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Long noncoding RNA ROR1-AS1 overexpression predicts poor prognosis and promotes metastasis by activating Wnt/β-catenin/EMT signaling cascade in cervical cancer

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Abstract. – OBJECTIVE: Several long noncoding RNAs (lncRNAs) display functional effects in the tumorigenesis and progression of cervical cancer (CC). We aimed to investigate the roles of lncRNA tyrosine protein kinase transmembrane receptor 1 antisense RNA 1 (ROR1-AS1) in the development of CC patients.

PATIENTS AND METHODS: Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was performed for the determination of ROR1-AS1 levels in both CC tissues and cell lines. The clinical value of ROR1-AS1 expression in CC patients was statistically analyzed. After transfection with si-ROR1-AS1 in SiHa and HeLa cells, cellular growth and apoptosis were examined by Cell Counting Kit (CCK-8) assay, cell colony formation, and flow cytometry. Then, wound-healing assays and transwell assays were performed to evaluate cell migration and invasion, respectively. The related proteins of epithelial-mesenchymal transition (EMT) markers and Wnt/β-catenin signaling pathway was assessed using Western blot assays.

RESULTS: We found that that the expressions of ROR1-AS1 were distinctly increased in CC tissues and cell lines. Clinical study revealed that high ROR1-AS1 expression was associated with distant metastasis, FIGO stage, and shorter five-year survival. Functional assays by performing *in vitro* assays revealed that inhibition of ROR1-AS1 distinctly suppressed CC cell proliferation, colony formation, migration and invasion, and promoted apoptosis. Based on results of Western blot, we showed that the downregulation of ROR1-AS1 inhibited the levels of N-cadherin and vimentin. In addition, the distinctly decreased levels of c-myc, β-catenin, and cyclin D1 were observed in CC cells transfected with si-ROR1-AS1.

CONCLUSIONS: Our results suggest that ROR1-AS1 is likely to serve as an efficient therapeutic approach in respect of CC treatment. Our results suggest that KLF5 may be a potential therapeutic target in laryngeal carcinoma.

Key Words:
LncRNA ROR1-AS1, Cervical cancer, Proliferation, Metastasis, Wnt/β-catenin pathway, EMT pathway.

Introduction

Cervical cancer (CC), the third leading cause of cancer mortality in women, brings about approximately 233,000 deaths per year worldwide[1]. The incidence of CC is on the rise with a median age < 40 at tumor confirmation[2]. In China, persistent infection of high-risk human papillomavirus (HPV) is believed as one reason for cervical carcinogenesis[3]. Radiotherapy and surgical operation could distinctly improve the clinical outcome only in limited, noninvasive CC patients. However, advanced CC still threatens the lives of women[4,5]. Thus, the identification of contributing factors involved in metastasis is still necessary to improve the long-term survival or clinical outcome of CC patients.

The extensive application of whole-genome sequencing technologies has demonstrated that more than 98% of the mammalian genome is not in protein-encoded regions, suggesting that noncoding RNAs (ncRNAs) may play an important role in biological progress[6]. Long noncoding RNA (lncRNA) is a class of ncRNAs, which are highly conserved in their secondary structures, generally longer than 200 nt and have no protein-coding ability[7,8]. The potential effects of lncRNAs have been functionally confirmed in a wide range of biological progress, such as gene transcription, miRNA degradation, cellular differentiation, and chromatin modification[9]. Many lncRNAs were demonstrated to be dysregulated in various tumors and functionally illustrated in

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several studies[10-12] showing that lncRNAs may be involved in the tumorigenesis and metastasis *via* acting as tumor suppressors or promoters. Given the important roles of lncRNAs in tumor progression, some special lncRNAs may be used as novel biomarkers for cancer diagnosis and prognosis and potential therapeutic targets for various tumor, including CC.

In this study, we identified a novel CC-related lncRNA, lncRNA tyrosine protein kinase transmembrane receptor 1 antisense RNA 1 (ROR1-AS1). With the use of RT-PCR, we revealed that ROR1-AS1 expression was increased in CC specimens compared to matched normal cervical tissues, which was consistent with the expression trend of ROR1-AS1 in liver cancer, mantle cell lymphoma, and colorectal cancer[13-15]. Then, we explored the clinical significance of ROR1-AS1 in CC patients, confirming that ROR1-AS1 may display negative influence on long-term survival. In addition, we performed *in vitro* assays for the exploration of ROR1-AS1 function and further preliminarily explored the potential mechanisms. Overall, our findings revealed that ROR1-AS1 was a tumor promoter in CC, and may be used as a novel prognostic factor and therapeutic target.

Patients and Methods

Clinical and Tissue Samples

Paired specimen samples (tumor and matched non-tumor specimens) were obtained from 174 patients with CC who underwent surgical resection from March 2011 to July 2014 at Cangzhou Central Hospital. Before participating in this study, written informed consents were obtained from the patients, and the Research Ethics Committee of Cangzhou Central Hospital approved the investigation. Tissue specimens were preserved at -80°C immediately after surgical resection for future use.

