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Numb confers to inhibit epithelial mesenchymal transition via β-catenin/Lin28 signaling pathway in breast cancer

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ABSTRACT

Objectives: This study aimed to investigate role of Numb in the epithelial mesenchymal transition (EMT) of breast cancer.
Methods: Numb and β-catenin were inhibited in MCF-7 cells using sh-RNA and overexpressed in T47D cells by pcDNA3.0-Numb, pcDNA3.0-β-catenin. Cell proliferation, invasion and migration were evaluated using MTT and Transwell assay, respectively. β-catenin, Lin28, and EMT related markers were determined using qRT-PCR and Western Blotting.
Results: Knockdown of Numb significantly promoted the proliferation, invasion and migration of MCF-7 cells, further increased the expression of β-catenin, Lin28, Snail-1, and N-cadherin, as well as decreased E-cadherin. In T47D cells transfected with pcDNA3.0-Numb, the results were quite the reverse.
Conclusions: Knockdown of Numb could promote the EMT of breast cancer cells via β-cateni/Lin28 signaling pathway.

1. Introduction

Due to the heterogeneous nature, breast cancer is one of the most challenging threaten for human health (Nooshinfar et al., 2016). Although the medical technology has been largely improved in recent decades, the prognosis of patients with breast cancer is still unsatisfied, especially for patients with advanced stage (Desantis et al., 2017). Therefore, it is important to explore the pathogenesis of breast cancer to improve the treatment of breast cancer in clinical. Despite multiple efforts to study the mechanism of breast cancer (Mittal et al., 2017; Jeselsohn et al., 2015; Hoyen et al., 2015), deeper insights for pathogenesis of breast cancer are still unclear.

As a tumor suppressor, Numb is originally identified as a cell fate determinant (Tosoni et al., 2017). Studies also show Numb, a critical negative regulatory factor of Notch signaling pathway which is abnormally expressed in many cancers, is down-regulated in many cancers and is associated with cell proliferation, metastasis and epithelial to mesenchymal transition (EMT) of cancer cells (Wang et al., 2015; Flores et al., 2014). Further investigations have revealed that Numb plays critical regulatory roles in several signaling pathways such as p53 signaling in modulating the cell fate of cancer cells (Lu et al., 2015; Faraldo and Glukhova, 2015). Some studies have already demonstrated relationship between Numb and breast cancer (Saha et al., 2017; T et al., 2009), however deeper understanding is still needed.

β-catenin signaling plays an important role in development of many cancers (Chesire et al., n.d.; Morin, 2015). Recently, it was reported β-catenin signaling was associated with Lin28, a RNA binding protein which was first identified in *Caenorhabditis elegans* as a development modulator, in nasopharyngeal carcinoma (Cai, 2018). It is reported Lin28 is up-regulated in 15% of cancers (Balzeau et al., 2017). Studies also showed that Lin28/let-7 pathway was associated with the development of cancers, including migration, invasion, and apoptosis of cancer cells, including breast cancer (Balzeau et al., 2017; Tu et al., 2015; Lv et al., 2012). However, up to now, no study focused on relationship between Numb and Lin28 in development of breast cancer.

In the present study, we aimed to investigate role of Numb in development of breast cancer and its relationship with β-catenin/Lin28 signaling pathway. For the first time we demonstrated that inhibition of Numb could boost EMT via facilitating β-catenin/Lin28 signaling pathway in breast cancer cells. This study could give deeper understanding for mechanisms of Numb in breast and provide some new research targets for breast cancer treatment.

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2. Materials and methods

2.1. Clinical samples

In the present study, a total of 30 breast cancer samples and adjacent normal tissues samples were collected from Affiliated Cancer Hospital of Xiangya School of Medicine for the following research. The tissues were immediately collected after resection and stored under −20 °C before used. This study was approved by the ethic committee of Affiliated Cancer Hospital of Xiangya School of Medicine hospital.

2.2. Immunohistochemistry (IHC)

Briefly, the tissues were immediately collected after resection and stored under -20 °C. All tissues were fixed, embedded and sectioned. Samples were then incubated with primary antibodies of anti-Numb and anti-Lin28 (Abcam, Cambridge, MA, USA) at 4 °C overnight, followed with incubation of corresponding second antibody at 37 °C for 30 min. Samples were then stained with diaminobenizidine (DAB). The samples were observed using an inverted microscopeand the pictures were photographed.

2.3. Cell culture and transfection

MCF-10A, PA-1, SK-BR-3, T47D and MCF-7 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Briefly, cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco) and 100 μg/ml penicillin-streptomycin at 37 °C in 95% air with 5% O_2. Cells were transfected with sh-NC, sh-Numb, sh-β-catenin, sh-Lin28, pcDNA3.0-NC, pcDNA3.0-Numb, pcDNA3.0-β-catenin or pcDNA3.0-Lin28 (all purchased from Shanghai GenePharma Co., Ltd., Shanghai, China) using the lipofectamine3000 (Life technologies) according to the manufacturer's instruction. After transfection for 48 h, cells were collected for the following investigation.

2.4. MTT (3-[4,5-dimethylthiazol-2yl] diphenyltetra-zolium brimide) assay

After transfection, cells viability was measured using MTT assay (Sigma-Aldrich, USA). Briefly, cells were seeded in 96-well microplate at a density of 1.5×10^4 cells/well, and incubated in a humiditiy incubator at 37 °C in 95% air with 5% O_2 for 24 h. Then, 10 μl MTT (with a concentration of 5 mg/ml) was added followed by further culture for 4 h at 37 °C and 5% CO_2. Then, medium was removed and 150 μl of DMSO was added. Absorbance at 490 nm was determined using a microplate reader (Bio-Rad 680, Hercules, CA, USA). Each experiment was performed in triplicate, and the average value was calculated.

