Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Hsa-miR-497-3p impedes the proliferation and invasion of triple-negative breast cancer cells by controlling epithelial-mesenchymal transition through ZEB1 targeting

Qian Dong[1], Hu Chen[2], Ying Li[1], Yan Kong[1], Cuizhi Geng[3*], Yibing Liu[1]

[1] Department of Oncology, The Forth Hospital of Hebei Medical University, Shijiazhuang, Hebei, 050000, China
[2] Department of Hepatological Surgery, The First Hospital of Hebei Medical University, Shijiazhuang, Hebei, 050000, China
[3] Department of Breast Surgery, The Forth Hospital of Hebei Medical University, Shijiazhuang, Hebei, 050000, China

ARTICLE INFO

Original paper

Article history:
Received: July 17, 2023
Accepted: August 24, 2023
Published: August 31, 2023

Keywords:

Epithelial-mesenchymal transition, MiR-497-3p, Triple-negative breast cancer, ZEB1

ABSTRACT

This study aimed to examine the hsa-miR-497-3p effect and mechanism on the behavior of triple-negative breast cancer (TNBC) cells. We evaluated the expression of Hsa-miR-497-3p in tissue samples obtained from patients diagnosed with TNBC or luminal breast cancer (BrCa), utilizing the quantitative fluorescence polymerase chain reaction (PCR) method. We transfected hsa-miR-497-3p mimics and NC into MDA-MB-231 cells, whilehsa-miR-497-3p inhibitor and NC into TNBC cells, respectively. To examine the impact of hsa-miR-497-3p expression level on TNBC cell proliferation, invasion, and migration, we employed MTT, clone formation, Transwell, and wound healing assays. We utilized both q-PCR and western blot to validate the role of hsa-miR-497-3p in the epithelial-mesenchymal transition (EMT) of TNBC cells. To confirm the targeting relationship between hsa-miR-497-3p and ZEB1, we performed luciferase assays, q-PCR, and western blot analysis. We found that the hsa-miR-497-3p expression was down-regulated in both TNBC tissues and cell lines in comparison to luminal BrCa tissues and cell lines. Furthermore, hsa-miR-497-3p overexpression hindered the cell function of TNBC cells MDA-MB-231, while downregulating the mRNA and protein expression of vimentin and N-cadherin, while simultaneously upregulating E-cadherin expression. Our results demonstrate that hsa-miR-497-3p regulates EMT in TNBC cells through ZEB1 targeting, as evidenced by the modulation of the expression of vimentin, N-cadherin, and E-cadherin via ZEB1 inhibition. Overall, our study suggests that hsa-miR-497-3p inhibits the proliferation and invasion of TNBC cells through the modulation of EMT via ZEB1 targeting.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.8.12

Introduction

Breast cancer (BrCa) is the foremost female malignancy across the globe and has witnessed 2,261,419 new cases and 684,996 reported deaths in 2020, contributing to 11.7% and 6.9% of all cancer, respectively (1). It can be divided into the luminal, HER-2(+), and Basal-like types according to molecular typing. Among these, the luminal type, or hormone receptor-positive BrCa, constitutes the most prevalent molecular subtype, representing over 70% of all BrCa (2). The Basal-like type, or triple-negative BrCa (TNBC), has highly expressed basal epithelial molecular markers (CK5/6 or 17, EGFR) and lowly expressed ER or ER-related genes, as well as HER-2 or HER-2 or HER-2 related genes.

Approximately 15-20% of all pathological types of BrCacan be attributed to TNBC, which is currently the one with the highest mortality rate and recurrence (3). With increasing research, the treatment of TNBC is now shifting towards the categorical treatment of subgroups (4,5) and targeted therapy for specific targets identified by testing (6,7). However, there is still some heterogeneity within subgroups of categorical therapy, the accuracy of existing targeted therapies is low and the detection and treatment are not effective and affordable. Therefore, searching for new biological targets for TNBC may help to develop more effective treatments for TNBC and thus prolong overall survival in patients.

MicroRNAs (miRNAs) could specifically target miRNA genes, resulting in the degradation of the target miRNA or inhibition of protein expression of its target gene (8). As the molecular mechanisms of BrCa development and progression are further explored, dysregulation of certain miRNAs has been shown to have prognostic value in TNBC patients (9). MiR-497-3p, a miRNA that is processed from the 3' arm of the human miR-497 precursor, is often expressed at low levels in various tumor tissues, and its reduced expression is associated with various malignant biological behaviors, including tumor migration and proliferation (10-12). Additionally, overexpression of miR-497-3p can inhibit BrCa cell invasion while promoting apoptosis by downregulating MUC1 (13). Furthermore, miR-497-3p can regulate the invasive and survival abilities of BrCa cells by targeting SMAD7 (14). However, the miR-497-3p effect and mechanisms on TNBC remain unclear.

