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Long non-coding RNA HEIH promotes breast cancer development via negative modulation of microRNA-200b

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This study aimed to investigate the effects and regulatory mechanism of long non-coding RNA HEIH in the development of breast cancer. The expression of HEIH in breast tumor tissues and breast cancer cells was determined, followed by investigating the effects and regulatory mechanism of HEIH dysregulation on breast cancer cell viability, apoptosis, migration and invasion. The expression of HEIH was upregulated in breast cancer tissue samples and cell lines. Suppression of HEIH inhibited cell viability, promoted cell apoptosis, and decreased migration and invasion in MDA-MB-231 cells. Moreover, a negative relationship existed between HEIH and miR-200b, and HEIH regulated breast cancer development *via* regulating miR-200b. Pre-leukemia transcription factor 3 (PBX3) was verified as a functional target of miR-200b, and miR-200b regulated the malignant behaviors of breast cancer cells through targeting PBX3. Furthermore, suppression of HEIH inhibited the activation of Wnt/β-catenin pathway, which was remarkably reversed after suppression of HEIH and inhibition of miR-200b synchronously. Our results reveal that HEIH may contribute to breast cancer development via modulation of microRNA-200b/axis and inducing the activation of Wnt/β-catenin pathway. Further studies are still required to confirm our findings.

1. Introduction

Breast cancer is the leading cause of cancer-related death among women worldwide. It is estimated that approximately 63,410 cases of female breast carcinoma *in situ* were diagnosed in 2017 (Siegel et al. 2017). The five-year survival rate of breast cancer is bleak (< 10%) due to bone metastasis (Bussard and Marini 2016). Moreover, current predictions and statistics reveal increases in both incidence of breast cancer and related mortality (Tao et al. 2015). Early detection of breast cancer is associated with reduced morbidity and mortality (Oeffinger et al. 2015). However, the mechanisms underlying breast cancer as well as the effective biomarkers for disease diagnosis are still poorly understood.

Long non-coding RNAs (lncRNAs) are a group of RNA transcripts with more than 200 bases in length but lack significant open reading frames (Iyer et al. 2015; Spizzo et al. 2012). LncRNAs have become a hotspot in the tumorigenesis and progression of breast cancer (Hansji et al. 2014; Sørensen et al. 2015; Tian et al. 2018). For instance, lncRNA HOXA11-AS can regulate epithelial-mesenchymal transition to promote the invasion and metastasis of breast cancer (Li et al. 2017b); overexpression of lncRNA PlncRNA-1 can suppress the growth of breast cancer through regulating TGF-β1 and PHGDH (Li et al. 2018); and the GAS5-miR-23a-ATG3 axis can play a key role in breast cancer via functioning as a key regulator of autophagy pathways (Gu et al. 2018). However, studies on lncRNAs in breast cancer are still in a preliminary stage. Recently, lncRNA-HEIH has been reported to be involved in the development of colorectal cancer (Cui et al. 2018), and whose expression in serum and exosomes may serve as a potential biomarker of hepatitis C virus (HCV)-related hepatocellular carcinoma (Zhang et al. 2018). Nevertheless, the roles of lncRNA HEIH in breast cancer have not been disclosed.

In the present study, we determined the expression of HEIH in breast tumor tissues and breast cancer cells, followed by investigating the effects and regulatory mechanism of HEIH dysregulation on breast cancer cell viability, apoptosis, migration and invasion. This study will lay a theoretical basis for the pathogenesis of breast cancer and provide new insight for its treatment.

2. Investigations and results

2.1. *HEIH expression is upregulated in breast cancer tissue samples and cell lines*

As presented in Fig. 1A, the expression of HEIH was significantly increased in breast tumor tissues in relation to that in adjacent non-tumor tissues (P < 0.01). Consistently, HEIH expression was also significantly increased in breast cancer cells (MCF-7 and MDA-MB-231) compared with that in normal mammary epithelial MCF10A cells (P < 0.01, Fig. 1B). Because the HEIH expression in MDA-MB-231 cells was the highest among the three cell lines, MDA-MB-231 cells were selected for subsequent analysis.

2.2. *Suppression of HEIH inhibits cell viability, promotes cell apoptosis, and decreases migration and invasion in MDA-MB-231 cells*

To investigate the role of HEIH in breast cancer, HEIH expression was suppressed in MDA-MB-231 cells by transfection with sh-HEIH. As shown in Fig. 1C, HEIH expression was suppressed in sh-HEIH-transfected MDA-MB-231 cells compared to that in sh-NC-transfected MDA-MB-231 cells (P < 0.05); Moreover, the transfection efficiency of sh-HEIH#1 was stronger than that of sh-HEIH#2, sh-HEIH#1 was used in the following experiment. Furthermore, the results of MTT assay showed that suppression of HEIH significantly inhibited the viability of MDA-MB-231 cells (P < 0.05, Fig. 1D); the results of western bolt showed that suppression of HEIH remarkably promoted the apoptosis of

