Original Paper

LncROR Promotes Bladder Cancer Cell Proliferation, Migration, and Epithelial-Mesenchymal Transition

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Key Words

Bladder cancer • lncRNA ROR • Proliferation • Migration • EMT

Abstract

Background: lncRNA ROR, a tumor oncogene associated with various human cancers, has been reported to be involved in regulating various cellular processes, such as proliferation, apoptosis and invasion through targeting multiple genes. However, the molecular biological function in bladder cancer has not been clearly elucidated. The aim of this study is to explore ROR expression levels and evaluated its function in bladder cancer. **Methods:** lncRNA ROR expression levels in the 36 pairs of bladder cancer tissues (and corresponding non-tumor tissues) and bladder cancer cells were assessed by qRT-PCR. MTT assay, colony formation assay, flow cytometric analysis, wound healing assay, cell transwell assays, attachment/detachment and western blotting were performed to assess the effects of ROR on proliferation, apoptosis, migration/invasion and epithelial-to-mesenchymal (EMT) phenotypes in BC cells *in vitro*. ZEB1 is target of ROR. Rescue assays were performed to further confirm that ROR contributes to the progression of BC cells through targeting ZEB1. **Results:** lncRNA ROR was up-regulated in bladder cancer tissues (compared to adjacent non-tumor tissues) and was almost overexpression in bladder cancer cells (compared with normal urothelial cell line SVHUC-1 cells). Increased lncRNA ROR expression significantly promoted tumor cells proliferation, inhibited cells apoptosis, facilitated cells metastasis and contributed to the formation of EMT phenotype. While down-regulated ROR could obviously inhibit cells proliferation, promote cells apoptosis, inhibit metastasis and reverse EMT to MET. ZEB1 was a target gene of ROR and was positive correlation with the level of ROR in cancer tissues. **Conclusion:** These results indicated that lncRNA ROR was associated with tumor progression in bladder cancer cells.

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Introduction

Bladder cancer (BC) is one of the most common malignancies over the world, and its incidence and mortality have drawn great attention in the past decade [1]. Despite the development of treatments for patients with bladder cancer, the 5-year cancer-specific survival rate remains unsatisfied [2]. Lacking sophisticated understanding of pathogenetic mechanism is one of the most crucial reasons for high case fatality rate of bladder cancer. So, further revealing the pathogenetic mechanism of bladder cancer is indispensable for developing an effective diagnosis or treatment.

Long non-coding RNAs (lncRNAs), generally defined as transcripts longer than 200 nucleotides have captured significant attention in many biology processes [3-7]. Many lncRNAs, such as MALAT1, H19, and HOTAIR have been demonstrated as critical regulator of carcinogenesis and development of many cancers [8-12]. lncROR was first discovered in induced pluripotent stem cells (iPSCs). Loewer et al. identified lncRNA-ROR as a major regulator of pluripotency by examining lncRNA expression following fibroblast reprogramming into induced pluripotent stem cells (iPSCs) [13]. Recently, lncROR has been documented dysregulated in many cancers such as breast cancer, lung cancer, hepatocellular cancer, prostate cancer, gastric cancer and pancreatic cancer [14-23]. However, the function of lncROR in BC still remains unclear.

In the current study, we comprehensively investigated the biologic functions of lncROR in BC cells. Our results showed that lncROR was upregulated in BC tissues and investigated the effects of lncROR expression on bladder cancer cells *in vitro*. Moreover, further experiments indicated that the overexpression of lncROR could promote proliferation, inhibit apoptosis, facilitate migration and induce EMT of the bladder cancer cell lines, and such function was main dependent on targeting ZEB1.

Methods and Materials

Patients and clinical samples collection

BC tissues (n=36) and pair-matched noncancerous tissues were obtained through tissue biopsy from patients diagnosed with BC at the Department of Urology, Affiliated Hospital of Guilin Medical University between March 2011 and September 2015. Informed consent was obtained from patients.

Cell lines

Bladder cancer UMUC3, SW780, 5637, T24 and normal urothelial cell line SVHUC-1 cells used in this study were purchased from the Tumor Cell Bank of the Chinese Academy of Medical Science (Shanghai, China). The UMUC3, T24 and SV-HUC-1 cells were cultured in Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, CA, USA) plus 10 % fetal bovine serum and ampicillin and streptomycin at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. The 5637 cells and SW780 cells were cultured in RPMI-1640 Medium (Invitrogen, Carlsbad, CA, USA) plus 10 % fetal bovine serum and ampicillin and streptomycin at 37 °C in a humidified atmosphere of 95% air and 5% CO₂.

