

Original Research Article

LncRNA-NEAT1/miR-148a-3p axis regulates cell viability, apoptosis and autophagy through wnt/ β -catenin signaling pathway in Breast Cancer

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Abstract

Purpose: Breast cancer has over the years been one of major acute carcinomas in women. This study investigated the fundamental mechanistic functions of the LncRNA-NEAT1/miR-148a-3p/Wnt/ β -catenin axis in moderating cell viability, apoptosis and autophagy in Breast Cancer (BC).

Methods: RT-qPCR measured expression of LncRNA NEAT1 and microRNA-148a-3p in human cell lines for Breast Cancer. Cell transfection upregulated or silenced the genes with CCK-8, western blot and FCM apoptosis assays determining the cellular growth, proliferation and protein expression related to autophagy biomarkers. Furthermore, luciferase assay analyzed the luciferase activity of LncRNA-NEAT1 and microRNA-148a-3p

Results: The outcomes indicated that LncRNA-NEAT1 was upregulated in BC cell lines and promoted cell viability, autophagy and inhibited Apoptosis in BC cells. However, LncRNA-NEAT1 knockdown inhibited cell viability, autophagy and enhanced apoptosis. In addition, LncRNA-NEAT1 directly targeted microRNA-148a-3p. And, it was found that microRNA-148a-3p overturns the cellular viability, autophagy and inhibitory effects on Apoptosis imposed by LncRNA-NEAT1 overexpression. Lastly, overexpressed LncRNA-NEAT1 activated the Wnt/ β -catenin regulatory network through sponging microRNA-148a-3p in BC cell lines.

Conclusion The present study showcased that LncRNA-NEAT1 could enhance tumor development in breast cancer via playing the role of molecular sponge to microRNA-148a-3p, and eventually hyper invigorating the Wnt/ β -catenin regulatory network.

Keywords: LncRNA-NEAT1; miR-148a-3p; Cellular Viability; Apoptosis; Autophagy; Breast Cancer

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INTRODUCTION

Breast Cancer (BC) is one of the malignant illness prevalent among women across the globe and is very fatal with an approximated 2.4 million

fresh incidences and 523 000 fatalities registered in 2015 [1]. In the USA, the American Cancer Society gave a summary of breast cancer statistics among women and it was approximated that by 2013 about 39,620 breast cancer deaths

were foreseen to happen, commonly among women of 40 years and older [2]. In China, a 5-year occurrence information shows that roughly 11 % of global BC takes place in China and that the prevalence has quickly become amplified than before over the past decades between 20-30% [3]. For instance, the China National Cancer Centre (2011) registered 248,620 fresh BC incidences among women, consistent with age-standardized occurrence rate at 29 for every 100,000 Chinese females. Recently, Ye *et al.*, established epidemiologic factors in BC and corresponding subtypes among Chinese females. In their study, they concluded that postmenopausal females had low risk of having BC but possessed non-luminal subtype, while females having an account of abortion had very high risk of having BC [4]. However, females having large breast density and Breast Imaging Reporting and Data System (BIRADS) scores of injuries were disposed to having advanced stage BC [4]. Furthermore, diagnosis, prognosis, prediction of therapy effectiveness and toxicity and reoccurrence have been hot subjects in recent years [5]. Thus, deaths related to BC have declined due to application of adjuvant chemotherapeutic and hormonal agents, employing biomarkers as a technique to help in diagnosing breast cancer, conducting prognosis, forecasting of therapeutic reaction, and disease examination throughout and post-therapy [5]. In addition, these diagnostic platforms and imaging approaches provide the base for conducting diagnostic examination viz; mammography, magnetic resonance imaging (MRI), positron-emission tomography (PET), Computed tomography (CT), and single-photon emission computed tomography (SPECT) that offer useful data related to BC cases [6]. Likewise, use of biochemical biomarkers for instance proteins, DNAs, mRNAs, and microRNAs has been widely accepted as novel diagnostic and treatment means such as radiation and chemo-therapy in BC patients [6].

Not long ago, scientists discovered the importance of utilizing long-non coding RNAs (lncRNAs) and miRNAs in diagnosis and treatment of breast cancer. Long noncoding RNAs (lncRNAs) are a class of non-encrypted RNAs comprising in excess of 200 nucleotides that act an essential role in numerous human cancers such as breast cancer [7], hepatoma [8], clear cell Renal cell carcinoma [9] and other cancers. In this study, we investigate the role of lncRNA-NEAT1/ miR-148a-3p in advancing breast cancer via several cellular functions such as viability, apoptosis and autophagy which still

remain unclear. A large body of evidence shows that lncRNA-NEAT1 has been involved in various studies related to breast cancer but the functions of lncRNA-NEAT1/ miR-148a-3p/wnt axis have never been investigated. For instance, lncRNA NEAT1 was identified to interact with miR-21/RRM2 axis in breast cancer and the axis was deemed as a novel biomarker in BC [10]. Again, the interplay between lncRNA H19/miR-675 versus lncRNA NEAT1/miR-204 axes was reported to be effective in breast cancer therapy [11]. In addition, silencing of lncRNA NEAT1 was discovered to repress colorectal cancer via regulation of microRNA miR-193a-3p/KRAS axis [12]. The migration and invasion of breast cancer cells was mediated and enhanced by miR-133b being sponged and silenced by lncRNA NEAT1 [11]. It has also been reported that NEAT1 was involved to have negatively regulated miR-218 expression [13], facilitated cellular development and invasiveness through the miR-211/HMG2A2 network [14] and promoted cellular proliferation and EMT in BC progression [15]. In this context the NEAT1 has been explored to enforce oncogenic functions in breast cancer.

However, miR-148a-3p has been reported to exhibit tumor suppressive roles in breast cancer. For example, miR-148a-3p was found to interact with Melatonin as therapeutic approach to Angiogenic aspects in breast cancer [16]. Several other functions of miR-148a-3p in breast cancer have reported in Refs. [17-21]. However, the interaction between lncRNA NEAT1 and miR-148a-3p and their mechanistic roles in breast cancer progression are yet to be known. Additionally, the Wnt/ β -catenin regulatory is a group of proteins involved several crucial cellular roles including stem cellular restoration and organ development. Numerous internal cell signal transduction networks are stimulated via the Wnt, particularly the Wnt/ β -catenin dependent regulatory network [22]. The dysregulation and induction of the Wnt/ β -catenin regulatory cascade has been characterized by a number of human cancers [23]. In this study, the miR-148a-3p was earmarked as a suppressor of the Wnt/ β -catenin network and breast cancer while the lncRNA NEAT1 was identified as an upstream molecule for miR-148a-3p that promoted the activation of Wnt/ β -catenin pathway by sponging the microRNA to advance breast cancer. However, this aforementioned was presumed to be suitable for understanding the progression of breast cancer which could eventually provide therapeutic benefits in breast cancers as a novel axis of biomarkers.