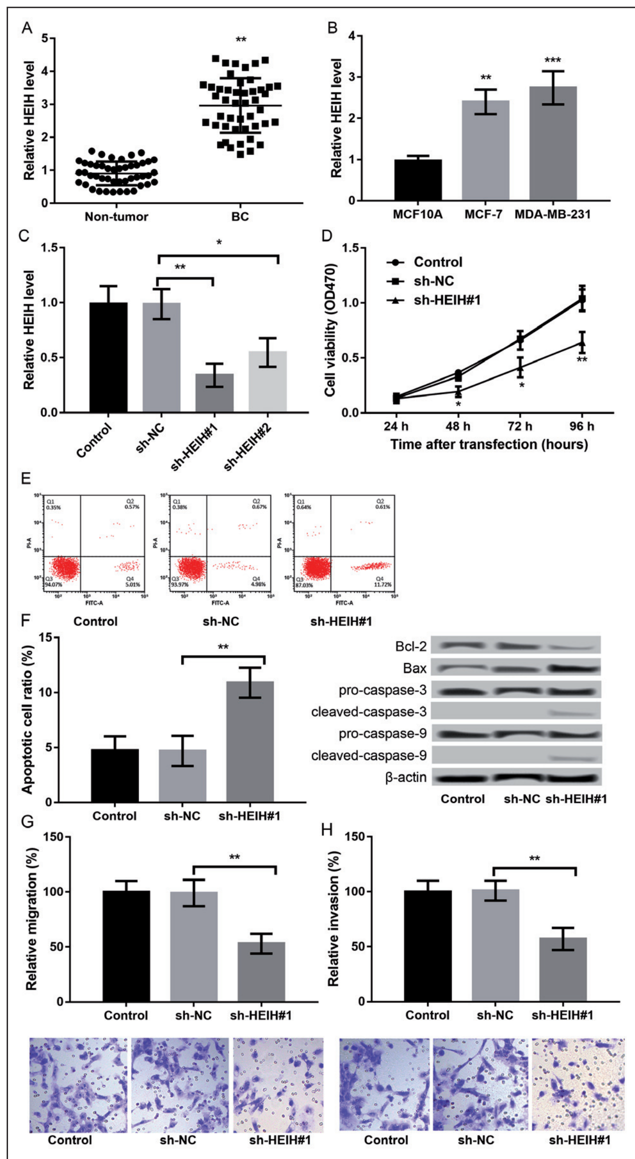

Fig. 1: HEIH expression is upregulated in breast cancer tissue samples (A) and cell lines (B) and suppression of HEIH inhibits cell viability, promotes cell apoptosis, and decreases migration and invasion in MDA-MB-231 cells. (C): The HEIH expression after transfection with sh-HEIH and si-NC. (D): Cell viability after transfection. (E): Cell apoptosis after transfection and the expression levels of pro-apoptotic proteins (Bax, cleaved-caspase-3 and cleaved-caspase-9) and anti-apoptotic protein Bcl-2. (F): Cell migration after transfection. (G): Cell invasion after transfection. * compared to respective control. * P < 0.05 and ** P < 0.01.

MDA-MB-231 cells (P < 0.01, Fig. 1E), and further western bolt showed that suppression of HEIH promoted the expression levels of pro-apoptotic proteins (Bax, cleaved-caspase-3 and cleaved-caspase-9) but inhibited the expression of anti-apoptotic protein Bcl-2 (Fig. 1F); and the results of migration and invasion assays revealed that suppression of HEIH obviously decreased the migration and invasion of MDA-MB-231 cells (P < 0.01, Fig. 1G-H).

2.3. HEIH regulates the malignant behaviors of breast cancer cells through miR-200b

It is reported that HEIH may be involved in regulating miR-200b in melanoma cells (Zhao et al. 2017). We thus investigated the relationship between HEIH and miR-200b in breast cancer. As revealed in Fig. 2A, the expression of miR-200b was significantly increased in sh-HEIH#1-transfected MDA-MB-231 cells compared to that in sh-NC-transfected MDA-MB-231 cells (P < 0.01), indicating a negative relationship between HEIH and miR-200b. Subsequently, we explored the expression of miR-200b in breast cancer tissues and cells. Expected results were obtained that miR-200b was remarkably downregulated in breast cancer tissues and cells (P < 0.01, Fig. 2B-C). Moreover, the expression of miR-200b was dysregulated in MDA-MB-231 cells by transfection. The results showed that miR-200b was distinctly overexpressed in MDA-MB-231 cells by transfection with miR-200b mimic and clearly suppressed by transfection with miR-200b inhibitor (P < 0.001, Fig. 2D). To further disclose whether HEIH regulated breast cancer development via miR-200b, MDA-MB-231 cells were cotransfected with sh-HEIH and a miR-200b inhibitor. The results showed that the effects of suppression of HEIH on cell viability (Fig. 2E), apoptosis (Fig. 2F), migration and invasion (Fig. 2G-H) were visibly reversed by inhibition of miR-200b at the same time (all P < 0.05).