Real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNA from tissues and cells was isolated with Trizol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. Reverse transcription was performed with PrimeScript RT reagent Kit (Takara, Japan) according to the manufacturer's protocol. qRT-PCR was performed with SYBR Prime Script RT-PCR Kits (Takara, Japan) based on the manufacturer's instructions. The ROR and ZEB1 level was calculated with the $2^{-\Delta\Delta C_t}$ method, which was normalized to GAPDH mRNA, respectively. All assays were performed in triplicate. The expression levels were relative to the fold change of the corresponding controls, which were defined as 1.0.

Cell viability

Cell viability was assessed via 3-(4,5-dimethylthiazol-2-yl)-5-diphenyl-tetrazolium bromide (MTT) assay. 5×10^3 cells/well were seeded in a 96-well flat-bottomed plate for 24 h, then transfected

with corresponding sh-RNA or pc-DNA3.1 and cultured in normal medium. At 0, 24, 48, 72 h and 96h after transfection, the MTT solution (5 mg/ml, 20 μ l) was added to each well. Following incubation for 4 h, the media was removed and 100 μ l DMSO were added to each well. The relative number of surviving cells was assessed by measuring the optical density (O.D.) of cell lysates at 560 nm. All assays were performed in triplicate.

Colony formation assay

Cells (500 cells/ well) were plated in 6-well plates and incubated in RPMI 1640 with 10% FBS at 37 °C. Two weeks later, the cells were fixed and stained with 0.1% crystal violet. The number of visible colonies was counted manually.

Flow cytometric analysis of apoptosis

Apoptosis were performed using flow cytometric analyses with Annexin V: FITC Apoptosis Detection Kits (BD Biosciences, USA), according to the manufacturer's instructions. All samples were assayed in triplicate.

Cell migration and invasion assays

Cell migration and invasion were measured by transwell chamber (8 μ m pore size, Corning) and Matrigel invasion (Bection Dickinson), respectively. 48 h after transfection, cells in serum-free media were placed into the upper chamber without or with 10 μ g/ml Matrigel. Media containing 10% FBS was added into the lower chamber. Following 48 h incubation, cells remaining in upper membrane were wiped off, while cells that migrated were fixed in methanol, stained with 0.1% crystal violet and counted under a microscope. Three independent experiments were carried out.

Cell attachment and detachment assay

For attachment assay, cells were seeded in 24-well plates at 5×10^4 cells per well. Unattached cells were removed after 1 h incubation, and the attached cells were counted after trypsinization. The data were presented as a percentage of the attached cells compared to total cells. For cell detachment assay, after 24 h incubation, the cells were incubated with 0.05 % trypsin for 3 min to detach the cells. Then, the culture medium was added to inactivate the trypsin and the detached cells were collected. The remaining cells were incubated with 0.25 % trypsin to detach and counted. The data were presented as a percentage of the detached cells to total cells.

Cell transfection

PcDNA3.1/ROR (pcDNA3.1 as control), sh-ROR (shRNA as control) and pcDNA3.1/ZEB1 (pcDNA3.1 as control) were purchased from Applied Biological Materials (ABM, Canada). Transfections were performed using the Lipofectamine 2000 kit (Invitrogen) according to the manufacturer's instructions.

Western bolt analysis and antibodies

Total protein lysates were separated in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and were electrophoretically transferred to polyvinylidene difluoride membranes (Roche). Protein loading was estimated using mouse anti-GAPDH monoclonal antibody. The membranes were blotted with 10% non-fat milk in TBST for 2 h at room temperature, washed and then probed with the rabbit anti-human E-cadherin (1: 2000 dilution), β -catenin (1: 2000 dilution), N-cadherin (1: 2000 dilution), vimentin (1: 2000 dilution), ZEB1 (1: 2000 dilution), activated caspase-3 (1: 2000 dilution), total caspase-3 (1: 2000 dilution), activated caspase-9 (1: 2000 dilution), total caspase-9 (1: 2000 dilution), activated PARP (1: 2000 dilution), total PARP (1: 2000 dilution), and GAPDH (1: 3000 dilution), overnight at 4 °C, followed by treatment with secondary antibody conjugated to horseradish peroxidase for 2 h at room temperature. The proteins were detected using an enhanced chemiluminescence system and exposed to x-ray film. All antibodies were purchased from Abcam (USA).