Cell Lines and Cell Transfection

Ect1/E6E7 (cervical epithelial cells) and five CC cells (SW756, ME-180, HeLa, C33A, and SiHa cells) were all preserved using Roswell-Park Memorial Institute-1640 medium (RPMI-1640; ZRBioRise, Songjiang, Shanghai, China) containing 10% fetal bovine serum (FBS; Xinshang Technology, Hangzhou, Zhejiang, China) and antibiotics (100 U/ml penicillin sodium; 100 μg/ml streptomycin) in an incubator with proper culture conditions (37°C; 5% CO_2). The cells were all purchased from Jenneo Biological Co., Ltd. (Nanjing, Jiangsu, China). Additionally, we used a transfection reagent (Sinofection; Sino Biological Inc., Tongzhou, Beijing, China) to carry out the small interfering RNAs (siRNAs) transfection. The siRNAs targeting ROR1-AS1 (si-1 and si-2) or control siRNAs (si-NC) were all designed and synthesized by STBio Co., Ltd. (Wuhan, Hubei, China).

Quantitative Reverse Transcription-Polymerase Chain Reaction Assays

Real Time-PCR assays were applied to detect the expressing levels of ROR1-AS1, β-catenin, and cyclin D1. Briefly, the total RNA was first extracted by TRIzol solution and subsequently reversely transcribed into cDNA using a cDNA transcription kit (Beijing Protein Innovation, Shunyi, Beijing, China). Afterwards, the qRT-PCR assays were carried out with the aid of a SYBR Green master kit (BerSinBio, Guangzhou, Guangdong, China) on a Real Time-PCR system (HealForce; Pudong, Shanghai, China). The relative levels of ROR1-AS1 were calculated by the $2^{-\Delta\Delta Ct}$ methods. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an endogenous control. The primers used in this study were presented in Table I.

Western Blot Assays

Seventy-two hours post-transfection, the culture medium was discarded and HeLa, as well as SiHa cells were washed using ice-cold phosphate-buffered saline (PBS). Later, 300 μl cell lysis buffer was added into the cells, and the cell lysates were collected using plastic cell scraper (Jinan Biological, Jiading, Shanghai, China). Afterwards, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; 8-12%) was conducted with 20 μg total protein per lane. Then, skim milk (with 5% concentration) was applied to block the membranes after the polyvinylidene difluoride (PVDF) membranes were used for the transfer of the proteins. The membranes were then separately incubated with anti-ROR1-AS1,

Table I. The primers for PCR.

Primer name	Sequences
ROR1-AS1: Forward	CCGGATCACTAGGTCTGGCAC
ROR1-AS1: Reverse	GCCTCACATCGAAACGTTATCC
GAPDH: Forward	CAATGACCCCTTCATTGACC
GAPDH: Reverse	GACAAGCTTCCCGTTCTCAG

anti-vimentin, anti-β-catenin, and anti-c-myc primary antibodies for 12 h at 4°C. On the second day, matched secondary antibodies were used to incubate with the membranes, and a ProtoGlow enhanced chemiluminescence kit (SangerBio, Hongkou, Shanghai, China) was utilized to examine the proteins. The integral optical density of all bands was calculated using Image J software (NIH, Bethesda, MD, USA).

Cell Proliferation Assays

Cell proliferation was evaluated by Cell Counting Kit-8 (CCK-8) and EdU (5-ethynyl-2'-deoxyuridine) assays. For CCK-8 assays, the ROR1-AS1 siRNAs-transfected CC cells (1×10^4 cells per well) were placed into 96-well plates, and the cells were then maintained at different periods of time (1, 2, 3, and 4 days). Afterwards, 10 μl CCK-8 reagent (ACMEC Biomedical, Fengxian, Shanghai, China) was added into each well. Following incubation for 2 h at 37°C, the absorbance was assessed at 450 nm using a microplate reader instrument (PT-3502PC; POTENOV, Tongzhou, Beijing, China).

For EdU assays, CC cells were placed into 48-well plates, and ROR1-AS1 siRNAs or NC siRNAs were used to transfect above the cells for the knockdown of ROR1-AS1. After culturing for 72 h, the EdU reagent (final concentration: 10 μM; iGeneBio, Guangzhou, Guangdong, China) was added into the cells, and the cells were cultured for an additional 2 h. Subsequently, the 4',6-diamidino-2-phenylindole (DAPI) solution was added into the cells and after incubation in the dark for 10 min, the medium was discarded. Finally, the cells were observed using an FRD-4C fluorescence microscope (COSSIM, Chaoyang, Beijing, China).