2.4. PBX3 is verified as a functional target of miR-200b

PBX3 is a member of the PBX family of transcription factors, which has been shown to function as an oncogene in the development of several cancers (Han et al. 2014; Ramberg et al. 2016). According to the information of Targetscan, we found that PBX3 was a potential target of miR-200b (http://www.targetscan.org/cgi-bin/targetscan/vert_71/view_gene.cgi?rs=ENST00000342287.5&taxid=9606&members=miR-200bc-3p/429&showcnc=0&shownc=0&showncf1=&showncf2=&subset=1, Fig. 3A), thus, the relationship between miR-200b and PBX3 was investigated. As shown in Fig. 3B, the results of dual-luciferase reporter assay indicated that the luciferase activity of PBX3-WT was significantly inhibited by miR-200b mimic (P < 0.05), confirming that PBX3 was a direct target of miR-200b (Fig. 3B). Moreover, the expression of PBX3 at both mRNA and protein levels was remarkably decreased in miR-200b mimic-transfected MDA-MB-231 cells, but distinctly increased in miR-200b inhibitor-transfected cells (P < 0.01, Fig. 3C-D), indicating that PBX3 expression was negatively regulated by miR-200b.

2.5. miR-200b regulates the malignant behavior of breast cancer cells through PBX3

Subsequently, we knocked down the expression of PBX3 by transfection with si-PBX3 and the high transfection efficiency was confirmed by qRT-PCR and western blot assays (P < 0.001, Fig. 4A). To confirm whether miR-200b regulated the malignant behaviors of breast cancer cells through PBX3, MDA-MB-231 cells were cotransfected with miR-200b inhibitor and si-PBX3. The results showed that the effects of inhibition of miR-200b on cell viability (Fig. 4B), apoptosis (Fig. 4C), migration and invasion (Fig. 4D-E) were overtly reversed by inhibition of miR-200b and knockdown of PBX3 at the same time (all P < 0.05).

2.6. Suppression of HEIH inhibits the activation of Wnt/β-catenin pathway in breast cancer cells

The activation of Wnt/β-catenin signaling is shown to play a key role in tumor development, including breast cancer (King et al. 2011). Thus, the relationship between HEIH and the activation of the Wnt/β-catenin signaling pathway was investigated. As shown in Fig. 4F, we found that suppression of HEIH significantly decreased the expression of wnt1, c-Myc, cyclin-D1, and β-catenin, which was remarkably reversed after suppression of HEIH and inhibition of miR-200b synchronously (P < 0.05). Further knockdown of PBX3 concurrently could reverse the effect of suppression of HEIH and inhibition of miR-200b synchronously on the expression of the aforementioned proteins again (P < 0.05).

3. Discussion

This study firstly investigated the role of HEIH in the development of breast cancer. The results showed that HEIH was upregulated in breast cancer tissue samples and cell lines. Suppression of HEIH