EXPERIMENTAL

Cell culture and transfection

We utilized these human cells for Breast Cancer (MDA-MB-231, MDA-MB-436, and MDA-MB-468) including the preserved normal human breast cell line (MCF-10A) that were provided by the cell bank of the Chinese Academy of Medical Science (Shanghai, China). All cell lines were multiplied in adapted RPMI-1640 substrate, with addition of 10 percent fetal bovine serum (FBS) and 200 µg/L of penicillin and 200 µg/L of streptomycin (Beyotime, Shanghai, China). The cells were nurtured at 37°C in a saturated environment of 5 percent CO₂. The manufacturer's guideline to perform all cell culture procedures was strictly followed.

The cells were injected with Lipofectamine 2000 (Beyotime, Shanghai, China) according to the manufacturer's instructions. Transfection of MDA-MB-436, and MDA-MB-468 cells was performed utilizing Lipofectamine 3000 (Beyotime, Shanghai, China) following manufacturer's guidelines. The transfection effectiveness was measured via qRT-PCR. The 100 nM pcDNA3.1- lncRNA-NEAT1 and 100 nM control pcDNA3.1 vector including the 100 nM shRNAs (sh-lncRNA-NEAT1 and sh-control) were designed and synthesized by Guangzhou Fulengen Co., Ltd, following to the manufacturer's guidelines. The miRNA mimic and respective negative controls were also provided by Guangzhou Fulengen Co., Ltd. The experiments were conducted after 48 hours following the transfection.

Real Time-quantitative Polymerase Chain Reaction (RT-qPCR)

Entire RNA was removed from human breast cancer cell lines using Beyozol mixture (#R0011, Beyotime, Shanghai, China) then reverse transcription of 1 µg RNA was done for each specimen to cDNA using BeyoRT™ cDNA First Chain Synthesis Kit (#D7166, Beyotime, Shanghai, China) following guidelines provided by manufacturer. The qRT-PCR experiments were conducted by use of Beyofast™ SYBR Green QPCR Mix (Beyotime, China) and associated miRNA qRT-PCR detection kit (Beyotime, China) to observe the expression of lncRNA-NEAT1 and microRNA-148a-3p expression, respectively. GAPDH was normalized control and the 2^{-ΔΔCT} procedure was used to estimate the comparative expression level of lncRNA-NEAT1 and microRNA-148a-3p. The experiments were conducted in triplicates Table 1 presents

sequences of the primers. All primers were produced by Genelily BioTech Co., Ltd (Shanghai, China).

Table 1: Primer sequences

Name	Sequences
lcnRNA-NEAT1 (forward)	TAATATATCCCCAGTCTAAGGCAT
lncRNA-NEAT1 (reverse)	AGCTTGCAGATGGAGCCC
microRNA-148a-3p (forward)	GAGGAAGACAGCACGTTTGGT
microRNA-148a-3p (reverse)	AAAGGCGCAGCGACGT
GAPDH (forward)	AGAAGGCTGGGGCTCATTTG
GAPDH (reverse)	AGGGGCCATCCACAGTCTTC

CCK-8 assay

The transfected cells at a density of 4x10³ in each well were planted into 96 well multiplying dishes, and multiplied from zero to 48 hours. Cellular viability was determined by use of CCK-8 kit number C0037 (Beyotime), following standard protocol specified by manufacturer. The microplate data capturing device (Molecular Devices, Sunnyvale, USA) was employed so as to quantify wave length optical density at 450 nm.

Flow Cytometry assay

Flow cytometry assay was performed to examine the apoptosis rate. Human breast cancer cell lines MDA-MB-231 and MDA-MB-468 were transiently transfected with pcDNA3.1-lncRNA NEAT1, control pcDNA3.1 vector, siRNAs (si-lncRNA-NEAT1 and si-control) including microRNA-148a-3p mimic and respective negative controls for 24 hours. Then, the preserved breast cancer cell lines MDA-MB-231 and MDA-MB-468 were cultured utilizing annexin V/PI mixture (Beyotime, Shanghai, China) for 30 minutes at room temperature, without any light.

Western blotting assay

Western blot determined the proteins concentration in autophagy related biomarkers. The obtained BC cells were lysed in RIPA lysate (strong, #P0013B, Beyotime, Shanghai, China) with protease inhibitor mixture (universal, 100X, #P1005, Beyotime, Shanghai, China) to obtain total protein. The protein quantification was analyzed using a BeyoECL Star (Ultra-Sensitive ECL Chemical Luminescent Kit) (#P0018AS, Beyotime, Shanghai, China), adhering to the manufacturer's protocol at 4°C for 10 minutes. Parts of protein were isolated by 20% SDS-PAGE electrophoresis and were moved to

polyvinylidene fluoride (PVDF) membranes (Beyotime, Shanghai, China). Then, membranes were congested in 10% scan milk/TBST (BD Biosciences, #BYL40422, USA) and were incubated with suitable primary antibodies targeting microRNA-148a-3p (Beyotime, Shanghai, China), anti-LC3B (LC3B Rabbit Polyclonal Antibody, 1:2000, #AF5225, Beyotime, Shanghai, China), anti- β -Catenin (β -Catenin Rabbit Polyclonal Antibody, 1:1000, #AF0066, Beyotime, Shanghai, China), anti-c-myc (Myc Tag Mouse Monoclonal Antibody, 1:1000, #AF0033, Beyotime, Shanghai, China), anti-cyclin-D1 (Cyclin D1 Rabbit Polyclonal Antibody, 1:2000, #AF0126, Beyotime, Shanghai, China) and anti GAPDH (GAPDH Rabbit Monoclonal Antibody, 1:100, #AF1186, Beyotime, Shanghai, China) at 25 °C temperature for 3 hours. The films were nurtured with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Dallas, TX, USA). The protein indication was examined using the boosted chemiluminescence reagents kit (Beyotime). The image J detection system was employed to determine the concentration of the bands. GAPDH was normalized control. The analysis of each group was repeated in three-fold.