Cell Colony Formation Assays

Firstly, the ROR1-AS1 siRNAs or NC siRNAs-transfected CC cells were placed into 6-well plates with 500 cells per well. Thereafter, the plates were kept in an incubator with proper culture conditions (37°C; 5% CO_2) for about 14 days. Then, the cell colonies were stained with crystal violet dye (0.1% concentration) and observed by an FRD-4C microscope (COSSIM, Chaoyang, Beijing, China).

Cell Apoptosis Assays

The cell apoptosis was determined by a Vazyme apoptosis detection kit (Vazym, Hangzhou, Zhanjiang, China). In brief, the tumor cells after transfection with ROR1-AS1 siRNAs or NC siRNAs were collected and stained with FITC-Annexin V, as well as propidium iodide (PI) in the Vazyme apoptosis detection kit (Vazym, Hangzhou, Zhanjiang, China). Then, the apoptotic rates of CC cells were analyzed by a Cytomics flow cytometer (Beckman, Pudong, Shanghai, China).

Wound Healing Assays

The CC cells were harvested and replaced in 6-well plates. When the cellular confluence reached to 70%-80%, ROR1-AS1 siRNAs or NC siRNAs were separately transfected into the cells. After the cells formed a confluent monolayer, a sterile 200 μl pipette tip was applied to scratch across the cell monolayer. The wounded areas were photographed at 0 h and 48 h after scraping using an FRD-4C microscope (COSSIM, Chaoyang, Beijing, China).

Transwell Invasion Assays

In short, 24-well plates were first inserted with 8-μm Corning transwell filters pre-treated with Matrigel. Then, the treated CC cells (200 μl, 2×10^4 cells per well) were plated in the upper chambers of the transwell filters. Meanwhile, the lower chambers were added with 500 μl complete culture medium (20% FBS). After culturing for 24 h, the cells that invaded through the membranes were fixed and stained using crystal violet dye (0.1% concentration), and subsequently photographed by an FRD-4C microscope (COSSIM, Chaoyang, Beijing, China).

Statistical Analysis

Statistical analysis was performed using SPSS software 17.0 (SPSS Inc., Chicago, IL, USA). The Student's *t*-test (two different groups) and Chi-square test were used to determine statistical significance. Overall survival curves were plotted according to the Kaplan-Meier methods, and the log-rank test was applied for comparison. Cox proportional hazards models were generated to assess the survival data. The difference was considered to be significant when p was less than 0.05.

Results

ROR1-AS1 was Highly Expressed in CC Tissues and Cell Lines

To screen whether ROR1-AS1 expression was dysregulated in CC, we firstly collected CC specimens and normal cervical tissues from 174 CC patients. As presented in Figure 1A, the results of RT-PCR showed that ROR1-AS1 expression was increased in CC specimens compared to matched normal specimens ($p<0.01$). We also observed that Ect1/E6E7 cells had decreased ROR1-AS1

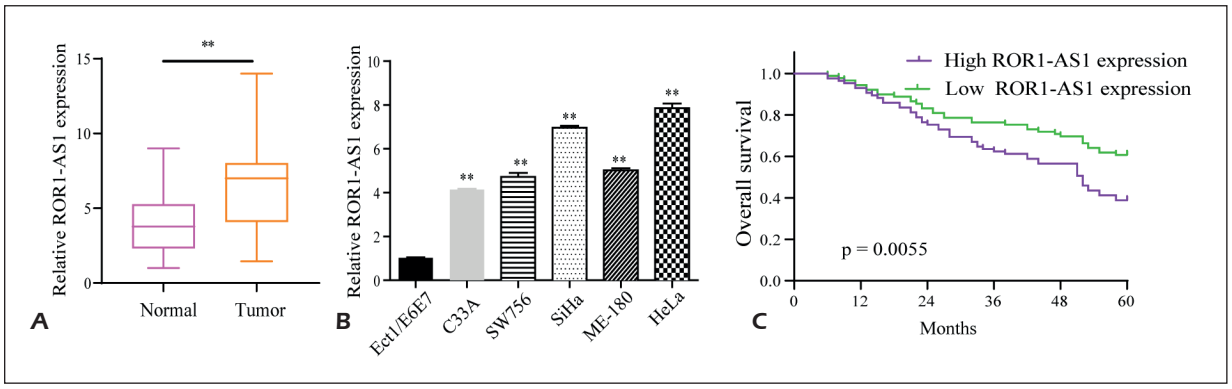

Figure 1. ROR1-AS1 was upregulated in CC and associated with poor survival of CC patients. **A**, The expressions of ROR1-AS1 in 174 pairs of CC specimens and matched normal specimens were detected using qRT-PCR. **B**, ROR1-AS1 levels in different CC cell lines were measured by qRT-PCR. **C**, Kaplan-Meier survival analysis of the correlation between ROR1-AS1 expression and cumulative survival of the 174 CC patients. *p<0.05, **p<0.01.

expression compared to CC cell lines SW756, ME-180, HeLa, C33A and SiHa cells (Figure 1B). Thus, our findings revealed ROR1-AS1 as an overexpressed lncRNA in CC, and this lncRNA may be involved in CC progression.

