miR-105-3p acts an oncogene to promote proliferation and metastasis of breast cancer cell by targeting GOLIM4miR-105-3p acts an oncogene to promote proliferation and metastasis of breast cancer cell by targeting GOLIM4

Bo Lin
Huai'an Key laboratory of Gastric Cancer
Enyi Shi
Suzhou Health College
Qiu Jin
Huai'an Key laboratory of Gastric Cancer
Wenhui Zhao
Huai'an Key laboratory of Gastric Cancer
Juan Wang
Huai'an Key laboratory of Gastric Cancer
Runyuan Ji (christ777735@163.com)
Huai'an Key laboratory of Gastric Cancer, Jiangsu College of Nursing https://orcid.org/0000-0002-3783-6229

Research article

Keywords: miR-105-3p; breast cancer; GOLIM4; proliferation; migration

Posted Date: June 29th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-36644/v1

Version of Record: A version of this preprint was published on March 15th, 2021. See the published version at https://doi.org/10.1186/s12885-021-07909-2.

Abstract

Background: Dysregulation of miRNAs is involved in carcinogenesis of breast cancer and may be used to prognostic biomarkers and therapeutic target during cancer process. The purpose of this study was to explore the effects of miR-105-3p on tumourigenicity of breast cancer and its underlying molecular mechanisms.

Methods: Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was applied to detect the miR-105-3p in breast cancer tissues. The impacts of miR-105-3p on proliferation, migration, invasion and apoptosis of human breast cancer lines (MCF-7 and ZR-75-30) were evaluated by CCK-8, transwell chamber assay, TUNEL assay and western blot assay, respectively. Besides, bioinformatics and luciferase reporter assay were used find out the targeted gene of miR-105-3p.

Results: The expression of miR-105-3p was elevated in breast cancer tissues and increased with tumor severity. Downregulation of miR-105-3p could inhibit cell proliferation, suppress cell migration/invasion, and promote cell apoptosis in MCF-7 and ZR-75-30 cells. Furthermore, Golgi integral membrane protein 4 (GOLIM4) was identified to be the direct target gene of miR-105-3p by bioinformatics and luciferase reporter assay. In addition, silencing of GOLIM4 could restore the anti-breast cancer effects induced by miR-105-3p downregulation.

Conclusions: miR-105-3p acts an oncogene to promote proliferation and metastasis of breast cancer cell by targeting GOLIM4, which provides a new target for the prevention and treatment of breast cancer.

Background

Breast cancer, the most common female malignant tumor around the world, has the second highest rate of mortality rate among all female malignancies, the incidence of which is increasing at a rate of 3% per year in China [1–3]. The great advances have been achieved in the early diagnosis and molecular-targeted therapy of breast cancer thanks to the development of modern molecular diagnostic technology [4]. The high postoperative recurrence and low postoperative survival rate of patients with advanced breast cancer still remained to be resolved. Therefore, further researches should focus on the molecular mechanism in the occurrence and development of breast cancer so as to find new therapeutic targets for breast cancer.

As an important single-stranded non-coding RNAs which are familiarly 18–25 nucleotides in length, miRNAs have gained much attention for their associations with the growth and invasion of tumors, including breast cancer, stomach cancer and so on[5, 6]. miRNA mainly regulates gene expression by binding to the 3’ untranslated region of messenger RNA during the post-transcriptional stage[7]. It should be noted that more than half of miRNAs can serve as oncogenic or tumor suppressor genes depending on their targeted mRNAs, and have impacts on a great deal of biological processes in cancer, including cell proliferation, cell cycle, apoptosis, invasion, metastasis, glucose and lipid metabolism and immune

responses [8]. Given that different levels of miRNAs expression have been identified in all stages of breast cancer, miRNAs have becoming early diagnosis biomarkers and potential therapeutic targets for breast cancer [9]. Bahena *et al* find that overexpression of miR-10b in breast cancer cells could inhibit PTEN expression, and promote epithelial-mesenchymal-transition (EMT) and stem cell marker expression, which thereby promotes breast cancer invasion and metastasis [10]. Damiano and his colleagues identify that the down-regulation of miR-200 in breast cancer could account for EMT and stem-like features of breast cancer by targeting ZEB1 [11]. All these studies reveal the great importance of miRNA in breast cancer.

MiR-105-3p is a species highly conserved miRNA molecules among humans, cattle, horses and many other species, which heralds its multiple potential biological effects. Recent studies reveal that miR-105-3p is closely related to the occurrence and development of tumors, such as ovarian cancer, prostate cancer, colon cancer and hepatocellular carcinoma [12, 13]. At the meanwhile, miR-105-3p could act as an oncogene that affects multiple biological behaviors of tumor growth by regulating different protein expression. However, little information about the expression and biological characteristics of miR-105-3p, as well as its targeted mRNA in breast cancer, was known so far. In this study, we systematically assess the expression characteristics of miR-105-3p both in the tissues of breast cancer and various breast cancer cell lines. We found that the expression level of miR-105-3p was obviously elevated in the tumor tissue. The *in vitro* experiments showed that downregulation of miR-105-3p repressed cellular proliferation, migration and invasion of MCF-7 and ZR-75-30 cells, indicating that it was an oncogene in breast cancer. In addition, our findings confirmed that miR-105-3p could directly bind to the 3'UTR of GOLIM4 and thus played a tumor suppressive role in breast cancer.

Methods

Cell culture and transfection

Human breast cancer cell line in this study, including MCF-7 and ZR-75-30, was purchased from China Infrastructure of Cell Line Resource and cultured in RPMI 1640 medium containing 10% FBS, 100 mg/ml penicillin and streptomycin at 37°C in 5% CO_2. Hsa-miR-105-3p inhibitor and its corresponding negative control (NC inhibitor) were synthesis by Sangon Biotech (Shanghai Co., Ltd) and transfected into these MCF-7 and ZR-75-30 cell lines by Lipofectamine 2000 (Thermo Fisher Scientific, Inc.; USA) according to the manufacturer's instructions. The successfully transfected cell lines were used in the following experiments. To silence the expression of GOLIM4 in MCF-7 and ZR-75-30 cell lines, shRNA targeted for GOLIM4, also synthesized by Sangon Biotech (Shanghai, Co., Ltd), and was also transfected into these MCF-7 and ZR-75-30 cell lines by Lipofectamine 2000.