This study detached the expression of miR-497-3p in various BrCa and its impact on TNBC cell proliferation, invasion, and migration. Moreover, we conducted a comprehensive functional analysis to elucidate the mechanisms of miR-497-3p on TNBC, addressing a gap in our understanding of miR-497-3p's role in TNBC pathogenesis.

* Corresponding author. Email: gengcuizhisci@163.com

Materials and Methods

Patients and cell culture

We recruited a total of 36 female BrCa patients who received treatment at our hospital between January 2016 and December 2017 and obtained their tumor tissue samples. The average age of the patients was 53.4 years. We classified the molecular typing of BrCa in all the patients based on their pathological features and immunohistochemical reports after surgery, in accordance with the diagnosis and treatment guidelines of BrCain China (2019 edition). Among these patients, 22 were Luminal-type cases and 14 were TNBC-type cases. All patients were staged according to the American Joint Committee on Cancer 8th edition TNM staging system for BrCa, and they were all diagnosed with TNM stage I-III BrCa, with tumors ranging from 0.1 to 3.5 cm in diameter. All 36 patients had complete clinical data, and their post-operative pathological histology reports were reviewed by senior pathologists. Patient demographics are summarized in Table 1. Each patient provided written consent after receiving complete information. We purchased MDA-MB-231, BT549, and MDA-MB-468 and MCF-7 from Procell Co.

Fluorescence quantitative PCR

According to the instructions of the RNA gent Isolation System (Promega, USA), 2 μg of total RNA was extracted with Trizol reagent. Subsequently, cDNA was reverse transcribed using the reverse transcription kit (Promega).

We performed the amplification reactions as per the instructions provided with the Trans Start Top Green qPCR Supermix. An initial denaturation step at 95°C for 10 minutes was followed by 42 amplification cycles at 95°C for 15 seconds and 60°C for 1 minute. Additionally, we quantified the mRNA expression of miR-497-3p, ZEB1, and EMT-associated proteins using the $2^{-\Delta\Delta Ct}$ method, and the sequences of primers are listed in Table 2.

Cell transfection

TNBC cells with low miR-497-3p expression (MDA-MB-231) and high miR-497-3p expression (BT-549) were cultured in RPMI-1640 medium with 5% CO_2 at 37°C and until they reached a confluence of 60-70%, which took 24 hours. miR-497-3p mimics, miR-497-3p NC plasmids and miR-497-3p inhibitors were procured from Guangzhou Ruibo Biotechnology Co., Ltd. The transfection was performed using 1 μL of Lipo6000TM Cell Transfection Reagent.

MTT assay

Seeded cells into 96-well plates (1-3 × 10^3 cells/well) and cultured for 0, 24, 48, and 72 hours. Following this, added 20 μl MTT solution to each well at the appropriate time. The liquid in the wells was removed with an empty needle (1 ml), added 10 μl DMSO to each well after a further 4 h of growth in the dark. The cells were gently stirred for 10 min, after which cell activity was assessed. Then, the absorbance was examined at 450nm using a multifunctional microplate reader.

Table 1. Patient demographics.

		n	miR-497 expression		*P* value
			low	high	
Age (years)	<40	2	2	0	0.124
	≥40	34	11	23	
Clinical stage	I	17	6	11	1
	II	18	7	11	
	III	1	0	1	
Lymph nodes	positive	10	3	7	0.716
	negative	26	10	16	
Molecular typing	lumina	22	6	16	0.286
	TNBC	14	7	7	
Tumor size	≤2cm	26	12	14	0.06
	>2cm≤5cm	10	1	9	
	>5cm	0	0	0	
Vascular invasion	Yes	3	1	2	1
	No	33	12	21	

Table 2. List of primer sequences for q-PCR.

Gene	Primer sequences (forward/reverse)
E-cadherin	5'- CCGCCATCGCTTACA -3' 5'- GGCACCTGACCCTTGTA -3'
N-cadherin	5'- GAAAGACCCATCCACG -3' 5'- CCTGCTCACCACCACTA -3'
Vimentin	5'- AAGGCGAGGAGAGCAGGATT -3' 5'- GGTCATCGTGATGCTGAGAAG-3'
GAPDH	5'-CAGATACTGGCTAAATGGGGAT-3' 5'-ACCTTGGCTGGTTTGATGAC-3'

Clone formation assay

Fixed cells in 4% paraformaldehyde for 20 min, removed it and stained cells for 30 min. The stained cells were washed three times using PBS. After drying, a microscope was applied to count visible colonies greater than or equal to 50 cells.