Statistical analysis

Data are shown as the means $\pm$ standard error of at least three independent experiments. The SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Two group comparisons

were performed with a Student t test. Multiple group comparisons were analyzed with one-way ANOVA. Statistically significant positive correlation between ROR and ZEB1 expression levels in 36 cases of bladder cancer tissues was analyzed by Spearman's correlation analysis. Statistically significant positive correlation between ROR and ZEB1 expression levels in 36 cases of bladder cancer tissues was analyzed by Spearman's correlation analysis. All tests performed were two-sided. $P < 0.05$ was considered statistically significant.

Results

lncROR is up-regulated in bladder cancer tissues and cancer cells

To investigate the function of lncROR in BC, we first examined the expression of lncROR in 36 pairs of BC tissues and pair-matched histologically normal tissues by quantitative reverse-transcription polymerase chain reaction (qRT-PCR). Compared with matched noncancerous tissue (Fig. 1A), lncROR levels were increased in cancerous tissues ($p < 0.01$). Next, we examined the expression of lncROR in four BC cell lines UMUC3, SW780, 5637, T24 and normal urothelial cell line SVHUC-1 cells. As presented in Fig. 1B, obviously increased expression of lncROR was observed in 5637 cell lines, and the level of lncROR in UMUC3 was nearly as low as that in normal urothelial cell line SVHUC-1. These results indicated that lncROR might be involved in the progression of the BC.

Lower lnc-ROR expression correlates with poor prognosis in patients with BC

As shown in Table 1, lnc-ROR expression was found to be correlated with tumor range, metastasis, lymphatic Nodes and pathological stage ($P = 0.000, 0.000, 0.001$ and 0.000 , respectively). Proportional hazards method analysis revealed that low lnc-ROR expression ($P = 0.011$) was an adverse prognostic factor; in addition to the independent prognostic impact of Lymph Nodes (Table 2, $P = 0.030$). These results suggest that lnc-ROR plays a critical role in BC and may be considered as a specific biomarker of poor prognosis.

lncROR contribute to the proliferation of bladder cancer cells

To examine the biological functions of lncROR in BC cells, we overexpressed or down-regulated lncROR in UMUC3 or 5637 cells through transfected with lncROR expression vector pcDNA3.1/lncROR or lncROR-specific shRNA, respectively, using the empty vector or control shRNA as a negative control (NC). Satisfactory transfection efficiency was obtained at 48 h post-transfection (Fig. 2A). Results from MTT assays showed increased proliferation

Fig. 1. lncRNA ROR is up-regulated in bladder cancer tissues and cancer cells. A. Differences in ROR expression levels between bladder cancer tissues and pair-matched noncancerous tissues. The expression of ROR was normalized to that of GADPH. Statistical differences between samples were analyzed with paired samples t-test ($n = 36, p < 0.01$). B. Expression level of ROR in four BC cell lines UMUC3, SW780, 5637, T24 and normal urothelial cell line SVHUC-1 cells. Data are presented as mean $\pm$ standard error based on at least three independent experiments. * $p < 0.05$, ** $p < 0.01$.

Table 1. Correlation between LncROR Expression and Clinical Features. (n=36). Low/high by the sample median. Pearson χ^2 test. P<0.05 was considered statistically significant

Variable	Lnc-ROR Expression		P-value
	low	high	
Age			
<60	4	8	0.289
≥60	14	10	
Gender			
Male	16	15	1.000
Female	2	3	
Smoking			
No smoking	5	3	0.691
Smoking	13	15	
Tumor Range			
T1-T3	18	4	0.000
≥T4	0	14	
Metastasis			
negative	18	2	0.000
positive	0	16	
Lymph Nodes			
negative	11	1	0.001
positive	7	17	
Pathological Stage			
<IV	17	2	0.000
≥IV	1	16	

Table 2. Multivariate analysis of prognostic parameters in patients with bladder cancer by Cox regression analysis. Proportional hazards method analysis showed a positive, independent prognostic importance of LncROR expression (P = 0.011), in addition to the independent prognostic impact of Lymph Nodes (P = 0.030). P < 0.05 was considered statistically significant