RT-qPCR assay

Cancer tissues and paired adjacent tissues of 80 patients with breast cancer were collected from our hospital. MagMAX™ RNA isolation kit and VetMAX™-Plus One-Step RT-PCR Kit (USA; Thermo Fisher

Scientifc, Inc.) were applied to isolate total RNA and miRNAs according to the instruments. After the total RNA was extracted from tumor tissues, TaqMan microRNA assay (Applied Biosystems; Thermo Fisher Scientific, Inc.) was carried out to measure the expression of miR-105-3p. The reagent components in the reaction system were showed as follows: 20× TaqMan miRNA assay (1μL), 2× TaqMan Universal PCR Master mix (10 μL; USA; Thermo Fisher Scientific, Inc.), cDNA (1.33 μL), forward primer (1 μL;) and reverse primer (1 μL) and double distilled water (5.67 μL). The RT-qPCR was performed in the ABI 7500 Real-Time PCR System and the results of the threshold cycle (Ct) were calculated by $2^{-\Delta\Delta Ct}$ method after being normalized by the endogenous controls U6 snRNA(Forward primer: 5'-ATTGGAACGATACAGAGAAGATT-3'; Reverse primer:5' GGAACGCTTCACGAATTTG3'). The expression level of GOLIM4 was detected with the methods as described above in breast cancer tissue and its cell line. . U6:Forward primer: 5'-ATTGGAACGATACAGAGAAGATT-3'; Reverse primer:5' GGAACGCTTCACGAATTTG3'. miR-105-3p: Forward primer: 5'-CAGTCTCACACCAGCACC-3'; Reverse primer:5'-TATGGTTGTTCACGACTCCTTCAC-3'. GOLIM4: Forward primer: 5'-AAACTCTATGCTCCCACCC-3'; Reverse primer: 5'-GCTGCTCTTTCCACTCCC-3'. GAPDH: Forward primer: 5'-TGACTTCAACAGCGACACCCA-3'; Reverse primer: 5'CACCCTGTTGCTGTAGCCAAA-3'

Colony formation assay

ZR-75-30 and MCF-7 cells were cultured into logarithmic growth phase, and then the cells were collected and suspended into a cell suspension. The concentration of suspended cells was adjust to 1×10^4 cell/mL, and a density of 500 cells was inoculated into 10 cm dish. The cells were incubated for 2 weeks and then methanol was added to fix the formed cells colonies. Subsequently, the cells were with crystal violet dye. The number of colonies containing more than 50 cells was counted.

CCK-8 assay

Cell Counting Kit-8 (Dojindo, Inc.; Japan) was used to detected the proliferation capacity of the transfected ZR-75-30 and MCF-7 cells according to the instruments. Briefly, a density of 5×10^3 cells was seeded into the 96-well plates with 10 μL CCK-8 solution. The cells were incubated for 24, 48 and 72 h, and then the absorbance was measured at 490 nm with a microplate reader.

Scratch test

The ZR-75-30 and MCF-7 cells were transfected with miR-105-3p inhibitor and cultured into the logarithmic growth phase. The bottom of 6-well plate was rowed with five straight lines with 0.5 cm intervals. After the cells were suspended and adjusted into 1×10^5 cells/mL, 2 mL cell suspension was added into the well and cultured for 24 h. Subsequently, the scratch that was perpendicular to the base lines above was made by 10-μl pipetting spear. The cells were cultured for 48 h with serum-free medium, and wound closure (%) was finally calculated.

Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay

TUNEL assay was performed to detect the apoptosis of transfected cells using One Step TUNEL Apoptosis Assay Kit (Beyotime Biotechnology Co., Ltd; China) following the manufacturer's instructions. Briefly, the cells were cultured in the 6-well plate, and then fixed with 4% paraformaldehyde for 30 min at room temperature. Subsequent to washing by PBS twice, 0.1% TritonX-100 was added to permeabilize the cell membrane. The cells were successively treated with TUNEL reaction mixture, converter-POD and DAPI substrate. The cell images were then obtained under the fluorescence microscope (Olympus, Tokyo, Japan).

Transwell chamber assay

Transwell chamber assay was applied to detect the invasion ability of ZR-75-30 and MCF-7 cells after transfected with NC or miR-105-3p inhibitor. The upper and lower chamber were added with FBS-free culture medium and culture medium containing 10% FBS, respectively. The transfected cells were cultured in FBS-free culture medium for 12 h, and then a density of 2×10^4 cells was seeded into the upper chamber with 10 mg/mL matrigel. After cultured for 48 h, the top of the filter was carefully wiped with cotton swabs to remove the remained cells. Those cells that migrated through the membrane were fixed with 95% methanol, stained with 0.5% crystal violet and finally counted with microscope (magnification, ×200; Olympus Corporation, Tokyo, Japan).

Western blot assay

After the transfected cells were cultured for 72 h, the cells was washed with PBS for three times and then centrifuged to obtain cell pellet. ProteoPrep® Total Extraction Sample Kit was used to extracted total protein according to the instruments followed by the detection of protein concentration using a BCA assay kit (USA; Thermo Fisher Scientifc, Inc.). Equal quality of protein were separated on 12 % SDS-PAGE and subsequently transferred to polyvinylidene difluoride (PVDF) membranes (Thermo Fisher Scientifc, Inc.; USA). After blocked with 5% milk in PBST for 1 h at room temperature, the membrane was incubated with proper dilution of primary antibodies for 1 h at 37 ℃ including anti-BAX, anti-Bcl-2, anti-Cleaved caspase-3, anti-Cleaved caspase-9, anti-ICAM-1 and anti-VCAM-1. β-actin was chosen as an internal control in the assay. Subsequent to washing with TBST for three times, HRP labeled secondary antibodies corresponding to primary antibodies were used to probe the expression of target protein in the membrane. The protein bands were visualized using Novex™ ECL Chemiluminescent Substrate Reagent Kit (Thermo Fisher Scientifc, Inc. USA). Densitometry quantification of protein bands was conducted using Image J pro plus software and then normalized with β-actin.

Luciferase reporter assay

In order to study the relationship between miR-105-3p and GOLIM4, the ZR-75-30 and MCF-7 cells were co-transfected with miR-NC or miR-105-3p and the psiCHECK2 vector containing the wild type or mutant GOLIM4 fragment (psiCHECK2- GOLIM4-3’ UTR WT or psiCHECK2- GOLIM4-3’ UTR MUT) with lipofectamine2000 (Thermo Fisher Scientific, Inc.; USA). After cultured for 48 h and three times washes,

the cells were lysed with harvest buffer for 10 min at 0℃. The mixture of ATP buffer and luciferin buffer (1:3.6) was added into the cell lysate followed by the detection of the absorbance.

Statistical analysis

The measurement data that conforms to the normal distribution was showed as mean ± standard deviation. The difference between two groups was analyzed by Student's t test. One-Way ANOVA was used to analyze the differences among group followed by Bonferroni post-hoc analysis. The 80 patients was divided into the high and low miR-105-3p expression group according to the median miRNA expression, and then life table methods were used to analyze the difference of survival data between the two groups, after which the differences in survival time were tested by the *Mantel-Cox log-rank method*. All the data were analyzed by SPSS software, version 20 (SPSS, Inc., Chicago, IL, USA). A P value < 0.05 was considered to indicate a significant effect.

Results

The expression levels of miR-105-3p in breast cancer tissues and cell lines.