Bioinformatics analysis

The target connecting locations microRNA-148a-3p including lncRNA-NEAT1 were analyzed Starbase.

Luciferase reporter assay

The target sequence of lncRNA-NEAT1 and microRNA-148a-3p connecting locations were predicted and replicated into a pGL3 Dual-luciferase Target Vector (Promega, USA), to construct Wild Type and Mutant Type lncRNA-NEAT1 plasmids. These Wild Type or Mutant Type lncRNA-NEAT1 membranes were transfected together into MDA-MB-231 and MDA-MB-468 cell lines along with mimic-NC or microRNA-148a-3p mimic (Guangzhou Fulengen Co., Ltd, Guangzhou, China) using Lipofectamine 3000 following manufacturer's protocol. Following elapsing of 48hrs, we conducted a luciferase assay via the dual-luciferase reporter method (Promega, USA), following protocol specified by the manufacture.

Statistical analysis

The trials were conducted in three-fold and separately. The experimental data has been exhibited as average and standard error. The GraphPad Prism 5 software and SPSS 18.0

version analyzed the statistics of the study. Student's t-test and ANOVA analysis were applied. The level of significance was P-value less than 0.05 to show a difference in statistical significance.

RESULTS

lncRNA NEAT1 was upregulated in BC cell lines and promoted cell viability, autophagy and inhibited Apoptosis in BC cells

The RT-qPCR measured the expression of NEAT1 which was analyzed in three separate human breast cancer cell lines (MDA-MB-231, MDAMB-436, and MDA-MB-468) and a single normal breast epithelial cell line (MCF-10A). The outcomes indicated elevated expressions of NEAT1 in the human BC cell lines (MDA-MB-231, MDAMB-436, and MDA-MB-468) in contrast to the epithelial normal breast cell line which indicated very low expression (Figure 1A, $p < 0.5$). The increments in expression by NEAT1 in human BC cell lines were notably higher in the MDAMB-436, and MDA-MB-468 cell lines that were adopted for further experiments. Then, the lncRNA-NEAT1 was overexpressed by pcDNA3.1 and RT-qPCR measured the efficacy of the expression in both adopted cell lines (Figure 1B, $p < 0.5$). The results if Fig.1B showed that lncRNA-NEAT1 was upregulated in the pcDNA3.1 lncRNA-NEAT1 in contrast to the negative control. Then, cellular viability, apoptosis and autophagy were detected via CCK-8, FCM and western blot assays respectively in both MDAMB-436, and MDA-MB-468 cell lines. Thereafter, the outcomes indicated increased cellular viability in the NEAT1 overexpressed group in contrast to the control group (Figure 1C, $p < 0.5$). However, the apoptosis rate was decreased after NEAT1 was upregulated in comparison to the negative control (Fig.1D, $p < 0.5$). For autophagy, the western blot results indicated increased level of expression in related biomarkers (LC3B-I and LC3B-II) in both adopted cell lines for upregulated NEAT1 compared to negative control group (Figure 1E, $p < 0.5$). In general, these outcomes signify the influence of lncRNA-NEAT1 on cellular viability, apoptosis and autophagy when upregulated.

lncRNA NEAT1 knockdown inhibited cell viability, autophagy and enhanced apoptosis

In order to understand further the effect of low levels of NEAT1 on cell viability, autophagy and apoptosis, NEAT1 was silenced and the knockdown efficacy was determined by RT-qPCR (Figure 2A, $p < 0.5$). As shown in Figure 2A,