Wound-healing assay

Marker lines were drawn through the diameter of the wells on the back of the 6-well plates. A total of 5×10^5 well-grown cells were inoculated in the 6-well plates and cultivated in an incubator at 37°Covernight until they formed a monolayer. Next, the bottom of the 6-well plates was scored with a sterile gun in the center perpendicular to the marker lines. Following this, washed cells three times using PBS and added to a 1% serum-supplemented medium for growth. Photographs of cell migration were taken under the microscope at 0 h and 24 h, and wound healing rates were measured by the ImageJ software.

Western blotting (WB)

Cells from each group were collected and total cellular protein was extracted via PMSF and RIPA. The BCA kit was applied to measure the protein concentration. Blocked membranes with 10% skimmed milk powder in TBS for 1 hour, incubated with primary antibodies against ZEB1, Vimentin, N-cadherin, E-cadherin, and GAPDH overnight at 4°C. After washing, incubated for 1 hour at 37°C with IR fluorescence-labeled secondary antibody diluted in TBST. The PVDF membranes were immersed in 1mL of color development solution for approximately 10 min away from light and the results were observed. The ImageJ software was utilized to analyze the protein expression levels. In the experiments, GAPDH was used as a control.

Luciferase reporter assay

The primer 5'-GTGGTTTA-3' was employed to construct the wild-type vector 3'-UTR (h-ZEB1-WT) for ZEB1. The mutant vector 3′-UTR (h-ZEB1-MUT) of ZEB1 was constructed using a point mutation introduced in the 3`-UTR sequence of ZEB1 mRNA (5'-CACCAAAT-3'). The wild-type vector, mutant vector of ZEB1, MiR-497-3p mimics, and miR-NC were mixed with Lipofectamine 2000™ and transfected in MDA-MB-231 cells. Luciferase activity was detected after 48h via the luciferase reporter gene assay kit.

Statistical analysis

Statistical analysis was performed using the SPSS 22.0 software. Independent samples t-test was utilized for measurement data, and the χ^2 test was applied for comparing count data. The comparison among multiple groups was conducted using One-way ANOVA.

Results

Low expression of miR-497-3p in TNBC

A marked reduction in miR-497-3pexpression level in TNBC tissues when compared to luminal BrCa tissues (Figure 1a). Then, we conducted a comparative analysis of miR-497-3pexpression in various BrCa cell lines. As expected, there was a significantly lower miR-497-3p expression in TNBC cell lines than in luminal BrCa cell lines (Figure 1b). Additionally, miR-497-3p in BT-549 was found to be the highest expression in TNBC cell lines, whereas the lowest was in MDA-MB-231.Consequently, BT549 and MDA-MB-231 were employed for the follow-up experiments below. It should be noted that miR-497-3p expression level did not correlate with age, TNM stage, lymph node metastasis, molecular staging, tumor size, and vascular invasion of BrCa patients (Table 1).

miR-497-3p regulates the proliferative, invasive, and migrative abilities of TNBC cells

After transfecting MDA-MB-231 and BT549 cells with miR-497-3p mimics, NC plasmid, and miR-497-3p inhibitor for 48 h, we conducted MTT, cell clone formation, wound healing, and Transwell assays to measure cell proliferation and metastasis. Results indicated that in MDA-MB-231 cells, the miR-497 mimic group significantly inhibited proliferation (Figure 2a, b), invasion (Figure 3a), and migration (Figure 3c) compared to the NC group. In BT549 cells, the miR-497 inhibitor group significantly increased proliferation (Figure 2c), invasion (Figure 3b), and migration (Figure 3d) compared to the NC group. These results suggest that miR-497-3p inhibits TNBC cell proliferation, invasion, and migration.

ZEB1 is the target gene of miR-497-3p

The qPCR experiments indicated successful transfec-

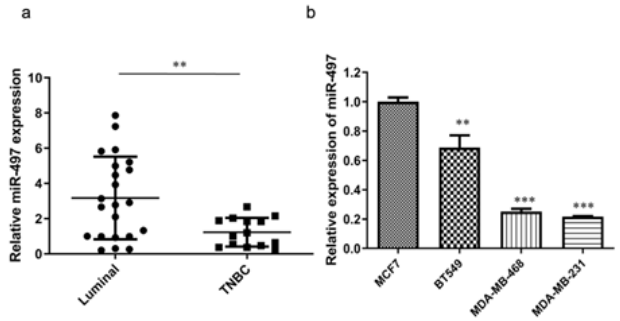

Figure 1. (A) Luminal BrCa tissues had significantly lower miR-497-3p expression levels than TNBC tissues. (B) TNBC cell lines had significantly lower miR-497-3p expression levels than MCF7 luminal BrCa cell line. **P<0.01; *** P<0.001.

