Tumor Biol. (2016) 37:12867–12875
DOI 10.1007/s13277-016-5210-z

ORIGINAL ARTICLE

Overexpression of LncRNA-ROR predicts a poor outcome in gallbladder cancer patients and promotes the tumor cells proliferation, migration, and invasion

Shou-Hua Wang[1] · Ming-Di Zhang[1] · Xiao-Cai Wu[1] · Ming-Zhe Weng[1] · Di Zhou[1] · Zhi-Wei Quan[1]

Received: 19 February 2016 / Accepted: 13 July 2016 / Published online: 23 July 2016

Abstract LncRNA-ROR has been reported to be involved in many kinds of human cancers. However, whether LncRNA-ROR is involved in gallbladder cancer progression remains largely unknown. The objective of this study is to investigate the role of LncRNA-ROR in gallbladder cancer. We found that LncRNA-ROR expression level was upregulated in gallbladder cancer tissues ($P < 0.05$) and was significantly associated with tumor sizes ($P < 0.05$) and lymph node metastasis ($P < 0.05$). High expression of LncRNA-ROR was significantly associated with poor prognosis in gallbladder cancer patients ($P < 0.05$). Moreover, knockdown of LncRNA-ROR inhibited cell proliferation, migration, and invasion. The epithelial-mesenchymal transition (EMT) phenotype induced by TGF-β1 was reversed after LncRNA-ROR knocking down in SGC-996 and Noz cells. LncRNA-ROR plays an important role in the development of gallbladder cancer and mediates the EMT in gallbladder cancer. LncRNA-ROR might act as a marker of prognosis and therapeutic target for gallbladder cancer.

Keywords Gallbladder cancer · LncRNA-ROR · Epithelial-mesenchymal transition · Tumor invasion

Introduction

Gallbladder carcinoma (GBC) is a highly malignant disease and rank as the fifth most common gastrointestinal malignancy worldwide [1]. Lack of reliable biomarkers for early diagnosis also contributed to poor prognosis [2, 3]. Therefore, early diagnostic markers and novel therapeutic targets are urgently needed to improve clinical management of gallbladder cancer.

Long non-coding RNAs (LncRNAs), are genomically transcribed noncoding transcripts longer than 200 nucleotides, which have been shown to play a significant role in cancer development and progression [4, 5]. In gallbladder cancer, some studies reported that long noncoding RNA HOTAIR [6], CCAT1 [7], and Malat1 [8] were associated with poor prognosis and promoted cancer progression in gallbladder cancer. LncRNA-ROR was discovered in induced pluripotent stem cells (iPSCs), where it was regulated by the key pluripotency factors Oct4, Sox2, and Nanog [9, 10]. A recent study has demonstrated that LncRNA-ROR may function as a molecular sponge for miR-145 to upregulate miR-145, and modulated the expression of OCT4, SOX2, and Nanog [9]. LncRNA-ROR was highly expressed in HCC, knockdown of LncRNA-ROR enhanced chemotherapy-induced apoptosis, and cytotoxicity [11]. Compared to normal mammary tissues, LncRNA-ROR was upregulated in breast tumor tissues. Ectopic overexpression of LncRNA-ROR in immortalized human mammary epithelial cells induced epithelial-to-mesenchymal transition (EMT) [12]. Until now, the biological function of LncRNA-ROR in GBC is unclear.

Epithelial to mesenchymal transition (EMT) is a process where the conversion of epithelial cells to a mesenchymal phenotype characterized by lost cellular polarity and adhesion, which were proved to enhance invasive and migratory properties [13, 14]. In the process of EMT, the epithelial markers (E-cadherin, occludin, claudin) are repressed and aberrant mesenchymal markers (including N-cadherin, Vimentin, and fibronectin) are upregulated [15, 16]. Deeper understanding

✉ Zhi-Wei Quan
Quanzhiwei3@163.com

[1] Department of General Surgery, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200000, China

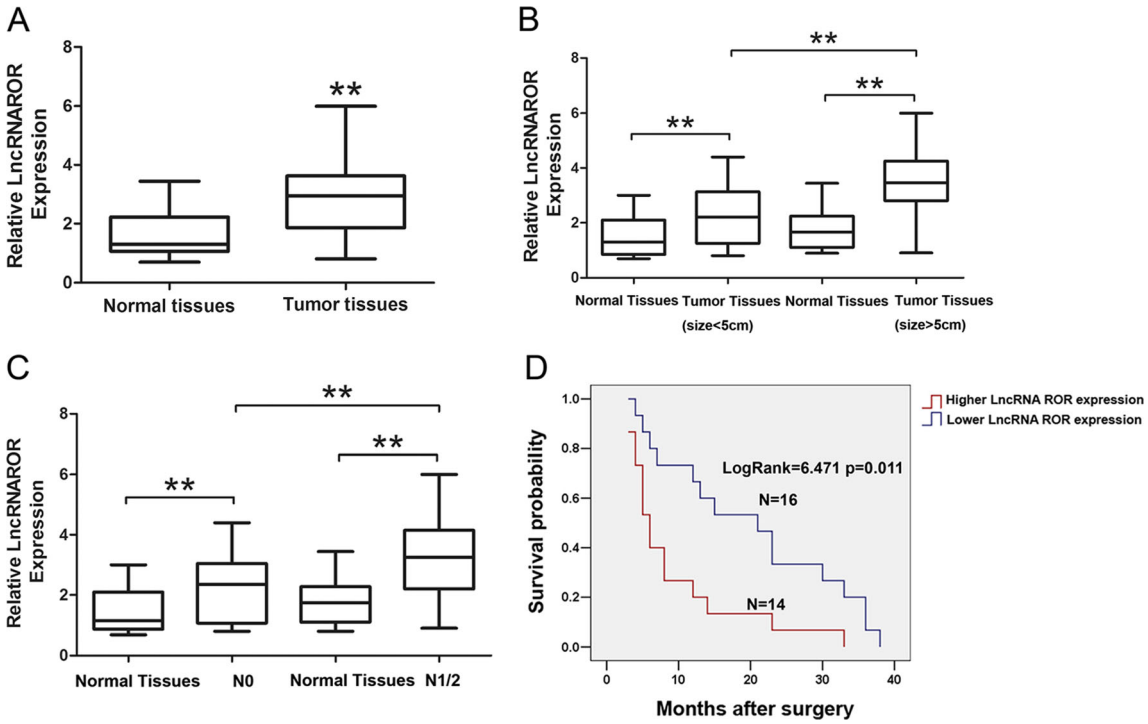

Fig. 1 Expression levels of LncRNA-ROR in GBC tissues. **a** LncRNA-ROR is significantly upregulated in gallbladder cancer tissues compared with matched normal tissues. LncRNA-ROR expression was examined by qRT-PCR and normalized to GAPDH expression. **b** LncRNA-ROR expression is significantly associated with tumor sizes (tumor size >5 cm vs. tumor sizes <5 cm, $P < 0.05$). **c** LncRNA-ROR expression is significantly associated with lymph node metastasis ($P < 0.05$). **d** The overall survival time is evaluated by Kaplan–Meier and log-rank test in gallbladder cancer patients. $^{**}P < 0.05$

