

Original Article

The HER2-targeted drug lapatinib inhibits the progression of breast cancer by regulating the miR-128-3p-BTG2 axis

Huihui Yang¹, Wei Wang²

¹Department of Pharmacy, Wenling Hospital of Traditional Chinese Medicine, Wenling, Taizhou, Zhejiang Province, China; ²Department of Pharmacy, Yantai Yuhuangding Hospital, Yantai, Shandong Province, China

Received October 18, 2020; Accepted November 24, 2020; Epub February 15, 2021; Published February 28, 2021

Abstract: Objective: To explore the molecular mechanism the HER2-targeted drug lapatinib uses to inhibit the progression of breast cancer (BC). Methods: CCK-8 and flow cytometry were used to measure the proliferation and apoptosis of BC cells after lapatinib intervention. qRT-PCR and Western blot were used to measure the changes in the miR-128-3p and BTG2 levels in the cells. The inhibitory or over-expression vectors of miR-128-3p and BTG2 were established and transfected into the BC cells to observe the changes in cell proliferation and apoptosis. A double luciferase report was used to confirm the relationship between miR-128-3p and BTG2. Results: The proliferation, migration, and invasion of MDA-MB-453 and BT-47 were significantly reduced, and the apoptosis rates increased in the lapatinib group (LG), the lapatinib + sh-miR-128-3p group (L-sh-G), and the sh-miR-128-3p group (sh-G). The proliferation, migration, and invasion abilities of MDA-MB-453 and BT-47 in the LG and sh-G were not significantly different and were significantly higher than they were in the L-sh-G, and the apoptosis rate was lower than it was in the L-sh-G. The MDA-MB-453 and BT-47 expressions in the normal control group (NCG) were significantly higher than they were in the LG and sh-G. There was no significant difference between the LG and the sh-G in terms of MDA-MB-453 and BT-47, but both groups were significantly higher than the L-sh-G. A double luciferase experiment revealed that there was a targeted relationship between miR-128-3P and BTG2. Conclusion: Lapatinib inhibits the proliferation of BC cells and promotes apoptosis, and its mechanism is related to its ability to regulate the miR-128-3p/BTG2 axis.

Keywords: Lapatinib, miR-128-3p, BTG2, breast cancer

Introduction

BC is one of the most familiar malignant tumors in women, ranking first among deaths from cancer among women [1]. Invasion and metastasis are the main causes of poor BC prognoses, but the molecular mechanism involved in BC's invasion and metastasis is still not completely clear [2].

MicroRNA (miR-NA) is a kind of non-coding small molecule RNA 19-25 nucleotides in length. It mainly participates in gene post-transcriptional regulation by binding to the 3'UTR region of the target gene mRNA [3]. Recent studies have revealed that miRNA can participate in biological processes such as the proliferation, differentiation, and apoptosis of tumor cells by

regulating the expressions of oncogenes or tumor suppressor genes [4-6]. miR-128-3p is a member of the miR-128 family. Studies by Block et al. have revealed that miR-128-3p is abnormal in BC patients, and it is related to the clinical prognosis [7]. BTG2 is a B cell translocation gene 2, so it is a members of the BTG gene family. BTG2 is a cell proliferation inhibitory gene. BTG2 plays a very vital role in the growth, proliferation, cell cycle, and apoptosis of gastric carcinoma cells, as well as in tumor formation, invasion, and metastasis [8, 9]. Some studies have shown that BTG2 can inhibit the proliferation and migration of BC cells [10]. Lapatinib is a small molecule tyrosine kinase inhibitor, and it has been approved by the U.S. Food and Drug Administration (FDA) to treat Her2 positive advanced or metastatic BC resis-

tant to anthracycline, taxanes, or trastuzumab [11]. Studies have shown that lapatinib affects the progression of BC through some signaling pathways, including the PI3K/AKT/mTOR, Ras/ERK, and HER2 pathways, but whether it affects the progression of BC through the miR-128-3p/BTG2 axis is unclear [12, 13].

TargetScan software has predicted that BTG2 is the targeted regulatory gene of miR-128-3p. Therefore, we speculated that miR-128-3p may promote the invasion and metastasis of BC through the targeted regulation of BTG2. This study aimed to investigate whether lapatinib can induce apoptosis by blocking the miR-128-3p/BTG2 signaling pathway and exerting an anti-HER-2-positive BC proliferation effect.

Materials and methods

Ethics

This study was approved by Wenling Hospital's Traditional Chinese Medicine ethics committee.

Main reagents and instruments

The reagents and instruments included human BC cells MDA-MB-453 and BT-474 (Beina Biology, BNCC311866, BNCC259970), lapatinib (GlaxoSmithKline (Tianjin) Co., Ltd., H201300-62), fetal bovine serum (Gibco, USA, 16000-044), Transwell and CCK-8 kits (Beyotime Biotechnology, C0038), T25 culture flask, six-well plate (Thermo Company, USA), Western and IP cell lysate, BTG2 protein quantitative kits (Shanghai Hushen Industry Co., Ltd., HZ-bTG-Mu), TUNEL detection kits (Solarbio, T2190), Annexin V/PI apoptosis detection kits (Solarbio, CA1020), ECL luminescence kit, enzyme-labeling instrument (BioTek Company, USA, 212-61000), and flow cytometer (BDBiosciences, USA, FACSCanto II).

Cell culture and transfection

MDA-MB-453 cells were cultivated in an L-15 medium comprising 10% fetal bovine serum. BT-474 cells were cultivated in an RPMI-1640 medium comprising 10% fetal bovine serum. In the experiment, they were divided into these groups: NCG, sh-G, LG, and L-sh-G. In the experiment, Lipofectamine™ 2000 kits (Invitrogen) were used to transfect the cells, and the opera-

tional steps were strictly carried out in accordance with the kit's instructions.