The expression of miR-105-3p in breast cancer and para-cancerous tissues of 80 breast cancer patients with different stages was detected with RT-qPCR. Compared with that of para-cancerous tissues, elevated expression level of miR-105-3p was observed in tumor tissues (Figure 1A). Of note, the tumor tissues within stage Ⅰ and Ⅱ expressed higher levels of miR-105-3p than those in tumor tissues at stage Ⅲ and Ⅳ (Figure 1B). As shown in Figure 1C, the survival curve analysis indicated that the survival time of patients with high expression level of miR-105-3p was shorter than that with low expression level (P 0.05). In order to choose suitable breast cancer cell lines to evaluate the biological function of miR-105-3p, the expression levels of miR-105-3p in several breast cancer cell, including MCF10A, MDA-MB-231, SKBr-3, MCF-7 and ZR-75-30, were also evaluate by RT-qPCR. We found that the expression levels of miR-105-3p in the MCF-7 and ZR-75-30 were relatively high among the five cell lines (Figure 1D). Thus, we chose these two cell lines for further research. To identify the role of miR-105-3p in the regulation of breast cancer progression, we firstly transfected hsa-miR-105-3p inhibitor and its corresponding negative control (NC inhibitor) into the indicated cells. Cellular immunofluorescence showed that the transfected cells contained green fluorescence (Figure 1E). Furthermore, RT-qPCR analysis identified the expression level of miR-105-3p was successfully downregulated by miR-105-3p inhibitor, which suggested that the miR-105-3p inhibitor could be used in the following experiments (Figure 1F).

Downregulation of miR-105-3p inhibits cell proliferation and promotes apoptosis in MCF-7 and ZR-75-30 cells

In order to evaluate the effect of miR-105-3p on the MCF-7 and ZR-75-30 cells, the two cell lines were transfected with miR-105-3p inhibitor or NC. We found that downregulation of miR-105-3p could suppress the cell proliferation as reflected by CCK-8 and colony formation assays (Figure 2A and B). In addition, the cell apoptosis was detected by TUNEL assay and the numbers of apoptotic cells in the MCF-7 and ZR-75-

30 cells transfected with miR-105-3p inhibitor was increased, when compared to those transfected with NC inhibitor (Figure 2C). At the molecular level, the results of western blot further revealed that miR-105-3p inhibitor increased the expression levels of Bax, cleaved-Caspase-3 and cleaved-Caspase-9, while decreased the Bcl2 levels (Figure 2D). These data suggested that the miR-105-3p knockdown inhibited the cell proliferation and triggered the cell apoptosis.

Downregulation of miR-105-3p suppresses the migration and invasion of MCF-7 and ZR-75-30 cells

To investigate the effects of miR-105-3p on the migration and invasion of MCF-7 and ZR-75-30 cells, the scratch test and transwell chamber assay were applied *in vitro*. As shown in Figure 3A, downregulation of miR-105-3p in MCF-7 and ZR-75-30 cells could inhibit healing area after 24 h scratch injury (Figure 3A). In addition, transwell chamber assay revealed that the numbers of the migrated cells transfected with miR-105-3p inhibitor were increased compared to these transfected with NC inhibitor (Figure 3B). Given the important function of ICAM-1 and VCAM1-1 in the regulation of cell migration and invasion, we detected the protein expression levels of these two proteins in response to knockdown of the miR-105-3p. As shown in Figure 3C, downregulation of miR-105-3p could suppress the expression of ICAM-1 and VCAM-1. All these data indicated that miR-105-3p was a pivotal regulator involved in the breast cancer migration and invasion.

MiR-105-3p modulates GOLIM4 expression by directly targeted its 3'UTR

By using TargetScan and miRanda, the GOLIM4 was identified as the potential target gene of miR-105-3p (Figure 4A). Hence, in order to identify the directly inhibition effects of miR-105-3p on the expression of GOLIM4, the luciferase reporter assay was carried out and we found that overexpression of miR-105-3p obviously increased the luciferase activities of GOLIM4-3’ UTR WT, while exhibited modest effects on GOLIM4-3’ UTR MUT (Figure 4B). Consistently, knockdown of miR-105-3p in MCF-7 and ZR-75-30 cells increased the expression level of GOLIM4 at both transcriptional and translational levels (Figure 4C, D). In addition, the expression levels of GOLIM4 in breast cancer tissues and cell lines were significantly declined (Figure 4E). Correlation regression analysis showed a negative correlation existed between the expression levels of miR-105-3p and GOLIM4 in breast cancer tissues (P 0.05, Figure 4F).

Silencing of GOLIM4 partially disrupts the miR-105-3p inhibitor-induced anti-breast cancer effects.

To confirm that miR-105-3p could act as an oncogene to promote proliferation and metastasis of breast cancer cell by targeting GOLIM4, the expression of GOLIM4 in the MCF-7 and ZR-75-30 cells transfected with miR-105-3p inhibitor was further silenced with sh-GOLIM4. As shown in Figure 5A, the expression of GOLIM4 in the MCF-7 and ZR-75-30 was successfully downregulated by transfection with sh-GOLIM4. Functionally, the miR-105-3p inhibitor-induced inhibition effects of cell proliferation and migration were partially impaired when the expression of GOLIM4 was also silenced in these two cell lines (Figure 5B and C). In the aspect of cell apoptosis, silencing of GOLIM4 could inhibit the apoptosis induced by the downregulation of miR-105-3p, as reflected by increased positive-TUNEL cells and increased apoptotic proteins, including Bax, cleaved-caspase-3 and cleave-caspase-9 (Figure 5D and E). In addition, silencing

of GOLIM could also increase cell migration and invasion of the MCF-7 and ZR-75-30 cells transfected with miR-105-3p inhibitors (Figure 5F and G). These data collectively indicated that GOLIM was an essential mediator in response to the miR-105-3p signals in the breast cancer progression.

Discussion

Breast cancer incidence rises sharply nowadays and has become the second most dangerous cancer among women although the great advantages have been made in diagnosis and treatments [14]. Up to date, miRNAs have been proved as master regulators of the tumor progression highlighted in various malignancies including breast cancer [15, 16]. In this study, it was validated that the elevated expression of miR-105-3p could be found in breast cancer tissues and increased with increasing tumor severity. Downregulation of miR-105-3p could inhibit cell proliferation, suppress cell migration and invasion and promote cell apoptosis in MCF-7 and ZR-75-30 cells, indicating it an oncogene in the carcinogenesis in breast cancer. Furthermore, this research provided evidence for GOLIM4 as a downstream gene for miR-105-3p since silencing GOLIM4 could restore the abilities of cell proliferation and migration and inhibit the apoptosis induced by the downregulation of miR-105-3p in MCF-7 and ZR-75-30 cells.