Table 1 The clinical correction between expression of LncRNA-ROR and clinical characteristics in 30 cases gallbladder cancer patients

	LncRNA-ROR expression			
Clinical characteristics	Case number	Low	High	*P* value
Gender				0.338
Female	21	10	11	
Male	9	6	3	
Age				0.626
≤60	17	9	8	
>60	13	7	6	
Tumor size				0.012**
<5 cm	14	11	3	
>5 cm	16	5	11	
Histological grade				0.261
Well and moderately	14	9	5	
Poorly and others	16	7	9	
N status				0.001**
N0	16	13	3	
N1/2	14	3	11	
Clinical stage				0.206
I-II	12	8	4	
III-IV	18	8	10	

$^{**}P < 0.05$

on the molecular mechanisms of EMT shed new light on early detection.

We designed the present study to explore the role of LncRNA-ROR in GBC. We found that LncRNA-ROR expression level was upregulated in gallbladder cancer tissues and cells, and high expression of LncRNA-ROR indicated a poor prognosis in GBC patients. In vitro, we found that LncRNA-ROR was involved in cell proliferation, migration, and invasion. These results demonstrated that LncRNA-ROR might serve as a biomarker and a potential therapeutic target for gallbladder cancer.

Materials and methods

Patients and samples

Thirty cases of GBC tissue samples and matched adjacent normal gallbladder tissue samples were obtained from patients who underwent surgery between Jan 2009 and Dec 2011 in Eastern Hepatobiliary Surgery Hospital (Second Military Medical University, Shanghai, China) and Xinhua Hospital (Shanghai Jiao tong University School of Medicine, Shanghai, China). The fresh tissue specimens were snap-frozen in liquid nitrogen and then stored at −80 °C. None of the patients had preoperative treatment history. The

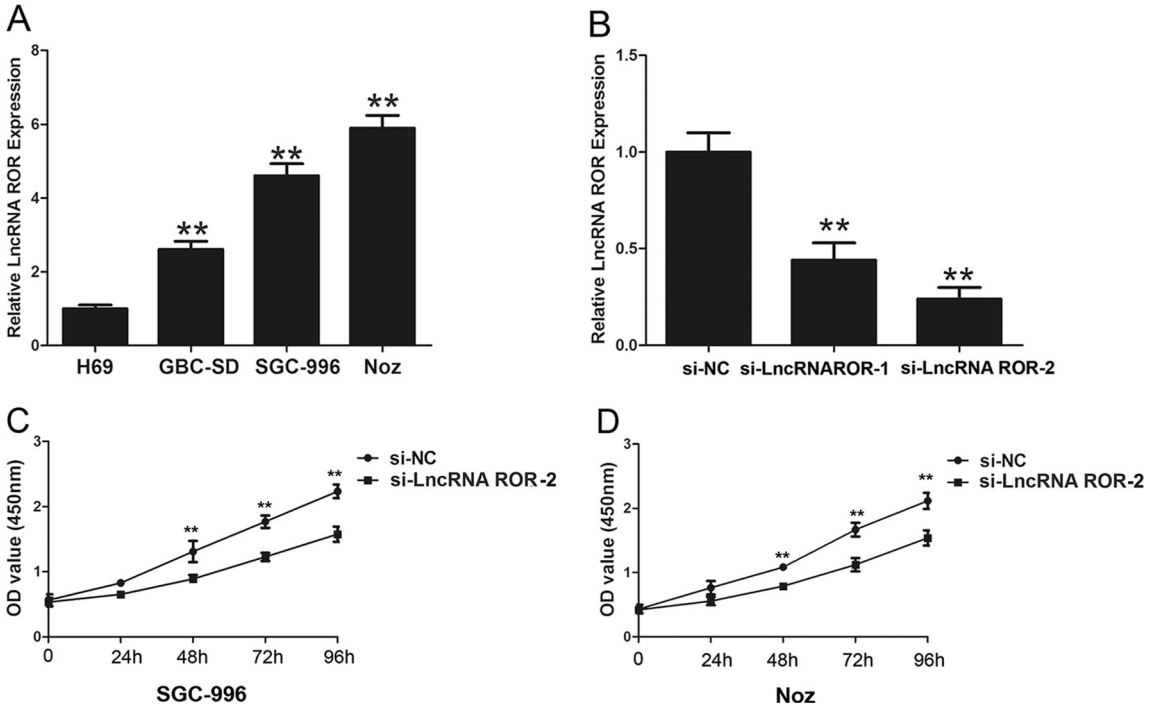

Fig. 2 Knockdown of LncRNA-ROR inhibits the cell proliferation in SGC-996 and Noz cells. **a** Expression levels of LncRNA-ROR in three GBC cell lines (SGC-996, GBC-SD, and Noz) and non-tumorigenic human intra-hepatic biliary epithelial cell line H69, data were normalized to GAPDH expression then presented as fold change vs. H19. **b** QRT-PCR analysis of the efficacy of LncRNA-ROR interference in NOZ cells. Si-NC was used as negative control. **c** After knockdown of LncRNA-ROR, cell growth was evaluated by CCK8 assay in SGC-996 cells. **d** After knockdown of LncRNA-ROR, cell growth was evaluated by CCK8 assay in Noz cells. *Error bars* represent the mean ± SD of three independent experiments, $^{**}P < 0.05$