Variable	Category	P-value
Age	<60	0.207
	≥60	
Gender	Male	0.741
	Female	
Smoking	No smoking	0.328
	Smoking	
Tumor Range	T1	0.449
	T2	
	T3	
	T4	
Metastasis	negative	0.884
	positive	
Lymph Nodes	negative	0.030*
	positive	
Pathological Stage	<IV	0.215
	≥IV	
Lnc-ROR expression	Low	0.011*
	High	

ability of UMUC3 cells transfected with LncROR compared with NC-transfected cells. On the contrary, weakened proliferation ability was observed in LncROR-downregulated 5637 cells compared with NC-transfected cells (p<0.01; Fig. 2B). Additionally, colony formation assays revealed higher proliferation of UMUC3 cells transfected with LncROR compared with NC-transfected cells (p<0.01; Fig. 2C). In contrast, suppression of proliferation was observed in LncROR-downregulated 5637 cells compared with NC-transfected cells (p<0.01; Fig. 2C). Furthermore, we used flow cytometry analyses to assess the effect of LncROR on apoptosis. Compared with negative controls, forced expression of LncROR caused a decrease in apoptosis in UMUC3 cells (p<0.01, Fig. 2D). Likewise, downregulation of LncROR resulted in increased apoptosis in 5637 cells compared with the NC group (Fig. 2D). Next, we measured the levels of apoptosis-related proteins (activated caspase-3, total caspase-3, activated caspase-9, total caspase-9, activated PARP and total PARP proteins). Overexpression of LncROR increased the levels of activated caspase-3, -9, and PRAP in UMUC3 cells while downregulation of LncROR in 5637 cells decreased it; however, total proteins levels remained unchanged (Fig. 2E). Therefore, LncROR might activate the caspase-3-dependent apoptosis pathway. Taken together, these data suggest that LncROR overexpression enhanced their proliferation while reducing apoptosis of BC cells.

LncROR promotes migratory and invasive capacities and enhances the epithelial-to-mesenchymal transition of bladder cancer cells

Metastasis/invasion is one of hallmarks of cancer. To investigate the function of ROR in regulating cell motility, we performed the migration /invasion assays and wound healing

Fig. 2. lncRNA ROR contribute to the proliferation of bladder cancer cells. A. qRT-PCR assay was performed to examine the expression of ROR 48 h in UMUC3 cells transfection with ROR expression vector pcDNA3.1/ROR (or control) and 5637 cells with ROR-specific shRNA (or shRNA control). B-C. MTT and colony formation assays on UMUC3 cells transfected with ROR, and 5637 cells transfected with sh-ROR. D. Flow cytometry of UMUC3 cells transfected with ROR, and 5637 cells transfected with sh-ROR. E. Western blot of apoptosis related proteins (activated caspase-3, total caspase-3, activated caspase-9, total caspase-9, activated PARP and total PARP). Error bars represent the mean $\pm$ SEM of at least three independent experiments. * $p < 0.05$, ** $p < 0.01$ vs. control group.

assays in BC cells. As is shown in Fig. 3A, overexpression of ROR enhanced the wound healing ability of UMUC3 cells, and deletion of ROR weakened the healing ability of 5637 cells. Additionally, the migration assay present that overexpression of ROR could facilitate the migration/invasion capacity of UMUC3 cells, and down-regulated ROR in 5637 cells could significant inhibit cells migration/invasion capability (Fig. 3B). Consistent with this, high-level of ROR promoted cell detachment and attachment in UMUC3 cells and down-expression of ROR inhibited cell detachment and attachment in 5637 cells (Fig. 3C). Furthermore, cell morphology and western blot assays revealed that high level of ROR in UMUC3 cells could induce formation of EMT and down-expression of ROR reverse the EMT to MET (Fig. 3D). These data together demonstrated that ROR was involved in the progression of BC cells.

ZEB1 was target of ROR and mediated the function of ROR in BC cells

It has been identified that ZEB1 was a target of ROR [16]. To detect whether ROR exerts its function in BC cells is dependent on ZEB1. We examined the level of ZEB1 in response to the level of ROR. As is shown in Fig. 4A and B, forced expression of ROR could increase the protein level of ZEB1 and depletion of ROR decreased the level of ZEB1 both in mRNA and protein level. Then, rescue assays were applied to further illuminate that the function of ROR was dependent on ZEB1. MTT assays and colony formation assays revealed that the proliferation ability of 5637 cells was rescued when co-transfected with sh-ROR and ZEB1 (Fig. 4C and D). Flow cytometry analyses showed that the pro-apoptosis function sh-ROR in

Fig. 3. lncRNA ROR facilitates the migration abilities. A-C., Wound healing, metastasis, and attachment/detachment assays were utilized to detect the migratory capability of BC cells transfected with ZEB1 or sh-ROR. D. Cell morphology and western blot of epithelial markers (E-cadherin, β-catenin) and mesenchymal markers (N-cadherin, vimentin). Error bars represent the mean ± SEM of at least three independent experiments. * $p < 0.05$, ** $p < 0.01$ vs. control group.