2.5. Cell cycle analysis

Cell cycle was measured using flow cytometry analysis. Briefly, cells were harvested by trypsinization, washed by PBS and fixed. Then cells were resuspended in 100 μl PBS containing 50 μg/ml RNase A for 30 min at room temperature. Propidium iodide (20 μg/ml) was used to stain the cells for 20 min using an FITC Annexin V kit (BD Biosciences, MA, USA). The cell cycle was analyzed using a flow cytometer (BD-LSR; BD Biosciences, San Jose, CA, USA).

2.6. Transwell assay

Cell invasion ability was measured using the transwell assay. Briefly, cells were seeded in the upper chamber of transwell membranes (Corning Costar, USA) coated with matrigel (BD Biosciences, USA) and maintained in DMEM without FBS. After addition of 500 μl of DMEM containing 10% FBS to the lower chamber, cells were further incubated for 24 h, stained with 0.1% crystal violet, and then counted and photographed with an optical microscope (Olympus, Center Valley, PA, USA). Transwell migration assay were performed with the similar approach without coating of Matrigel.

2.7. Quantitative real-time PCR(qRT-PCR)

Total RNA was extracted using TRIzol reagent (Takara, Dalian, China). Reserve transcription was conducted using a High Capacity RNA-to-cDNA kit (Applied Biosystems, USA) according to the manufacturers' protocols. Gene expression levels were quantified using SYBR Premix Dimer Eraser kit (Takara, Dalian, China) in an ABI prism 7500 (Applied Biosystems, Foster City, CA, USA). Primer sequences were listed below: Numb F 5′-AGGCCAGTCGTCCACATCA-3′, R 5′-GGTACT TAACCGGGAAGCTACAT-3′; β-catenin F 5′-GCTGACCTGACGGAGTT GGA-3′, R 5′-GCTACTTGCTCTTGCGTGAA-3′; Lin28 F 5′-CGGGCATCT GTAAGTGGTTC-3′, R 5′- CAGACCCTTGGCTGACTTCT-3′; N-cadherin F 5′-GTGCATGAAGGACAGCCTCT-3′, R 5′-CCACCTTAAAATCTGCA GGC-3′; E-cadherin F 5′-ATTCTGATTCTGCTGCTCTTG-3′, R 5′-TGGCA TTAGCAGTAGGTTCTTG-3′; Snail-1F 5′-GAGGCGGTGGCAGACTAG AGT-3′, R 5′-CGGGCCCCCAGAATAGTTC-3′; vimentin F 5′-GAGTCCAC TGAGTACCGGAGAC-3′, R 5′-TGTAGGTGGCAATCTCAATGTC-3′; GAPDH F 5′- ACAGCAACAGGGTGGTGGAC-3′, R 5′-TTTGAGGGTGCA GCGAACTT-3′. The relative expression of genes was assessed using $2^{-\Delta\Delta Ct}$ method.

2.8. Western blotting

Total protein in cells was extracted using the RIPA lysis buffer protein amount was quantified using a BCA method. Following this, supernatants were boiled with equal volume of loading buffer for 10 min, loaded for 10% SDS-PAGE, and transferred to PVDF membranes. Membranes were incubated with primary antibodies (all purchased from Abcam, Cambridge, MA, USA) after blocked with 5% non-fat milk. Primary antibodies used in this study was as follows: anti-Numb (anti-Numb antibody, ab14140, 1 μg/ml), Lin28 (anti-Lin28 antibody, ab46020, 1/1000), β-catenin (anti-β-catenin antibody, ab32572, 1/5000), Snail-1 (anti-Snail-1 antibody, ab53519, 3 μg/ml), N-cadherin (anti-N-cadherin antibody, ab18203, 1 μg/ml), vimentin (anti-vimentin antibody, ab137321, 1/500), and E-cadherin (anti-E-cadherin antibody, ab15148, 1/500) antibodies at 4 °C overnight, respectively. Protein bands were scanned with the Pierce ECL Western Blotting Substrate (Pierce, Shanghai, China). β-actin was served as an internal control.

2.9. TCGA data collection

Expression data of Numb was obtained from the publically available TCGA datasets which were directly downloaded from the TCGA Data Portal at https://tcga-data.nci.nih.gov/tcga/. Gene expression data were available for 1097 in breast cancer samples, and 114 in normal samples.

2.10. Statistical analysis

Student *t*-test and one-way analysis of variance (ANOVA) were used for comparison between two groups or comparison among three or more groups, respectively. The measurement data was expressed by mean ± SD. $P < .05$ was considered as significant difference. All calculations were made using SPSS 22.0 (SPSS Inc., USA).