The discovery of miRNAs provides new visions to investigate the pathogenesis of tumors, as well as new strategies for the diagnosis and treatment of tumors. Various studies have showed the tumor-specific miRNA contribute to the precision medicine in malignancies by serving as a potential therapeutic targets and early diagnosis indicator [9]. In the research filed of breast cancer, several miRNAs are identified and are extensively involved in the occurrence and development of various cancer diseases via modulating the expression of key proteins at the post-transcription level [9]. For example, the miR-10b, miR-200 and miR-21 have been demonstrated to be the important miRNAs, which could be upregulated in breast cancer. They also serve as oncogenes by targeting PTEN, TGF-β and so on [16]. Our study revealed that the elevated expression of miR-105-3p could be found in breast cancer tissues and the cancer tissues with higher tumor stages also contained higher level of miR-105-3p, indicating that miR-105-3p may become the potentially prognostic factors for breast cancer. These findings further confirmed the importance of miR-105-3p in breast cancer patients.

MiR-105-3p is a well-studied miRNA that could inhibit the carcinogenesis of hepatocellular carcinoma, ovarian, prostate, and colon cancer [12, 13, 17, 18]. MiR-105 expression was markedly downregulated in hepatocellular carcinoma (HCC) and acts as an oncogene to promote the proliferation of HCC by suppressing PI3K/AKT signaling [12]. Similarly, Honeywell D. Rice also reported that miR-105 was down-regulated in human prostate cancer cell lines (PC3 and DU145) and it was involved in tumor cell proliferation and growth via inhibition of the CDK6 expression [18]. Furthermore, miR-105 could also promote oncogenic transformation and tumorigenesis in colorectal cancer [17]. From this study, the proliferation, invasion and migration capabilities of breast cancer cells were significantly inhibited when downregulating the level of miR-105-3p, which is similar to the previous researches in other kinds of tumors. We further detected the cell apoptosis in the breast cancer cell lines. As respected, knockdown of

miR-105-3p in breast cancer cells attenuated the apoptosis of MCF-7 and ZR-75-30 cells. Taken together, the expression level of miR-105-3p was inversely correlated with the growth and metastatic potential of breast cancer cells, indicating its essential role in governing breast cancer cell progression.

For the molecular mechanism underlying the regulation effect of miR-105-3p on breast cancer, it is of great important to dissect its target gene. Thus, the two publicly available miRNA databases, named TargetScan and miRanda, were used and the results showed that GOLIM4 was the potential target gene of miR-105-3p. GOLIM4 is a membrane binding protein in Golgi apparatus and plays a vital role in transporting proteins between Golgi apparatus and endosomes [19]. Given the fact that dysfunction of Golgi and endosomes concentrated to the progress of various tumors, GOLIM4 was considered to be a tumor suppressor gene, which are identified in the carcinogenesis of human head and neck cancer [20]. The increased expression of GOLIM4 could inhibit the proliferation of neck cancer, promote cell apoptosis and induce G1 phase arrest in human head and neck cancer cell lines, such as FaDu and Tca-8113 cell lines [20]. Luciferase reporter assay in this study provided evidence for GOLIM4 as a potential target for miR-105-3p. The results revealed that miR-105-3p overexpression suppressed the luciferase activities of Wt 3'UTR of GOLIM4; however, no inhibition effect on the Mut 3'UTR of GOLIM4 could be detected, which indicates that GOLIM4 was the target gene for miR-105-3p. Most importantly, silencing of GOLIM4 reversed the promoting effect on the biological characteristics of breast cancer cell induced by downregulating the level of miR-105-3p in these cancer cells. Since GOLIM4 been identified as a tumor suppressor gene in other kinds of cancers, it was validated that the elevated expression of miR-105-3p could suppress the expression of GOLIM4 by binding to its 3’ UTR region in the carcinogenesis of breast cancer. These data provided the additional evidences for the idea that miR-105-3p acted an oncogene to promote proliferation and metastasis of breast cancer cell by targeting GOLIM4. However, due to the complexity of molecular mechanisms in tumor cells, it cannot be ruled out that other proteins are involved in the function of miR-105-3p which needs further researches.

In summary, miR-105-3p was upregulated in breast cancer tissue and was correlated to the tumor stage. The *in vitro* experiments verified the importance of miR-105-3p in tumor invasion process including the promotion of the cell proliferation, the enhancement of the cell migration, the facilitation of invasion processes, and the suppression of cell apoptosis. All these effects of miR-105-3p were partially mediated by its inhibitory impacts on the expression of GOLIM4. Our findings provided a promising evidence that miR-105-3p is a potential target for clinical treatment of breast cancer and might predict the prognosis of breast cancer patients.

Conclusion

The eleaveted expression level of miR-105-3p was correlated with the tumor stage. The *in vitro* experiments showed that downregulation of miR-105-3p repressed cellular proliferation, migration and invasion of MCF-7 and ZR-75-30 cells, indicating that it was an oncogene in breast cancer. Furthermore, miR-105-3p could directly bind to the 3'UTR of GOLIM4 and thus played a tumor suppressive role in

breast cancer. All these foundings indicated that miR-105-3p might serve as a potential therapeutic target in the precise treatment of breast cancer, and it could also predict the prognosis of breast cancer patients.

Abbreviations

Abbreviations miR: micro RNA; HCC: Hepatocellular Carcinoma; TGF-β: Transforming growth factor-β; PTENL: Phosphatase and tensin homologue deleted on chromosome ten; GOLIM4: EMT: Epithelial-mesenchymal-transition; GOLIM4: Golgi integral membrane protein 4.

Declarations

Ethics approval and consent to participate

This study has been approved in writing by our hospital's ethics committee, and we obtained the patient's tumor tissue after signing an informed consent form with the patient.

Consent for publication

All authors agree that articles published in this journal.

Availability of data and material

All authors agree that the data in this paper will be freely available to any scientist wishing to use them for non-commercial purposes.

Competing interests

The authors declare that there are no conflicts of interest.

Funding

Not applicable

Authors' contributions

Author contributions: RY Ji and B Lin conception and design of research; B Lin and EY Shi performed experiments; B Lin and RY Ji analyzed data; B Lin drafted manuscript; RY Ji edited and revised manuscript; B Lin approved final version of manuscript. All authors have read and approved the manuscript.

Acknowledgements

Not applicable

References

[1] Anastasiadi Z, Lianos G D, Ignatiadou E, Harissis H V, Mitsis M. Breast cancer in young women: an overview. Updates Surg, 2017, 69(3): 313-7.PMID:28260181. doi:10.1007/s13304-017-0424-1

[2] Harbeck N, Gnant M. Breast cancer. Lancet, 2017, 389(10074): 1134-50.PMID:27865536. doi:10.1016/S0140-6736(16)31891-8

[3] Li T, Mello-Thoms C, Brennan P C. Descriptive epidemiology of breast cancer in China: incidence, mortality, survival and prevalence. Breast Cancer Res Treat, 2016, 159(3): 395-406.PMID:27562585. doi:10.1007/s10549-016-3947-0

[4] Waks A G, Winer E P. Breast Cancer Treatment: A Review. JAMA, 2019, 321(3): 288-300.PMID:30667505. doi:10.1001/jama.2018.19323

