 2017;72(10):599–603.
12. Wang L, Liu D, Wu X, Zeng Y, Li L, et al. Long non-coding RNA (LncRNA) RMST in triple-negative breast cancer (TNBC): Expression analysis and biological roles research. Journal of cellular physiology. 2018;233(10):6603–12.
13. Wang S, Ke H, Zhang H, Ma Y, Ao L, et al. LncRNA MIR100HG promotes cell proliferation in triple-negative breast cancer through triplex formation with p27 loci. Cell death disease. 2018;9(8):805.
14. Wang PS, Chou CH, Lin CH, Yao YC, Cheng HC, et al. A novel long non-coding RNA linc-ZNF469-3 promotes lung metastasis through miR-574-5p-ZEB1 axis in triple negative breast cancer. Oncogene. 2018;37(34):4662–78.
15. Kopp F, Mendell JT. Functional Classification and Experimental Dissection of Long Noncoding RNAs. Cell. 2018;172(3):393–407.
16. Singh R, Gupta SC, Peng WX, Zhou N, Pochampally R, et al. Regulation of alternative splicing of Bcl-x by BC200 contributes to breast cancer pathogenesis. Cell death disease. 2016;7(6):e2262.
17. Chen Q, Cai J, Wang Q, Wang Y, Liu M, et al. Long Noncoding RNA NEAT1, Regulated by the EGFR Pathway, Contributes to Glioblastoma Progression Through the WNT/β-Catenin Pathway by Scaffolding EZH2. Clinical cancer research: an official journal of the American Association for Cancer Research. 2018;24(3):684–95.

18. Dey BK, Mueller AC, Dutta A. Long non-coding RNAs as emerging regulators of differentiation, development, and disease. Transcription. 2014;5(4):e944014.
19. Li X, Zhong W, Xu Y, Yu B, Liu H. Silencing of lncRNA LINC00514 inhibits the malignant behaviors of papillary thyroid cancer through miR-204-3p/CDC23 axis. Biochem Biophys Res Commun. 2019;508(4):1145–8.
20. Yu D, Xu X, Li S, Zhang K. LINC00514 drives osteosarcoma progression through sponging microRNA-708 and consequently increases URGCP expression. Aging. 2020;12(8):6793–807.
21. Ye Y, Gu B, Wang Y, Shen S, Huang W. E2F1-mediated MNX1-AS1-miR-218-5p-SEC61A1 feedback loop contributes to the progression of colon adenocarcinoma. Journal of cellular biochemistry. 2019;120(4):6145–53.
22. Feng K, Liu Y, Xu LJ, Zhao LF, Jia CW, et al. Long noncoding RNA PVT1 enhances the viability and invasion of papillary thyroid carcinoma cells by functioning as ceRNA of microRNA-30a through mediating expression of insulin like growth factor 1 receptor. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2018;104:686 – 98.
23. Chai L, Yuan Y, Chen C, Zhou J, Wu Y. The role of long non-coding RNA ANRIL in the carcinogenesis of oral cancer by targeting miR-125a. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2018;103:38–45.
24. Wu J, Shuang Z, Zhao J, Tang H, Liu P, et al. Linc00152 promotes tumorigenesis by regulating DNMTs in triple-negative breast cancer. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2018;97:1275-81.
25. Xu Y, Yao Y, Jiang X, Zhong X, Wang Z, et al. SP1-induced upregulation of lncRNA SPRY4-IT1 exerts oncogenic properties by scaffolding EZH2/LSD1/DNMT1 and sponging miR-101-3p in cholangiocarcinoma. Journal of experimental clinical cancer research: CR. 2018;37(1):81.
26. Papa A, Caruso D, Tomao S, Rossi L, Zaccarelli E, et al. Triple-negative breast cancer: investigating potential molecular therapeutic target. Expert Opin Ther Targets. 2015;19(1):55–75.
27. Schmadeka R, Harmon BE, Singh M. Triple-negative breast carcinoma: current and emerging concepts. Am J Clin Pathol. 2014;141(4):462–77.
28. Andreopoulou E, Schweber SJ, Sparano JA, McDaid HM. Therapies for triple negative breast cancer. Expert opinion on pharmacotherapy. 2015;16(7):983–98.
29. Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. Nature. 2014;505(7483):344–52.
30. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annual review of pathology. 2014;9:287–314.
31. Acunzo M, Romano G, Wernicke D, Croce CM. MicroRNA and cancer–a brief overview. Advances in biological regulation. 2015;57:1–9.
32. Yahya SM, Elsayed GH. A summary for molecular regulations of miRNAs in breast cancer. Clinical biochemistry. 2015;48(6):388–96.