Western blot was used to measure the expression levels of the related proteins

In each experimental group, the cells were lysed to extract the total proteins, and 12% SDS-PAGE gel electrophoresis was prepared and a membrane transfer was conducted. After sealing, the primary antibody (The dilution ratio of the primary antibody was 1:500) was added and stored at 4°C overnight. The membrane was rinsed with TBST 3 times, 10 min/time. The diluted secondary antibody (1:5000) was added and cultivated at room temperature for 2 h. The membrane was rinsed with TBST 3 times, 10 min/time. GAPDH was used as an internal reference. Chemiluminescence kits were used to color and glow the protein bands. The experiment was repeated for three times independently.

The cell proliferation, invasion, and migration experiments

(1) CCK-8 was used to test the growth of the transfected cells: the transfected TPC-1 cells were inoculated into 96-hole cell culture plates, and the blank control group was instituted at the same time. The blank control group was treated with untransfected TPC-1 with a cell concentration of 1×10^5 cells/mL. The cell suspension (100 μ L) was added to each well (a 6-well repeat was set up in each group). The cells were cultured in a CO₂ incubator at 37°C for 24 h. Then, the CCK-8 was added for the cell proliferation quantification, and the OD450 value was read within 2 hours. (2) Transwell was used to determine the cell invasion ability. After the cells transfected for 24 hours, the cells were assimilated and rinsed 3 times in a serum-free medium then counted to prepare a 5×10^5 cells/mL cell suspension. The cell suspension (100 μ L) (3 chambers) was added to the upper chamber. The complete culture medium (500 μ L) was added to the lower chamber. After culturing for 24 hours, paraformaldehyde was used to fix the solution. The matrix glue and cells in the upper chamber were wiped off. A crystal violet ethanol solution (1%) was used to incubate and stain. The numbers of cells under the membrane in 16 fields of vision ($\times 100$) were randomly counted under the microscope. (3) The scratch method was used to test

the cells' migration ability: First, a marker pen was used to draw a horizontal line behind the 6-hole plate with a ruler, 3 lines for each hole. Approximately 3.5×10^6 cells were added to the hole. After they adhered to the wall, the cells were scratched with a spear head and ruler perpendicular to the horizontal line at the back. The cells were rinsed with PBS for 2 to 3 times, and the scratched cells were removed. Then, a serum-free medium was added. The cells were placed in a 5% CO₂ incubator at 37°C, cultured for 0, 24, and 48 h, and then they were photographed, with 6 photos saved.

Measuring the apoptosis using flow cytometry

All the cells were processed using DMSO or lapatinib for 48 h, and then they were incubated in a 5% CO₂ incubator at 37°C for 48 h. After collecting the cells, the apoptosis was measured using an Annexin V-FITC/PI double staining apoptosis kit (eBioscience), following the kit's instructions. After preparing the sample, the apoptosis was measured using an up-flow cytometer, and the data was processed using FlowJo.

Statistical methods

SPSS 22.0 was used for the statistical processing. The measurement data conforming to a normal distribution were represented as the mean \pm standard deviation ($\bar{x} \pm s$). The comparisons between two groups were tested using T tests. The univariate comparisons among multiple groups were conducted using one-way ANOVA. A difference was statistically significant when $P < 0.05$.

Results

Comparison of the proliferation ability

By observing the results of the MTT test, we concluded that, compared with the NCG, the MDA-MB-453 and BT-47 proliferation abilities in the LG, L-sh-G, and sh-G were significantly reduced, while the proliferation abilities of MDA-MB-453 and BT-47 in the LG and sh-G were not significantly different, and they were significantly higher than they were in the L-sh-G (**Figure 1**).

Comparison of invasive ability

Our Transwell experiment showed that the invasive abilities of MDA-MB-453 and BT-47 in the

LG, L-sh-G, and sh-G were significantly lower than they were in the NCG by comparing the invasive abilities in each group. The invasive abilities of MDA-MB-453 and BT-47 in the LG and sh-G were not significantly different and were significantly higher than they were in the L-sh-G (**Figure 2**).

Comparison of migration ability

We measured the mobility of MDA-MB-453 and BT-47 in each group using scratch tests and found that the mobility of MDA-MB-453 and BT-47 in the NCG was significantly higher than it was in the LG, L-sh-G, and sh-G. The mobility of MDA-MB-453 and BT-47 in the LG and sh-G was not significantly different and was significantly higher than it was in the L-sh-G (**Figure 3**).

Comparison of the apoptosis rates

The cell apoptosis was analyzed using flow cytometry. It revealed that the MDA-MB-453 and BT-47 apoptosis rates in the NCG were significantly lower than they were in the LG, L-sh-G, and sh-G, but there was no significant difference between the LG and the sh-G, and they were significantly lower than they were in the L-sh-G (**Figure 4**).

Measuring the BTG2 expressions

In each group, the BTG2 in the MDA-MB-453 cells and BT-47 cells was measured using Western blot. The measurements revealed that the expressions of BTG2 in MDA-MB-453 and BT-47 in the NCG were significantly higher than they were in the LG and sh-G, but the BTG2 expressions in the LG and sh-G showed no significant differences, and they were significantly higher than they were in the L-sh-G (**Figure 5**).