The Prognostic Value of ROR1-AS1 Expressions in CC

For the study of the associations of ROR1-AS1 levels with clinical features of CC patients, 174 participants were divided into two groups: high-ROR1-AS1-expression group (n=85) and low-ROR1-AS1-expression group (n=89), according to the median value of ROR1-AS1. As shown in Table II, the results of Chi-square test revealed that high expression of ROR1-AS1 was associated with distant metastasis (p=0.001) and FIGO stage (p=0.021). However, there were no significant correlations of ROR1-AS1 expression with other clinical features (all p>0.05). Then, we performed Kaplan-Meier analyses to investigate the possible correlation between ROR1-AS1 expressions and

Table II. The association of ROR1-AS1 expression with clinicopathological features of patients with CC.

Clinicopathological features	No. of cases	ROR1-AS1-high	ROR1-AS1-low	*p*-value
Age				0.133
<45	92	40	52	
≥45	82	45	37	
Tumor size (cm)				0.072
<4.0	106	46	60	
≥4.0	68	39	29	
Histological type				0.559
Squamous carcinoma	94	44	50	
Adenocarcinoma	80	41	39	
Tumor differentiation				0.137
Well + moderate	106	47	59	
Poor	68	38	30	
Lymph node metastasis				0.090
Yes	123	55	68	
No	51	30	21	
Distant metastasis				0.011
Yes	124	53	71	
No	50	32	18	
FIGO stage				0.021
I/II	117	50	67	
III/IV	57	35	22	

Table III. Univariate and multivariate analyses for overall survival by Cox regression model.

Parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	0.862	0.487-1.775	0.237	-	-	-
Tumor size (cm)	1.273	0.675-1.986	0.359	-	-	-
Histological type	1.487	0.852-2.138	0.219	-	-	-
Tumor differentiation	1.988	1.129-2.525	0.091	-	-	-
Lymph node metastasis	2.987	1.365-4.428	0.021	2.566	1.187-4.119	0.031
Distant metastasis	3.322	1.462-5.217	0.006	3.019	1.218-4.775	0.013
FIGO stage	3.185	1.228-5.786	0.009	2.986	1.110-5.019	0.014
ROR1-AS1 expression	2.996	1.386-4.654	0.015	2.776	1.159-4.327	0.019

five-year survival of patients with CC. As shown in Figure 1C, patients with high levels of ROR1-AS1 had poorer survival than those with lower levels of ROR1-AS1 (p=0.0055). Finally, our group performed univariate and multivariate assays for further determination of the prognostic value of several clinical features in CC patients. Univariate assays confirmed distant metastasis, FIGO stage, and high ROR1-AS1 expression as possible factors involved in the clinical survival of CC patients. Of note, the results of multivariate assays demonstrated that ROR1-AS1 expression (HR=2.776, 95% CI: 1.159-4.327, p=0.019) was an independent poor prognostic factor for overall survival in CC (Table III).

Knockdown of ROR1-AS1 Contributed to the Inhibition of CC Cellular Growth

To evaluate the functional roles of ROR1-AS1 in CC, we next assessed the effects of ROR1-AS1 down-regulation on the proliferative rates of CC cells. Firstly, both the cells were transiently transfected with specific siRNAs against ROR1-AS1 (si-1 and si-2) or si-NC, and the data of qRT-PCR assays suggested that the mRNA levels of ROR1-AS1 in CC cells were remarkably reduced by about 70% (Figure 2A). Subsequently, the cellular function of ROR1-AS1 was examined by CCK-8 assays. According to the data, after transfection with ROR1-AS1 siRNAs, cell growth of CC cells was significantly suppressed (Figure 2B and C). We also performed EdU assays to explore the influences of ROR1-AS1 on cell proliferation. It was observed that down-regulation of ROR1-AS1 caused marked loss of cell proliferative abilities in CC cells (Figure 2D and E). Similarly, the results from colony formation assays proved that repressing the expression of ROR1-AS1 led to notably decreased cell colonies of CC cells (Figure 2F). Moreover, the CC cell apoptosis was next determined using flow cytometry. As the data presented in Figure 2G, the apoptotic CC cells transfected with ROR1-AS1 siRNAs were increased by more than two times compared with that of the si-NC-transfected cells. Collectively, these results clearly illustrated that ROR1-AS1 affected the apoptosis and cellular growth of CC cells.