Figures

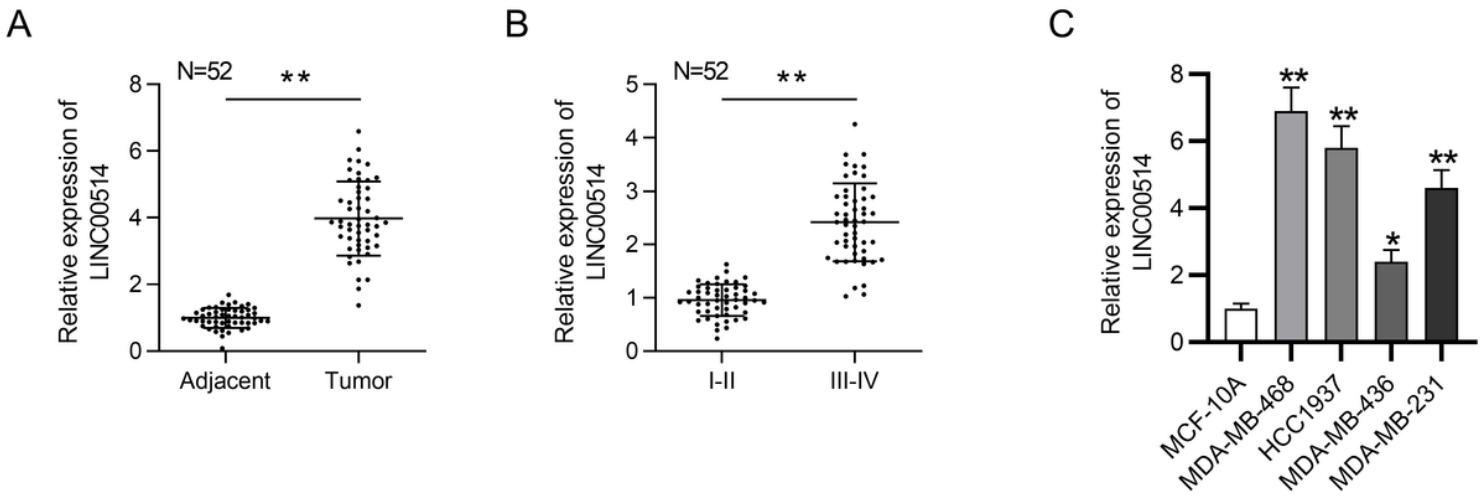

Figure 1

LINC00514 expression in TNBC tissues and cells. (A) RT-qPCR analysis of LINC00514 expression in TNBC tissues, with adjacent nontumor tissues as control. (B) Correlation of LINC00514 expression with clinical stage. (C) LINC00514 expression in TNBC cell lines was confirmed by RT-qPCR. *$p < 0.05$, **$p < 0.01$.