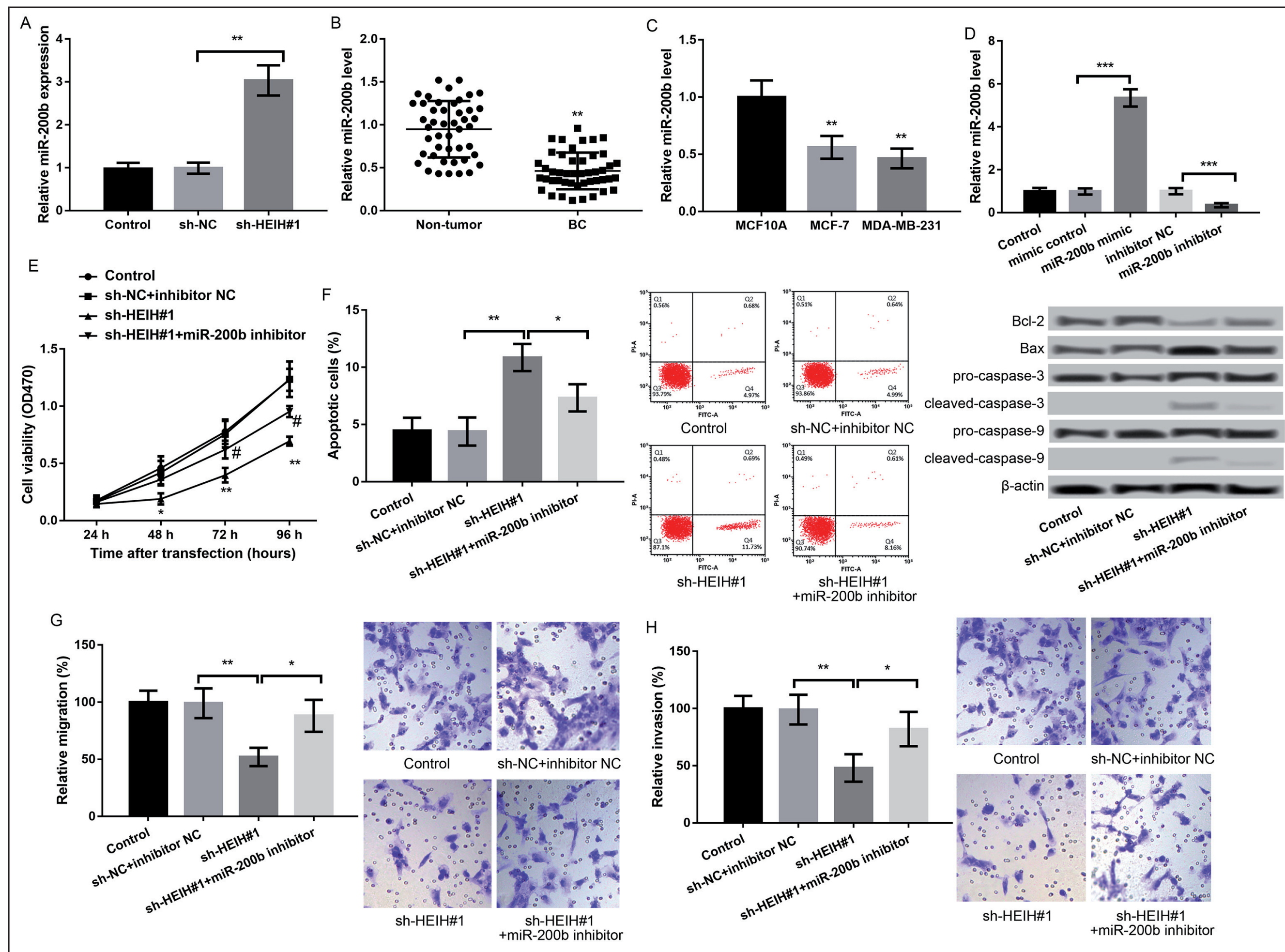

Fig. 2: HEIH regulates the malignant behavior of breast cancer cells through miR-200b. (A): The miR-200b expression after transfection with sh-HEIH and sh-NC. (B) and (C): The miR-200b expression in breast cancer tissue samples and cell lines. (D): The miR-200b expression after transfection with miR-200b mimic, miR-200b inhibitor, and respective controls. (E): Cell viability after transfection. (F): Cell apoptosis after transfection and the expression levels of pro-apoptotic proteins (Bax, cleaved-caspase-3 and cleaved-caspase-9) and anti-apoptotic protein Bcl-2. (G): Cell migration after transfection. (H): Cell invasion after transfection. * compared to respective control. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

remarkably inhibited cell viability, promoted cell apoptosis, and decreased migration and invasion of MDA-MB-231 cells, indicating that HEIH might function as an oncogene in breast cancer, which was in consistent with previous findings that HEIH was identified as an oncogenic lncRNA that promoted s tumor progression in hepatocellular carcinoma (Yang et al. 2011).

Moreover, we found that there was a negative relationship between HEIH and miR-200b, and HEIH modulated the malignant behaviors of MDA-MB-231 cells through negative regulation of miR-200b. In a previous study, miR-200b was found to act as an effective inhibitor of chemotherapy-induced chemotherapy-induced epithelial-mesenchymal transition and consequently regulates tumor metastasis in human tongue cancer (Sun et al. 2012). Moreover, miR-200 is shown to promote the colonization of mouse breast cancer cells to form distant metastases (Dykxhoorn et al. 2009). Ye et al. (2004) demonstrated that miR-200b was downregulated in breast cancer tissues and cell lines and the downregulation was correlated with poor outcome in patients with breast cancer. miR-200b has the potential to serve as prognostic biomarker and tumor suppressor for BC patients. Yao et al. also showed that miR-200b can act as tumor suppressor in breast cancer and has the potential to serve as prognostic biomarker (Yao et al. 2015). In this study, we also found that miR-200b was remarkably downregulated in breast cancer tissues and cells. Therefore, we assume that miR-200b may inhibit breast cancer development, and HEIH may contribute to breast cancer through downregulation of miR-200b.

Furthermore, PBX3 was confirmed as a target of miR-200b. A previous study has shown that PBX3 can regulate epithelial-mesenchymal transition and predicts poor outcome in colorectal cancer (Lamprecht et al. 2018). Additionally, it is reported that miR-144-3p can inhibit the progression of gastric cancer by targeting PBX3 (Li et al. 2017a); miRNA-33a-3p suppresses the migration and invasion of hepatocellular carcinoma cells *via* targeting PBX3 (Han et al. 2016). However, the role of PBX3 in breast cancer progression has not yet been fully revealed. In this study, we found that PBX3 was a target of miR-200b that could mediate the role of miR-200b in regulating the malignant behaviors of MDA-MB-231 cells. Given the oncogenic role of PBX3, we assume that miR-200b may suppress the development of breast cancer *via* targeting PBX3.