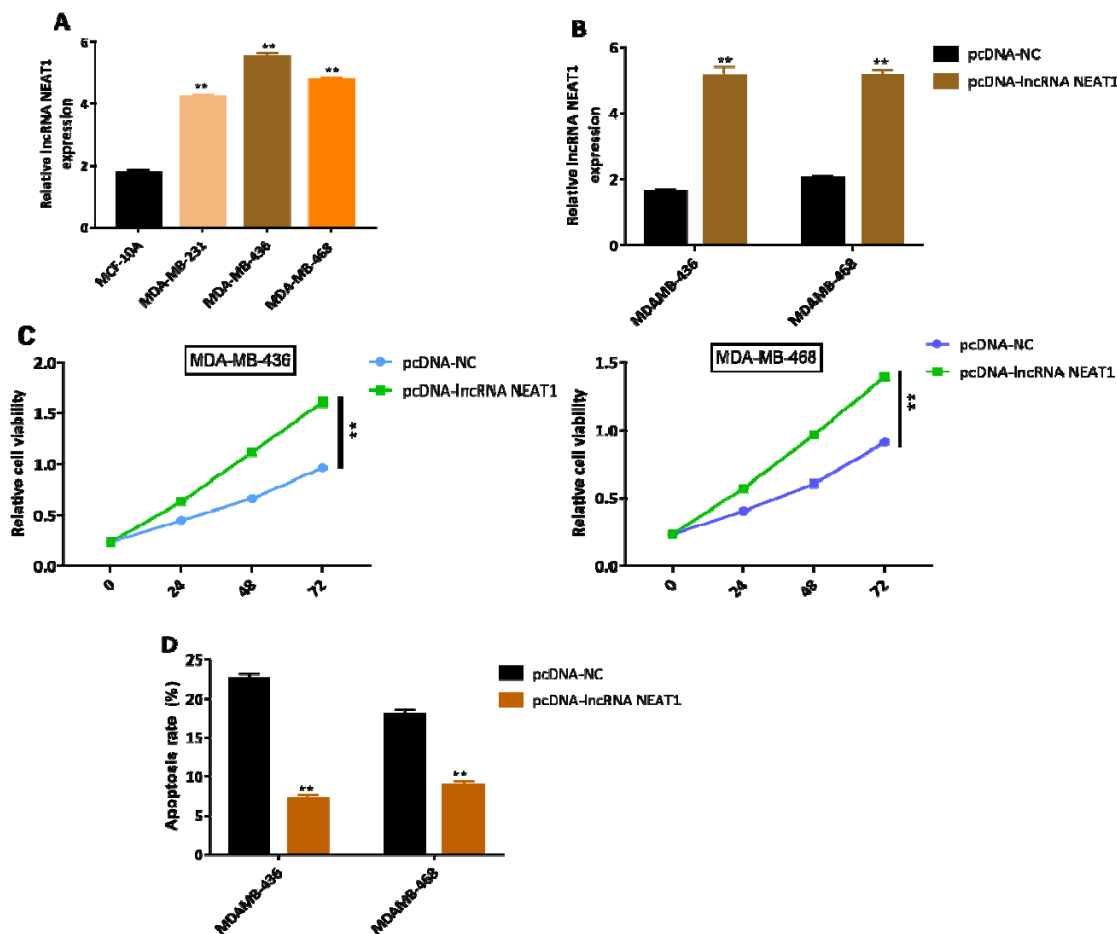


Figure 1: LncRNA NEAT1 was upregulated in BC cell lines and promoted cell viability, autophagy and inhibited Apoptosis in BC cells.

A) RT-qPCR assay measured lncRNA NEAT1 expression in three separate human Breast Cancer cell lines (MDA-MB-231, MDAMB-436, and MDA-MB-468) and a single normal breast epithelial cell line (MCF-10A) ($p < 0.05$). B) RT-qPCR detected overexpression efficacy of lncRNA NEAT1 in MDAMB-436 and MDA-MB-468 cell lines ($p < 0.05$). C) CCK-8 assay measure cellular viability of MDAMB-436 and MDA-MB-468 cells after lncRNA NEAT1-overexpression ($p < 0.05$). D) FCM assay measured apoptosis rate in MDAMB-436 and MDA-MB-468 cells after lncRNA NEAT1-overexpression ($p < 0.05$). All data were Mean and SD

the NEAT1 expression was remarkably reduced in the silenced group in comparison to the control group. Next, cellular viability, apoptosis and autophagy were detected via CCK-8, FCM and western blot assays respectively in both MDAMB-436, and MDA-MB-468 cell lines. The CCK-8 outcomes indicated remarkable decrease in cellular viability in the NEAT1 silenced group in contrast to the control group in both cell lines (Figure 2B, $p < 0.5$). Further, the apoptosis rate dramatically increased following NEAT1 knockdown compared with negative control in both cell lines (Figure 2C, $p < 0.5$). For autophagy, western blot outcomes indicated dramatic decline in the autophagy related biomarkers (LC3B-I and LC3B-II) for the silenced NEAT1 group contrasting to the negative control in both adopted cell lines (Figure 2D, $p < 0.5$). In general,

these outcomes imply that knocking down NEAT1 exhibits inhibitory effects on cellular viability and autophagy but enhances apoptosis.

LncRNA NEAT1 directly targets microRNA-148a-3p

To comprehend the relationship among lncRNA-NEAT1 and microRNA-148a-3p, we performed a prediction analysis via bioinformatics tool Starbase. It was predicted that these two genes have some binding locations in which microRNA-148a-3p was connecting with lncRNA-NEAT1 (Figure 3A). To further validate this interaction, the luciferase reporter assay was performed. First, we created and generated luciferase reporter for wild type lncRNA-NEAT1 (NEAT1-WT) or mutant type lncRNA-NEAT1 (NEAT1-