Determining the relationship between miR-128-3P and BTG2

In order to further explore the connection between miR-128-3P and BTG2, we used Target-Scan 7.2 to predict the target genes downstream of miR-128-3P, and the prediction revealed that there was a targeted binding site for miR-128-3P and BTG2. Further, the fluorescence activity of BTG2-Wt was significantly reduced according to the double luciferase report measurement. The cell transfection showed that the BTG2 protein expression was

HER2-targeted drugs inhibit the progression of breast cancer

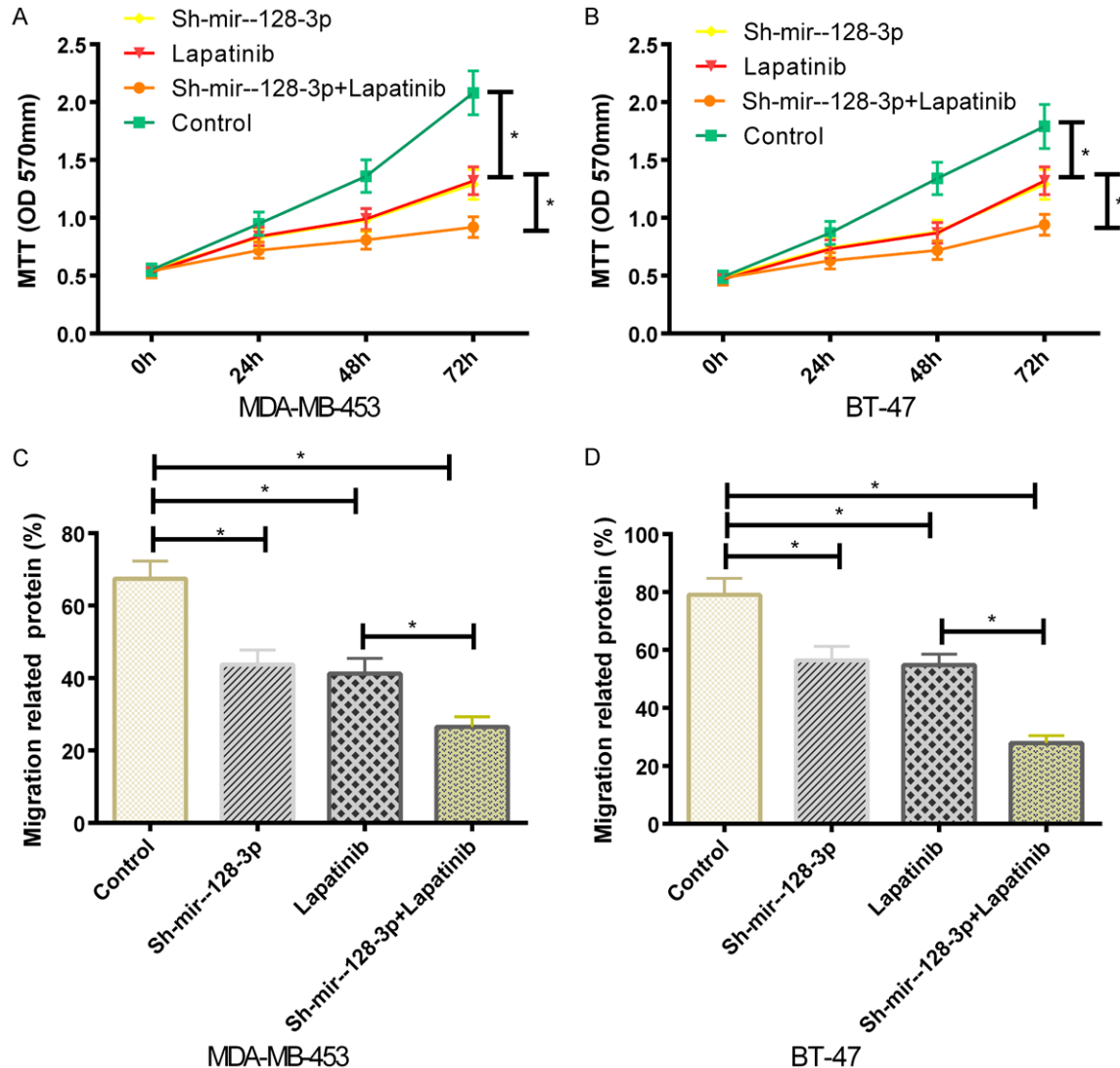


Figure 1. Comparison of cell proliferation and invasion ability. In terms of MDA-MB-453 (A) and BT-47 (B), compared with the normal control group, the lapatinib group, lapatinib + sh-miR-128-3P group, and sh-miR-128-3P group showed significantly lower proliferation ability, and there was no significant difference in the proliferation ability between the lapatinib group and the sh-miR-128-3P group, but the proliferation ability of the two groups was significantly higher than that of the lapatinib + sh-miR-128-3P. Additionally, in terms of MDA-MB-453 (C) and BT-47 (D), compared with the normal control group, the lapatinib group, lapatinib + sh-miR-128-3P group, and sh-miR-128-3P group showed significantly lower invasion ability, and there was no significant difference in the invasion ability between the lapatinib group and the sh-miR-128-3P group, but the invasion ability of the two groups was significantly higher than that of the lapatinib + sh-miR-128-3P. Note: * indicates $P < 0.05$.

up-regulated after the miR-128-3P inhibitor, but the BTG2 protein expression was down-regulated after the miR-128-3P mimics transfection ($P < 0.05$) (Figure 6).

Discussion

BC is a serious threat to human health. Although the current treatment level is continuously improving, its incidence rate is still high [14].

Studies have shown that invasion and metastasis are the key factors leading to death in BC patients [15, 16]. Therefore, research on BC's mechanism has attracted much attention in recent years, especially research on how to control the invasion and metastasis of BC, how to prevent and treat BC, and how to improve BC patients' survival rates and quality of life [17, 18]. Therefore, it is very important to study the molecular mechanism of BC [19].