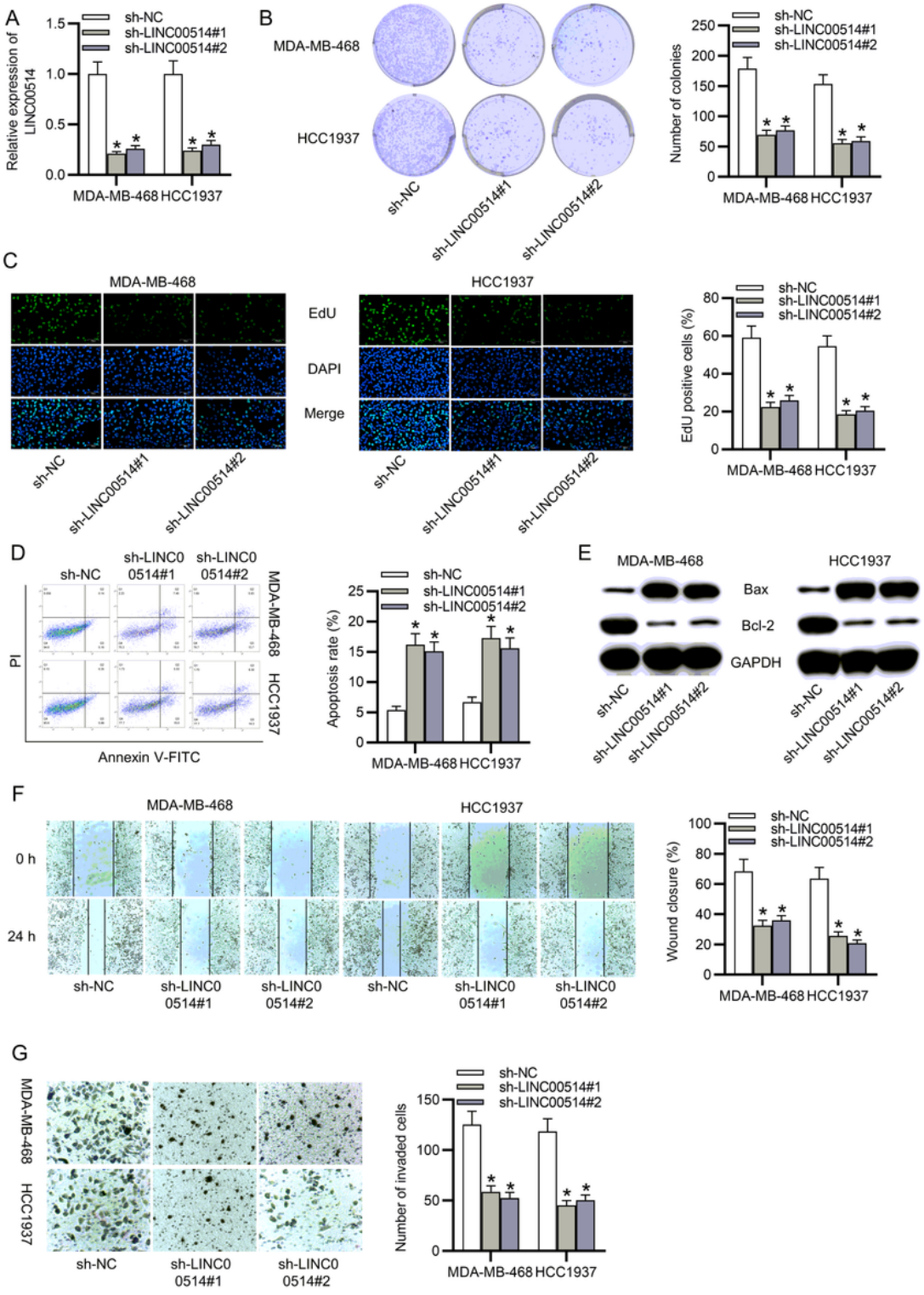

Figure 2

LINC00514 knockdown played inhibitory role in TNBC cell growth and migration. (A) Transfection efficiency of sh-LINC00514 in MDA-MB-468 and HCC1937 cells was evaluated by RT-qPCR. (B-C) Colony formation assay and EdU assay were carried out to assess cell (MDA-MB-468, HCC1937) proliferative ability with LINC00514 silencing. (D) Apoptosis of sh-LINC00514 transfected TNBC cells was detected by flow cytometry analysis. (E) Impact of LINC00514 deficiency in levels of apoptosis-associated proteins

was evaluated with western blot. (F) Wound healing assay verified migratory ability of LINC00514-depleted TNBC cells. (G) Transwell assay was performed detecting cell invasive ability under LINC00514 knockdown. *p < 0.05.