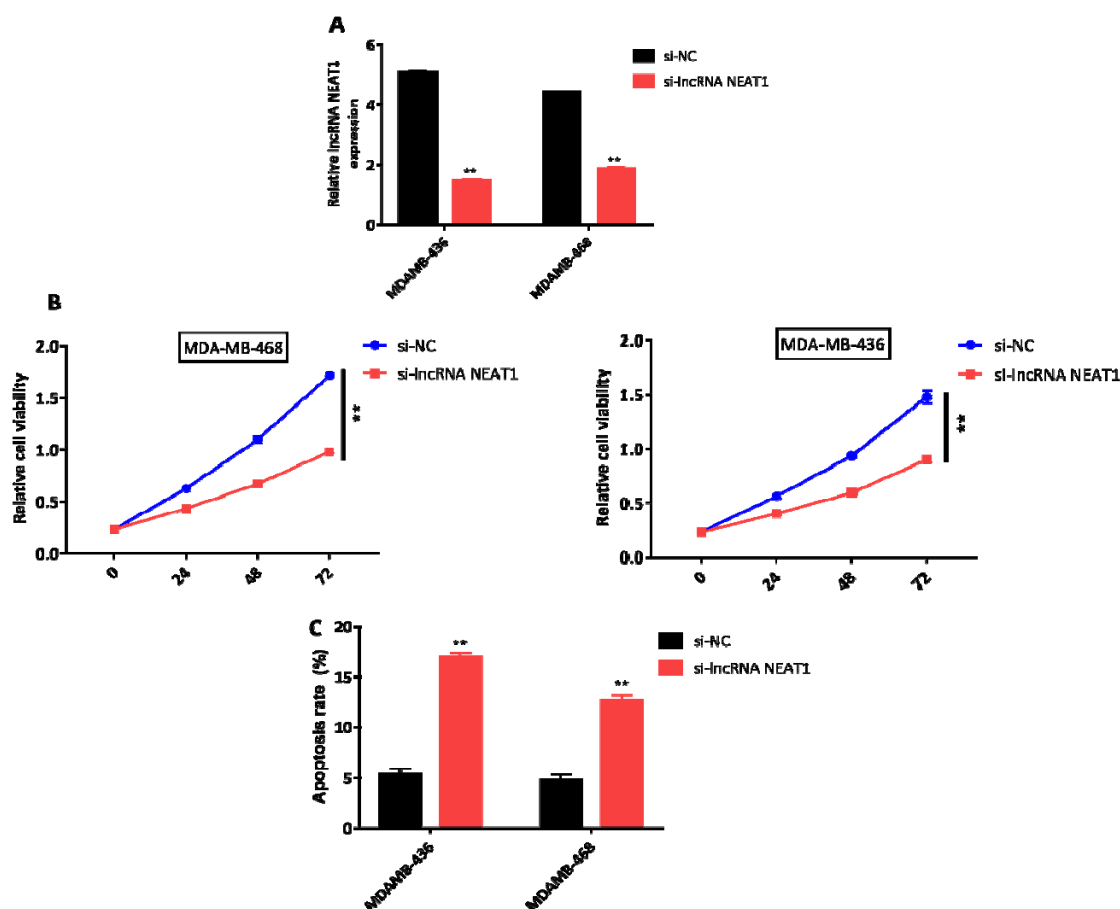


Figure 2: LncRNA NEAT1 knockdown inhibited cell viability, autophagy and enhanced apoptosis. A) RT-qPCR assay measured lncRNA NEAT1 knock down expression efficacy in human Breast Cancer cell lines (MDAMB-436 and MDA-MB-468) ($p < 0.05$). B) CCK-8 assay measure cellular viability of MDAMB-436 and MDA-MB-468 cells after lncRNA NEAT1-knock down ($p < 0.05$). C) FCM assay measured apoptosis rate in MDAMB-436 and MDA-MB-468 cells after lncRNA NEAT1-knock down ($p < 0.05$). All data were Mean and SD

MUT) followed by transfection with either negative controls or microRNA-148a-3p mimic in BC cell lines. The results revealed that silenced lncRNA-NEAT1 led to decreased luciferase activity in NEAT1-WT transfected with microRNA-148a-3p mimic compared with negative control in both BC cells (Figure 3B, $p < 0.05$). However, with no activity observed in NEAT1-MUT transfected with microRNA-148a-3p mimic compared with negative control in both BC cells (Figure 3C, $p < 0.05$). These data implied that lncRNA-NEAT1 might directly sponge microRNA-148a-3p in BC. Lastly, the expression of microRNA-148a-3p was detected via RT-qPCR in three separate human Breast Cancer cell lines (MDA-MB-231, MDAMB-436, and MDA-MB-468) and a single normal breast epithelial cell line (MCF-10A). The outcomes indicated significantly lowered expressions of microRNA-148a-3p in the human BC cell lines (MDA-MB-231, MDAMB-436, and MDA-MB-468) in contrast

to the epithelial normal breast cell line which indicated very high expression (Figure 3D, $p < 0.05$). The decrease in expression by microRNA-148a-3p in human BC cell lines were notably lower in the MDAMB-436 and MDA-MB-468 cell lines that were adopted for further experiments.

MicroRNA-148a-3p overturns the cellular viability, autophagy and inhibitory effects on Apoptosis by lncRNA-NEAT1

To further understand the interplay between lncRNA-NEAT and microRNA-148a-3p on how they influence cell viability, autophagy and apoptosis if they are interacting, we performed CCK-8, western blot and FCM assays respectively. Firstly, the BC cell lines were transfected with either pcDNA-NC, pcDNA-lncRNA-NEAT1+microRNA-148a-3p mimic or pcDNA-lncRNA-NEAT1 followed by RT-qPCR to

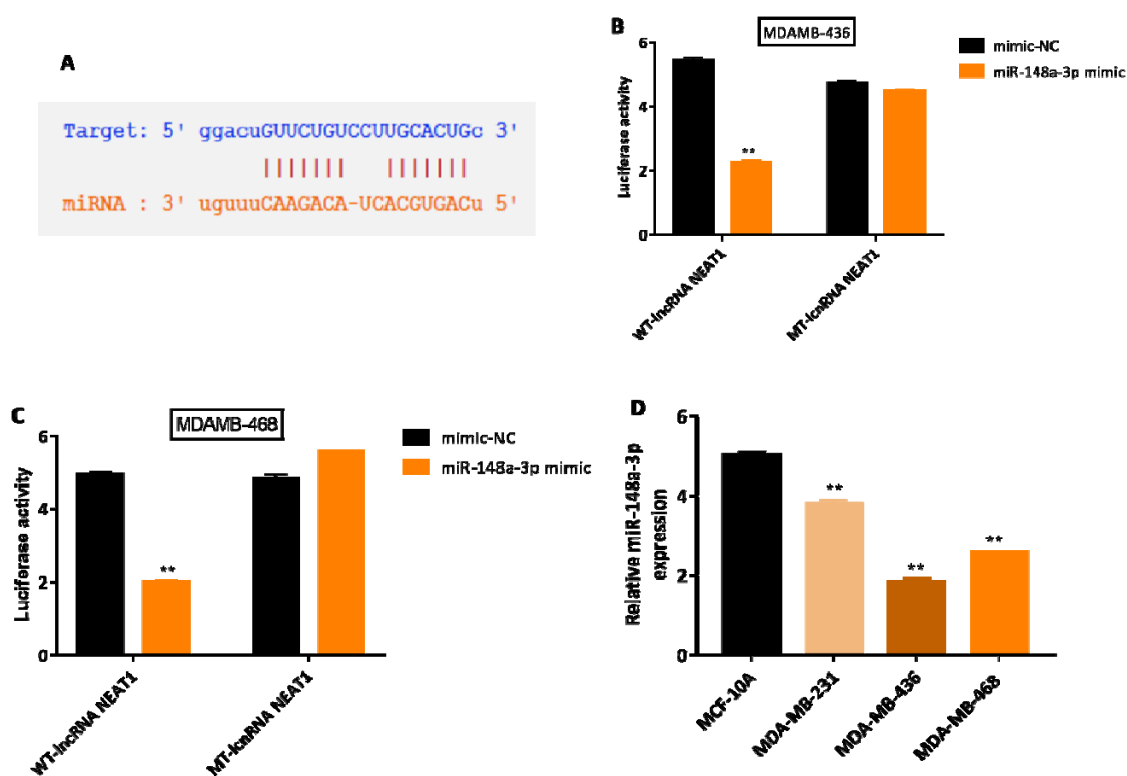


Figure 3: LncRNA NEAT1 directly targets microRNA-148a-3p

A) Bioinformatics tool Starbase predicted the connecting location of lncRNA-NEAT1 and microRNA-148a-3p. B) Luciferase reporter assay to measure luciferase activity in MDAMB-436 cell line ($p < 0.05$). C) Luciferase reporter assay to measure luciferase activity in MDAMB-468 cell line ($p < 0.05$). D) RT-qPCR assay measured microRNA-148a-3p expression in three separate human Breast Cancer cell lines (MDA-MB-231, MDAMB-436, and MDA-MB-468) and a single normal breast epithelial cell line (MCF-10A) ($p < 0.05$). All data were Mean and SD