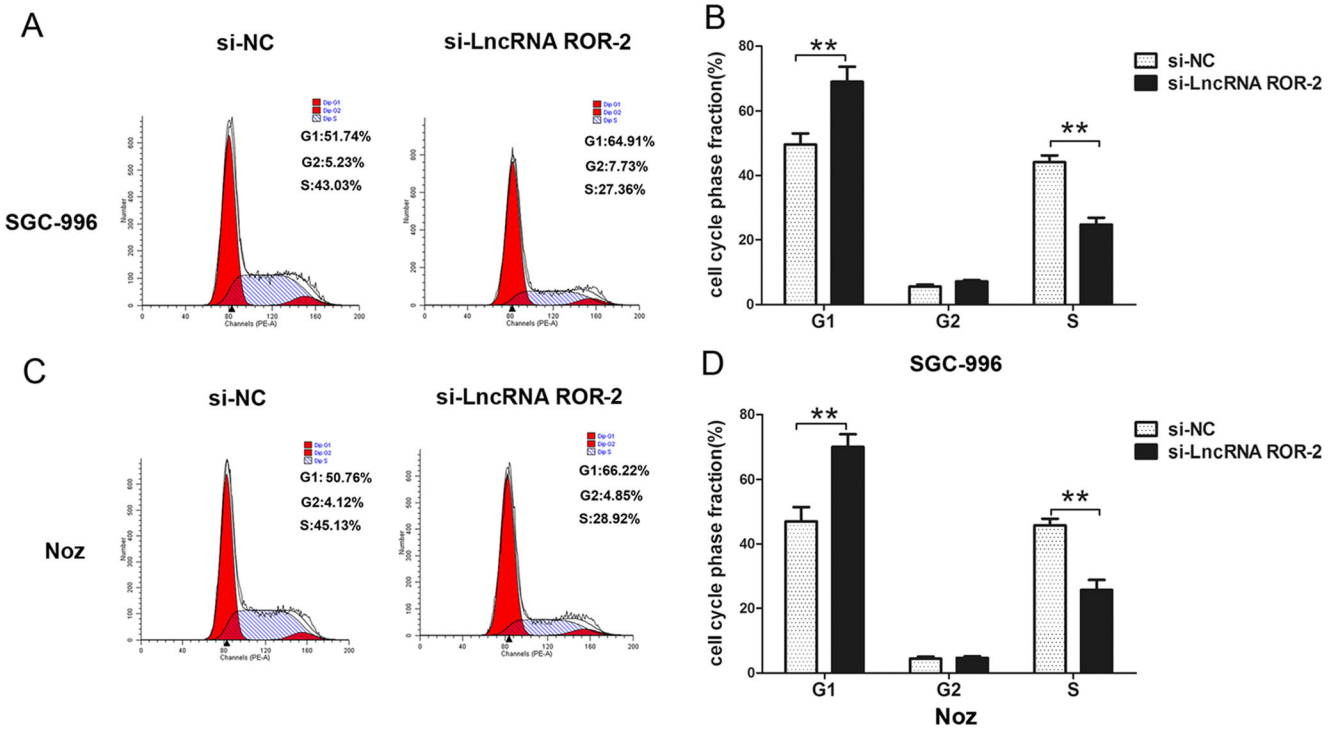

Fig. 3 Cell-cycle analyses after knockdown of LncRNA-ROR in SGC-996 and Noz cells. **a** Cell-cycle analyses after knockdown of LncRNA-ROR in SGC-996 cells. **b** Statistical analyses of cell-cycle analyses after knockdown of LncRNA-ROR in SGC-996 cells. **c** Cell-cycle analyses after knockdown of LncRNA-ROR in Noz cells. **d** Statistical analyses of cell-cycle analyses after knockdown of LncRNA-ROR in Noz cells. *Error bars* represent the mean ± SD of three independent experiments, $^{**}P < 0.05$