[5] Qadir M I, Faheem A. miRNA: A Diagnostic and Therapeutic Tool for Pancreatic Cancer. Crit Rev Eukaryot Gene Expr, 2017, 27(3): 197-204.PMID:29199604. doi:10.1615/CritRevEukaryotGeneExpr.2017019494

[6] Sun Z, Shi K, Yang S, Liu J, Zhou Q, Wang G, Song J, Li Z, Zhang Z, Yuan W. Effect of exosomal miRNA on cancer biology and clinical applications. Mol Cancer, 2018, 17(1): 147-.PMID:30309355. doi:10.1186/s12943-018-0897-7

[7] Vishnoi A, Rani S. MiRNA Biogenesis and Regulation of Diseases: An Overview. Methods Mol Biol, 2017, 1509(1-10.PMID:27826912. doi:10.1007/978-1-4939-6524-3_1

[8] Zou Q, Tang Q, Pan Y, Wang X, Dong X, Liang Z, Huang D. MicroRNA-22 inhibits cell growth and metastasis in breast cancer via targeting of SIRT1. Exp Ther Med, 2017, 14(2): 1009-16.PMID:28781618. doi:10.3892/etm.2017.4590

[9] Nassar F J, Nasr R, Talhouk R. MicroRNAs as biomarkers for early breast cancer diagnosis, prognosis and therapy prediction. Pharmacol Ther, 2017, 172(34-49.PMID:27916656. doi:10.1016/j.pharmthera.2016.11.012

[10] Bahena-Ocampo I, Espinosa M, Ceballos-Cancino G, Lizarraga F, Campos-Arroyo D, Schwarz A, Garcia-Lopez P, Maldonado V, Melendez-Zajgla J. miR-10b expression in breast cancer stem cells supports self-renewal through negative PTEN regulation and sustained AKT activation. EMBO Rep, 2016, 17(7): 1081-.PMID:27371635. doi:10.15252/embr.201642700

[11] Damiano V, Brisotto G, Borgna S, Di Gennaro A, Armellin M, Perin T, Guardascione M, Maestro R, Santarosa M. Epigenetic silencing of miR-200c in breast cancer is associated with aggressiveness and is modulated by ZEB1. Genes Chromosomes Cancer, 2017, 56(2): 147-58.PMID:27717206. doi:10.1002/gcc.22422

[12] Shen G, Rong X, Zhao J, Yang X, Li H, Jiang H, Zhou Q, Ji T, Huang S, Zhang J, Jia H. MicroRNA-105 suppresses cell proliferation and inhibits PI3K/AKT signaling in human hepatocellular carcinoma.

Carcinogenesis, 2014, 35(12): 2748-55.PMID:25280563. doi:10.1093/carcin/bgu208

[13] Sirotkin A V, Lauková M, Ovcharenko D, Brenaut P, Mlyncek M. Identification of microRNAs controlling human ovarian cell proliferation and apoptosis. J Cell Physiol, 2010, 223(1): 49-56.PMID:20039279. doi:10.1002/jcp.21999

[14] Merino Bonilla J A, Torres Tabanera M, Ros Mendoza L H. Breast cancer in the 21st century: from early detection to new therapies. Radiologia, 2017, 59(5): 368-79.PMID:28712528. doi:10.1016/j.rx.2017.06.003

[15] Inns J, James V. Circulating microRNAs for the prediction of metastasis in breast cancer patients diagnosed with early stage disease. Breast, 2015, 24(4): 364-9.PMID:25957467. doi:10.1016/j.breast.2015.04.001

[16] Serpico D, Molino L, Di Cosimo S. microRNAs in breast cancer development and treatment. Cancer Treat Rev, 2014, 40(5): 595-604.PMID:24286642. doi:10.1016/j.ctrv.2013.11.002

[17] Hamfjord J, Stangeland A M, Hughes T, Skrede M L, Tveit K M, Ikdahl T, Kure E H. Differential expression of miRNAs in colorectal cancer: comparison of paired tumor tissue and adjacent normal mucosa using high-throughput sequencing. PLoS One, 2012, 7(4): e34150-e.PMID:22529906. doi:10.1371/journal.pone.0034150

[18] Honeywell D R, Cabrita M A, Zhao H, Dimitroulakos J, Addison C L. miR-105 inhibits prostate tumour growth by suppressing CDK6 levels. PLoS One, 2013, 8(8): e70515-e.PMID:23950948. doi:10.1371/journal.pone.0070515

[19] Witkos T M, Lowe M. Recognition and tethering of transport vesicles at the Golgi apparatus. Curr Opin Cell Biol, 2017, 47(16-23.PMID:28237810. doi:10.1016/j.ceb.2017.02.003

[20] Bai Y, Cui X, Gao D, Wang Y, Wang B, Wang W. Golgi integral membrane protein 4 manipulates cellular proliferation, apoptosis, and cell cycle in human head and neck cancer. Biosci Rep, 2018, 38(4): BSR20180454.PMID:30068697. doi:10.1042/BSR20180454