evaluate expression efficiency of microRNA-148a-3p (Figure 4A, $p < 0.5$). As shown in the Figure, the results showed highest expression in the overexpressed group (pcDNA-lncRNA-NEAT1) compared to the lower expression in the control group (pcDNA-NC) in both cell lines. However, Figure 4A also shows that combined interplay between co-transfected overexpressed-lncRNA-NEAT1 with microRNA-148a-3p mimic led to a remarkable decline in expression contrasting to the overexpressed group (pcDNA-lncRNA-NEAT1) in both cell lines. Furthermore, the CCK-8 assay outcomes demonstrated dramatic decreased cell viability in the co-transfected overexpressed-lncRNA-NEAT1 with microRNA-148a-3p mimic compared to the lncRNA-NEAT1 overexpressed group and negative control group in both cell lines (Figure 4B, $p < 0.5$). In addition, the apoptosis rate was remarkably higher in the co-transfected overexpressed-lncRNA-NEAT1 with microRNA-148a-3p mimic compared to the lncRNA-NEAT1 overexpressed group and negative control group in both cell lines (Figure 4C, $p < 0.5$). For

autophagy, the western blot results indicated lower levels of LC3B-I and LC3B-II in the co-transfected overexpressed-lncRNA-NEAT1 with microRNA-148a-3p mimic compared to the lncRNA-NEAT1 overexpressed group and negative control group in both cell lines (Figure 4D, $p < 0.5$). Generally, these results suggest that microRNA-148a-3p reversed the cellular viability, autophagy and inhibitory effects on Apoptosis by lncRNA-NEAT1.

LncRNA NEAT1 activated the Wnt/ β -catenin regulatory network through sponging microRNA-148a-3p in BC cell lines

The Wnt/ β -catenin regulatory pathway is an essential moderator of tumor development and advancement. Unsurprisingly, the microRNA and protein quantities in various lower-level targets of the Wnt/ β -catenin regulatory network, as well as β -catenin, c-myc plus cyclinD1, were dramatically increased in the lncRNA NEAT1-overexpressed in both MDAMB-436 and MDA-

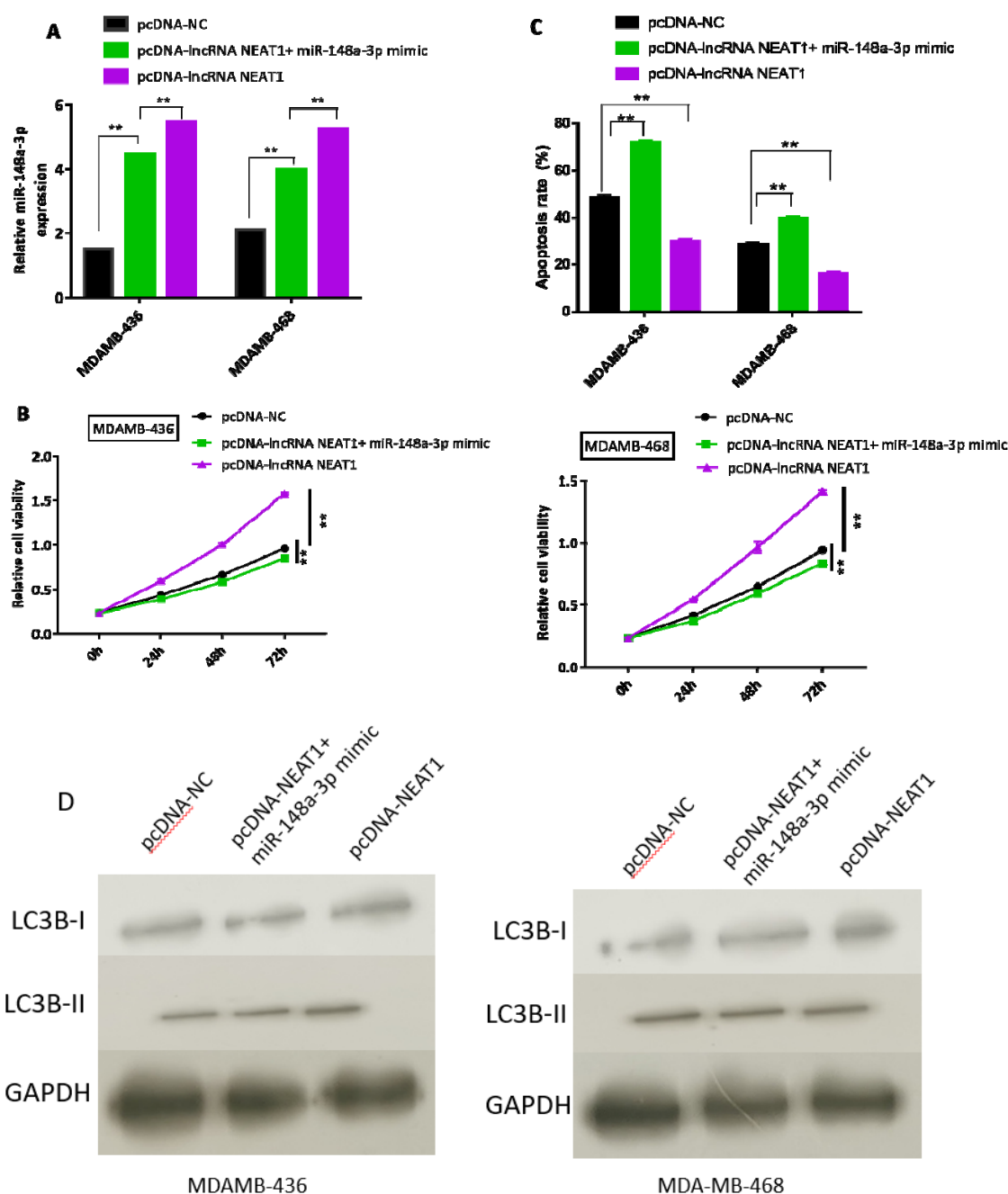


Figure 4: MicroRNA-148a-3p overturns the cellular viability, autophagy and inhibitory effects on Apoptosis by lncRNA-NEAT1.