3. Results

3.1. Numb was down-regulated and Lin28 were up-regulated in breast cancer

The expression of Numb and Lin28 in the breast cancer and adjacent

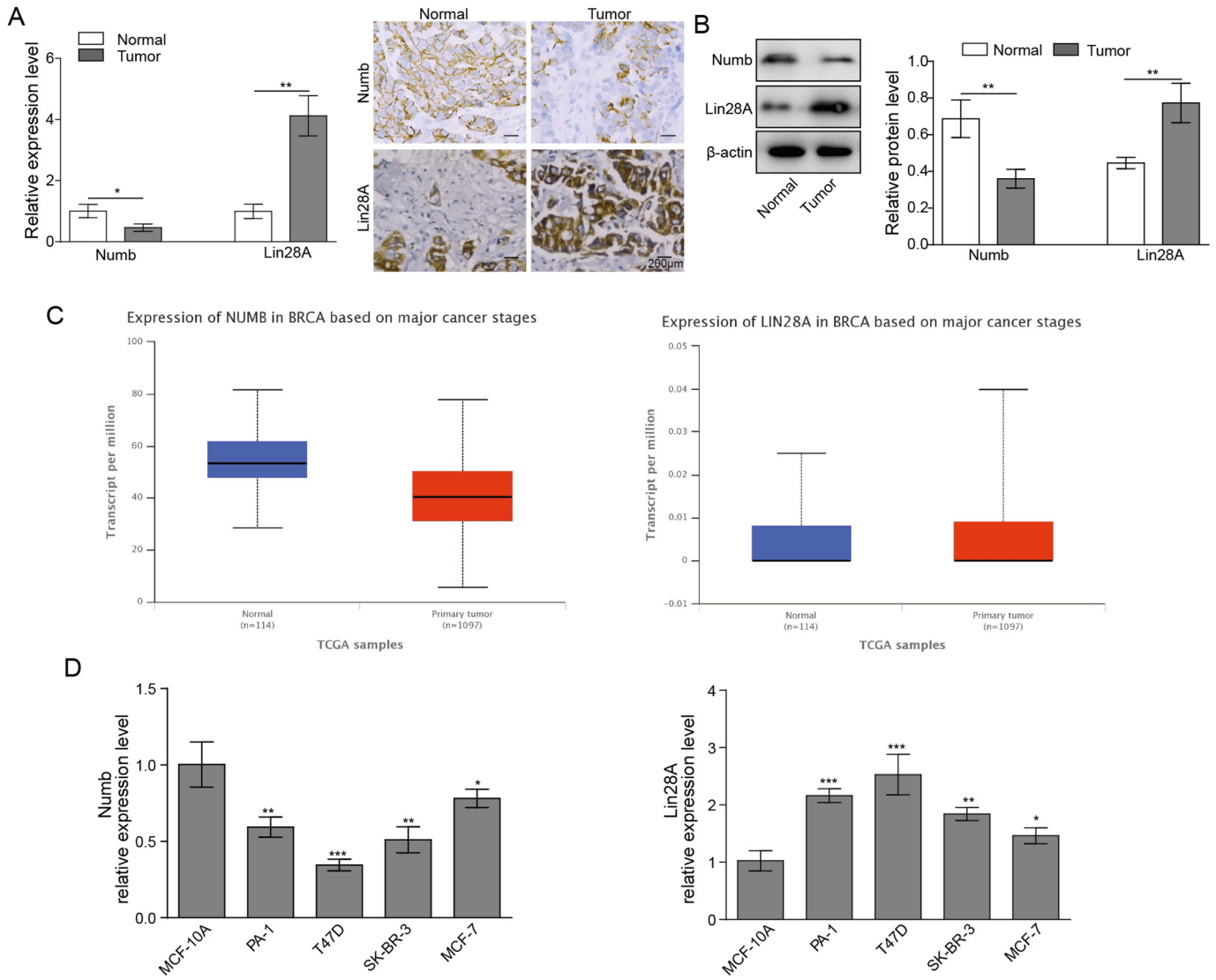

Fig. 1. Numb was down-regulated and Lin28 were significantly up-regulated in breast cancer. A. Expression of Numb and Lin28 in breast cancer and normal tissues by qRT-PCR and IHC; B. Expression of Numb and Lin28 in breast cancer and normal tissues by Western blotting. C. The relative Numb and LIN28A expression level in breast tumor tissues and normal tissues from TCGA datebase. D. The relative Numb and LIN28A expression level in MCF-7, PA-1, T47D and SK-BR-3 cells was determined by qRT-PCR $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, compared with human normal mammary epithelial cells MCF-10A. All experiments were conducted in triplicate.

normal tissues was determined by IHC, as well as qRT-PCR and Western blotting methods. Results showed that Numb was down-regulated while expression of Lin28 was significantly up-regulated for both mRNA and protein levels in breast cancer tissues ($P < .05$, Fig. 1A and B). Then, to understand the association between clinical and expression of Numb and Lin28 in breast cancer patients, analysis of TCGA database revealed that the relative Numb expression levels in tumor tissues ($n = 1097$) were significantly lower than in adjacent normal tissues ($n = 114$), while Lin28A expression levels were significantly higher than in tumor tissues ($P < .01$; Fig. 1C). In addition, we further examined the Numb and LIN28A expression in normal human mammary epithelial cells MCF-10A and 4 breast cancer cell lines, including MCF-7, T47D, PA-1, and SK-BR-3 cells. qRT-PCR showed that Numb was down-regulated, while LIN28A was up-regulated in all 4 breast cancer cell lines (Fig. 1D). Among these cell lines, the expression level of Numb was significantly higher in MCF-7 cells, while lower in T47D cells, and opposite trend was observed in the expression of LIN28A. Thus, MCF-7 and T47D cells were selected for the subsequent experiments.