HER2-targeted drugs inhibit the progression of breast cancer

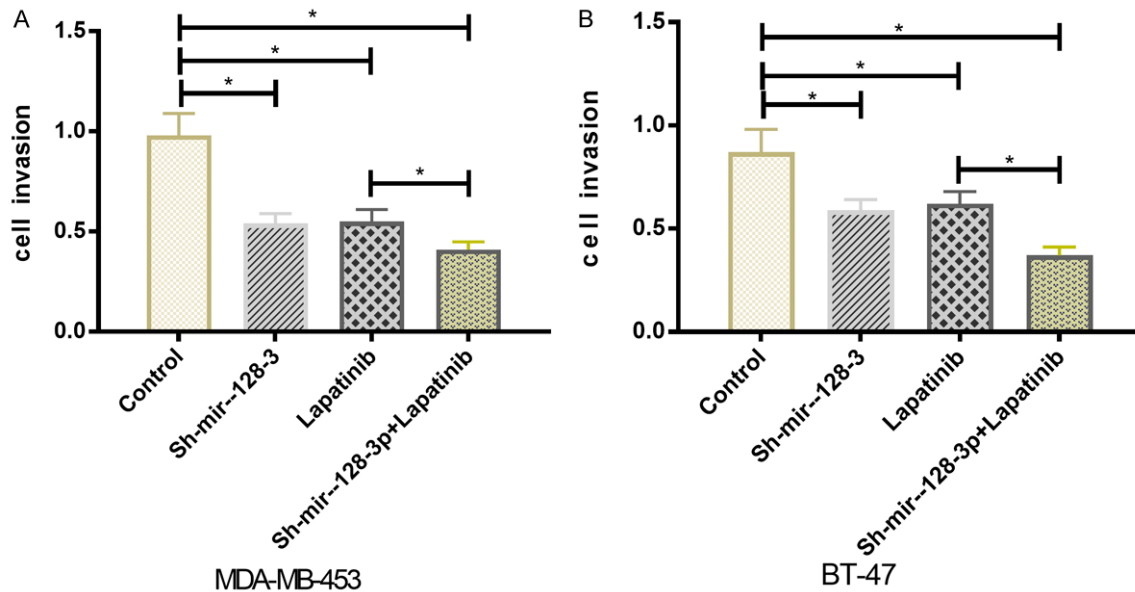


Figure 2. Comparison of the invasive ability. A. The invasive abilities of MDA-MB-453 in the LG, L-sh-G, and sh-G were significantly lower than they were in the NCG. There was no obvious difference in the invasive abilities between the LG and the sh-G, and they all were significantly higher than they were in the L-sh-G. B. The invasive abilities of BT-47 in the LG, L-sh-G, and sh-G were significantly lower than they were in the NCG. There was no significant difference in invasive ability between the LG and the sh-G, and they all were significantly higher than they were in the L-sh-G. *indicates $P < 0.05$.

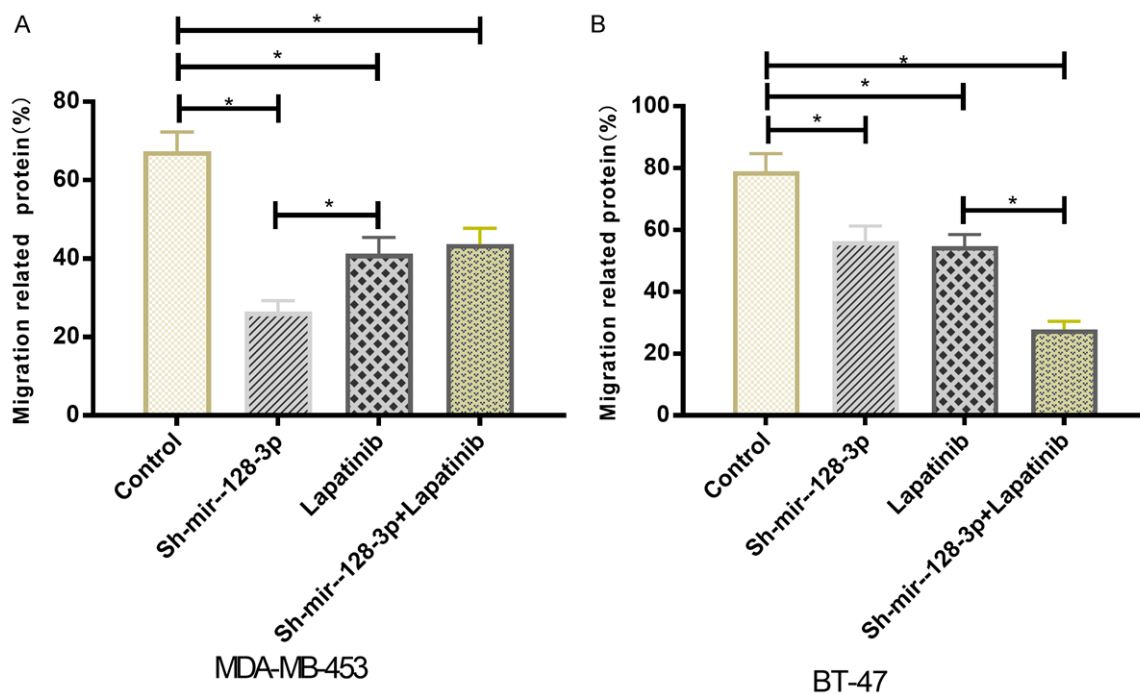


Figure 3. Comparison of the migration ability. A. The migration rate of MDA-MB-453 in the normal control group was significantly higher than it was in the lapatinib group, the lapatinib + sh-miR-128-3P group, and the sh-miR-128-3P group. There was no significant difference in the migration rate of MDA-MB-453 between the lapatinib group and the sh-miR-128-3P group, and the migration rate of MDA-MB-453 in the two groups was higher than it was in the lapatinib + sh-miR-128-3P group. B. The migration rate of BT-47 in the normal control group was significantly higher than it was in the lapatinib group, the lapatinib + sh-miR-128-3P group, and the sh-miR-128-3P group. There was

HER2-targeted drugs inhibit the progression of breast cancer

no significant difference in the migration rate of BT-47 between the lapatinib group and the sh-miR-128-3P group, and the migration rates of BT-47 in the two groups were both higher than the rate in the lapatinib + sh-miR-128-3P group. *indicates $P < 0.05$.