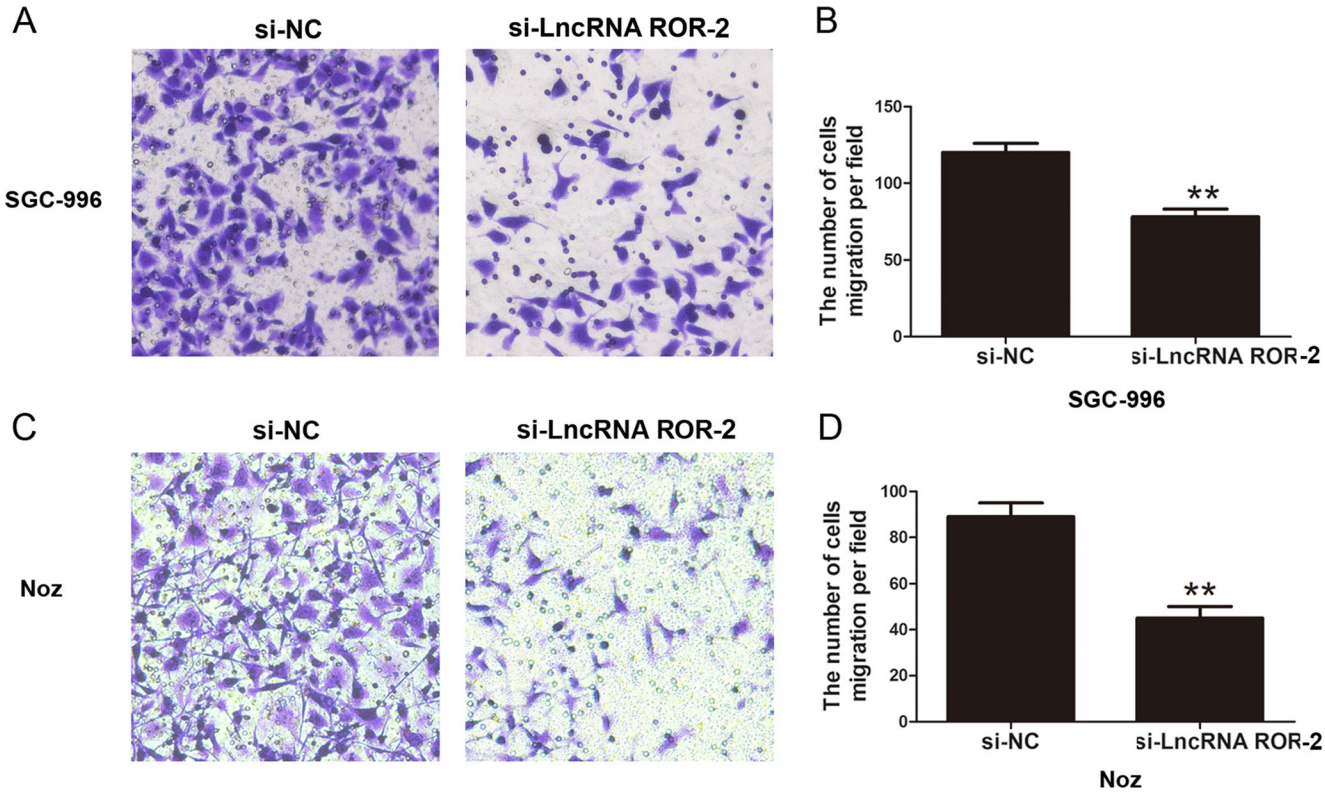

Fig. 4 Cell migration analyses after knockdown of LncRNA-ROR in SGC-996 and Noz cells. **a** After knockdown LncRNA-ROR, cells migration were evaluated by trans-well assay in SGC-996 cells. **b** Statistical analyses of cells migration after knockdown of LncRNA-ROR in SGC-996 cells. **c** After knockdown LncRNA-ROR, cells migration is evaluated by trans-well assay in Noz cells. **c** Statistical analyses of cells migration after knockdown of LncRNA-ROR in Noz cells. *Error bars* represent the mean ± SD of three independent experiments, $^{**}P < 0.05$

pathological stage was classified according to the tumor node metastasis (TNM) staging system (7th edition) of the American Joint Committee on Cancer (AJCC) staging system. Complete clinicopathological follow-up data of the GBC patients were collected. Informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Human Ethics Committee of Xinhua Hospital at Shanghai Jiao tong University (Shanghai, China).

Cell culture

Three human GBC cell lines (SGC-996, GBC-SD, Noz) were used in this study. GBC-SD and SGC-996 were purchased from Cell Bank of the Chinese Academy of Science (Shanghai, China). Noz was purchased from the Health Science Research Resources Bank (Osaka, Japan). The non-tumorigenic human intra-hepatic biliary epithelial cell line H69 was purchased from the Health Prescience Resources Bank. Cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10 % fetal bovine serum (Invitrogen), 100 U/ml penicillin and 100 U/ml streptomycin (Invitrogen) and were incubated at 37 °C with 5 % CO_2.

Cell growth assays

Cell growth was measured using CCK8 assay (Beyotime, Shanghai, China) in SGC-996 and Noz cells. In brief, cells were plated at 3×10^3 cells per well in 96-well plates with three wells for each condition. After treatment, 450 nm absorbance was measured every 24 h to assess cell viability.

Recombinant human TGF-β1 treatment

Recombinant human TGF-β1 was purchased from PeproTech Inc. (Rocky Hill, NJ). SGC-996 and Noz cells were cultured in plates to 70 % confluence. Medium were replaced with DMEM without serum, and then incubated for an additional 24 h to synchronized cells in a non-activating and non-proliferating phase. All serum-starved cells were incubated in DMEM supplement with TGF-β1 (20 ng/ml) for 72 h, cells were then harvested for further analyses.