Figures

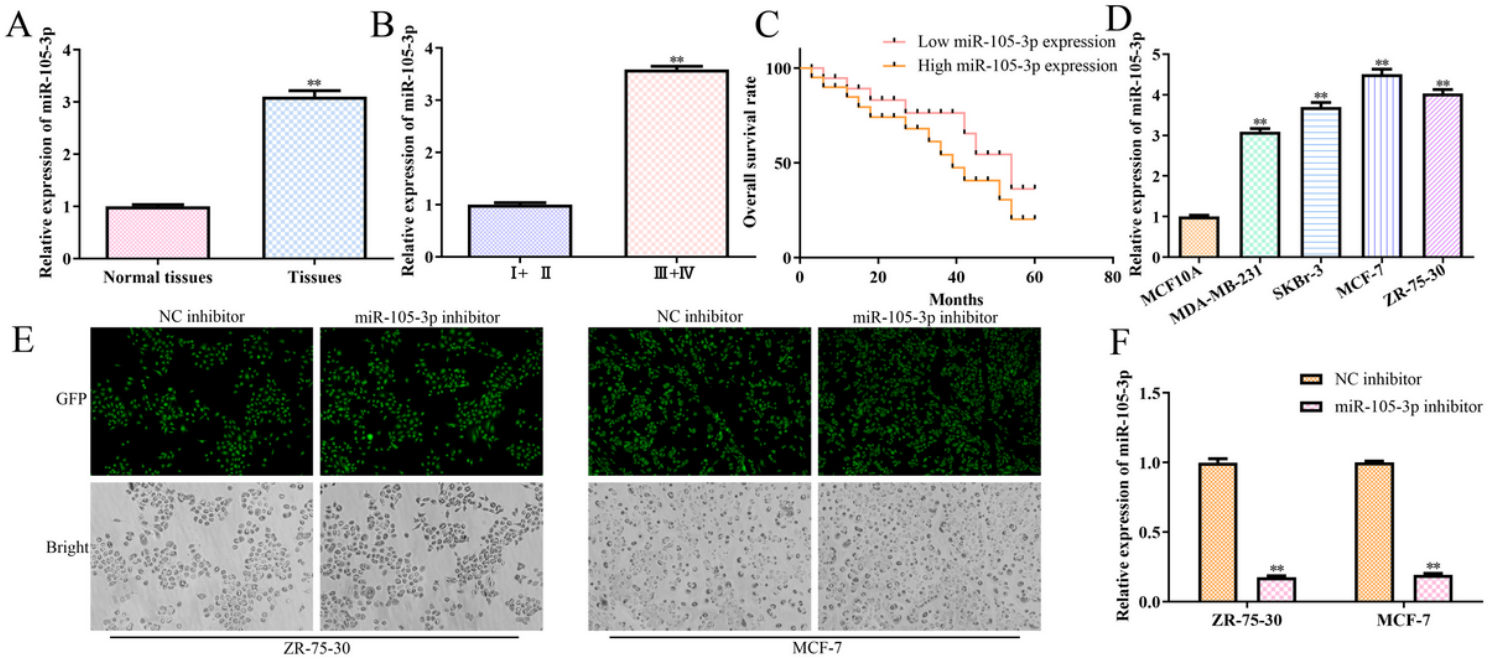

Figure 1

The expression of miR-105-3p in breast cancer tissues and its cell lines. (A) The expression of miR-105-3p in breast cancer and its para-carcinoma tissues. (B) The expression of miR-105-3p in breast cancer tissues with different tumor stages. (C) Kaplan–Meier survival curves analyses among breast cancer patients with different expression level of miR-105-3p. (D) The expression level of miR-105-3p in breast cancer cell lines named MCF10A, MDA-MB-231, SKBr-3, MCF-7 and ZR-75-30 respectively. (E) Transfection efficiency of miR-105-3p miRNA inhibitor and NC inhibitor in MCF-7 and ZR-75-30 cells. (F) The expression level of miR-105-3p in MCF-7 and ZR-75-30 cells transfected with miR-105-3p miRNA inhibitor and NC inhibitor. Asterisks indicated significant differences from the control (**P < 0.01, Student t-test, compared to Normal tissues, breast cancer of stage Ⅲ+Ⅳ, MCF-10A group or NC inhibitor group).

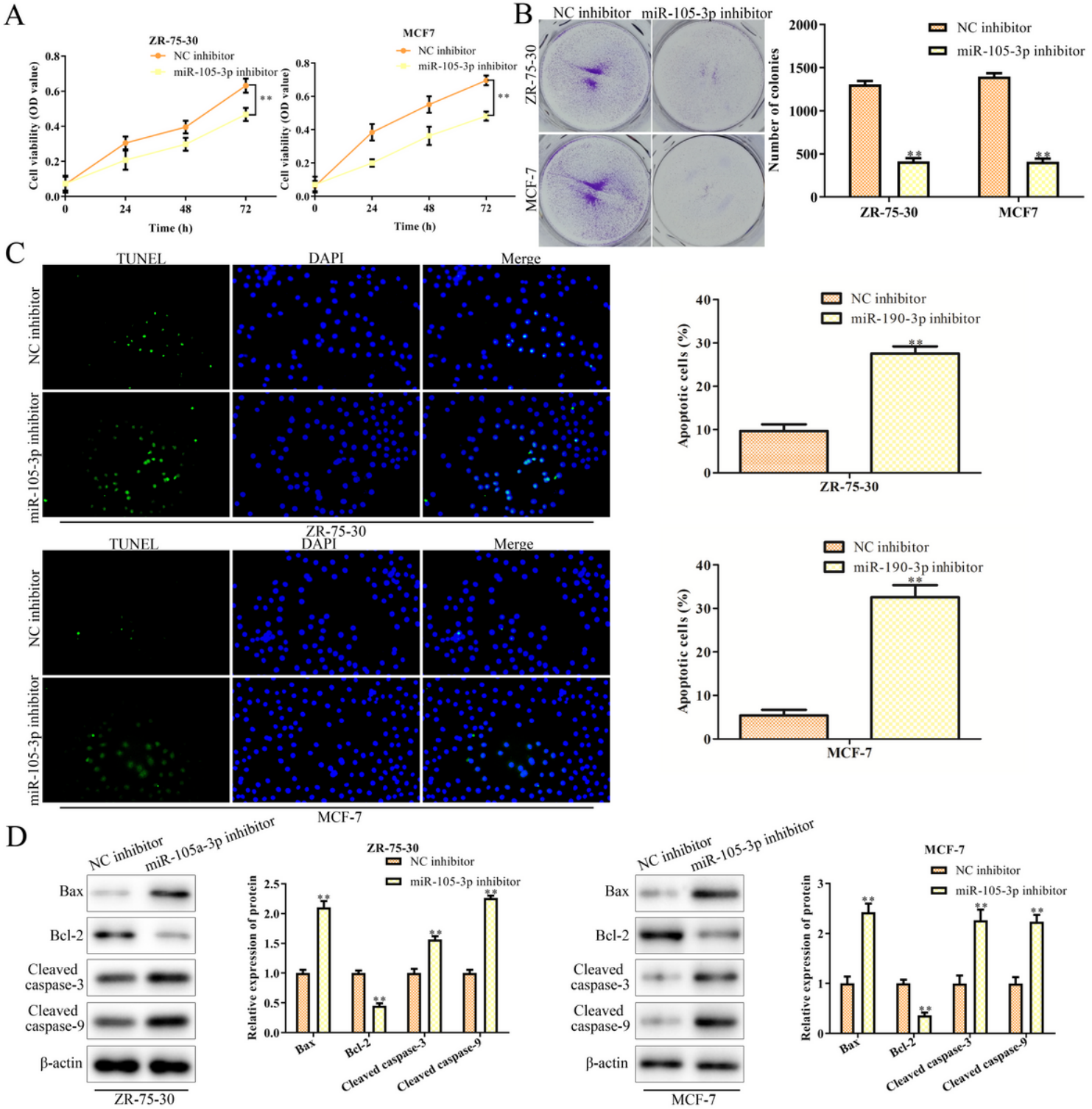

Figure 2

Downregulation of miR-105-3p inhibits cell proliferation and promote apoptosis in MCF-7 and ZR-75-30 cells. (A) MCF7 and ZR-75-30 cells were transfected with either NC inhibitor or miR-105-3p inhibitor for 72 h. (A) CCK-8 assay of cell viability of MCF-7 and ZR-75-30 cells. (B) Scratch test. (C) TUNEL assay. (D) Western blot analysis for the related proteins involved in cell apoptosis, including Bax, Bcl-2, Cleaved caspase-3, Cleaved caspase-9. Asterisks indicated significant differences from the control (**P< 0.01, Student's t-test, compared to NC inhibitor).