A) RT-qPCR detected expression efficacy of microRNA-148a-3p in MDAMB-436 and MDA-MB-468 cell lines for either of these groups (pcDNA-NC, pcDNA-lncRNA NEAT1+ miR-148a-3p mimic or pcDNA-lncRNA NEAT1) ($p < 0.05$). B) CCK-8 assay measure cellular viability of microRNA-148a-3p expression in MDAMB-436 and MDA-MB-468 cell lines for either of these groups (pcDNA-NC, pcDNA-lncRNA NEAT1+ miR-148a-3p mimic or pcDNA-lncRNA NEAT1) ($p < 0.05$). C) FCM assay measured apoptosis rate of microRNA-148a-3p expression in MDAMB-436 and MDA-MB-468 cell lines for either of these groups (pcDNA-NC, pcDNA-lncRNA NEAT1+ miR-148a-3p mimic or pcDNA-lncRNA NEAT1) ($p < 0.05$). D) Western blot examined autophagy based on microRNA-148a-3p expression in MDAMB-436 and MDA-MB-468 cell lines for either of these groups (pcDNA-NC, pcDNA-lncRNA NEAT1+ miR-148a-3p mimic or pcDNA-lncRNA NEAT1) ($p < 0.05$). All data were Mean and SD

MB-468 BC cell lines and reduced in the negative control group in both MDAMB-436 and MDA-MB-468 BC cell lines. Nonetheless, co-transfecting overexpressed-lncRNA-NEAT1 with microRNA-148a-3p mimic group overturned the hyper invigorating of the Wnt/ β -catenin regulatory network in the overexpressed-lncRNA-NEAT1 transfected cells (Figure 5, $p < 0.05$). To sum up, these outcomes evidently proved that overexpressing lncRNA-NEAT1 hyper stimulated the Wnt/ β -catenin regulatory network via sponging the microRNA-148a-3p in breast cancer cells.

DISCUSSION

The breast cancer has been reported to be among the extremely belligerent melanomas resulting from numerous factors [24]. By means of speedy advances in molecular biotechnology, biochemical motivated treatment has been increasingly employed to aid in crucial therapy for breast cancer [25]. A large body of evidence suggests that dysregulated expression of lncRNAs is involved in several malignancies, and the lncRNA motivated target therapies have been applied as new detection, prediction and treatment tools in BC. For example other researchers have investigated the mechanistic roles of lncRNAs such as NKILA [26], DANCR [27], GIHCG [28], TTN-AS1 [29], LINC02273 [30] and DLEU1 [31] in breast cancer among others. To the best of the authors' knowledge, this study offers firsthand knowledge and information for the first time in relation to the novel mechanistic link among NEAT1, microRNA-148a-3p and the Wnt/ β -catenin regulatory network in human Breast Cancer. We discovered that lncRNA-NEAT1 expression was dramatically elevated in BC cell lines. In addition, upregulation of lncRNA-NEAT1 expression accelerated cellular viability, autophagy and prevented apoptosis while down-regulation of lncRNA-NEAT1 expression repressed, Breast Cancer cellular viability, autophagy and promoted apoptosis in vitro. Furthermore, it has been shown in this study that lncRNA-NEAT1 targeted microRNA-148a-3p which was lowly expressed in BC. And, the interaction among lncRNA NEAT1 and microRNA-148a-3p elucidated that this interplay results in dramatic reduces expression levels in lncRNA which in turn reverses the effects of cellular proliferation, autophagy enhancement and apoptosis inhibition as influenced by lncRNA NEAT1 overexpression. Moreover, the authors have demonstrated that overexpressed lncRNA-NEAT1 hyper invigorated the Wnt/ β -catenin regulatory network through acting as a direct sponge to microRNA-148a-3p. Jointly, these outcomes have showcased that lncRNA NEAT1

acts as malignant promoter or tumor oncogene in breast cancer and may perhaps be considered as an essential therapeutic target for BC in medical treatment.

The recognized Wnt/ β -catenin network is activated through the released Wnt ligands that connect with Frizzled (FZD) group conduit including LRP5/LRP6 co-receptor to incite the β -catenin network structure, eventually expedites the expression of c-myc and cyclin-D1 [32]. De-regulated invigoration of the Wnt/ β -catenin regulatory network is frequently discovered in several human melanomas and may possibly advance tumor growth with BC inclusive [33]. Thus, comprehending the fundamental mechanisms is essential for the advancement and perfection in cancer treatment. Cui *et al.* discovered that lncRNA Neat1 mediated microRNA-124-stimulated activation of Wnt/ β -catenin network to regulate neuronal differentiation, apoptosis and migration in spinal cord neural progenitor [34]. Thus, their study gave precedential illustrative fundamental molecular mechanistic functions of lncRNA and Wnt/ β -catenin network that could be regarded a potential treatment approach even for cancer. Nonetheless, so far, the upstream features of the Wnt/ β -catenin regulatory network together with the fundamental roles in the regulation of carcinogenesis and metastasis in breast cancer is yet to be clarified extensively. De-regulated lncRNAs' expressions play essential functions in initiation of cancer and subsequent advancement, and the controlling mechanisms of lncRNAs in carcinomas are far much appealing to the scientific community worldwide. Preceding papers have recognized the oncogenic role of lncRNA NEAT1 when it is overexpressed in several human cancers including Hepatocellular Carcinoma [35, 36]. Intriguingly, silenced lncRNA NEAT1 moderates autophagy to increase 5-FU reaction in colorectal cancer through targeting microRNA-34a [37]. In this investigation, it has been demonstrated through the outcomes that overexpressed lncRNA-NEAT1 might enhance cellular viability, autophagy and prevent apoptosis in breast cancer cells via invigorating the Wnt/ β -catenin regulatory network by sponging microRNA-148a-3p. Other studies have showcased that lncRNA-NEAT1 played the role of a competitive endogenous RNA to; microRNA-410 in bladder cancer [38], microRNA-214 in multiple myeloma [39] including microRNA-124 and microRNA-125a in acute ischemic stroke [40]. Some of the functional molecular roles in these studies indicated regulation of M2 macrophage polarization, enhancement of disease risk, poor recurrence-free survival of patients and