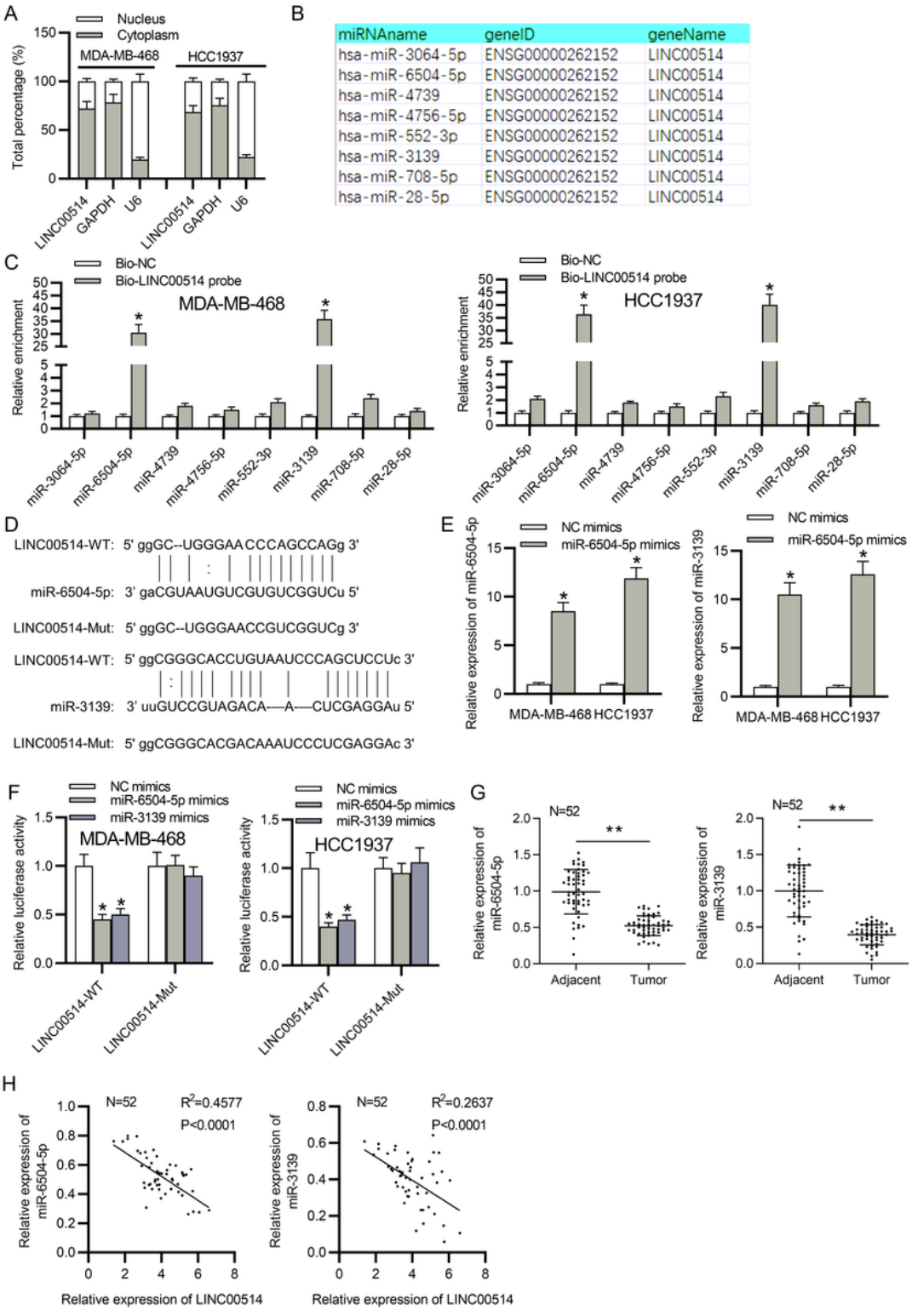

Figure 3

MiR-6504-5p and miR-3139 were the downstream miRNAs for LINC00514. (A) Subcellular fractionation assay determined LINC00514 location in TNBC cells. (B) StarBase predicted miRNAs binding to

LINC00514. (C) RNA pull-down assay was utilized for validating the binding of LINC00514 to potential miRNAs. (D) The miR-6504-5p/miR-3139 binding sites for LINC00514 were predicted by starBase and their matched mutated sites were designed. (E) RT-qPCR analysis of miR-6504-5p/miR-3139 expression in MDA-MB-468 and HCC1937 cells transfected with miR-6504-5p/miR-3139 mimics. (F) Luciferase reporter LINC00514-WT/Mut was built to verify the binding of LINC00514 to miR-6504-5p/miR-3139. (G) RT-qPCR analysis of miR-6504-5p expression or miR-3139 expression in TNBC tissues and paired adjacent tissues. (H) Correlation analysis of LINC00514 expression and miR-6504-5p/miR-3139 expression in TNBC tissues. *$p < 0.05$, **$p < 0.01$.