Besides, activation of the Wnt/β-catenin pathway has been found to be enriched in basal-like breast cancers and indicates poor outcome (Khramtsov et al. 2010). Wnt/β-catenin signaling is also found activated in triple negative breast cancer and may serve as promising a therapeutic target for treatment (King et al. 2012). Jang et al. (2015) also revealed that blockade of this signaling pathway can represses breast cancer metastasis. Furthermore, diallyl disulfide is shown to suppress the activation of the Wnt-1 signaling pathway by upregulation of miR-200b, and consequently inhibit proliferation and promote apoptosis of gastric cancer cells (Tang et al. 2013). In this study, we also found a significant association between dysregulation of HEIH/miR-200b/PBX3 axis and activation of the Wnt/β-catenin pathway. Therefore, we assume that

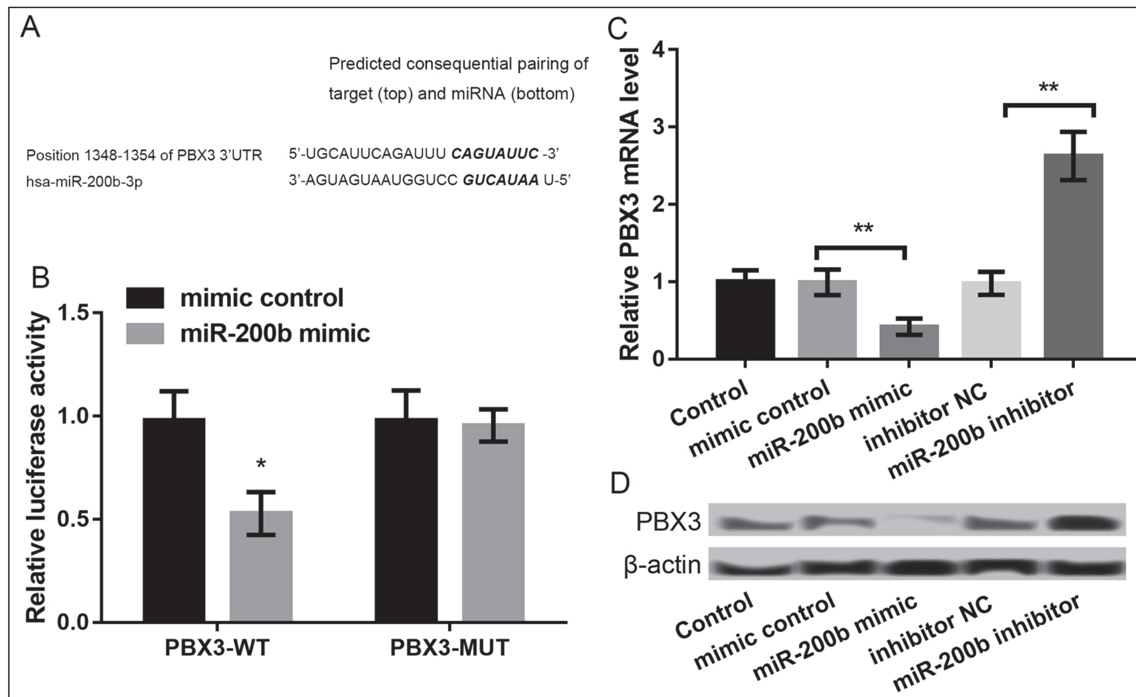

Fig. 3: PBX3 is verified as a functional target of miR-200b. (A): The predicted binding sequence between miR-200b and PBX3. (B): The luciferase activity of PBX3-WT and PBX3-MUT after cotransfection with miR-200b mimic or mimic control. (C) and (D): The PBX3 expression after transfection with miR-200b mimic, miR-200b inhibitor, and respective controls. * compared to respective control. * $P < 0.05$ and ** $P < 0.01$.

Wnt/β-catenin pathway is a key mechanism of HEIH/miR-200b/PBX3 axis in mediating breast cancer development.
In conclusion, our data reveal that HEIH may contribute to breast cancer development *via* modulation of the miR-200b/PBX3 axis and inducing the activation of the Wnt/β-catenin pathway.

4. Experimental

4.1. Human tissue samples and cell lines

In total, 46 pairs of breast tumor tissues and corresponding adjacent normal tissues were obtained from 46 patients with breast cancer by surgical resection at our hospital between 2011 and 2017. These samples were preserved in liquid nitrogen for 30 min and saved at -80 °C. Informed consent was obtained from all subjects and this study was approved by the local ethic committee.
Human breast cancer cell lines MDA-MB-23 and MCF-7 as well as normal human mammary epithelial cell line MCF10A were purchased from the American Type Culture Collection (ATCC). They were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO, USA) containing 10 % heat-inactivated fetal bovine serum (FBS, GIBCO), and maintained in a 37 °C humidified incubator with 5% CO_2. To maintain exponential growth, cells were passaged every 2-3 days.

4.2. Cell transfection

To explore the role of HEIH, miR-5095, and pre-leukemia transcription factor 3 (PBX3) in breast cancer, MDA-MB-231 cells were transfected with the sh-HEIH, sh-NC, miR-200b mimic, mimic NC, inhibitor NC, miR-200b inhibitor, si-NC or si-PBX3 (GenePharma, China) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

4.3. Quantitative real-time reverse-transcription PCR (qRT-PCR)

Total RNA was isolated from tissues or cells using Trizol (Invitrogen), and reverse transcription into to cDNA was performed using the Omniscript RT Kit (Qiagen, Hilden, Germany). qRT-PCR was then performed using a standard SYBR Green PCR kit (Toyobo, Osaka, Japan) protocol in an StepOnePlus™ (Applied Biosystems) with the following PCR reaction: 20 s at 95 °C, and then 40 cycles of 3 s at 95 °C and 30 s at 60 °C. The internal controls for miRNAs and RNAs were U6 and β-actin, respectively. For relative quantification, the threshold cycle values were normalized against that of internal controls.