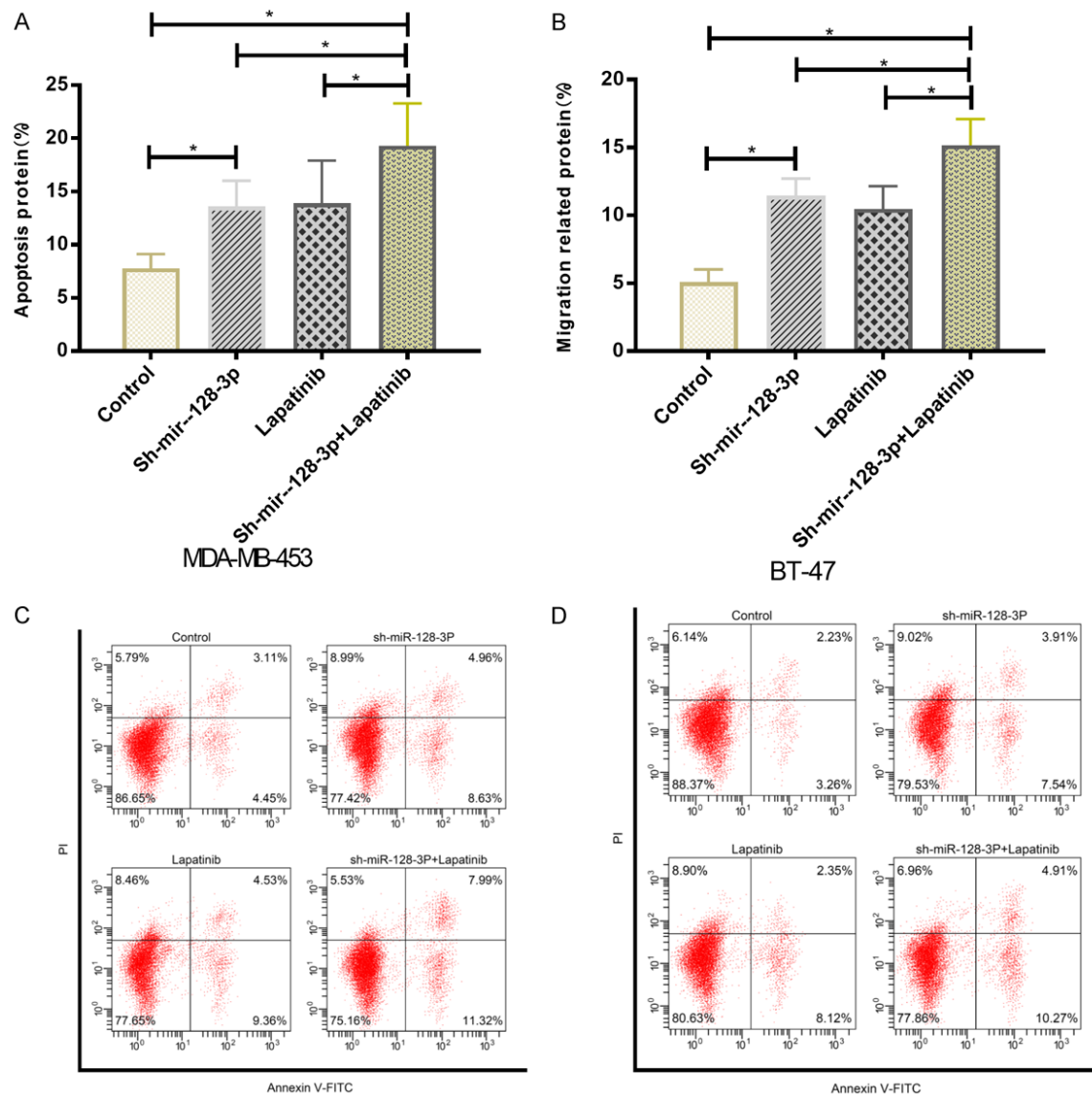


Figure 4. Comparison of the apoptosis rates. A. The apoptosis rate of MDA-MB-453 in the NCG was significantly lower than that in the LG, L-sh-G, and sh-G. There was no significant difference between the LG and the sh-G, and they were significantly lower than those in the L-sh-G. B. The apoptosis rate of BT47 in the NCG was significantly lower than that in the LG, L-sh-G, and sh-G. There was no significant difference between the LG and the sh-G, and they were significantly lower than those in the L-sh-G. C. Profiling of apoptosis of MDA-MB-453 cells after transfection. D. Profiling of apoptosis of BT-47 cells after transfection. *indicates $P < 0.05$.

Lapatinib is a dual inhibitor of the epidermal growth factor receptor (EGFR) and Her2, and it can inhibit tumor cell growth and induce cell apoptosis. It has achieved a better clinical efficacy in advanced metastatic HER2 positive BC that has failed trastuzumab therapy [20-22]. miRNAs are non-coding small RNAs with a

length of about 19-24 nucleotides, and they play the roles of clearing the mRNA or inhibiting the translation process [23]. Many studies have revealed that miR-128-3p is closely related to a variety of diseases, such as non-small cell lung carcinoma [24], liver carcinoma [25], etc. Zhu et al. [26] have revealed that the miR-128-3p/