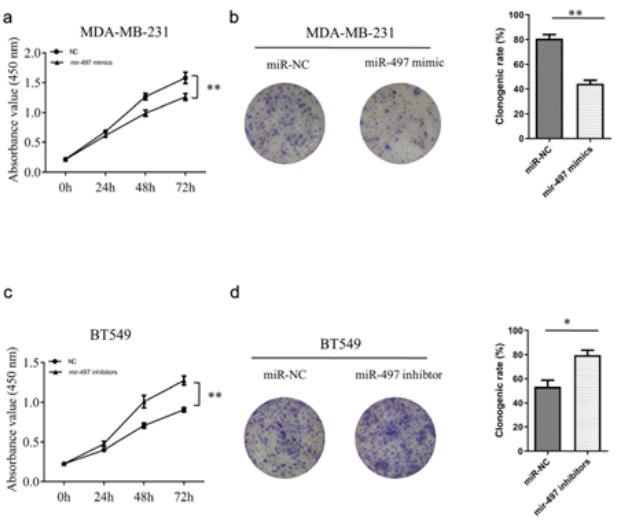

Figure 2. The effect of miR-497-3p on TNBC cell proliferation. Overexpression of miR-497-3p inhibited MDA-MB-231 cell proliferation (A-B), while inhibition of miR-497-3p promoted BT-549 cell proliferation (C-D). * P<0.05; ** P<0.01.

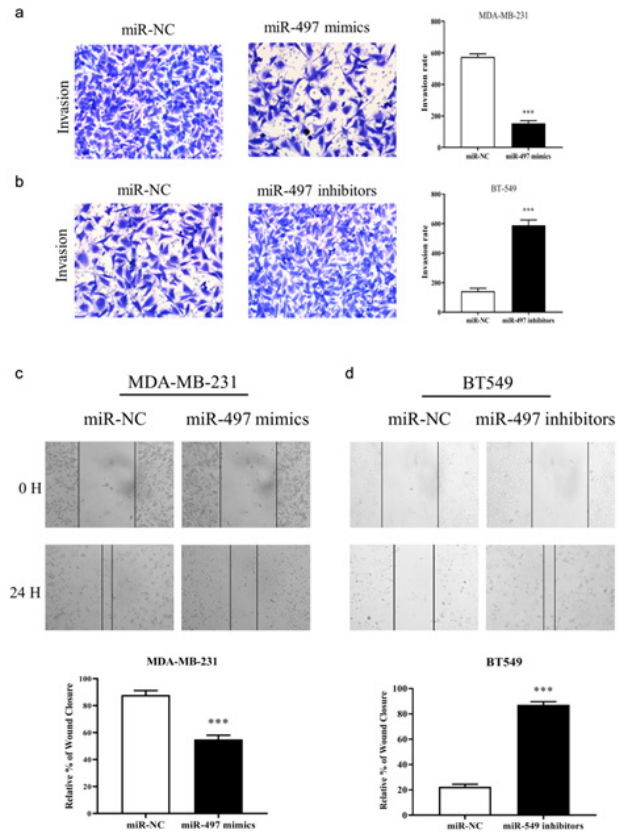

Figure 3. The effect of miR-497-3p on TNBC cell invasion and migration. (A) Overexpressed miR-497-3p hindered MDA-MB-231 cell invasion. (B) Knockdown of miR-497-3p promoted BT-549 cell invasion. (C-D) The wound healing assay was used to assess the migratory ability of MDA-MB-231 cells with overexpressed miR-497-3p and BT-549 cells with low miR-497-3p expression. *** P<0.001.

tion of MDA-MB-231 cells with miR-497 mimics (Figure 4a) and significantly lower ZEB1 expression in the miR-497 mimics group compared to miR-NC group (Figure 4b). WB experiments also showed a remarkable decrease in ZEB1 protein expression in the miR-497 mimics group (Figure 4c, d). Bioinformatics software predicted a binding site for miR-497-3p in the ZEB1 3'-UTR region (Figure 4e), which was confirmed by a luciferase reporter gene assay showing that miR-497-3p significantly inhibited luciferase activity in the ZEB1 3'-UTR wild type in MDA-MB-231 (Figure 4f). Thus, miR-497-3p was found to target ZEB1 and regulate its expression.