3.2. Knockdown of Numb promoted the proliferation of breast cancer cells

To further study effect of Numb in breast cancer, Numb was knocked down in MCF-7 cells and was over-expressed in T47D cells. As shown in Fig. 2A, expression of Numb was significantly down-regulated (almost 1/2 by qRT-PCR and 1/2 by western blot) in MCF-7 cells transfected with sh-Numb compared with the control cells ($P < .05$); while expression of Numb was significantly up-regulated (almost 3.5 folds by qRT-PCR and 2.5 folds by western blot) in T47D cells with over-expressed Numb ($P < .05$, Fig. 2B), indicating the successful establishment of Numb knockdown and overexpression cell models. Meanwhile, cell viability analysis by MTT assay showed that knockdown of Numb in MCF-7 cells significantly enhanced the cell viability compared with the control cells ($P < .05$). However, cell viability was significantly inhibited when T47D cells were transfected with pcDNA3.0-Numb compared with the control ($P < .05$, Fig. 2C and 2D). Two first-line chemotherapy drugs, 5-FU and cisplatin, were added for the experiments, we found that the cell viability of Numb-over-expression cells were remarkably reduced compared with control cells upon 5-FU or cisplatin treatment, while cell viability of shNumb cells

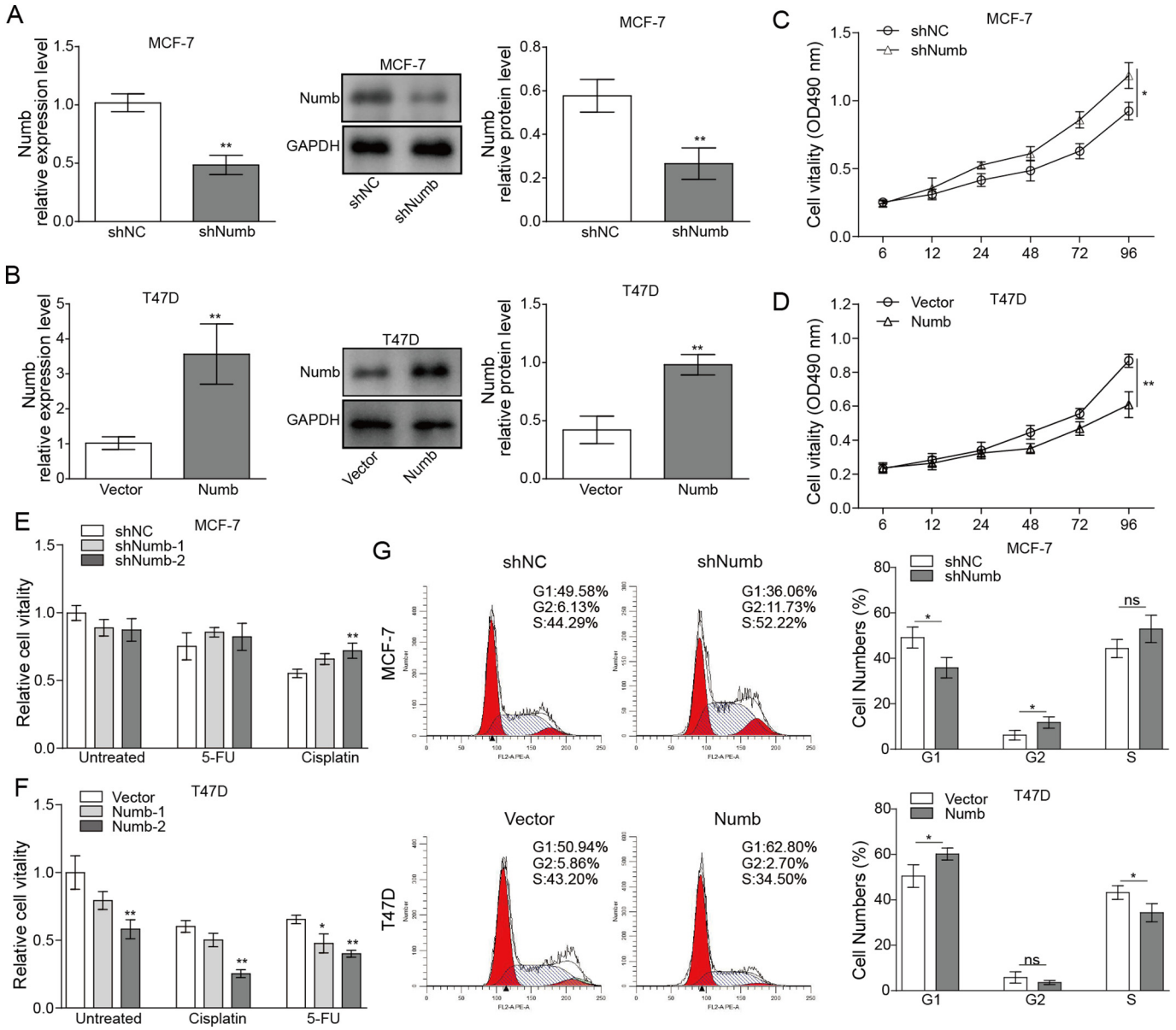

Fig. 2. Knockdown of Numb significantly promoted the proliferation while overexpression of Numb significantly inhibited the proliferation of breast cancer cells. A. Relative mRNA expression of Numb in MCF-7 cells transfected with sh-Numb or sh-NC by qRT-PCR; B. Relative mRNA expression of Numb in T47D cells transfected with pcDNA3.0-Numb or NC; C. Cell viability of MCF-7 transfected with sh-Numb or sh-NC by MTT assay; D. Cell viability of T47D transfected with pcDNA3.0-Numb or NC. E. Cell proliferation of MCF-7 transfected with sh-Numb or sh-NC was determined, followed by 5-FU and cisplatin treatment. F. Cell proliferation of T47D transfected with pcDNA3.0-Numb or NC was determined, followed by 5-FU and cisplatin treatment. G. Cell cycle analysis for MCF-7 transfected with sh-Numb and T47D transfected with pcDNA3.0-Numb or NC. All experiments were conducted in triplicate. *$P < .05$, **$P < .01$, compared with the control.

showed resistance towards drugs ($P < .05$, Fig. 2E–F). Additionally, we also analyzed the cell cycle of MCF-7 cells transfected with sh-Numb and T47D cells transfected with pcDNA3.0-Numb. Results showed when Numb was knocked down in MCF-7 cells, the cell number in G1 phase significantly decreased while cell number in G2 and S phases significantly increased compared with the NC. However when Numb was over-expressed in T47D cells, the effects were opposite($P < .05$, Fig. 2G). Thus, these results suggested inhibition of Numb could significantly promote the proliferation of breast cancer cells.