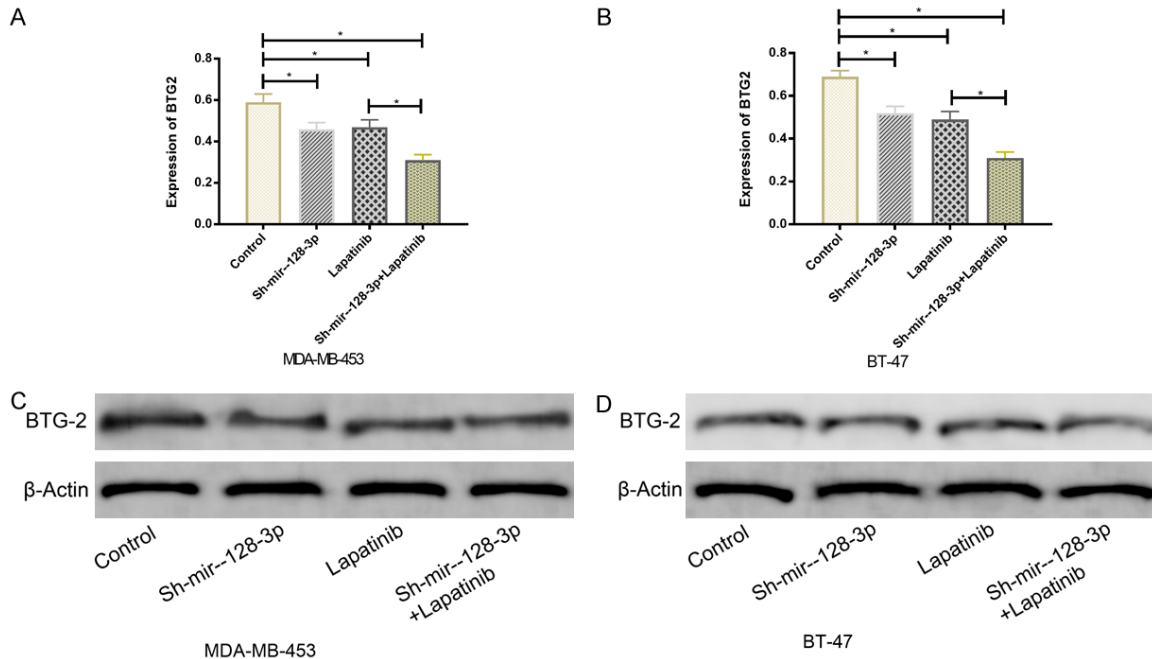


Figure 5. Quantification of the BTG2 expression. A. The BTG2 expression of MDA-MB-453 in the NCG was significantly higher than it was in the LG and sh-G. There was no significant difference in the MDA-MB-453 between the LG and the sh-G, and the MDA-MB-453 was significantly higher than it was in the L-sh-G. B. The BTG2 expression of BT-47 in the NCG was significantly higher than it was in the LG and the sh-G. There was no significant difference in the BT-47 between the LG and the sh-G, and the BT-47 was significantly higher than it was in the L-sh-G. C. Quantification of the BTG2 expression in the MDA-MB-453 cells using Western blot. D. Quantification of the BTG2 expression in the BT-47 cells using Western blot. *indicates $P < 0.05$.

CKD14 axis is associated with the progression of ovarian cancer. Zhao [27] showed that miR-128-3p inhibits the progression of BC cells by targeting LIMK1, but whether lapatinib promotes BC progression by targeting the regulation of miR-128-3p and BTG2 has not been investigated. MDA-MB-453 and BT-47 BC cells were studied. A growth inhibition experiment, flow cytometry and western blotting were used to analyze the influences of lapatinib on the cell growth, cell cycle distribution, apoptosis, and miR-128-3p-BTG2 axis of BC. The results showed that, compared with the NCG, the growth and invasion capabilities of MDA-MB-453 and BT-47 in the LG, L-sh-G, and sh-G were significantly reduced, while the proliferation and invasion capabilities of MDA-MB-453 and BT-47 in the LG and sh-G were not significantly different and were significantly higher than they were in the L-sh-G. This showed that lapatinib can effectively inhibit the growth and invasion of MDA-MB-453 and BT-47 in BC cells. By determining the apoptosis rate of each group using flow cytometry, we found that the MDA-MB-453 and BT-47 apoptosis rates in the NCG was sig-

nificantly lower than it was in the LG, L-sh-G, and sh-G. Compared with the sh-G, the LG had no significant difference, and it was significantly lower than the L-sh-G, a finding similar to the findings of the studies by Kalous et al. [28], showing that lapatinib can promote the apoptosis of BC cells. After that, we detected the BTG2 protein expression in each group using Western blot and found that the BTG2 in MDA-MB-453 and the BT-47 in the NCG was significantly higher than it was in the LG and sh-G, but the expressions of BTG2 in the LG and sh-G had no significant differences, and they were significantly higher than they were in the L-sh-G. This also suggested that lapatinib might also inhibit BTG2 using miR-128-3p to inhibit the growth, invasion, and migration of BC cells and promote their apoptosis.

In order to further verify the relationship of miR-128-3P with BTG2, we conducted double luciferase experiments. It was found that the fluorescence activity of BTG2-Wt was significantly reduced. The transfection of cells revealed that the expression of the BTG2 protein was up-regulated after the miR-128-3P inhibitor, but the