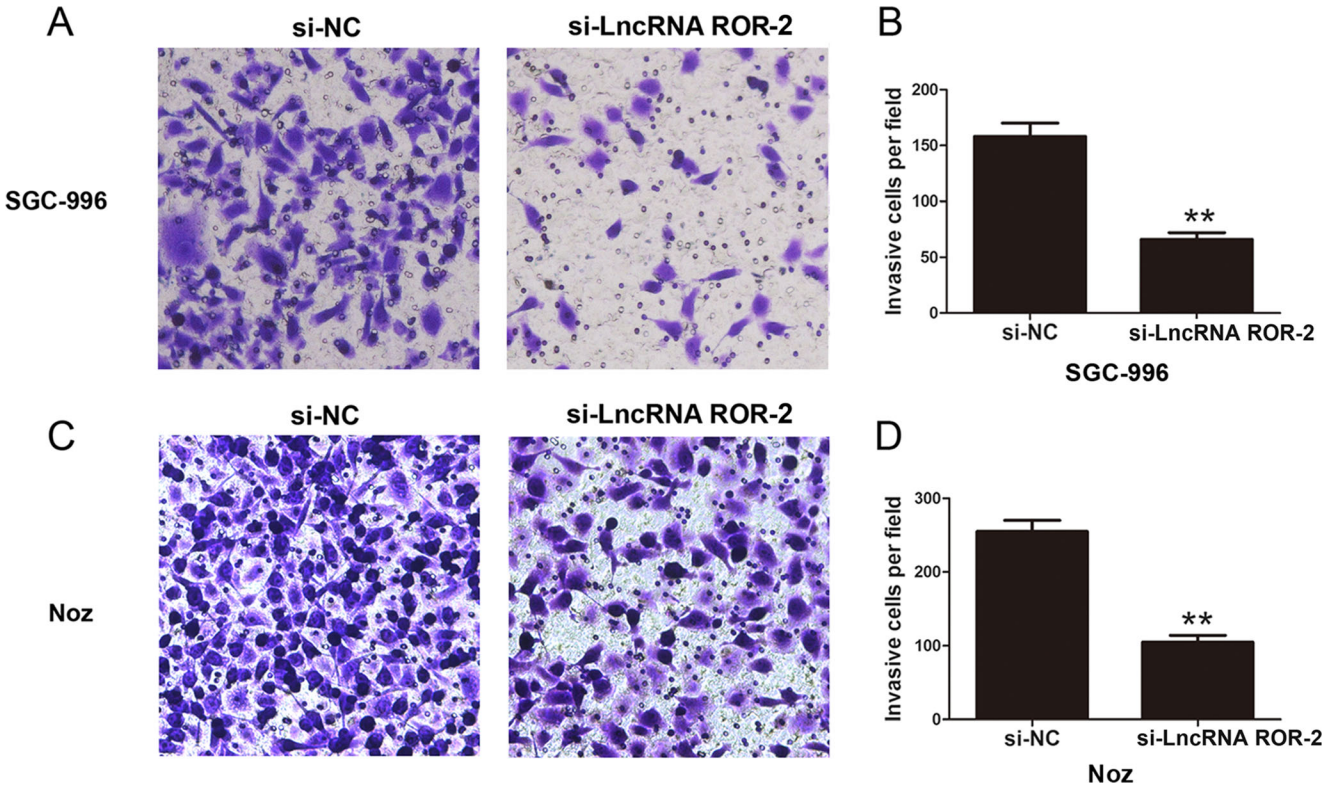

Fig. 5 Cells invasion analyses after knockdown of LncRNA-ROR in SGC-996 and Noz cells. **a** After knockdown LncRNA-ROR, cells invasion were evaluated by trans-well assay in SGC-996 cells. **b** Statistical analyses of cells invasion after knockdown of LncRNA-ROR in SGC-996 cells. **c** After knockdown LncRNA-ROR, cells invasion is evaluated by trans-well assay in Noz cells. **d** Statistical analyses of cells migration after knockdown of LncRNA-ROR in Noz cells. *Error bars* represent the mean ± SD of three independent experiments, $^{**}P < 0.05$

RNA extraction and real-time quantitative PCR (qRT-PCR)

Total RNA from tissue and cells was extracted using Trizol reagent (TaKaRa, Dalian, China). RNA was reverse transcribed into cDNA using the cDNA template according to the manufacturer's manual. Gene expression in each sample was normalized to GAPDH expression. The primer sequences used were as follows: for GAPDH-F, 5′-CGGAGTCAACGGATTTGGTCGTAT-3′, GAPDH-R, 5′-AGCCTTCTCCATGGTGGTGAAGAC-3′; LncRNA-ROR-F, 5′-TCCCTACTGTTCGTTCACCA-3′; LncRNA-ROR-R, 5′-CAGGTTTCCAGATGCGATTT-3′; Twist1-F, 5′-GGAGTCCGCAGTCTTACGAG-3′, Twist1-R, 5′-CCAGCTTGAGGGTCTGAATC-3′; E-cadherin-F, 5′-GACCGAGAGAGTTTCCCTACG-3′; E-cadherin-R, 5′-TCAGGCACCTGACCCTTGTA-3′; Vimentin-F, 5′-AGATGGCCCTTGACATTGAG-3′; Vimentin-R, 5′-CCAGAGGGAGTGAATCCAGA-3′. The reactions were incubated at 95 °C for 60 s, followed by 40 cycles (95 °C for 5 s plus 60 °C for 34 s). Real-time reactions were performed by the ABI7500 system (Applied Biosystems, Carlsbad, CA, USA). Real-time PCRs was performed in triplicate. The relative expression fold change of mRNAs was calculated by the $2^{-\Delta\Delta Ct}$ method.

Cell transfection

Two siRNA sequences for LncRNA-ROR specifically targeting LncRNA-ROR were synthesized by Shanghai Gene Pharma Co, Ltd., si-LncRNA-ROR-1, 5′-GGAAGCCTGAGAGTTGGCATGAAT-3′, si-LncRNA-ROR-2,5′-GGTTAAAGACACA-GGGGAA-3′,si-NC,sense,5′-UUCUUCGAACGUGUCACGUTT-3′,antisense,5′-ACGUGACACGUUCGGAGAATT-3′. Lipofectamine TM 2000 (Invitrogen, Carlsbad, CA, USA) was used for cell transfection according to the manufacturer's instructions.

Cell-cycle analysis

Cells were harvested and adjust to 1×10^5 cells per/sample for further analysis. Cells were washed in PBS and fixed in ice-cold 70 % ethanol overnight at 4 °C. Cells were then washed twice in PBS and incubated in 500 μL propidium iodide for 30 min in dark at 37 °C. Cell cycle was examined by a flow cytometer (FACSort; Becton). Cell cycle assay was performed three times independently. Data were expressed as percentage distribution of cells in G0/G1, S, and G2/M phases of the cell cycle.