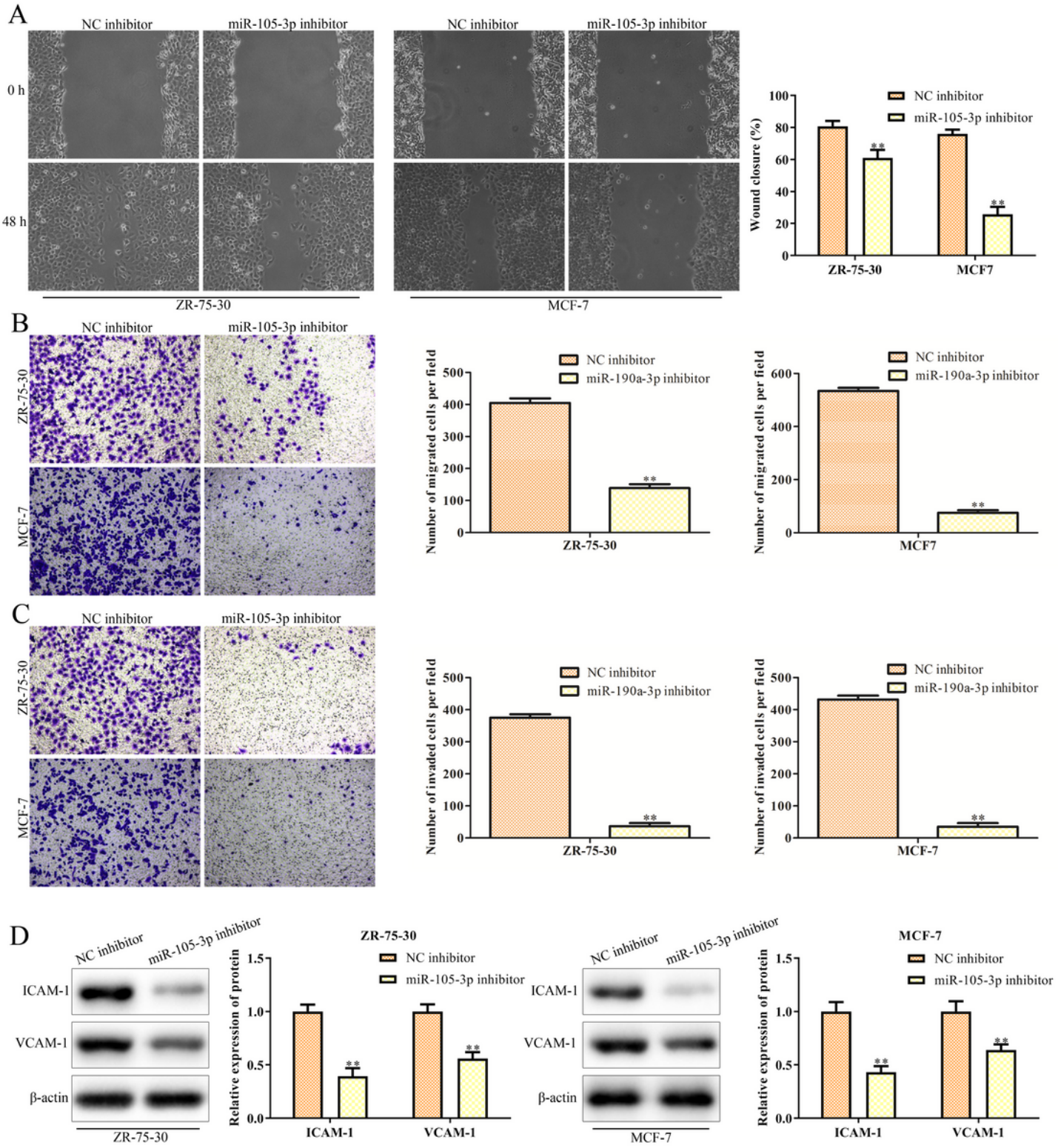

Figure 3

Downregulation of miR-105-3p suppresses the migration and invasion of MCF-7 and ZR-75-30 cells. (A) Scratch test for cell migration. (B, C) Cell migration and invasion were detected by transwell chamber assays. (D) Western blot analysis for ICAM-1 and VCAM-1 that were involved in cell migration and invasion. Asterisks indicated significant differences from the control (**P < 0.01, Student's t-test, compared to NC inhibitor group).