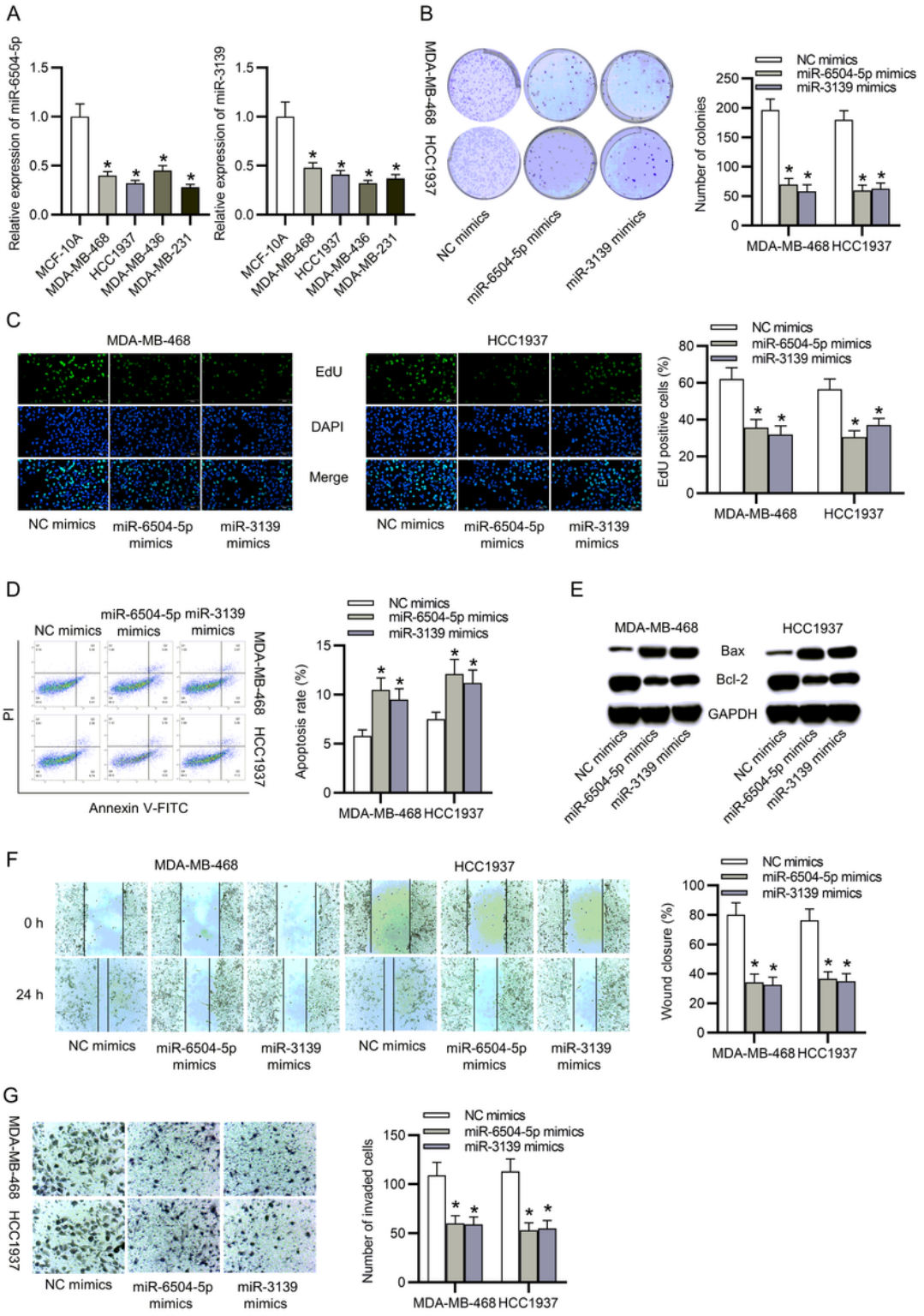

Figure 4

The inhibitive effect of overexpressed miR-6504-5p or miR-3139 on TNBC cellular process. (A) RT-qPCR of miR-6504-5p and miR-3139 expression in TNBC cells and MCF-10A cells. (B-C) The proliferation in TNBC cells with miR-6504-5p/miR-3139 mimics transfection was evaluated through colony formation and EdU assays. (D) Flow cytometry analysis demonstrated the function of upregulated miR-6504-5p and miR-3139 on cell apoptosis. (E) Bax and Bcl-2 protein levels in TNBC cells with transfection of miR-6504-

5p/miR-3139 mimics or NC mimics. (F) Impact of upregulated miR-6504-5p/miR-3139 on TNBC cell migratory ability. (G) Transwell assay evaluated the invasion in miR-6504-5p/miR-3139 overexpressed cells. *p < 0.05.