The role of miR-497-3p in EMT of TNBC cells

MiR-497-3p overexpression resulted in decreased expression of Vimentin and N-cadherin mRNA and increased E-cadherin mRNA in MDA-MB-231 cells, as demonstrated by q-PCR and Western blot analysis (Figure 5a, b). This indicates that miR-497-3p can regulate the EMT process by targeting ZEB1.

miR-497-3psuppressesEMT, proliferation, and invasion of TNBC cells by targeting ZEB1

Previous studies have indicated that miR-497 suppresses the TNBC cells function through YAP1 (15). However, our research has discovered that miR-497-3p targets the ZEB1 gene and regulates the EMT of TNBC. In order to investigate whether the inhibitory effects of miR-497-3p on TNBC cells' EMT, proliferation, and invasion were mediated by ZEB1, we conducted Western blot experiments, MTT assays, and Transwell invasion assays. The Western blot experiments showed that the protein expression levels of vimentin, N-cadherin, and ZEB1 were increased, while the expression of E-cadherin was drastically decreased in miR-497-overexpressing MDA-MB-231 cells after restoring ZEB1 expression levels compared to the Vector group (Figure 6b). The inhibitory effects of miR-497-3p on MDA-MB-231 cell proliferation (Figure 6a) and invasion (Figure 6c) were reversed after restoring ZEB1 expression levels compared to the miR-mimics group, as observed in the MTT and Transwell invasion assays. All these suggest that miR-497-3p inhibits the malignant phenotype of TNBC cells by targeting ZEB1.

Discussion

MiRNAs are vital regulators of cancer development and progression. Here, we found that miRNAs regulate TNBC cells. Specifically, we observed miR-497-3p was a lower expression in TNBC, and it could regulate EMT and inhibit the proliferative, invasive, and migrative abilities

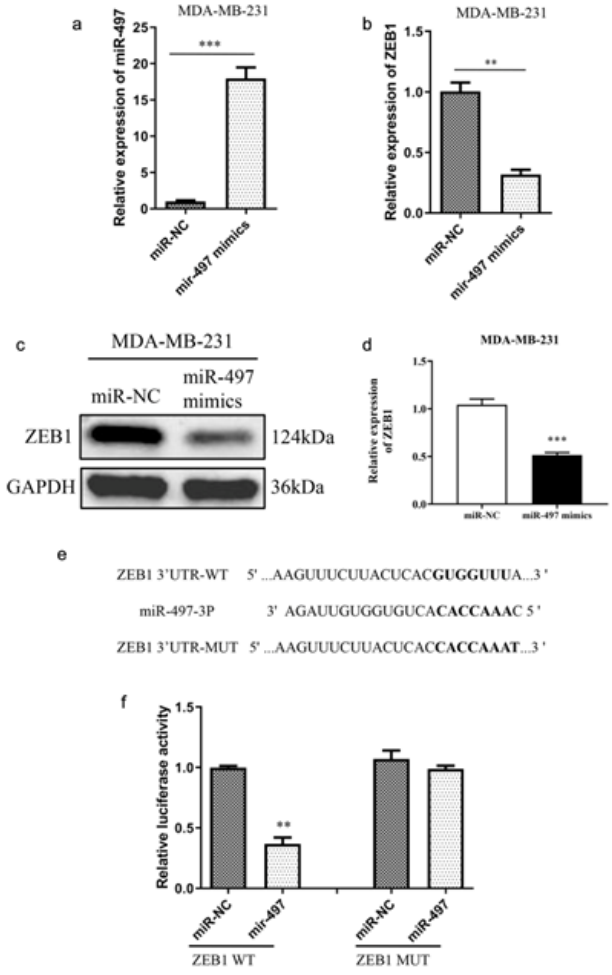

Figure 4. ZEB1 is the target gene of miR-497-3p. (A) miR-497-3p mimics transfection efficiency detection using q-PCR. (B) The overexpression of miR-497-3p resulted in a downregulation of the mRNA (C) and protein (D) expression of ZEB1. (E) By utilizing Target Scan and miRbase software, it was found that miR-497-3p is a complementary binding site in the ZEB1 3'-UTR region. (F)The wild type of ZEB1 demonstrated a significant decrease in luciferase activity following transfection with the miR-497-3p gene. ** P<0.01, *** P<0.001.