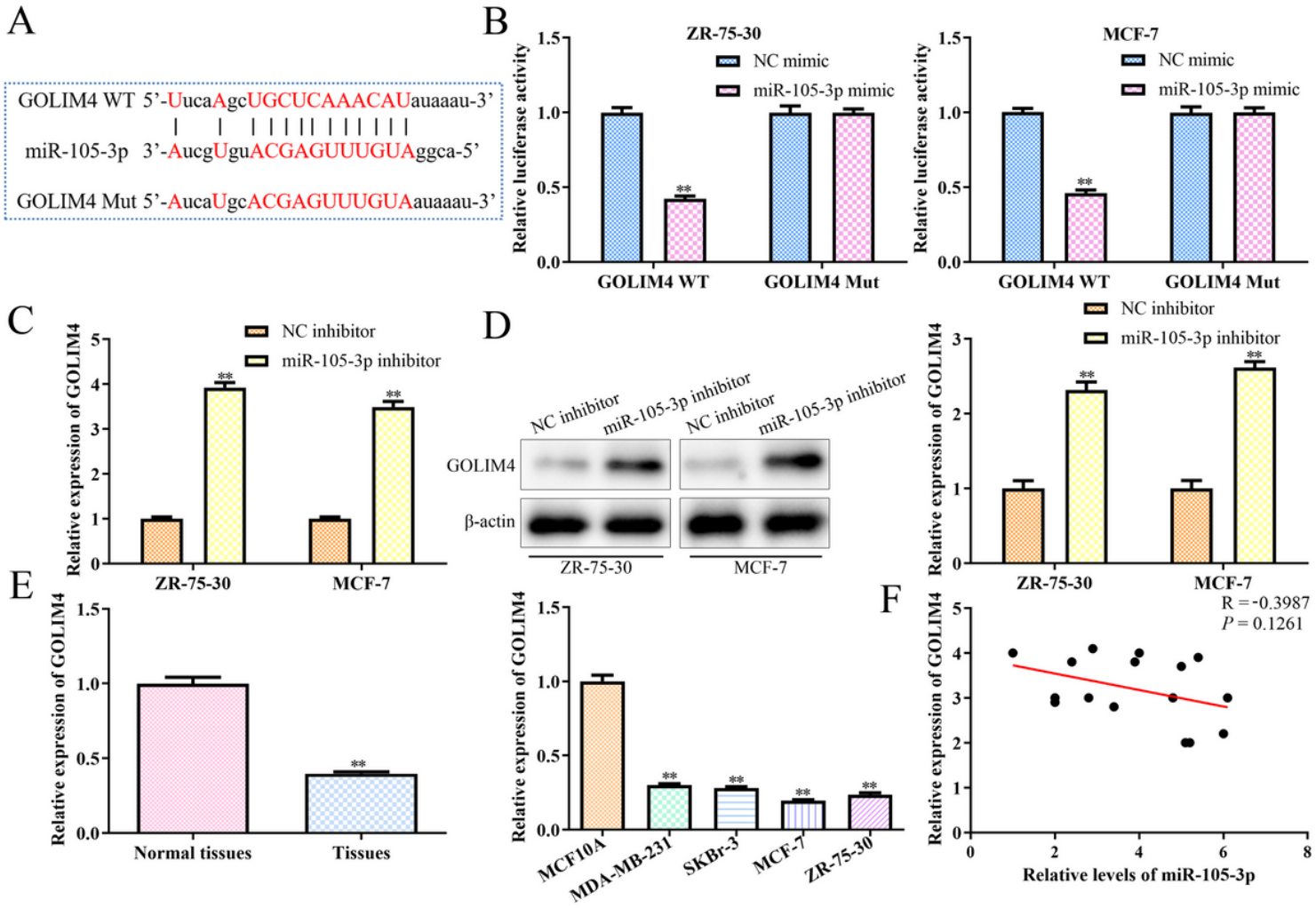

Figure 4

miR-105-3p modulates GOLIM4 expression in MCF-7 and ZR-75-30 cells by directly targeted its 3'UTR. (A) The predicted miR-105-3p target sequence in the 3'UTR of GOLIM4 and a Mut containing altered nucleotides in the 3'UTR of GOLIM4. (B) Luciferase reporter assay for luciferase activities in MCF-7 and ZR-75-30 cells synchronously transfected with miR-105-3p mimic or NC mimic together with psiCHECK2-GOLIM4-3' UTR WT or psiCHECK2- GOLIM4-3' UTR MUT. (C, D) RT-qPCR and western blot analysis for GOLIM4 in MCF-7 and ZR-75-30 cells after transfected with miR-105-3p inhibitor. Asterisks indicated significant differences from the control (E) The expression of GOLIM4 in tumor and its para-carcinoma tissues as well as the breast cancer cell lines. (F) Correlation analysis of the expression of GOLIM4 and miR-105-3p in breast cancer tissues. (**P < 0.01, Student's t-test, compared to NC mimic group or NC inhibitor group).

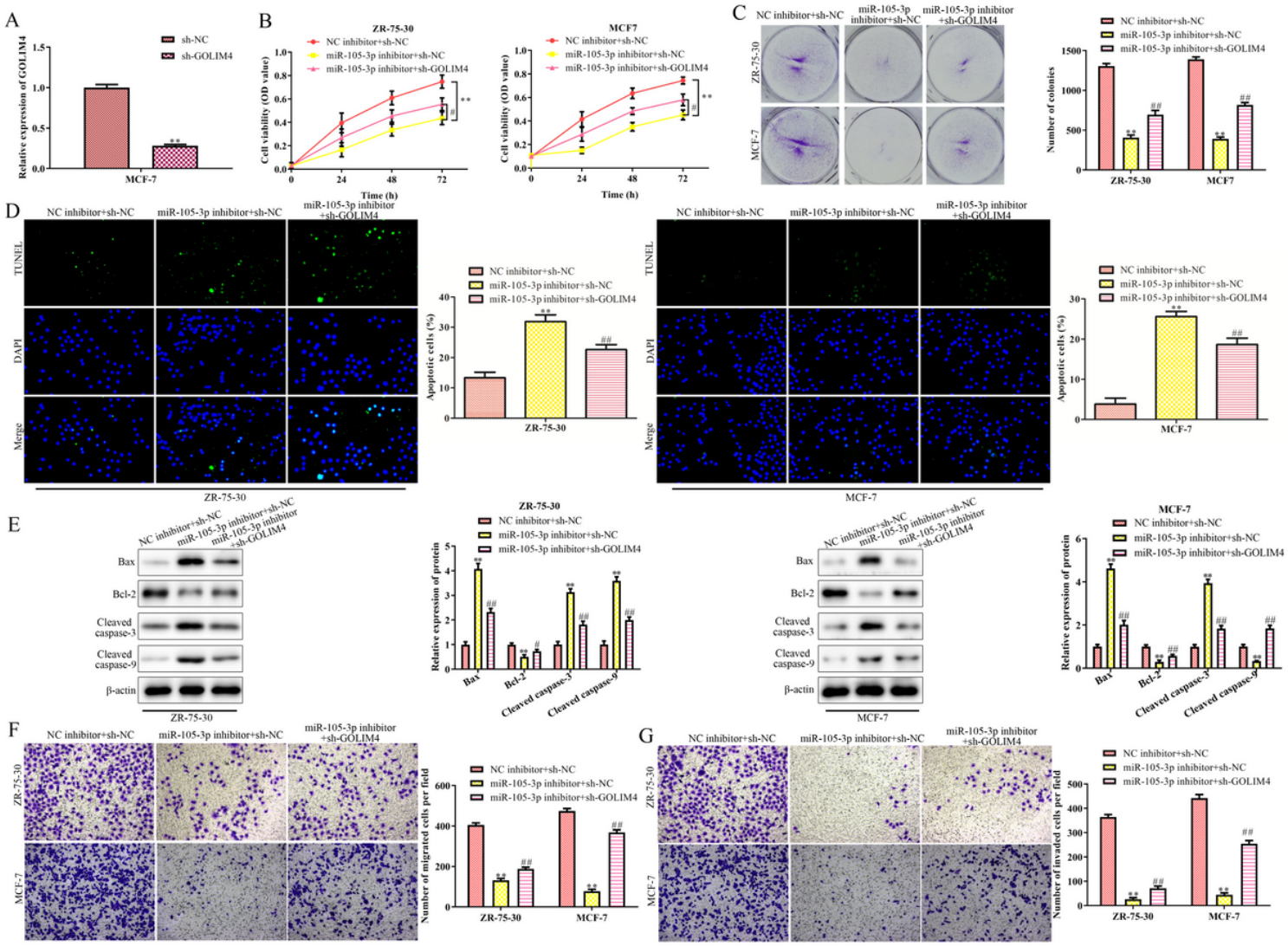

Figure 5

Silencing of GOLIM4 restores the abilities of cell proliferation and migration and inhibits the apoptosis induced by the downregulation of miR-105-3p. Either sh-NC or sh- GOLIM4 were transfected into the miR-105-3p-downregulation MCF7 and ZR-75-30 cells, and the cells co-transfected with NC inhibitor and sh-NC were chosen as control. (A) RT-qPCR analysis for the expression level of GOLIM4 in MCF-7 and ZR-75-30 cells after transfected with sh-NC or sh-GOLIM4. (B, C) CCK-8 and colony formation assays for cell viability and proliferation. (D) TUNEL assay for cell apoptosis. (E) Western blot analysis for related proteins that were involved in cell apoptosis. (E) Transwell chamber assay for cell migration and invasion. Asterisks indicated significant differences from the control (**P < 0.01, compared to sh-NC, NC inhibitor+ sh-NC group. ##P < 0.01, compared to miR-105-3p miRNA inhibitor + sh-GOLIM4).