3.3. Knockdown of Numb promoted the invasion and migration of breast cancer cells

Invasion and migration for breast cancer cells transfected with sh-Numb or pcDNA3.0-Numb were determined using transwell assay. When expression of Numb was inhibited, both cell invasion and migration were significantly promoted in MCF-7 cells compared with the negative control (NC) group ($P < .05$, Fig. 3A). On the contrary, overexpression of Numb significantly inhibited cell migration and invasion in T47D cells compared with the NC group ($P < .05$, Fig. 3A), indicating that knockdown of Numb could promote invasion and migration of breast cancer cells.

3.4. Knockdown or overexpression of Numb regulated the expression of β-catenin, N-cadherin, Snail-1, vimenitin and E-cadheri

To further investigate mechanisms of effects of Numb on breast cancer cells, expression of β-catenin, Lin28, N-cadherin, Snail-1, vimentin and E-cadherin was determined in different cells using both qRT-PCR and Western blotting. As shown in Fig. 4, expressions of β-

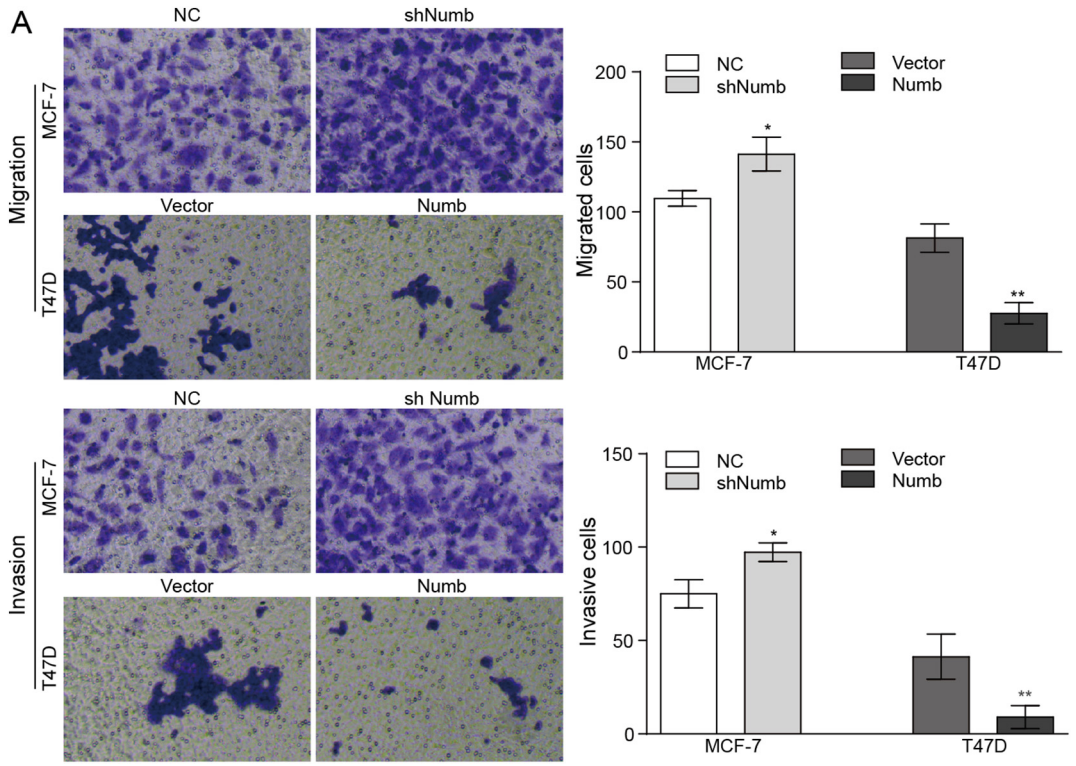

Fig. 3. Knockdown of Numb significantly promoted the migration and invasion while overexpression of Numb significantly suppressed the migration and invasion of breast cancer cells. A. Cell invasion and migration of MCF-7 cells transfected with sh-Numb or sh-NC and T47D cells transfected with pcDNA3.0-Numb or NC by transwell assay; All experiments were conducted in triplicate. *$P < .05$, **$P < .01$, compared with the control.

catenin, Lin28, N-cadherin, vimentin and Snail-1 all significantly increased, while expression of E-cadherin decreased significantly in MCF-7 cells transfected with sh-Numb compared with the NC ($P < .05$). However, when transfected with pcDNA3.0-Numb, expression of β-catenin, Lin28, N-cadherin, and Snail-1 was significantly decreased, while E-cadherin was significantly up-regulated in T47D cells compared with the control ($P < .05$). These results indicated that knockdown of Numb could promote invasion and migration of breast cancer cells through up-regulating β-catenin and EMT related proteins.