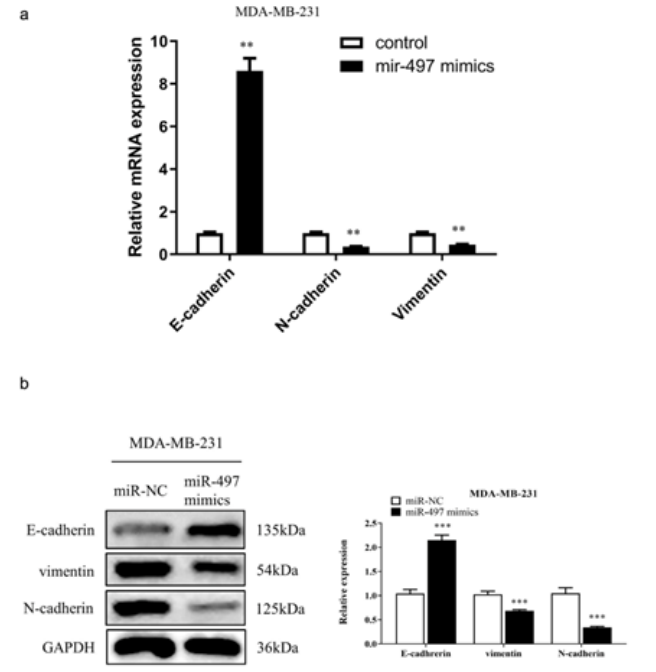

Figure 5. The role of miR-497-3p in EMT of TNBC cells. The expression of miR-497-3p mimics significantly inhibited mRNA(A) and (B) protein expression of Vimentin and N-cadherin, while promoting mRNA (A) and protein(B) expression of E-cadherin. ** P<0.01, *** P<0.001.

of TNBC cells. Our mechanistic analysis further suggests that miR-497-3p regulates EMT by targeting ZEB1 and thereby suppressing TNBC proliferation and invasion. Thus, our study indicates that miR-497-3p may function as a promising target for TNBC prevention.

The research conducted by Jiang et al. shed light on the miR-497-3p role in BrCa by revealing that low levels of ER in ER-negative BrCa led to reduced miR-497-3p expression. This, in turn, resulted in increased expression of MIF and MMP9, leading to upregulation of ERRα and ultimately promoting the cancer cells growth. Similarly, Tao et al. demonstrated that miR-497-3p can regulate BrCa by targeting specific genes. However, the differential expression of miR-497-3p in different molecular types of BrCa cells and the mechanism of miR-497-3p in TNBC cell invasion and metastasis remain unclear. Here, the TNBC cell lines had notably lower miR-497-3p expression levels than Luminal cell lines, as demonstrated by real-time fluorescence quantitative PCR and WB assays. Further analyses indicated that transfecting miR-497-3p mimics and inhibitors in cells with low and high miR-497-3p expression, respectively, significantly inhibited or enhanced the proliferative, invasive, and migratory capacities of TNBC cells. To elucidate its mechanism in TNBC cell invasion and migration, q-PCR and WB experiments were conducted to confirm its regulatory role of it in the EMT process of TNBC. The findings revealed that miR-497-3p targets the gene ZEB1. Consequently, it was hypothesized that miR-497-3p could regulate EMT by targeting ZEB1 expression, ultimately impeding TNBC cell invasion and migration (16).

ZEB1 belongs to the ZEB transcription factor family and binds to target DNA sequences to suppress the expression of cell adhesion molecules as well as genes related to cell polarity, ultimately inducing EMT. Previous studies have shown that DNAJB9 can inhibit TNBC metastasis by promoting FBXO45-mediated degradation of ZEB1 (5), while CHFR can modulate chemoresistance in TNBC by disrupting ZEB1 (17). MiR-128-3p could regulate colon cancer cell invasion and migration by targeting and regulating ZEB1 expression (18). Collectively, ZEB1 plays a critical role in the overall mechanism of tumor invasion and migration, while targeting ZEB1 with miR-497-3p may also be pivotal in TNBC development. To test this hypothesis, a rescue assay was designed in which ZEB1 was transfected into miR-497-overexpressing MDA-MB-231 cells, and the biological characteristics were observed. The results indicate that miR-497-3p can regulate EMT by targeting ZEB1, thereby hindering TNBC invasion and migration.

It is worth noting that, however, this study did not further analyze the role of the miR-497-3p-ZEB1 signaling pathway in the mechanism of TNBC development and progression due to experimental constraints. This does not mean that further studies on the miR-497-3p-ZEB1 signaling pathway are not clinically and biologically significant.

Acknowledgements
Not applicable.