4.4. Cell proliferation assays

Cell proliferation was assessed by a Cell Proliferation Reagent Kit I (MTT) (Roche Applied Science, Penzberg, Germany). After different transfections, the MDA-MB-231 cells were grown in 96-well plates, followed by assessing cell proliferation every 24 h following the manufacturer's protocol.

4.5. Cell apoptosis

The MDA-MB-231 cells were harvested 48 h after transfection. Cells were then double stained with FITC-Annexin V and propidium iodide (PI) using the FITC-Annexin V Apoptosis Detection Kit (BD Biosciences) following the manufacturer's recommendations. The apoptotic cells were then analyzed with a flow cytometry (FACScan; BD Biosciences) equipped with a CellQuest software (BD Biosciences).

4.6. Cell migration and invasion assays

For cell migration, $5x10^4$ cells 48 h after transfection were suspended in serum-free media and then seeded into the upper chamber of an insert (8-mm pore size; Millipore, Billerica, MA, USA). For cell invasion, 1×10^5 cells 48 h after transfection were also suspended in serum-free medium, but placed into the upper chamber of an insert coated with Matrigel. In both migration and invasion assays, medium containing 10 % FBS was added to the lower chamber. After 24 h of incubation, the migrated or invaded cell through the membrane were stained with methanol and 0.1 % crystal violet, imaged, and counted using an IX71 inverted microscope (Olympus, Tokyo, Japan).

4.7. Western blot

After transfection, cells were lysed with cell lysis buffer (Beyotime, Haimen, China). The protein concentration determination was performed, followed by SDS-polyacrylamide gel electrophoresis for separating protein bands. The bands were transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA) and incubated overnight with the primary antibodies to Bax, Bcl-2, pro-caspase-3, cleaved-caspase-3, pro-caspase-9, cleaved-caspase-9, PBX3, wnt1, c-myc, cyclin D1, β-catenin, and β-actin (1:1,000; Abcam, Cambridge, UK) at 4 °C. β-Actin was used as the control. After incubation with the appropriate secondary antibodies, the protein signals were revealed by means of an Odyssey Infrared Imaging System (LI-COR, Lincoln, NE, USA).

4.8. Dual-luciferase reporter assay

To construct the reporter vectors PBX3-wild-type (PBX3-WT) and PBX3-mutated-type (PBX3-MUT), the fragments from PBX3 containing the predicted miR-200b binding site and flanking sequence were respectively inserted into the pmirGlO Dual-luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA). Subsequently, cells were transfected with the constructed reporter vectors, along with miR-200b mimic or mimic control. After 48 h of transfection, the luciferase activity was the detected by the Dual-Luciferase Reporter Assay System (Promega).

4.9. Statistical analysis

Experiments were independently repeated three times. Differences between groups were analyzed using Student's t test or ANOVA that was performed using SPSS version 18.0 (SPSS, Chicago, IL). $P < 0.05$ was considered statistically significant.