5637 cells was rescued when introduction of ZEB1 (Fig. 4E). Additionally, as shown in Fig. 5A-C wound healing assays, migration/invasion assays and attachment/detachment assays present that anti-metastasis function of sh-ROR could be partially rescued by introduction of ZEB1. Furthermore, ZEB1 could reverse the MET induced by sh-ROR to EMT (Fig. 5D). These data indicates that ROR exerts its function in BC cells is dependent on ZEB1.

ZEB1 was up-regulated in bladder cancer tissues and was positive correlation with the level of lncRNA ROR

To better investigate the correlations between ROR and ZEB1 in BC, we performed qRT-PCR to detect the expression of ZEB1 in 36 tumor tissue samples. The relative level of ZEB1 expression in the tumor tissue samples was significantly higher than that in pair-matched histologically normal tissues ($p < 0.01$, Fig. 6A). Moreover, there was a significant positive correlation between ZEB1 expression level and ROR expression level (2-tailed Spearman's correlation, $r = 0.713$, $p < 0.01$; Fig. 6B). Additionally, the level of lncROR in metastasis cancer tissues was higher than that in non-metastasis cancer tissues (Fig. 6C). Consistent, in metastasis cancer tissues, the expression of ZEB1 both in mRNA and protein level was higher than that in non-metastasis cancer tissues (Fig. 6D). Collectively, these findings indicated that lnc-ROR/ZEB1 signaling pathway was involved in the proliferation and metastasis of BC.

Discussion

Accumulating evidence has shown that ncRNAs play a pivotal role in tumorigenesis [24-26]. Currently, the participation of lncRNAs in a wide repertoire of biological processes has

Fig. 4. ZEB1 was target of ROR and contribute to the proliferation of BC cells. A-B. qRT-PCR and western blot assays were utilized to detect the level of ZEB1 in response to the level of ROR both in mRNA and protein level. C-D. MTT and colony formation assays on 5637 cells co-transfected with sh- ROR and ZEB1. E. Flow cytometry of 5637 cells co-transfected with sh-ROR and ZEB1. Error bars represent the mean $\pm$ SEM of at least three independent experiments. * $p < 0.05$, ** $p < 0.01$ vs. control group.

been a topic of intense research, and dysregulation of lncRNAs has indicated a vital function of these molecules in cancer pathogenesis and provided novel insights into therapeutics against cancers [27, 28]. Despite these advances, the roles of lncRNAs in the carcinogenesis of BC are far from fully elucidated. Investigating the molecular mechanism by which lncRNAs function in BC might facilitate the exploitation of novel therapeutic targets. Besides, EMT, a biological process in which cancer cells lose their epithelial polarity and undergo transition into a mesenchymal phenotype, plays a key role in cancer cell malignant transformation. The emergence of EMT contributes to the malignancy of BC. Therefore, investigating the mechanism underlying the generation of BC and the EMT phenotype in BC is especially important for the development of novel treatment methods and improving patient survival rate.

The long non-coding RNAs (lncRNAs) are RNA molecules over 200 nt in length with little protein-coding potential [29]. lncRNAs have been documented to regulate gene expression by a variety of mechanisms, for example, lncRNA H19 regulates glioma angiogenesis and the biological behavior of glioma-associated endothelial cells by targeting microRNA-29a, lncRNA ANRIL promotes the metastasis of thyroid cancer cells via regulating TGF- β /Smad signaling pathway, and lncRNA DUXAP8 promotes gastric cancer cell proliferation and migration via epigenetically silencing PLEKH01 expression [30-32].