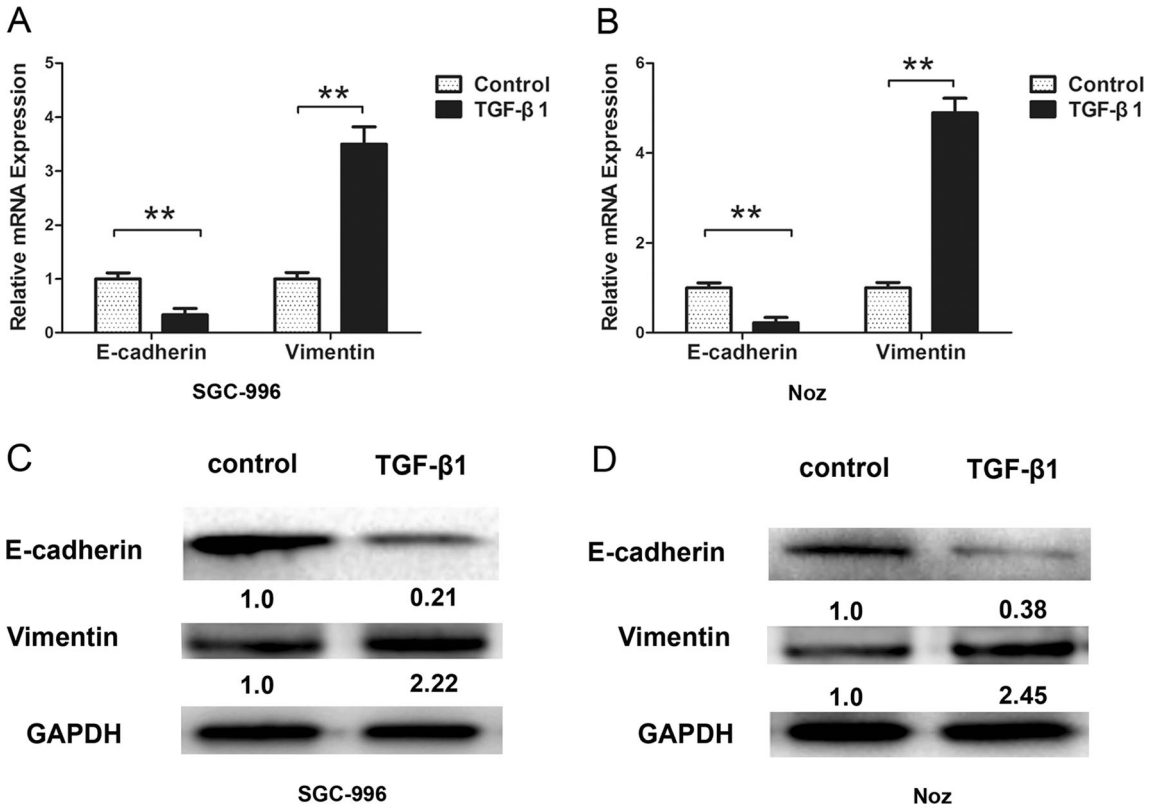

Fig. 6 Established an EMT model in GBC cells that induced by TGF-β1. **a** The RNA expression level of epithelial marker gene E-cadherin and mesenchymal marker gene Vimentin were evaluated by qPCR in SGC-996 cells after TGF-β1 treatment for 72 h. **b** The RNA expression level of E-cadherin and Vimentin were evaluated by qPCR in Noz cells after TGF-β1 treatment for 72 h. **c** The protein expression level of E-cadherin and Vimentin were evaluated by qPCR in SGC-996 cells after TGF-β1 treatment for 72 h. **d** The protein expression level of E-cadherin and Vimentin were evaluated by qPCR in Noz cells after TGF-β1 treatment for 72 h. *Error bars* represent the mean ± SD of three independent experiments, $^{**}P < 0.05$

Trans-well migration and invasion assay

Cell migration and invasion was performed by trans-well migration and invasive assay and used without and with the Matrigel-coated (BD, Franklin Lakes, NJ, USA) filters in 24-well plates according to the manufacturer's instruction. And 1×10^5 cells/well had been transfected to seed onto the upper chambers of the trans-wells in serum-free DMEM medium for an invasion assay. DMEM medium including 10 % fetal bovine serum was added to the lower chambers. The plates were incubated at 37 °C with 5 % CO_2 for 24 h, cells were viewed and photographed under a phase-contrast microscope (Olympus, Tokyo, Japan) and counted in 10 randomly microscopic fields.

Protein extraction and western blot analysis

Cells were washed and then lysed in RIPA buffer containing fresh protease and phosphatase inhibitor cocktails (Sigma) incubated at 4 °C for 10 min. Equal quantities of protein were electrophoresed through a 10 % SDS/polyacrylamide gel and transferred to a PVDF membrane (Millipore, Billerica, MA). The membranes were incubated with antibody against GAPDH, (1:1500, Cell Signaling Technology), Twist1 (1:2000, Abcam), E-cadherin, Vimentin (both 1:600, Santa Cruz Biotechnology Inc) and incubated at 4 °C overnight on a shaker. Blots were incubated in Horseradish peroxidase (HRP)-conjugated secondary antibody (1:2000, Abcam) at room temperature for 1.5 h. The protein concentration was determined using the Bio-Rad assay system (Bio-Rad, Hercules, CA, USA). Blots were developed using enhanced chemiluminescence detection reagents and scanned with a Molecular Imager system (Bio-Rad).

Statistical analysis

Derived values were presented as the means ± SD. Comparisons between two groups were conducted using two-tail Student's *t* test with SPSS 13.0 software. Kaplan-Meier analysis and the log-rank test were used to assess the survival plots. $P < 0.05$ was considered to be significant.