3.5. Knockdown of β-catenin inhibited EMT of breast cancer cells through down-regulating Lin28

At last, we used sh-β-catenin to knockdown β-catenin in MCF-7 cells and used pcDNA3.0-β-catenin to overexpress β-catenin in T47D cells. As shown in Fig. 5A, expression of β-catenin was significantly inhibited to almost 1/3 in MCF-7 cells transfected with sh-β-catenin compared with the NC ($P < .05$); while expression of β-catenin was significantly up-regulated to almost 2 folds in T47D cells with over-expressed β-catenin compared with the NC ($P < .05$). Meanwhile, when cells were transfected with sh-β-catenin, Lin28 level was also significantly decreased to almost 40% ($P < .05$); while when β-catenin was over-expressed, expression of Lin28 was also significantly enhanced to almost 3 folds compared with the NC ($P < .05$). These results indicated Lin 28 was regulated by β-catenin in breast cancer cells.

Then the cell invasion and migration were determined in different cancer cells. Results showed when cells were transfected with sh-β-catenin, both cell invasion and migration were significantly suppressed ($P < .05$, Fig. 5B). On the contrary, the cell invasion and migration were significantly promoted by overexpression of β-catenin compared with the NC ($P < .05$). Besides, overexpression (knockdown) of Lin28 significantly rescue the decreased (increased) invasion and migration induced by sh-β-catenin (pcDNA3.0-β-catenin), which further confirmed the relationship between Lin28 and β-catenin. Further determination for β-catenin, Lin28 and EMT related proteins showed when β-catenin was inhibited, all expressions of Lin28, Snail-1 and N-cadherin significantly decreased while expression of E-cadherin was significantly up-regulated compared with the NC ($P < .05$, Fig. 5C). Meanwhile, transfection of pcDNA3.0-Lin28 could significantly reduce the effects by knockdown of β-catenin. The expressions of Lin28, Snail and N-cadherin were all significantly up-regulated and expression of E-cadherin was remarkably down-regulated in cells transfected with sh-β-catenin + LIN28 compared with cells transfected with sh-β-catenin ($P < .05$), indicating that LIN28 could regulate the expression of Snail and N-cadherin and E-cadherin. Similar results were observed in cells over-expressing β-catenin. When β-catenin was over-expressed in T47D cells, expressions of Lin28, Snail-1 and N-cadherin all significantly increased while expression of E-cadherin significantly decreased compared with the control ($P < .05$), while inhibition of Lin28 could significantly release the effects by overexpression of β-catenin. All these results suggested inhibition of β-catenin could suppress the migration and invasion of breast cancer cells through down-regulation of Lin28 and EMT.

4. Discussion

Despite numerous studies on breast cancer, the underlying mechanisms for carcinogenesis and development are still unclear. Recently, role of Numb in cancer development has attracted scholars' attention. Several studies have already shown effect of Numb in breast cancer (Zhang et al., 2016). However, up to now, no study focused on relationship of Numb with β-catenin/Lin28 signaling pathway and EMT in breast cancer cells. In the present study, we first demonstrated that inhibition of Numb could promote EMT via β-catenin/Lin28 signaling pathway in breast cancer cells (Fig. 6).

The expression of Numb was found to be associated with cancer cell proliferation and metastasis. A Danish study found Numb expression was associated with hormone receptor status in breast cancer patients