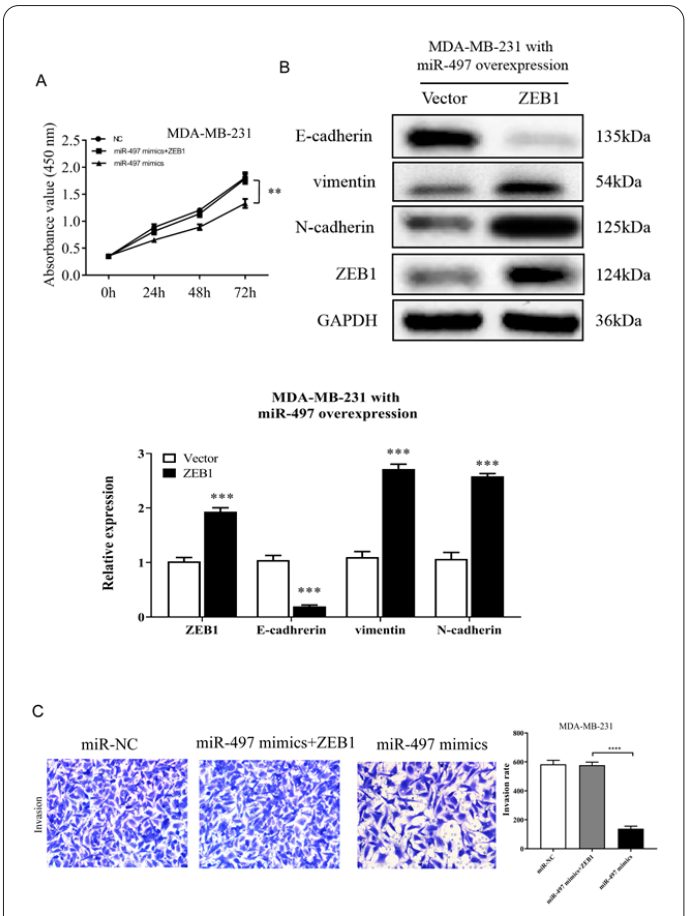

Figure 6. miR-497-3p suppresses EMT, proliferation, and invasion of TNBC cells by targeting ZEB1. (A) Overexpression of ZEB1 promoted the proliferation of MDA-MB-231 cells, whereas knockdown of ZEB1 inhibited cell proliferation. (B)Overexpression of miR-497-3p in MDA-MB-231 cells significantly upregulated the protein expression levels of vimentin and N-cadherin and downregulated the E-cadherin. (C)Overexpression of ZEB1 enhanced the invasive ability of MDA-MB-231 cells, while knockdown of ZEB1 hindered cell invasion. The statistical analysis revealed that the differences were significant with ** P<0.01, ***P<0.001, and ****P<0.0001.

Competing Interests
The authors have no relevant financial or non-financial interests to disclose.

Author Contributions
Conceptualization, methodology, and formal analysis: Qian Dong. Data curation and writing-original draft preparation: Hu Chen. Visualization and investigation: Ying Li.Supervision and formal analysis: Yan Kong.Writing-reviewing and editing and project administration: Cuizhi Geng. Resources and validation: Yibing Liu.

Data Availability
The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval
All protocols were approved by the Medical Ethics Committee of the Fourth Hospital of Hebei Medical University (2015MEC074).

Consent to participate
Informed consent was obtained from all individual participants included in the study.