Silence of ROR1-AS1 Impaired the Metastatic Potentials of CC Cells

To explore the potential roles of ROR1-AS1 in the modulation of the mobility of CC cells, we next assessed the migratory and invasive abilities of CC cells after treatment of ROR1-AS1 siRNAs or si-NC. For HeLa cells, our group firstly performed transwell invasion assays to assess the cellular invasion. According to the results, there were significant changes of HeLa cellular invasive capacities when the cells were transfected with ROR1-AS1 siRNAs (Figure 3A). Subsequently, wound healing assays were conducted and the data revealed that the migratory abilities of ROR1-AS1 siRNAs-transfected HeLa cells were also remarkably decreased as the wounded areas were significantly wider in these groups (Figure 3B). Accordingly, the invasive SiHa cell number was remarkably reduced after the cells were transfected with ROR1-AS1 siRNAs (Figure 3C). Similarly, as confirmed by wound healing assays, knockdown of ROR1-AS1 dramatically resulted in losing the migratory capacities of SiHa cells (Figure 3D). Besides, as epithelial-mesenchymal transition (EMT) was in regard to cancer cell metastasis, we next carried out Western blot assays to examine the expressions of vimentin and N-cadherin. As the data shown in Figure 3E and F, depressed expression of ROR1-AS1 in CC cells distinctly reduced the protein expressing levels of vimentin and N-cadherin. Hence, our data con-