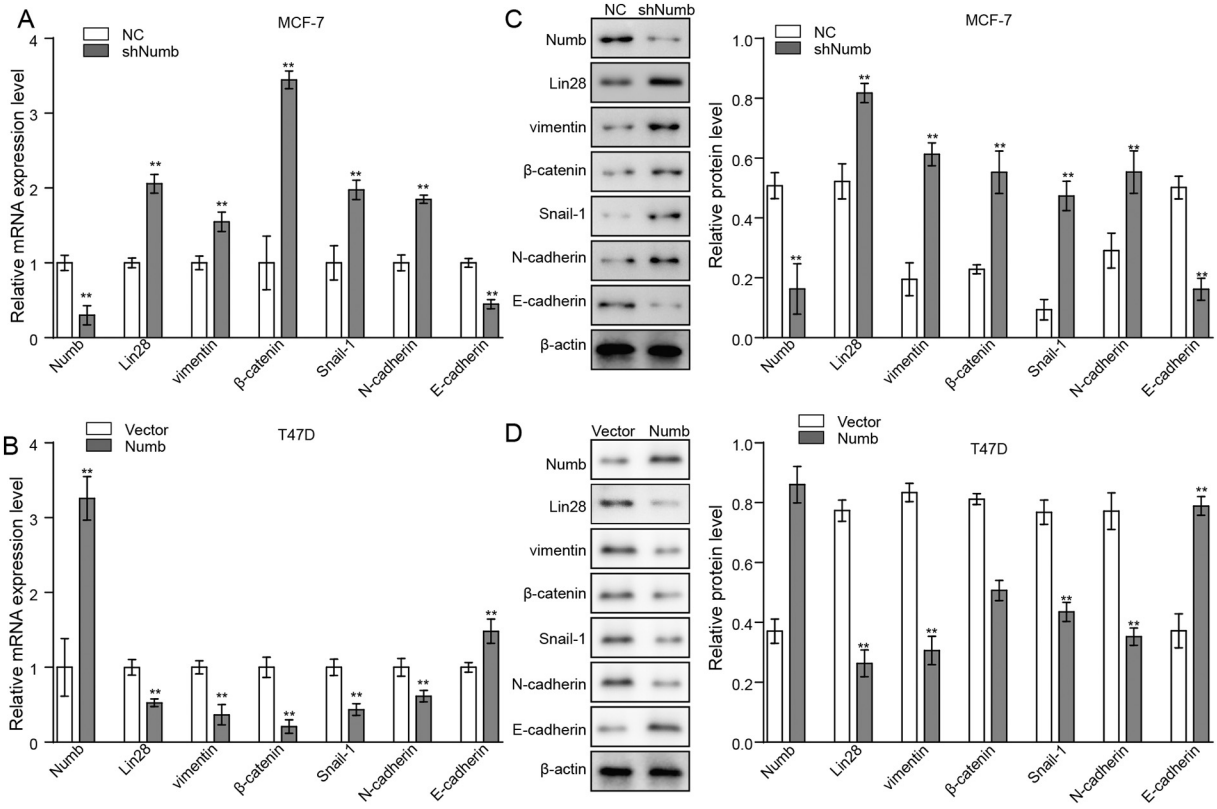

Fig. 4. Knockdown of Numb significantly increased the expression of β-catenin, N-cadherin, vimentin and Snail-1, and significantly decreased the expression of E-cadherin; while overexpression of Numb significantly decreased the expression of β-catenin, N-cadherin, vimentin and Snail-1, and significantly increased the expression of E-cadherin. A. Expression of Numb, β-catenin, N-cadherin, E-cadherin, vimentin and Snail-1 by both qRT-PCR in MCF-7 cells transfected with sh-Numb or shNC; B. Expression of Numb, β-catenin, N-cadherin, E-cadherin, vimentin and Snail-1 by both qRT-PCR in T47D cells transfected with pcDNA3.0-Numb or shNC. C. Expression of Numb, β-catenin, N-cadherin, E-cadherin, vimentin and Snail-1 by Western blotting in MCF-7 cells transfected with sh-Numb or shNC; D. Expression of Numb, β-catenin, N-cadherin, E-cadherin, vimentin and Snail-1 by Western blotting in T47D cells transfected with pcDNA3.0-Numb or shNC. All experiments were conducted in triplicate. $^{*}P < .05$, $^{**}P < .01$, compared with the control.

(Zhou et al., 2010). However few other studies demonstrated the clinical significance for Numb in breast cancer. Flores et al. demonstrated Numb could be a therapeutic target in prostate cancer (Flores et al., 2014). In a recent study, Jin et al. found overexpression of Numb could inhibit cell proliferation and metastasis in renal cell carcinoma cells (Jin et al., 2015). And in the present study, we further confirmed found Numb was down-regulated in breast cancer tissues and cells, which was also in consistent with the result of TCGA database, meanwhile knockdown of Numb promoted cell proliferation and cell metastasis, which was in consistent with other studies.

Effects of Numb in cancer development are associated with many signaling pathways. The correlation of Numb and β-catenin has been demonstrated in several studies. Saha et al. found β-catenin/RAC1 complex had an interaction with Numb-dependent Notch signaling pathway in breast cancer (Saha et al., 2017). Liu et al. Wnt/β-catenin signaling was involved in the process of up-regulation of Numb (Liu et al., 2013). However, few studies reported relationship between Numb and Lin28. In our research, Numb was found to modulate expression of among β-catenin and Lin28, as well as EMT related proteins.

Relationship between Numb and EMT was also demonstrated in many studies. Zhang et al. showed up-regulation of Numb could suppress EMT in pulmonary fibrosis (Zhang et al., 2017). Bocci et al. demonstrated Numb could prevent a complete EMT by modulating Notch signaling, and in this research, it was Numb could negatively regulate ZEB1 (Bocci et al., 2017). What's more, in a recent study, it was shown ZEB could also regulate the LIN28/let-7 axis (Mohit Kumar et al., 2015). Despite these researches, the role of β-catenin and Lin28 in effects of Numb on EMT was firstly demonstrated in the present study. In our research, we found that Numb could negatively regulate the β-catenin/Lin28 signaling.

Many studies already showed role of Lin28 and β-catenin in cancer development. In a recent study, it was considered Lin28 positivity was significantly associated with more advanced stage and lymph node involvement in breast cancer patients (Park et al., 2012). It was also found Lin28 immunostaining was increased in tumors compared with the adjacent tissues and was associated with poor differentiation, advanced-stage disease, and Ki67-positive status in breast cancer patients (Xie et al., 2014). All these findings are in consistent with our results, that Lin28 was up-regulated in breast cancer tissues and cells. Cai et al. showed Wnt/β-catenin signaling could be activated by miR-374a and further promoted metastasis of breast cancer cells (Cai et al., 2013). King et al. found Lin28 promoted colon cancer migration, invasion, and transformation (King et al., 2011). However, only a few studies demonstrated interaction between β-catenin and Lin28. In a recent study, it was found Wnt/β-catenin-Lin28/let7-PDK1 signaling has played an important role in aerobic glycolysis and proliferation of nasopharyngeal carcinoma (Cai, 2018). Cai et al. also showed let-7 microRNA expression could be suppressed by Wnt-β-catenin signaling through transactivation of Lin28 in breast cancer (WY et al., 2013). In this paper, we for the first time demonstrated Numb might influence EMT and metastasis of breast cancer cells by modulating expression of β-catenin through Lin28.