LncROR was first discovered in induced pluripotent stem cells (iPSCs), where it was regulated by the key pluripotency factors Oct4, Sox2 and Nanog. LncROR had a key role in the maintenance of iPSCs and embryonic stem cells (ESCs) by preventing the activation of cellular stress pathways, including the p53 response [13]. Subsequent studies unveiled that

Fig. 5. lncRNA ROR facilitates the migration dependent on ZEB1 and induce EMT. A-C. Wound healing, metastasis and attachment/detachment assays were utilized to detect the migratory capability of BC cells co-transfected with sh-ROR and ZEB1. D. Western blot of epithelial markers (E-cadherin, β-catenin) and mesenchymal markers (N-cadherin, vimentin). Error bars represent the mean ± SEM of at least three independent experiments. * $p < 0.05$, ** $p < 0.01$ vs. control group.

lncROR could act as a p53 repressor in response to DNA damage [33]. Moreover, it has been demonstrated that lncROR may function as a molecular sponge for mir-145 to upregulate the mir-145 targets, such as OCT4, SOX2 and Nanog expression [34]. Despite the implications of lncROR in the regulation of a variety of transcription factors and physiological processes, the role of lncROR in BC metastasis and EMT remains unexplored. Our results confirmed the oncogenic role of lncROR in BC, and identified ZEB1 as potential target gene of lncROR. Overexpression of ZEB1 increased the migration and invasion capacity and reversed MET to EMT in 5637 cells transfected with sh-ROR, indicating that ZEB1 was a potential target gene of lncROR, mediated the function exert of lncROR in BC cells.

In conclusion, our study was the first to investigate the role of lncROR in BC. The results indicated that lncROR was a novel potential oncogene in BC, and was up-regulated in BC tissues. lncROR can promote proliferation, inhibit apoptosis, facilitate cell migration/invasion and induce EMT. ZEB1 was a potential target gene of lncROR, and mediated the function exert of lncROR in BC cells, implicating the potential application of ROR in the prognosis and treatment of BC.

Despite we revealed that the lncROR/ZEB1 signal pathway involves in regulating the progression of BC cells, the current research is still flawed. The main limitation of the present study is the exact regulatory mechanism between lncROR and ZEB1. Mechanisms of lncRNA regulating gene expression are mainly at three different levels: the transcriptional level, the post-transcriptional level and the epigenetic regulation level [35]. It has been reported that lncROR could function as competing endogenous RNA, therefore release the miRNA-

Fig. 6. ZEB1 was up-regulated in bladder cancer cells and was positive correlation with the level of lncRNA ROR. A. The expression of ZEB1 in 36 paired samples of bladder cancer tissues and pair-matched noncancerous tissues ($p < 0.01$), B. showing positive correlation between ZEB1 expression and ROR expression in 36 BC tissues (2-tailed Spearman's correlation, $r = 0.779$, $p < 0.01$). C. level of lncROR in metastasis cancer tissues was higher than that in non-metastasis cancer tissues. D. the expression of ZEB1 in metastasis cancer tissues and non-metastasis tissues (left), the expression level of ZEB1 proteins in three pairs of non-metastasis and metastasis tissues (right). * $p < 0.05$, ** $p < 0.01$ vs. control group

combined target genes [36-38]. Moreover, lncROR has also been documented as a necessary decoy oncoRNA that plays an important regulatory role in tumorigenesis and represents a novel style of histone modification [39]. In addition to these reported regulating mechanisms of lncROR, another regulating mechanism of most well-defined lncRNAs, such as *HOTAIR*, *lncRNA-p21*, *ANRIL*, and *MALAT-1*, sharing a common functionality in the formation of RNA-protein complexes with chromatin regulatory factors [40]. In the future, we will focus on the regulating mechanism between lncROR and ZEB1. We will first knockdown Dicer enzyme—key molecular in the process of miRNA maturation, to study whether miRNAs involves in lncROR-mediated function in BC cells. If knockdown Dicer enzyme, the function of lncROR vanishes or significantly decreases, we speculate that lncROR may function as a ceRNA in regulating ZEB1. If knockdown Dicer enzyme, the function of lncROR still exists, we will examine the methylation status of ZEB1 promoter or the relationship between lncROR and RNA-protein complexes with chromatin regulatory factors like PRC2 to study whether lncROR regulating ZEB1 through other pathways. In addition, in the future, we will also investigate the relationship of lncROR function and chemoresistant in BC cells, since chemotherapy is critical in treating BC patients.

In summary, the present study revealed that lncROR contributes to the progression of BC cells through targeting ZEB1. However, the regulatory mechanism between lncROR and ZEB1 remains uncovered. Therefore, in the future, we will further study the regulatory mechanism between lncROR and ZEB1. Additionally, we will also investigate the role of lncROR in regulating BC cells chemoresistant. Our ultimate goal is to provide a new train of thought for developing more efficient therapeutic agents.

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Disclosure Statement

The authors declare no conflict of interest

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