Consent to publish
Not applicable.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71(3): 209-249. https://doi.org/10.3322/caac.21660
2. Howlader N, Altekruse SF, Li CI, Chen VW, Clarke CA, Ries LA, Cronin KA. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. J Natl Cancer Inst 2014; 106(5): dju055. https://doi.org/10.1093/jnci/dju055
3. Garrido-Castro AC, Lin NU, Polyak K. Insights into molecular classifications of triple-negative breast cancer: improving patient selection for treatment. Cancer Discov 2019; 9(2): 176-198. https://doi.org/10.1158/2159-8290.CD-18-1177
4. Liu YR, Jiang YZ, Xu XE, Yu KD, Jin X, Hu X, Zuo WJ, Hao S, Wu J, Liu GY, Di GH, Li DQ, He XH, Hu WG, Shao ZM. Comprehensive transcriptome analysis identifies novel molecular subtypes and subtype-specific RNAs of triple-negative breast cancer. Breast Cancer Res 2016; 18(1): 33. https://doi.org/10.1186/s13058-016-0690-8
5. Kim HY, Kim YM, Hong S. DNAJB9 suppresses the metastasis of triple-negative breast cancer by promoting FBXO45-mediated degradation of ZEB1. Cell Death Dis 2021; 12(5): 461. https://doi.org/10.1038/s41419-021-03757-x
6. Adams S, Diéras V, Barrios CH, Winer EP, Schneeweiss A, Iwata H, Loi S, Patel S, Henschel V, Chui SY, Rugo HS, Emens LA, Schmid P. Patient-reported outcomes from the phase III IMpassion130 trial of atezolizumab plus nab-paclitaxel in metastatic triple-negative breast cancer. Ann Oncol 2020; 31(5): 582-589. https://doi.org/10.1016/j.annonc.2020.02.003
7. Im SA, Xu B, Li W, Robson M, Ouyang Q, Yeh DC, Iwata H, Park YH, Sohn JH, Tseng LM, Goessl C, Wu W, Masuda N. Olaparib monotherapy for Asian patients with a germline BRCA mutation and HER2-negative metastatic breast cancer: OlympiAD randomized trial subgroup analysis. Sci Rep 2020; 10(1): 8753. https://doi.org/10.1038/s41598-020-63033-4
8. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, Zeng YX, Shao JY. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA 2008; 14(11): 2348-2360. https://doi.org/10.1261/rna.1034808
9. Piasecka D, Braun M, Kordek R, Sadej R, Romanska H. MicroRNAs in regulation of triple-negative breast cancer progression. J Cancer Res Clin Oncol 2018; 144(8): 1401-1411. https://doi.org/10.1007/s00432-018-2689-2
10. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, Kellokumpu-Lehtinen P, Bono P, Kataja V, Desmedt C, Piccart M, Loibl S, Denkert C, Smyth M, Joensuu H, Sotiriou C. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol 2014; 25(8): 1544-1550. https://doi.org/10.1093/annonc/mdu112
11. Tu Y, Liu L, Zhao D, Liu Y, Ma X, Fan Y, Wan L, Huang T, Cheng Z, Shen B. Overexpression of miRNA-497 inhibits tumor angiogenesis by targeting VEGFR2. Sci Rep 2015; 5: 13827. https://doi.org/10.1038/srep13827
12. Solinas C, Ceppi M, Lambertini M, Scartozzi M, Buisseret L, Garaud S, Fumagalli D, de Azambuja E, Salgado R, Sotiriou C, Willard-Gallo K, Ignatiadis M. Tumor-infiltrating lymphocytes in patients with HER2-positive breast cancer treated with neoadjuvant chemotherapy plus trastuzumab, lapatinib or their combination: A meta-analysis of randomized controlled trials. Cancer Treat Rev 2017; 57: 8-15. https://doi.org/10.1016/j.ctrv.2017.04.005
13. Tao S, Li H, Ma X, Lian B, He J, Gao Y, Li J. Methylation-mediated silencing of microRNA-497 promotes breast cancer progression through up-regulation of mucin1. Front Oncol 2020; 10: 552099. https://doi.org/10.3389/fonc.2020.552099
14. Liu J, Zhou Y, Shi Z, Hu Y, Meng T, Zhang X, Zhang S, Zhang J. MicroRNA-497 modulates breast cancer cell proliferation, invasion, and survival by targeting SMAD7. DNA Cell Biol 2016; 35(9): 521-529. https://doi.org/10.1089/dna.2016.3282
15. Li Y, Hua K, Jin J, Fang L. MiR-497 inhibits proliferation and invasion in triple-negative breast cancer cells via YAP1. Oncol Lett 2021; 22(2): 580. https://doi.org/10.3892/ol.2021.12841
16. Han L, Liu B, Jiang L, Liu J, Han S. MicroRNA-497 downregulation contributes to cell proliferation, migration, and invasion of estrogen receptor alpha negative breast cancer by targeting estrogen-related receptor alpha. Tumour Biol 2016; 37(10): 13205-13214. https://doi.org/10.1007/s13277-016-5200-1
17. Luo H, Zhou Z, Huang S, Ma M, Zhao M, Tang L, Quan Y, Zeng Y, Su L, Kim J, Zhang P. CHFR regulates chemoresistance in triple-negative breast cancer through destabilizing ZEB1. Cell Death Dis 2021; 12(9): 820. https://doi.org/10.1038/s41419-021-04114-8
18. Dong N, Li X, Xue C, Zhang L, Wang C, Xu X, Shan A. Astragalus polysaccharides alleviates LPS-induced inflammation via the NF-κB/MAPK signaling pathway. J Cell Physiol 2020; 235(7-8): 5525-5540. https://doi.org/10.1002/jcp.29452