In conclusion, we conducted an in vitro study to investigate role of Numb in development of breast cancer and its relationship with β-catenin/Lin28 signaling pathway. Results showed Numb could inhibit EMT and metastasis of breast cancer cells by modulating expression of

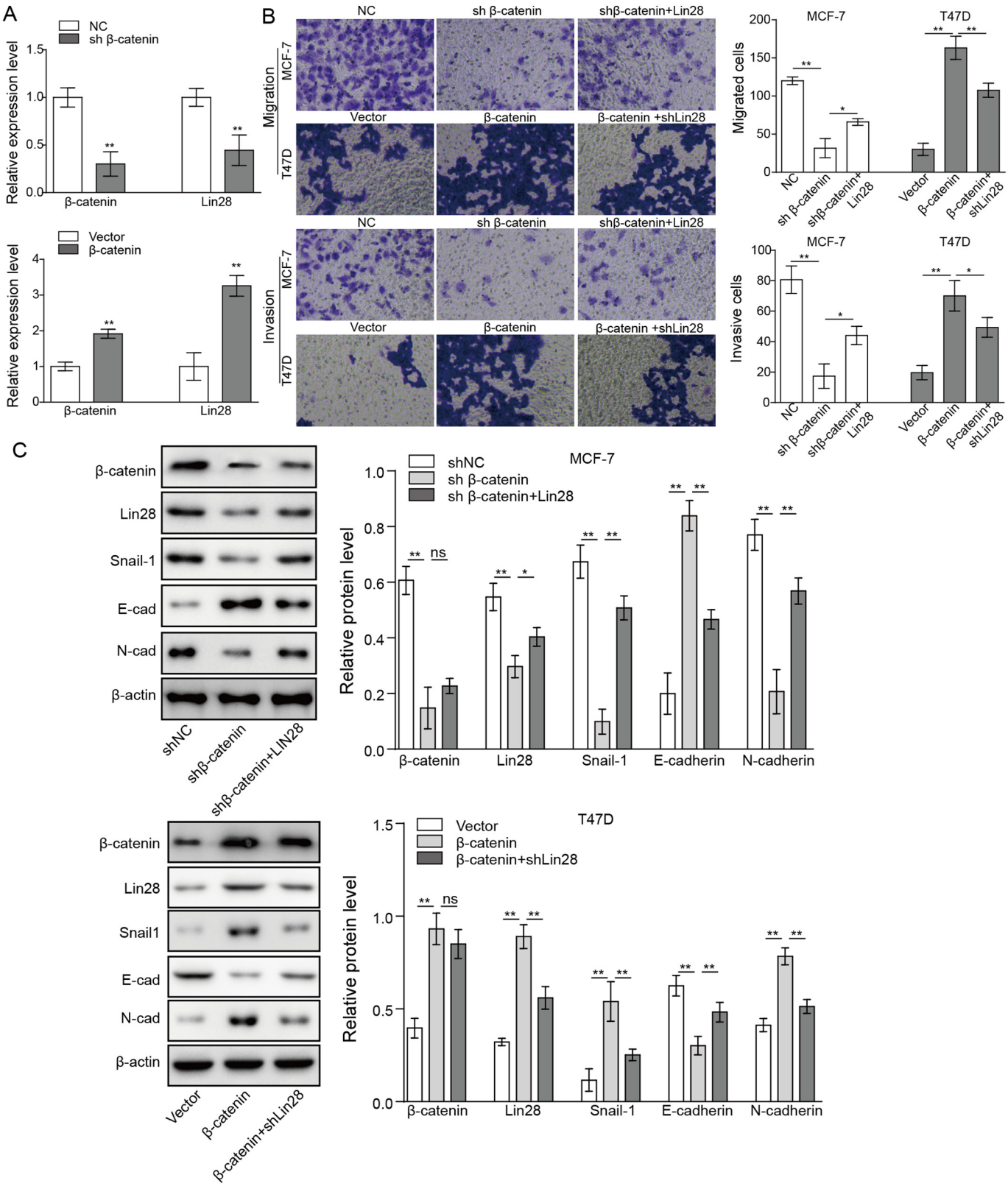

Fig. 5. Knockdown of β-catenin significantly inhibited EMT of breast cancer cells through down-regulating Lin28; while up-regulation of β-catenin significantly increased EMT of breast cancer cells through up-regulating Lin28. A. Expression of β-catenin and Lin28 in MCF-7 cells transfected with sh-β-catenin or sh-NC, or T47D cells transfected with pcDNA3.0-β-catenin or NC by qRT-PCR; B. Cell invasion and migration by transwell assay in MCF-7 cells transfected with sh-β-catenin, sh-β-catenin and pcDNA3.0-Lin28 or sh-NC, or T47D cells transfected with pcDNA3.0-β-catenin, pcDNA3.0-β-catenin and sh-Lin28 or NC; C. Expression of Numb, β-catenin, Lin28, N-cadherin, E-cadherin, and Snail-1 by both RT-qPCR and Western blotting, in MCF-7 cells transfected with sh-β-catenin or sh-NC, or T47D cells transfected with pcDNA3.0-β-catenin or NC. All experiments were conducted in triplicate. $*P < .05$, $**P < .01$, compared with the control.

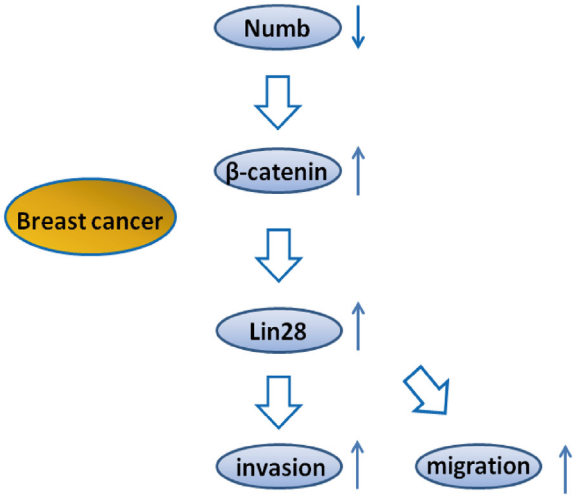

Fig. 6. The summary circuit of the study.

β-catenin through Lin28. This study could give deeper understanding for mechanisms of Numb in breast and provide some new research targets for breast cancer treatment.

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