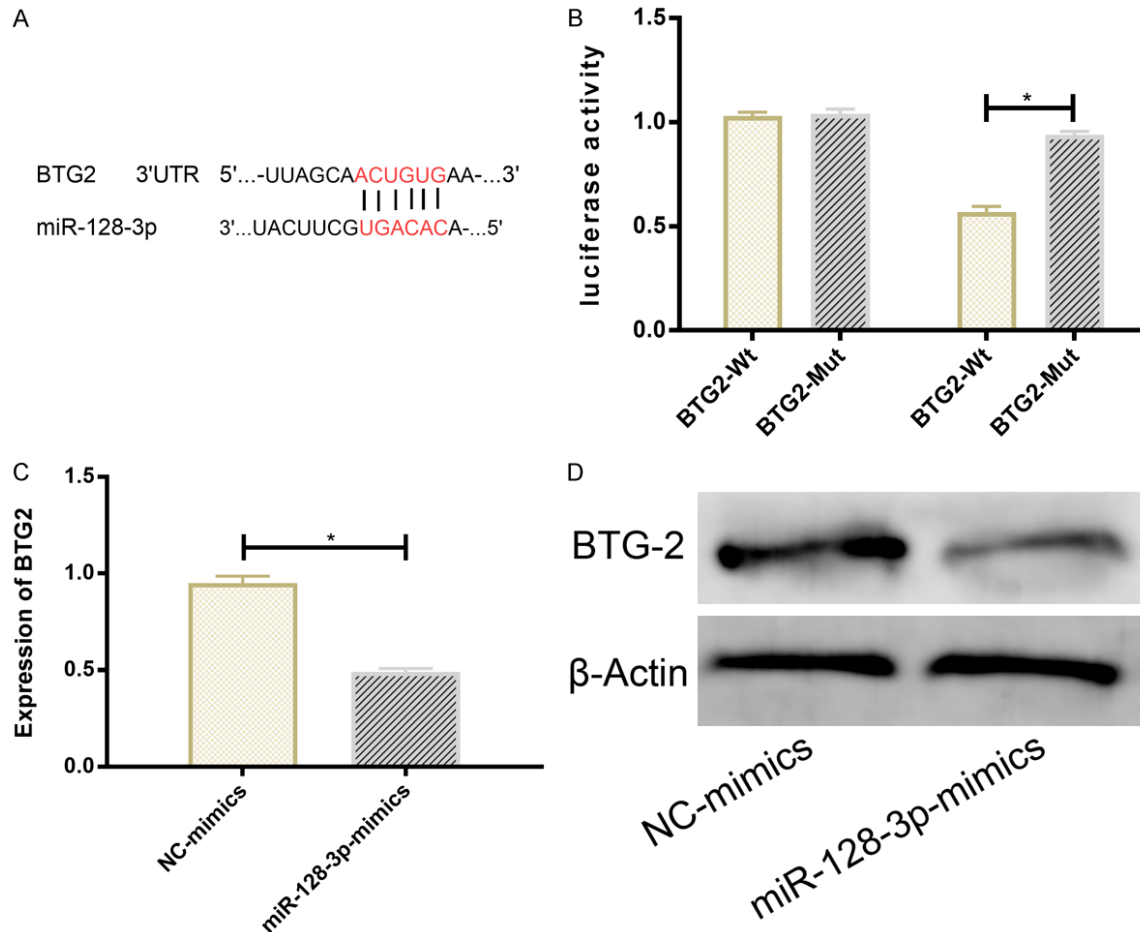


Figure 6. Targeting the relationship between miR-128-3p and BTG2. A. The targeted binding site of miR-128-3P and BTG2. B. Double luciferase report quantification. C. The effects of the different transfections on the BTG2 expressions in the cells. D. The effects of the different transfections on the BTG2 expressions in the cells. Note: *indicates the comparison with mir-NC, *P<0.05.

expression of the BTG2 protein was decreased after miR-128-3P mimics transfection, which further revealed that miR-128-3p had a negative regulatory effect on BTG2.

Although we have provided a new direction for studying the mechanism of lapatinib in the treatment of BC, the current studies are mostly limited to cell and animal experiments, so there are still some limitations. It is necessary for us to further carry out population research and clinical research to provide more applications for clinical practice.

To sum up, lapatinib can inhibit the proliferation, invasion, and migration and promote the apoptosis of BC cells by regulating the miR-128-3p-BTG2 axis.

Address correspondence to: Wei Wang, Department of Pharmacy, Yantai Yuhuangding Hospital,

No. 20 Yuhuangding East Road, Yantai, Shandong Province, China. Tel: +86-15553596381; E-mail: wangwei666w@126.com

References

- [1] Wormann B. Breast cancer: basics, screening, diagnostics and treatment. *Med Monatsschr Pharm* 2017; 40: 55-64.
- [2] Hollingsworth AB. Is invasion a necessary step for metastases in breast cancer? *Narod SA, Sopik V. Breast Cancer Res Treat* 2018; 169: 633-637.
- [3] Pearce L. Breast cancer. *Nurs Stand* 2016; 30: 15.
- [4] Leblanc N, Harquail J, Crapoulet N, Ouellette RJ and Robichaud GA. Pax-5 inhibits breast cancer proliferation through miR-215 up-regulation. *Anticancer Res* 2018; 38: 5013-5026.
- [5] Li L, Jia L and Ding Y. Upregulation of miR-375 inhibits human liver cancer cell growth by modulating cell proliferation and apoptosis via tar-