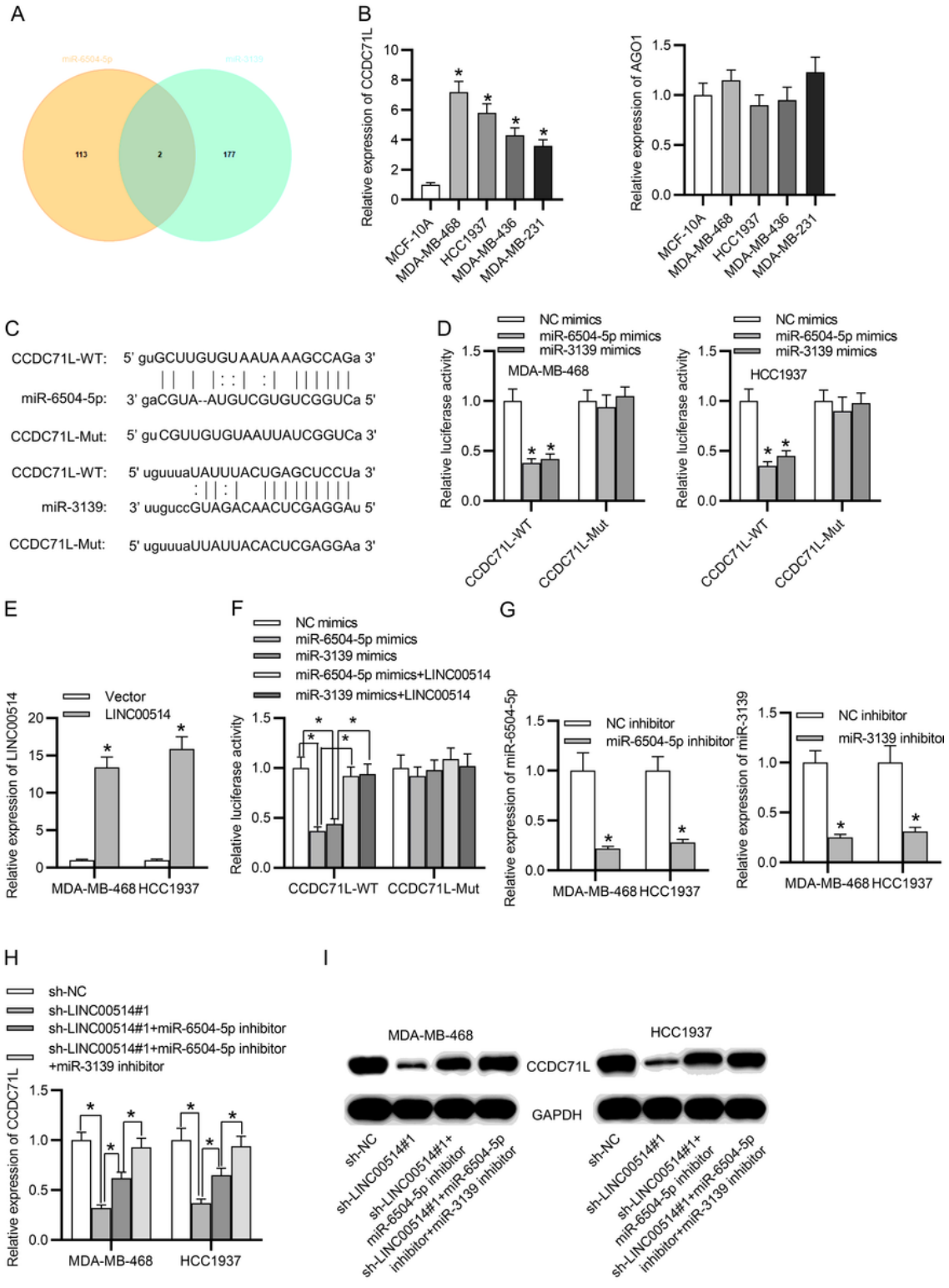

Figure 5

CCDC71L was targeted by miR-6504-5p and miR-3139. (A) Predicted targets for both miR-6504-5p and miR-3139 via starBase. (B) Expressions of CCDC71L and AGO1 in TNBC cells was analyzed by RT-qPCR.

(C) The predicted binding sites and constructed mutant sites between CCDC71L and miR-6504-5p/miR-3139. (D) Luciferase activity of CCDC71L-WT/Mut in TNBC cells transfected with miR-6504-5p/miR-3139 mimics. (E) LINC00514 expression in TNBC cells with pcDNA3.1/LINC00514 transfection. (F) YAP1-WT/Mut luciferase activity with indicated transfection in HEK293T cell was verified by luciferase reporter assay. (G) Transfection efficiency of miR-6504-5p/miR-3139 inhibitor in TNBC cells was evaluated by RT-qPCR. (H-I) Detection of CCDC71L mRNA and protein levels in TNBC cells with transfection of indicated plasmids. *p < 0.05.