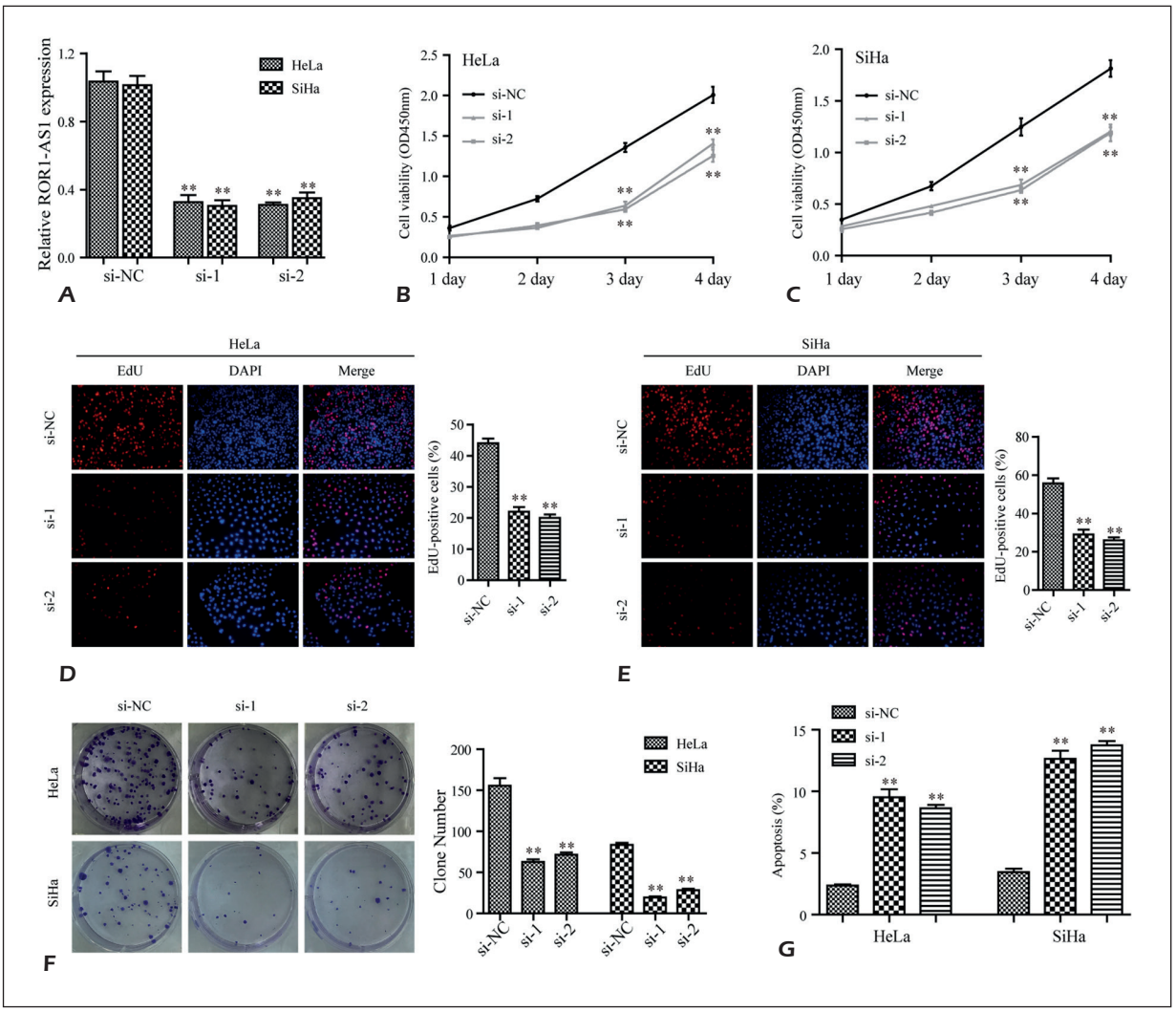

Figure 2. The influence of ROR1-AS1 on HeLa and SiHa cellular proliferation and apoptosis. **A**, Relative mRNA expression of ROR1-AS1 in HeLa and SiHa cells after transfection with negative control siRNAs (si-NC) or ROR1-AS1 siRNAs (si-1 and si-2). **B**, and **C**, Cell viability of cells was determined by CCK-8 assays. **D**, and **E**, The cellular proliferation was evaluated by EdU assays. The proliferative cells were labeled with EdU (red); nuclei were labeled with DAPI (blue) (magnification: 100×). **F**, Colony formation abilities of HeLa and SiHa cells were evaluated by cell colony formation assays (magnification:10×). **G**, Flow cytometry assays evaluated the apoptosis of cells. *p<0.05, **p<0.01.

firmed that ROR1-AS1 promoted the mobility of CC cells via activating EMT progress.

ROR1-AS1 Downregulation Depressed the Activation of Wnt/β-Catenin Signaling in CC Cells

As the above data demonstrated that ROR1-AS1 affected the cellular growth and metastatic potentials of CC cells, we then tried to unravel the detail molecular mechanisms behind that. Considering Wnt/β-catenin signaling could serve as important regulators in multiple biological processes of diverse cancer types, we therefore carried out qRT-PCR and Western blot assays to evaluate the influence of ROR1-AS1 on this signaling. We found that repression of ROR1-AS1 in CC cells dramatically attenuated the mRNA levels of β-catenin, as well as its two downstream factors: c-myc and cyclin D1 (Figure 4A). In accordance with the results from qRT-PCR assays, remarkable depressed protein levels of above factors were also observed in CC cells by the use of Western blot assays after the transfection of ROR1-AS1 siRNAs (Figure 4B). Therefore, our data suggested that the activity of Wnt/β-catenin pathway was suppressed by ROR1-AS1 knockdown in CC cells.