- geting ErbB2. *Oncol Lett* 2018; 16: 3319-3326.
- [6] Yang H, Zhang H, Ge S, Ning T, Bai M, Li J, Li S, Sun W, Deng T, Zhang L, Ying G and Ba Y. Exosome-derived miR-130a activates angiogenesis in gastric cancer by targeting C-MYB in vascular endothelial cells. *Mol Ther* 2018; 26: 2466-2475.
- [7] Block I, Burton M, Sorensen KP, Andersen L, Larsen MJ, Bak M, Cold S, Thomassen M, Tan Q and Kruse TA. Association of miR-548c-5p, miR-7-5p, miR-210-3p, miR-128-3p with recurrence in systemically untreated breast cancer. *Oncotarget* 2018; 9: 9030-9042.
- [8] Shuai Y, Ma Z, Liu W, Yu T, Yan C, Jiang H, Tian S, Xu T and Shu Y. TEAD4 modulated LncRNA MNX1-AS1 contributes to gastric cancer progression partly through suppressing BTG2 and activating BCL2. *Mol Cancer* 2020; 19: 6.
- [9] Devanand P, Oya Y, Sundaramoorthy S, Song KY, Watanabe T, Kobayashi Y, Shimizu Y, Hong SA, Suganuma M and Lim IK. Inhibition of TNF- α -interacting protein α (Tipalpha)-associated gastric carcinogenesis by BTG2 (/TIS21) via downregulating cytoplasmic nucleolin expression. *Exp Mol Med* 2018; 50: e449.
- [10] Choi JA, Jung YS, Kim JY, Kim HM and Lim IK. Inhibition of breast cancer invasion by TIS21/BTG2/Pc3-Akt1-Sp1-Nox4 pathway targeting actin nucleators, mDia genes. *Oncogene* 2016; 35: 83-93.
- [11] Voigtlaender M, Schneider-Merck T and Trepel M. Lapatinib. *Recent Results Cancer Res* 2018; 211: 19-44.
- [12] Veeraraghavan J, De Angelis C, Reis-Filho JS, Pascual T, Prat A, Rimawi MF, Osborne CK and Schiff R. De-escalation of treatment in HER2-positive breast cancer: determinants of response and mechanisms of resistance. *Breast* 2017; 34 Suppl 1: S19-S26.
- [13] D'Amato V, Raimondo L, Formisano L, Giuliano M, De Placido S, Rosa R and Bianco R. Mechanisms of lapatinib resistance in HER2-driven breast cancer. *Cancer Treat Rev* 2015; 41: 877-883.
- [14] Shull JD, Dennison KL, Chack AC and Trentham-Dietz A. Rat models of 17 β -estradiol-induced mammary cancer reveal novel insights into breast cancer etiology and prevention. *Physiol Genomics* 2018; 50: 215-234.
- [15] Liu D, Xiong H, Ellis AE, Northrup NC, Rodriguez CO Jr, O'Regan RM, Dalton S and Zhao S. Molecular homology and difference between spontaneous canine mammary cancer and human breast cancer. *Cancer Res* 2014; 74: 5045-5056.
- [16] Sanders J and Samuelson DJ. Significant overlap between human genome-wide association-study nominated breast cancer risk alleles and rat mammary cancer susceptibility loci. *Breast Cancer Res* 2014; 16: R14.
- [17] Homer-Bouthiette C, Zhao Y, Shunkwiler LB, Van Peel B, Garrett-Mayer E, Baird RC, Rissman AI, Guest ST, Ethier SP, John MC, Powers PA, Haag JD, Gould MN and Smits BMG. Deletion of the murine ortholog of the 8q24 gene desert has anti-cancer effects in transgenic mammary cancer models. *BMC Cancer* 2018; 18: 1233.
- [18] Quang CT, Leboucher S, Passaro D, Fuhrmann L, Nourieh M, Vincent-Salomon A and Ghysdael J. The calcineurin/NFAT pathway is activated in diagnostic breast cancer cases and is essential to survival and metastasis of mammary cancer cells. *Cell Death Dis* 2015; 6: e1658.
- [19] Jin L, Han B, Siegel E, Cui Y, Giuliano A and Cui X. Breast cancer lung metastasis: molecular biology and therapeutic implications. *Cancer Biol Ther* 2018; 19: 858-868.
- [20] Mu Y and Sun D. Lapatinib, a dual inhibitor of epidermal growth factor receptor (EGFR) and HER-2, enhances radiosensitivity in mouse bladder tumor line-2 (MBT-2) cells in vitro and in vivo. *Med Sci Monit* 2018; 24: 5811-5819.
- [21] Sartore-Bianchi A, Trusolino L, Martino C, Ben-cardino K, Lonardi S, Bergamo F, Zagonel V, Leone F, Depetris I, Martinelli E, Troiani T, Ciardiello F, Racca P, Bertotti A, Siravegna G, Torri V, Amatu A, Ghezzi S, Marrapese G, Palmieri L, Valtorta E, Cassingena A, Lauricella C, Vanzulli A, Regge D, Veronese S, Comoglio PM, Bardelli A, Marsoni S and Siena S. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol* 2016; 17: 738-746.
- [22] De Angelis C, Nagi C, Hoyt CC, Liu L, Roman K, Wang C, Zheng Y, Veeraraghavan J, Sethunath V, Nuciforo P, Wang T, Tsimelzon A, Mao S, Hilsenbeck SG, Trivedi MV, Cataldo ML, Pavlick A, Wolff AC, Weigelt B, Reis-Filho JS, Prat A, Gutierrez C, Osborne CK, Rimawi MF and Schiff R. Evaluation of the predictive role of tumor immune infiltrate in patients with HER2-positive breast cancer treated with neoadjuvant anti-HER2 therapy without chemotherapy. *Clin Cancer Res* 2020; 26: 738-745.
- [23] He X, Guo S, Wang Y, Wang L, Shu S and Sun J. Systematic identification and analysis of heat-stress-responsive lncRNAs, circRNAs and miRNAs with associated co-expression and ceRNA networks in cucumber (*Cucumis sativus* L.). *Physiol Plant* 2020; 168: 736-754.
- [24] Cai J, Fang L, Huang Y, Li R, Xu X, Hu Z, Zhang L, Yang Y, Zhu X, Zhang H, Wu J, Huang Y, Li J,

HER2-targeted drugs inhibit the progression of breast cancer

- Zeng M, Song E, He Y, Zhang L and Li M. Simultaneous overactivation of Wnt/beta-catenin and TGFbeta signalling by miR-128-3p confers chemoresistance-associated metastasis in NSCLC. *Nat Commun* 2017; 8: 15870.
- [25] Huang CY, Huang XP, Zhu JY, Chen ZG, Li XJ, Zhang XH, Huang S, He JB, Lian F, Zhao YN and Wu GB. miR-128-3p suppresses hepatocellular carcinoma proliferation by regulating PI-K3R1 and is correlated with the prognosis of HCC patients. *Oncol Rep* 2015; 33: 2889-2898.
- [26] Zhu L, Wang A, Gao M, Duan X and Li Z. LncRNA MIR4435-2HG triggers ovarian cancer progression by regulating miR-128-3p/CKD14 axis. *Cancer Cell Int* 2020; 20: 145.
- [27] Zhao J, Li D and Fang L. MiR-128-3p suppresses breast cancer cellular progression via targeting LIMK1. *Biomed Pharmacother* 2019; 115: 108947.
- [28] Kalous O, Conklin D, Desai AJ, O'Brien NA, Gintner C, Anderson L, Cohen DJ, Britten CD, Taylor I, Christensen JG, Slamon DJ and Finn RS. Dacomitinib (PF-00299804), an irreversible Pan-HER inhibitor, inhibits proliferation of HER2-amplified breast cancer cell lines resistant to trastuzumab and lapatinib. *Mol Cancer Ther* 2012; 11: 1978-1987.