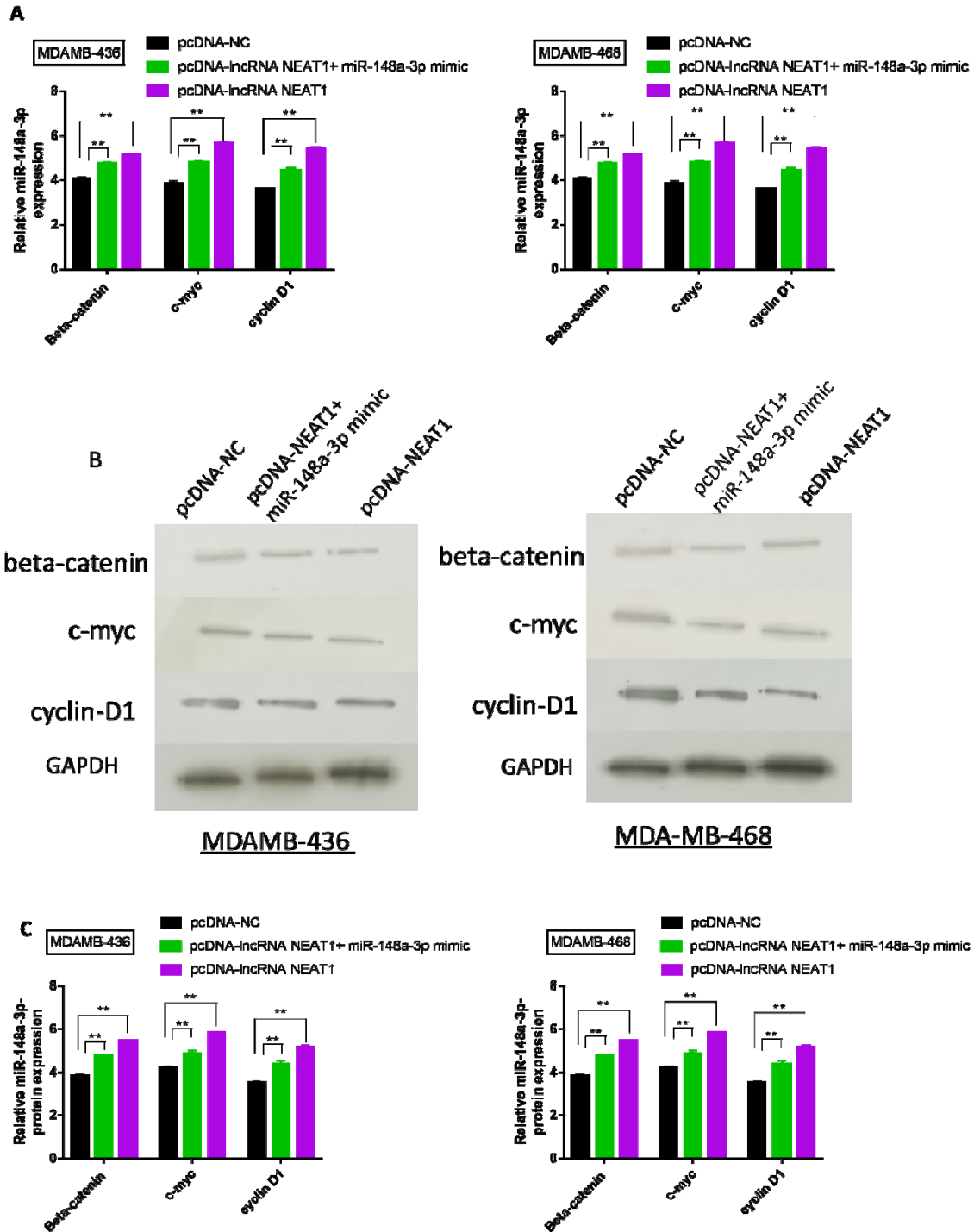


Figure 5: LncRNA NEAT1 activated the Wnt/ β -catenin regulatory network through sponging microRNA-148a-3p in BC cell lines

A) RT-qPCR detected relative expression of miRNA in beta-catenin, c-myc and cyclin-D1 in MDAMB-436 and MDA-MB-468 cell lines for either of these groups (pcDNA-NC, pcDNA-lncRNA NEAT1+ miR-148a-3p mimic or pcDNA-lncRNA NEAT1) ($p < 0.05$). B) Western blot assay measured relative miRNA protein expression in beta-catenin, c-myc and cyclin-D1 in MDAMB-436 and MDA-MB-468 cell lines for either of these groups (pcDNA-NC, pcDNA-lncRNA NEAT1+ miR-148a-3p mimic or pcDNA-lncRNA NEAT1) ($p < 0.05$). C) RT-qPCR detected protein expression of miRNA in beta-catenin, c-myc and cyclin-D1 in MDAMB-436 and MDA-MB-468 cell lines for either of these groups (pcDNA-NC, pcDNA-lncRNA NEAT1+ miR-148a-3p mimic or pcDNA-lncRNA NEAT1) ($p < 0.05$). All data were Mean and SD

development and progression of these diseases. Thus, direct target of lncRNA-NEAT1 was further explored in BC.

In this study the binding location of lncRNA – NEAT1 and microRNA-148a-3p were predicted by application of bioinformatics analytics using Starbase second version (2.0). Meanwhile, the outcomes from the experiments showed that overexpression of lncRNA-NEAT1 prevent the expression of microRNA-148a-3p while its silencing led it increased expression of microRNA-148a-p. In addition, microRNA-148a-3p expression co-transfected with overexpressed lncRNA-NEAT1 reversed proliferation effects and overturned enhanced autophagy coupled with improved apoptosis. Thus, we suggest that lncRNA-NEAT1 might directly sponge the microRNA-148a-3p and eventually activates the Wnt network in breast cancer. To this end, this paper has preliminarily elucidated the fundamental correlation among lncRNA-NEAT1 and microRNA-148a-3p. So far, the molecular mechanistic roles fundamental to the pathogenesis of breast cancer still remain vague.

CONCLUSION

The present study showcased that lncRNA-NEAT1 could enhance tumor development in breast cancer via playing the role of molecular sponge to microRNA-148a-3p, and eventually hyper invigorating the Wnt/ β -catenin regulatory network. These outcomes unearth a novel biomarker of the Wnt network hyper stimulation in Breast Cancer, and lncRNA NEAT1 could be a prospective treatment tool for BC therapy in the immediate time ahead.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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