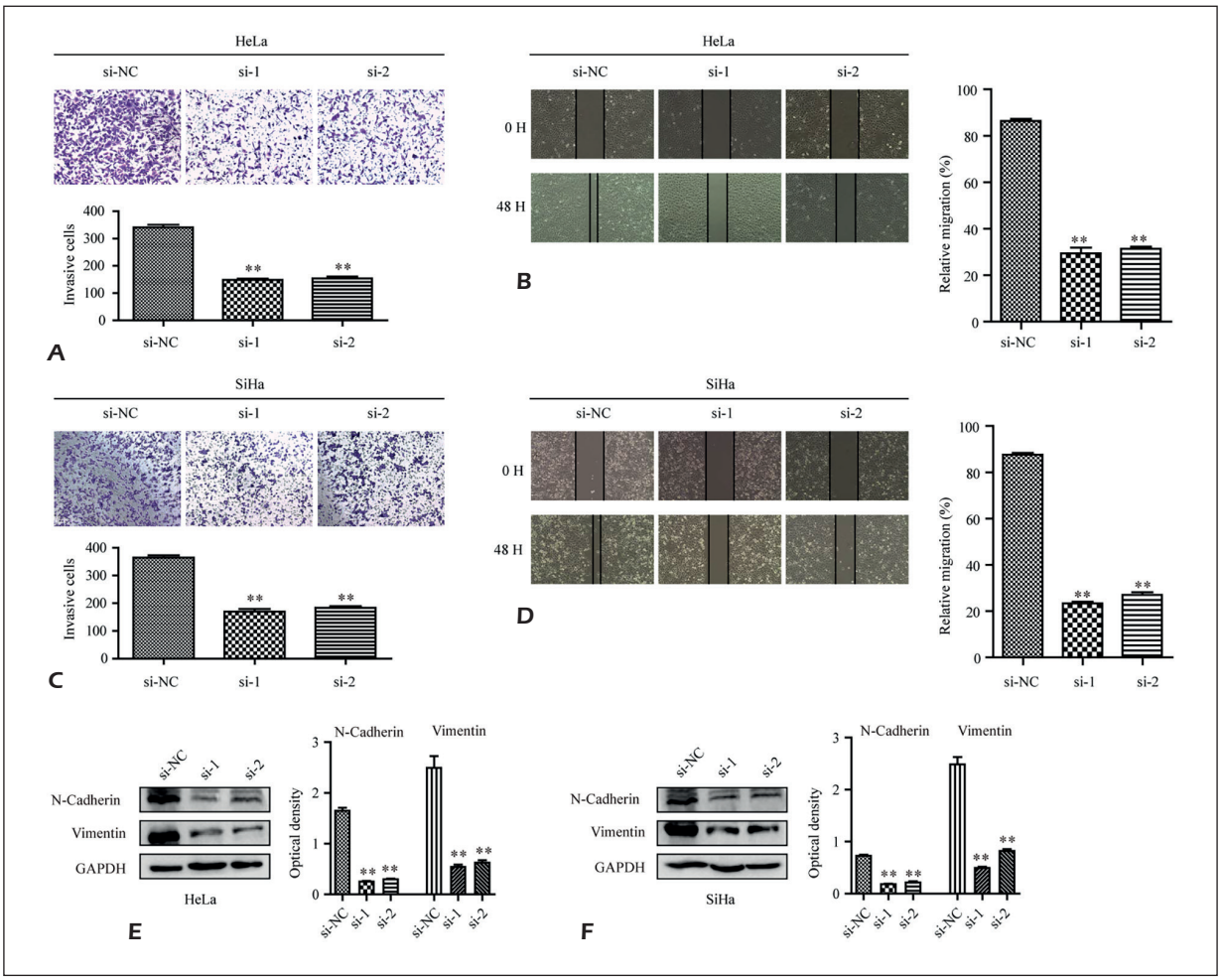

Figure 3. ROR1-AS1 affected the invasion and migration of HeLa and SiHa cells. **A**, and **B**, The invasion and migration of HeLa cells were evaluated by transwell invasion assays (magnification: 40×) and wound healing assays (magnification: 10×), respectively. **C**, and **D**, Transwell invasion assays determined the invasive abilities of SiHa cells after treatment with ROR1-AS1 siRNAs (magnification: 40×), and wound healing assays assessed the migratory capacities of SiHa cells after transfection with ROR1-AS1 siRNAs (magnification: 10×). **E**, and **F**, Western blot assays detected the protein expression of N-cadherin and vimentin. The expression of N-cadherin and vimentin was decreased in HeLa and SiHa cells transfected with ROR1-AS1 siRNAs. *$p<0.05$, **$p<0.01$.

Discussion

CC is a grievous tumor in the genital system with a morality rate > 35% within five years of diagnosis[16,17]. Despite efforts using pap smear screening and other diagnostic techniques, the prognosis of CC patients with metastasis remains poor. The processes of metastasis inducing and stimulating pathway are complex and still not well understood. Therefore, understanding of the molecular mechanisms involved in the progression of CC may provide novel insights, novel cancer biomarker for diagnosis and prognosis and therapeutic targets. In this study, we identified a new dysregulated lncRNA ROR1-AS1, a tumor-related lncRNA whose tumor-promotive function had been demonstrated in liver cancer. Then, we also found that ROR1-AS1 expression was distinctly up-regulated in both CC specimens and cell lines. Thus, we also observed that high expression of ROR1-AS1 was associated with distant metastasis, FIGO stage and shorter overall survival, suggesting ROR1-AS1 as a positive regulator in the clinical progress of CC. Notably, multivariate survival assays demonstrated that ROR1-AS1 could be an independent prognostic marker for CC patients. To the best of our best knowledge, this is the first time to report the expression pattern and prognostic value of ROR1-AS1 in CC. Previously, higher expression of ROR1-AS1 in liver cancer