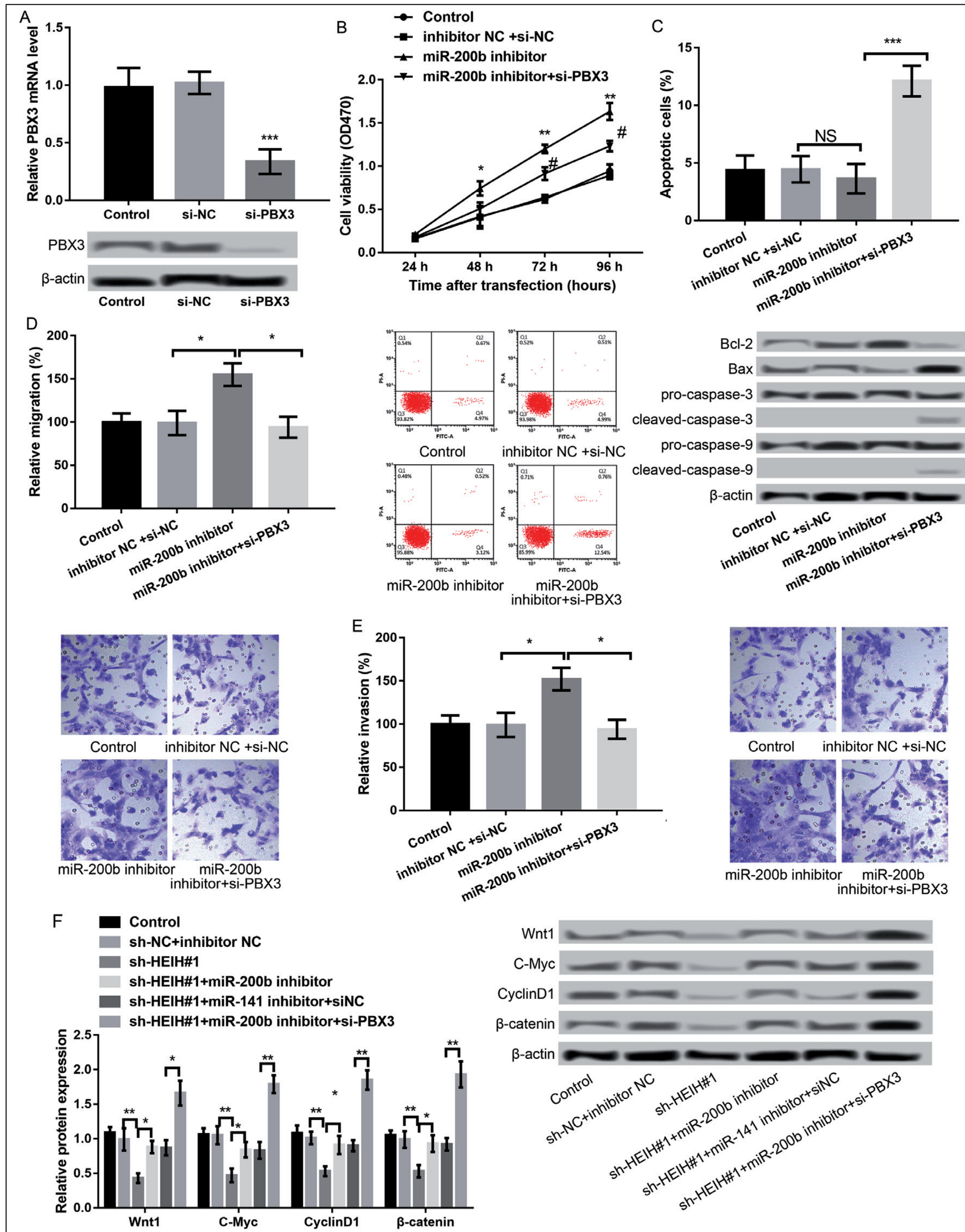

Fig. 4: miR-200b regulates the malignant behaviors of breast cancer cells through PBX3. (A): The PBX3 expression after transfection with si- PBX3 and si-NC. (B): Cell viability after transfection. (C): Cell apoptosis after transfection and the expression levels of pro-apoptotic proteins (Bax, cleaved-caspase-3 and cleaved-caspase-9) and anti-apoptotic protein Bcl-2. (D): Cell migration after transfection. (E): Cell invasion after transfection. (F) The protein expression levels of Wnt/β-catenin pathway-related proteins including wnt1, c-Myc, cyclin-D1, and β-catenin after dysregulation of HEIH, miR-200b, and PBX3.* compared to respective control. * $P < 0.05$ and ** $P < 0.01$. * compared to respective control. * $P < 0.05$ and ** $P < 0.01$.

Conflicts of interest: None declared.

References

Bussard KM, Marini FC (2016) Tumor-associated osteoblasts are major mediators in facilitating bone metastatic breast cancer cell quiescence, AACR.

Cui C, Zhai D, Cai L, Duan Q, Xie L, Yu J (2018) Long noncoding RNA HEIH promotes colorectal cancer tumorigenesis via counteracting miR-939-mediated transcriptional repression of Bcl-xL. Cancer Res Treat 50: 992-1008.

Dykxhoorn DM, Wu Y, Xie H, Yu F, Lal A, Petrocca F, Martinvalet D, Song E, Lim B, Lieberman J (2009) miR-200 enhances mouse breast cancer cell colonization to form distant metastases. Plos One 4: e7181.

Gu J, Wang Y, Wang X, Zhou D, Zhou M, He Z (2018) Effect of the lncRNA GAS5-MiR-23a-ATG3 axis in regulating autophagy in patients with breast cancer. Cell Physiol Biochem 48: 194-207.

Han H-B, Gu J, Ji D-B, Li Z-W, Zhang Y, Zhao W, Wang L-M, Zhang Z-Q (2014) PBX3 promotes migration and invasion of colorectal cancer cells via activation of MAPK/ERK signaling pathway. World J Gastroenterol 20: 18260-18270.

Han S-Y, Han H-B, Tian X-Y, Sun H, Xue D, Zhao C, Jiang S-T, He X-R, Zheng W-X, Wang J (2016) MicroRNA-33a-3p suppresses cell migration and invasion by directly targeting PBX3 in human hepatocellular carcinoma. Oncotarget 7: 42461-42473.

Hansji H, Leung EY, Baguley BC, Finlay GJ, Askarian-Amiri ME (2014) Keeping abreast with long non-coding RNAs in mammary gland development and breast cancer. Front Genet 5: 379.

Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, Barrette TR, Prensner JR, Evans JR, Zhao S, Poliakov A, Cao X, Dhanasekaran SM, Wu YM, Robinson DR, Beer DG, Feng FY, Iyer HK, Chinnaiyan AM (2015) The landscape of long noncoding RNAs in the human transcriptome. Nature Genet 47: 199-208.

Jang G-B, Kim J-Y, Cho S-D, Park K-S, Jung J-Y, Lee H-Y, Hong I-S, Nam J-S (2015) Blockade of Wnt/β-catenin signaling suppresses breast cancer metastasis by inhibiting CSC-like phenotype. Sci Rep 5: 12465.