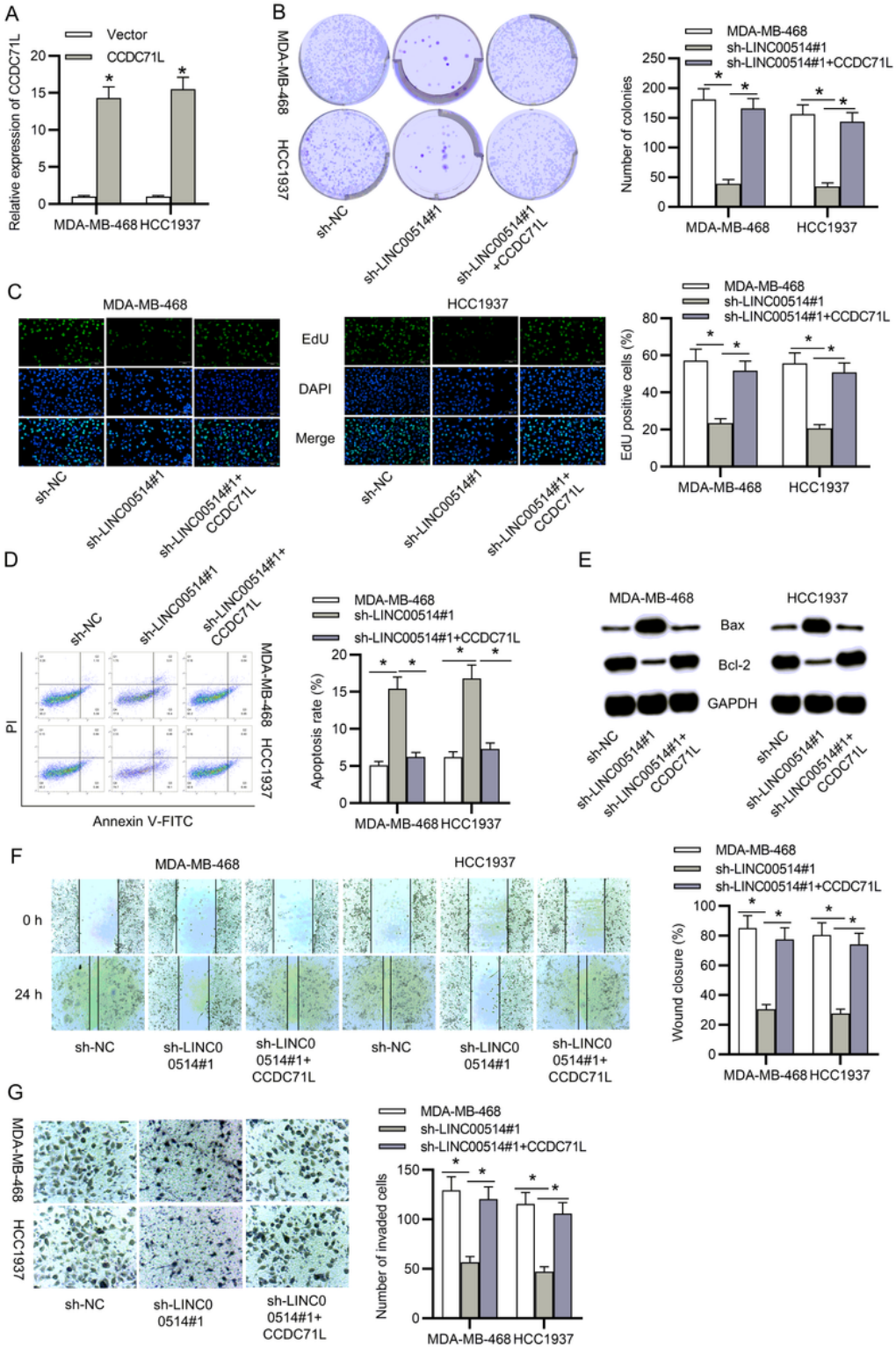

Figure 6

LINC00514 exerted regulatory functions on cellular activities via CCDC71L. (A) Transfection efficiency of pcDNA3.1/CCDC71L was confirmed. (B-C) Colony formation assay and EdU assay using MDA-MB-468 and HCC1937 cells with appointed transfection. (D-E) Cell apoptosis rate and proteins associated with apoptosis in each group were measured. (F-G) Cell migratory and invasive abilities with indicated transfection were assessed. *$p < 0.05$.