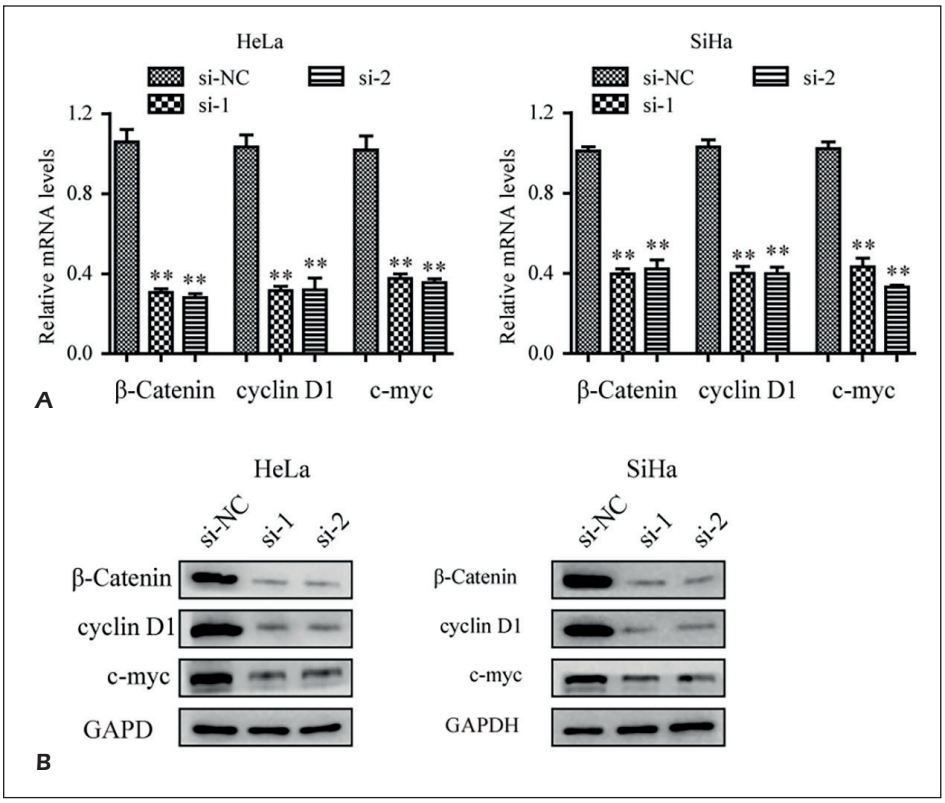

Figure 4. ROR1-AS1 modulated the activity of Wnt/β-catenin signaling in HeLa and SiHa cells. **A**, The relative mRNA levels of β-catenin, cyclin D1 and c-myc in HeLa and SiHa cells were examined by qRT-PCR assays. **B**, After transfection with ROR1-AS1 siRNAs or NC siRNAs, Western blot assays were conducted to detect the protein expression of β-catenin, cyclin D1 and c-myc. *p<0.05, **p<0.01.

was also reported to predict poor clinical outcome of patients. Thus, our findings, together with previous evidence, indicated that ROR1-AS1 may be a novel prognostic biomarker for tumor patients. However, further studies with larger sample sizes are needed to demonstrate these findings.

Several lncRNAs have been reported to be involved in CC progression via complex mechanisms. LncRNA ZNF667-AS1, a newly functionally reported lncRNA, was found to suppress the metastasis of CC cells via neutralizing miRNA-93-dependent PEG3 downregulation[18]. Upregulation of lncRNA POU3F3 induced by SP1 promoted the proliferation and metastasis of CC cells by modulating miRNA-127-5p/FOXD1[19]. These findings provided conclusive evidence that some functional lncRNA may influence the cellular behaviors of CC cells. Thus, we wondered whether ROR1-AS1 may also display an oncogenic role due to its frequent upregulation in CC. In this study, we used si-ROR1-AS1 to down-regulate the levels of ROR1-AS1 and performed a series of cellular experiments to explore the influence of ROR1-AS1 on CC cells proliferation and metastasis. As expected, we found that knockdown of ROR1-AS1 significantly suppressed CC cells proliferation, migration and invasion, and promoted apoptosis, which confirmed that ROR1-AS1 may act as an oncogenic lncRNA in CC progression. Besides, the results of Western blot revealed that down-regulation of ROR1-AS1 distinctly suppressed the protein levels of mesenchymal markers (N-Cadherin and Vimentin), indicating that ROR1-AS1 exhibited its promotive roles in the metastatic ability of CC cells via activating EMT pathway. In addition, the tumor-promotive roles of ROR1-AS1 in colorectal cancer were also confirmed, which was in line with our findings[15]. However, due to the limitation of time and funds, *in vivo* assays were not performed. Further research was needed to add more evidence.

Wnt/β-catenin pathway, also called canonical Wnt pathway, is highly conserved from nematodes to humans and crucial to adult tissue homeostasis[20]. This pathway can be divided into three main branches: the Wnt/Ca^{2+} pathway, the non-canonical PCP pathway and Wnt/β-Catenin pathway[21,22]. This tumor-related pathway has been implicated in a wide range of cellular biological, such as cellular growth, differentiation and cellular migration. Dysregulation of Wnt/β-catenin signaling was shown to be involved in pathogenesis of different kinds of diseases and its important values playing vital roles in carcinogenesis by

regulating cell growth, cell cycling, cell survival and invasion were also confirmed[23-25]. Recently, Liu et al[26] and Ma et al[27] showed that some lncRNAs exhibited their tumor-associated functions via modulating Wnt/β-catenin signaling. In this study, we explored the mechanisms by which ROR1-AS1 modulates malignant CC phenotypes. The results of Western blot showed that the levels of c-myc, cyclin D1 and β-catenin were decreased when ROR1-AS1 expression was suppressed, suggesting that ROR1-AS1 displayed its carcinogenic function via regulating Wnt/β-catenin signaling.

Conclusions

Overall, our research demonstrated that ROR1-AS1 acted as a key regulator in human cervical carcinogenesis and revealed the roles of ROR1-AS1 in regulating Wnt/β-catenin signaling. ROR1-AS1 may have potential as a novel biomarker and treatment target for this type of cancer. However, the underlying molecular mechanisms through which ROR1-AS1 contributes to regulation of CC cell proliferation and metastasis require further investigation.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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