Khramtsov AI, Khramtsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH (2010) Wnt/β-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. Am J Pathol 176: 2911-2920.

King TD, Suto MJ, Li Y (2012) The wnt/β-catenin signaling pathway: A potential therapeutic target in the treatment of triple negative breast cancer. J Cell Biochem 113: 13-18.

King TD, Suto MJ, Yonghe L (2011) The Wnt/β-catenin signaling pathway: a potential therapeutic target in the treatment of triple negative breast cancer. J Cell Biochem 113: 13-18.

Lamprecht S, Kaller M, Schmidt EM, Blaj C, Schiergens TS, Engel J, Jung A, Hermeking H, Grünewald TG, Kirchner T (2018) PBX3 is part of an EMT regulatory network and indicates poor outcome in colorectal Cancer. Clin Cancer Res 24: 1974-1986.

Li B, Zhang S, Shen H, Li C (2017a) MicroRNA-144-3p suppresses gastric cancer progression by inhibiting epithelial-to-mesenchymal transition through targeting PBX3. Biochem Biophys Res Comm 484: 241-247.

Li Q, Gao H, Zhou S, Liao Y (2018) LncRNA PlncRNA-1 overexpression inhibits the growth of breast cancer by upregulating TGF-β1 and downregulating PHGDH. Breast Cancer: 1-7.

Li W, Jia G, Qu Y, Du Q, Liu B (2017b) Long non-coding RNA (LncRNA) HOXA11-AS promotes breast cancer invasion and metastasis by regulating epithelial-mesenchymal transition. Med Sci Monitor 23: 3393-3403.

Oeffinger KC, Fontham ETH, Ruth E, Abbe H, Michaelson JS, Ya-Chen Tina S, Walter LC, Church TR, Flowers CR, Lamonte SJ (2015) Breast cancer screening for women at average risk: 2015 Guideline update from the American Cancer Society. JAMA 314: 1599-1614.

Ramberg H, Grytli HH, Nygård S, Wang W, O Ö, Zhao S, Løvf M, Katz B, Skotheim RI, Bjartell A (2016) PBX3 is a putative biomarker of aggressive prostate cancer. Int J Cancer 139: 1810-1820.

Sørensen KP, Thomassen M, Tan Q, Bak M, Cold S, Burton M, Larsen MJ, Kruse TA (2015) Long non-coding RNA expression profiles predict metastasis in lymph node-negative breast cancer independently of traditional prognostic markers. Breast Cancer Res 17: 55.

Siegel RL, Miller KD, Dvm AJ (2017) Cancer statistics, 2017. Cancer J Clinicians 67: 7-30.

Spizzo R, Almeida MIe, Colombatti A, Calin GA (2012) Long non-coding RNAs and cancer: a new frontier of translational research? Oncogene 31: 4577-4587.

Sun L, Yao Y, Liu B, Lin Z, Lin L, Yang M, Zhang W, Chen W, Pan C, Liu Q (2012) MiR-200b and miR-15b regulate chemotherapy-induced epithelial-mesenchymal transition in human tongue cancer cells by targeting BMI1. Oncogene 31: 432.

Tang H, Kong Y, Guo J, Tang Y, Xie X, Yang L, Su Q, Xie X (2013) Diallyl disulfide suppresses proliferation and induces apoptosis in human gastric cancer through Wnt-1 signaling pathway by up-regulation of miR-200b and miR-22. Cancer Lett 340: 72-81.

Tao ZQ, Shi A, Lu C, Song T, Zhang Z, Zhao J (2015) Breast cancer: epidemiology and etiology. Cell Biochem Biophys 72: 333-338.

Tian T, Wang M, Lin S, Dan Z, Liu K, Zhu W, Additionals S (2018) The impact of lncRNA dysregulation on clinicopathology and survival of breast cancer: a systematic review and meta-analysis. Mol Ther Nucleic Acids 12: 359-369.

Yang F, Zhang L, Huo Xs, Yuan Jh, Xu D, Yuan Sx, Zhu N, Zhou Wp, Yang Gs, Wang Yz (2011) Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. Hepatology 54: 1679-1689.

Yao Y, Hu J, Shen Z, Yao R, Liu S, Li Y, Cong H, Wang X, Qiu W, Yue L (2015) MiR-200b expression in breast cancer: a prognostic marker and act on cell proliferation and apoptosis by targeting Sp1. J Cell Mol Med 19: 760-769.

Ye F, Tang H, Liu Q, Xie X, Wu M, Liu X, Chen B, Xie X (2014) miR-200b as a prognostic factor in breast cancer targets multiple members of RAB family. J Transl Med 12: 17.

Zhang C, Yang X, Qi Q, Gao Y, Wei Q, Han S (2018) lncRNA-HEIH in serum and exosomes as a potential biomarker in the HCV-related hepatocellular carcinoma. Cancer Biomark 21: 651-659.

Zhao H, Xing G, Wang Y, Luo Z, Liu G, Meng H (2017) Long noncoding RNA HEIH promotes melanoma cell proliferation, migration and invasion via inhibiting miR-200b/a/429. Biosci Rep BSR20170682.