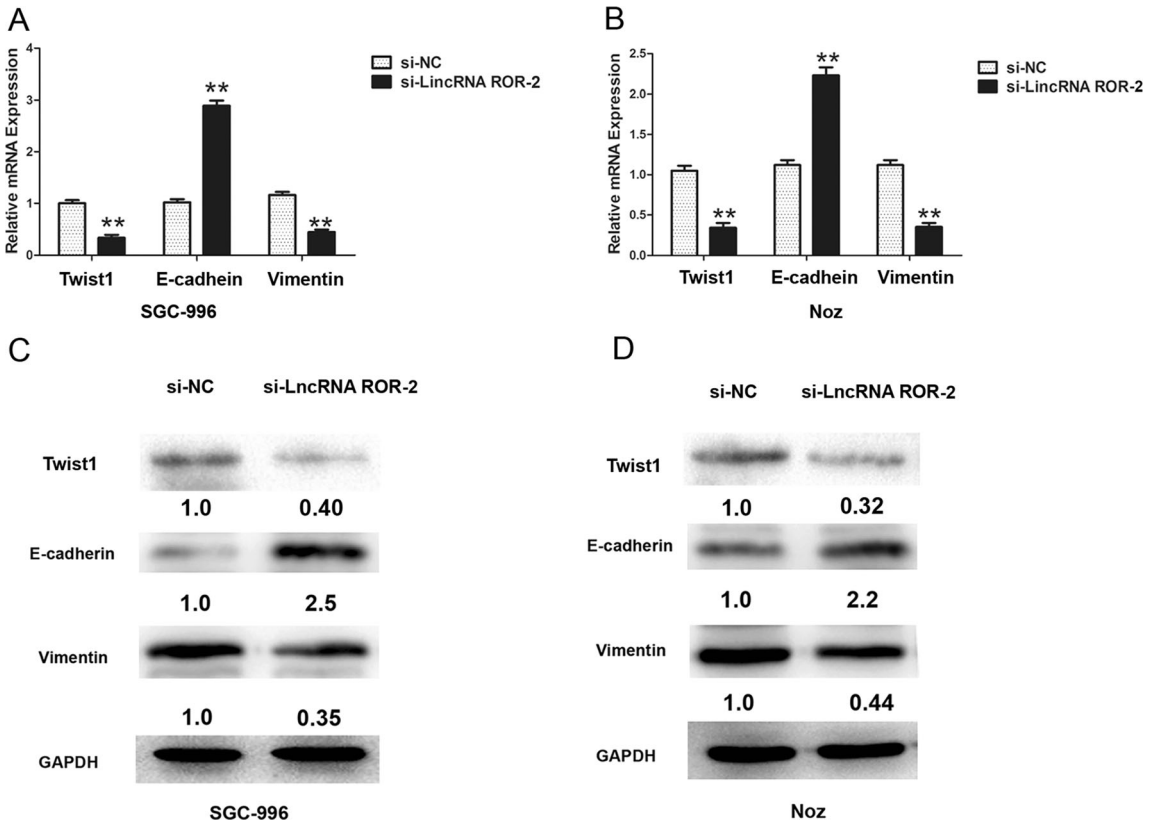

Fig. 7 Knockdown of LncRNA-ROR inhibits EMT phenotype in GBC cell's EMT model. **a** After knockdown of LncRNA-ROR, the RNA expression level of EMT related transcription factor Twist1, epithelial marker gene E-cadherin and mesenchymal marker gene Vimentin were evaluated by qPCR in TGF-β1 induced EMT model of SGC-996 cells. **b** After knockdown of LncRNA-ROR, the RNA expression level of Twist1, E-cadherin and Vimentin were evaluated by qPCR in TGF-β1 induced EMT model of Noz cells. **c** After knockdown of LncRNA-ROR, the protein expression level of Twist1, E-cadherin and Vimentin were evaluated by qPCR in TGF-β1 induced EMT model of SGC-996 cells. **d** After knockdown of LncRNA-ROR, the protein expression level of Twist1, E-cadherin, and Vimentin were evaluated by qPCR in TGF-β1 induced EMT model of Noz cells. *Error bars* represent the mean ± SD of three independent experiments, $^{**}P < 0.05$

Results

LncRNA-ROR is upregulated in gallbladder cancer tissues

We measured the expression level of LncRNA-ROR in 30 cases of gallbladder cancer tissues by qRT-PCR. Compared to matched normal tissues, LncRNA-ROR was significantly overexpressed in gallbladder cancer tissues ($n = 30$; $P < 0.01$; Fig. 1a). Furthermore, the expression level of LncRNA-ROR was significantly associated with tumor sizes ($P = 0.012$, Fig. 1b, Table 1) and lymph node metastasis ($P = 0.001$, Fig.1c, Table 1), but was not significantly associated with other characters, such as age, gender and so on (Table 1). According to median ratio of relative LncRNA-ROR expression (2.81) in the 30 cases of tissues, patients were classified into higher LncRNA-ROR group ($n = 14$, LncRNA-ROR expression ratio ≥ median ratio) and lower LncRNA-ROR group ($n = 16$; LncRNA-ROR expression ratio ≤ median ratio). Kaplan-Meier analysis and the log-rank test verified that higher expression of LncRNA-ROR had poorer overall survival time in gallbladder cancer patients (log-rank = 6.471, $P = 0.011$; Fig. 1d).

Knocking down LncRNA-ROR inhibits the proliferation in SGC-996 and Noz cells

We examined the expression levels of LncRNA-ROR in three GBC cell lines (SGC-996, GBC-SD, Noz) and the non-tumorigenic human intra-hepatic biliary epithelial cell line H69 (Fig. 2a). SGC-996 and Noz were used for loss-of-function studies for their high expression of LncRNA-ROR. Two siRNA-LncRNA-ROR were transfected to knocking down LncRNA-ROR, si-NC was used as negative control. LncRNA-ROR expression was effectively knocked down by si-LncRNA-ROR-1 (60 %) or si-LncRNA-ROR-2 (77 %) in Noz cell (Fig. 2b). The si-LncRNA-ROR-2 was used for the following experiments for its higher knockdown efficiency.

Then we evaluated the role of LncRNA-ROR in cell proliferation, migration and invasion in gallbladder cancer. LncRNA-ROR was silenced in SGC-996 and Noz cells.

CCK8 assay suggested that cell proliferation was inhibited by silencing LncRNA-ROR in SGC-996 and Noz cells (Fig. 2c and Fig. 2d). In addition, cell-cycle analyses suggested that the number of cells in S-phase was decreased significantly after LncRNA-ROR was knocked down, indicating inhibition of cell proliferation (Fig. 3a–d).

Silencing of LncRNA-ROR inhibits cell migration and invasion in SGC-996 or Noz cells

Trans-well assay was used to evaluate the migration and invasion ability of cells. After LncRNA-ROR knockdown, the number of cell migration was significantly reduced in SGC-996 and Noz cells (Fig. 4a–d). Next, the invasion phenotype was examined by trans-well assay. After LncRNA-ROR knockdown, the number of invasive cells was significantly decreased compared with control groups both with SGC-996 and Noz cells (Fig. 5a–d).

Silencing of LncRNA-ROR inhibits epithelial to mesenchymal transition (EMT) in SGC-996 and Noz cells

We established an in vitro EMT model in SGC-996 or Noz cells by a well-characterized EMT inducer transforming growth factor-β1 (TGF-β1) (20 ng/ml). After 72 h induction by transforming growth factor-β1 (TGF-β1), the mRNA and protein level of E-cadherin, an epithelial marker was downregulated, meanwhile the mesenchymal maker Vimentin was upregulated in SGC-996 and Noz cells (Fig. 6a–d).

Then we asked whether LncRNA-ROR was involved in the epithelial to mesenchymal transition (EMT) in gallbladder cancer cells. LncRNA-ROR was silenced by siRNA in a TGF-β1 treatment model, the mRNA expression of E-cadherin was significantly increased in the SGC-996 and Noz cells, but Twist1 and Vimentin were markedly decreased (Fig. 7a, b). Western-blot results also confirmed that after LncRNA-ROR knockdown, the protein level of E-cadherin was significantly upregulated. Meanwhile, the protein expressions of Twist1 and Vimentin were markedly downregulated in SGC-996 and Noz cells (Fig. 7c, d).

Discussion

In the present study, we reported that LncRNA-ROR was upregulated in gallbladder cancer tissues and cells. Further we confirmed that LncRNA-ROR was involved in cells proliferation, migration and invasion in SGC-996 and Noz cells. So we put forward that LncRNA-ROR might play important role in tumor progression in gallbladder cancer cells.

LncRNAs have been reported to regulate gene expression by a variety of mechanisms [17]. The LncRNA-ROR has been shown to be involved in modulation of hypoxia signaling pathways in hepatocellular carcinoma (HCC) cells [18]. In breast tumor samples, LncRNA-ROR was upregulated and ectopic overexpression of LncRNA-ROR in immortalized human mammary epithelial cells induced epithelial-to-mesenchymal transition and prevented the degradation of mir-205 target genes, including the EMT related transcription factor ZEB2 [12]. Moreover, LncRNA-ROR was dramatically upregulated in Triple-negative (ER (−), HER2 (−), PR (−)) breast cancer (TNBC), and E-cadherin localization and affects cell-cell adhesion was regulated by LncRNA-ROR/miR-145/ARF6 axis [19]. In another report, knockdown of LncRNA-ROR in pancreatic cancer could inhibit cell proliferation, invasion, and tumorigenicity by modulating Nanog [20].

In contrast to tumor promotion function in majority publications, different expression pattern has been reported in glioma [21], where LncRNA-ROR exerted a tumor suppressing function. There has not been any report about the role of LncRNA-ROR in GBC so far. Although we recently reported that lncRNA H19 was upregulated and promoted cell proliferation in GBC [22], relative research about H19 was broad and deep. In contrast, LncRNA-ROR is relatively new and deserves further study.

In conclusion, we found that high expression of LncRNA-ROR indicated a poor prognosis in gallbladder cancer patients. Knockdown of LncRNA-ROR inhibited proliferation, migration, and invasion in GBC cells. These findings suggest that LncRNA-ROR might function as an oncogene in gallbladder cancer, and could be served as a potential prognostic marker and therapeutic target for gallbladder cancer.

Acknowledgments This work was supported by the National Natural Science Foundation of China (Grant number 81272747 and 81572297) and Doctorial innovation funds of Shanghai Jiao Tong University School of Medicine. We thank Dr. QIU LEI, helped us with language editing.

Compliance with ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Human Ethics Committee of Xinhua Hospital at Shanghai Jiao tong University (Shanghai, China).

Conflicts of interest None.

Reference

1. Kanthan R, Senger JL, Ahmed S, Kanthan SC. Gallbladder cancer in the 21st century. J Oncol. 2015;2015:967472.
2. Goetze TO. Gallbladder carcinoma: prognostic factors and therapeutic options. World J Gastroenterol. 2015;21(43):12211–7.
3. Aloia TA, Jarufe N, Javle M, Maithel SK, Roa JC, Adsay V, et al. Gallbladder cancer: expert consensus statement. HPB (Oxford). 2015;17(8):681–90.

4. Liz J, Esteller M. Lncrnas and micrornas with a role in cancer development. Biochim Biophys Acta. 2016;1859(1):169–76.
5. Managadze D, Lobkovsky AE, Wolf YI, Shabalina SA, Rogozin IB, Koonin EV. The vast, conserved mammalian lincrnome. PLoS Comput Biol. 2013;9(2):e1002917.
6. Ma MZ, Li CX, Zhang Y, Weng MZ, Zhang MD, YY Qin, et al. Long non-coding RNA HOTAIR, a c-myc activated driver of malignancy, negatively regulates miRNA-130a in gallbladder cancer. Mol Cancer. 2014;13:156.
7. Ma MZ, Chu BF, Zhang Y, Weng MZ, Qin YY, Gong W, et al. Long non-coding RNA CCAT1 promotes gallbladder cancer development via negative modulation of miRNA-218-5p. Cell Death Dis. 2015;6:e1583.
8. Wu XS, Wang XA, Wu WG, Hu YP, Li ML, Ding Q, et al. Malat1 promotes the proliferation and metastasis of gallbladder cancer cells by activating the ERK/MAPK pathway. Cancer Biol Ther. 2014;15(6):806–14.
9. Wang Y, Xu Z, Jiang J, Xu C, Kang J, Xiao L, et al. Endogenous miRNA sponge lincRNA-RoR regulates oct4, Nanog, and sox2 in human embryonic stem cell self-renewal. Dev Cell. 2013;25(1):69–80.
10. Loewer S, Cabili MN, Guttman M, Loh YH, Thomas K, Park IH, et al. Large intergenic non-coding rna-ror modulates reprogramming of human induced pluripotent stem cells. Nat Genet. 2010;42(12):1113–7.
11. Takahashi K, Yan IK, Kogure T, Haga H, Patel T. Extracellular vesicle-mediated transfer of long noncoding RNA RoR modulates chemosensitivity in human hepatocellular cancer. FEBS Open Bio.2014;4:458–67.
12. Hou P, Zhao Y, Li Z, Yao R, Ma M, Gao Y, et al. LincRNA-RoR induces epithelial-to-mesenchymal transition and contributes to breast cancer tumorigenesis and metastasis. Cell Death Dis. 2014;5:e1287.
13. Mitra A, Mishra L, Li S. Emt, ctcs and cscs in tumor relapse and drugresistance. Oncotarget. 2015;6(13):10697–711.
14. Brivio S, Cadamuro M, Fabris L, Strazzabosco M. Epithelial-to-mesenchymal transition and cancer invasiveness: what can we learn from cholangiocarcinoma? J Clin Med. 2015;4(12):2028–41.
15. Creighton CJ, Gibbons D, Kurie JM. The role of epithelial-mesenchymal transition programming in invasion and metastasis: a clinical perspective. Cancer Manag Res. 2013;5:187–95.
16. Peinado H, Olmeda D, Cano A. Snail, zeb and bhlh factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer. 2007;7(6):415–28.
17. Guil S, Esteller M. RNA-RNA interactions in gene regulation: the coding and noncoding players. Trends Biochem Sci. 2015;40(5):248–56.
18. Takahashi K, Yan Ik, Haga H, Patel T. Modulation of hypoxia-signaling pathways by extracellular lincror. J Cell Sci. 2014;127:1585–94.
19. Eades G, Wolfson B, Zhang Y, Li Q, Yao Y, Zhou Q. LincRNA-RoR and mir-145 regulate invasion in triple-negative breast cancer via targeting arf6. Mol Cancer Res. 2015;13(2):330–8.
20. Gao S, Wang P, Hua Y, Xi H, Meng Z, Liu T, et al. Ror functions as a cerna to regulate nanog expression by sponging mir-145 and predicts poor prognosis in pancreatic cancer. Oncotarget. 2016;7(2):1608–18.
21. Feng S, Yao J, Chen Y, Geng P, Zhang H, Ma X, etal. Expression and functional role of reprogrammingrelated long noncoding RNA (lincRNA-RoR) in glioma. J Mol Neurosci. 2015;56(3):623–30.
22. Wang SH, Wu XC, Zhang MD, Weng MZ, Zhou D, Quan ZW. Long noncoding RNA h19 contributes to gallbladder cancer cell proliferation by modulated mir-194-5p targeting akt2. Tumour Biol. 2016. doi:10.1007/s13277-016-4852-1.