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Original Article

Overexpression of lincRNA-ROR predicts poor prognosis in patients with gastric cancer

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Abstract: Introduction: lincRNA-ROR has been discovered to be associated with tumor progression in many tumor entities. However, the role of lincRNA-ROR in gastric cancer is still unclear. The aim of this study is to explore the clinicopathological and prognostic implication of lincRNA-ROR in gastric cancer patients. Methods: Expression of lincRNA-ROR expression was detected by quantitative real-time PCR (qRT-PCR) in 137 pairs of gastric cancer tissues and adjacent normal tissues. The correlation between lincRNA-ROR expression and clinicopathological factors was analyzed. Overall survival was evaluated using the Kaplan-Meier method with log-rank test. Multivariate analysis was performed using the Cox proportional hazard model to identify independent prognostic factors. Results: lincRNA-ROR expression was significantly higher in gastric cancer tissues compared with adjacent non-tumor tissues. Moreover, lincRNA-ROR expression level was significantly associated with tumor size, tumor depth, differentiation, lymph node metastasis and TNM stage. In addition, the expression of lincRNA-ROR was remarkably correlated with gastric cancer patients' overall survival. Patients with high lincRNA-ROR expression were presented with shorter overall survival. Multivariate analysis indicated that lincRNA-ROR level was an independent prognostic factor for gastric cancer patients. In conclusion, our results indicate that lincRNA-ROR plays an important role in gastric cancer progression, and might be considered as a potential molecular biomarker for predicting the prognosis of patients.

Keywords: Gastric cancer, lncRNA, lincRNA-ROR, prognosis

Introduction

Gastric cancer is one of the most common human malignant diseases and the second leading cause of cancer-related deaths worldwide [1]. In spite of the significant improvements that have been made in the treatment of early gastric cancer, the prognosis for advanced stage gastric cancer is still poor [2]. The characteristic progressive overgrowth and distant metastasis may contribute to the overall poor prognosis in gastric cancer. Although the TNM stage can act as an indicator to predict prognosis of patients, recent studies have suggested that the criteria alone may be insufficient to estimate the clinical outcomes [3, 4]. Therefore, it is urgently needed to identify novel biomarkers for the improvement of diagnostic and prognostic techniques, and for the development of more efficient therapeutic strategies for patients with gastric cancer.

With the rapid development of whole-genome sequencing technology, it has been found that no more than 2% of the mammalian genome are in protein-encoded regions, while others are in non-coding RNAs (ncRNAs) [5]. Among them are long non-coding RNAs (lncRNAs). lncRNAs are defined as transcribed RNA longer than 200 nt and lacking an open reading frame of significant length [6]. Recently, many lncRNAs are shown to play important roles in the regulation of gene transcription and translation, genetic, epigenetic, cell differentiation, ontogenetic and other cellular activities [7-9]. They also may play critical roles in the development and progression of cancers in a way similar to those played by oncogenes or tumor suppressors. lncRNAs have been proved to be new approaches of tumor biomarkers for early cancer diagnosis and prognosis. Mounting studies have identified a number of lncRNAs that are aberrantly expressed in tumors. For instance, Kogo et al.

found that HOTAIR expression was higher in colorectal cancer tissues compared with adjacent noncancerous tissues, and increased HOTAIR expression significantly associated with the liver metastasis in patients with colorectal cancer patients [10]. Lai et al. showed increased expression level of MALAT1 in both hepatocellular carcinoma tissue samples and cell lines. Moreover, they found that patients with high level of MALAT1 presented a significantly higher risk of tumor recurrence [11]. Yang et al. indicated that lncRNA H19 was up-regulated in gastric cancer tissues and cells compared with normal controls. In addition, ectopic expression of H19 could promote cell growth [12]. More recently, Sun et al. revealed that the expression of GAS5 was markedly decreased in gastric cancer, and low expression of GAS5 was associated adverse disease-free survival and overall survival of patients with gastric cancer. In addition, ectopic expression of GAS5 could inhibit gastric cancer cell proliferation and meanwhile induce apoptosis both in vitro and in vivo [13]. However, to our knowledge, the expression pattern and prognostic role of lincRNA-ROR expression has not been reported in gastric cancer. In this study, we detected the expression level of lincRNA-ROR in clinical gastric cancer and adjacent non-tumor tissues. In addition, we analyzed the association of lincRNA-ROR expression and clinicopathological parameters and overall survival of gastric cancer patients. Our results revealed that lincRNA-ROR is involved in the progression of gastric cancer.

Materials and methods

Patients and tissue samples

This study was approved by the Research Ethics Committee of Cancer Center of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent was obtained from all patients, and tissue specimens were anonymized and handled according to ethical and legal standards. Human gastric cancer and adjacent non-tumor tissues were obtained from 137 patients who underwent gastrectomy at the Department of Cancer Center of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. The specimens were snap frozen in liquid nitrogen and stored until further use. None of the patients had received any chemotherapy or radiotherapy before surgery. All patients were followed up regularly with an interval of three months. A comprehensive set of clinicopathological parameters (including age, gender, tumor size, tumor depth, differentiation, T stage, lymph node invasion, and Peritoneal dissemination) were recorded. Overall survival time was calculated from the date of the surgery to the date of death or last contact.

Real-time PCR analysis

Total RNA from tissues was extracted by using Trizol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized with 10 μg total RNA using the Primer-Script one step RT-PCR kit (Promega, Madison, WI, USA). The cDNA template was then amplified by real-time PCR using the SYBR Premix Dimmer Eraser kit (Takala, Dalian, China). GAPDH was used as an reference, and lincRNA-ROR level were normalized to GAPDH. qRT-PCR analysis was performed by the ABI7900 system (Applied Biosystems, Foster City, USA). The primers (Invitrogen) were designed as follows: for human lincRNA-ROR, the forward primer was 5'-CCAGGACAATGAAACCAC-3' and the reverse primer was 5'-AGGAGCCCAAAGTAACAG-3'; for human GAPDH, the forward primer was 5'-CCCACTCCTCCACCTTTGAC-3' and the reverse primer was 5'-ATGAGGTCCACCACCCTGTT-3. Relative quantification of RNA expression was calculated by using the $2^{-\Delta\Delta CT}$ method. Each sample was tested in triplicate.

Statistical analysis

All statistical analyses were performed using SPSS version 16.0 and GraphPad 5.0 software. Data was presented as mean ± SD. The Wilcoxon signed rank test was applied to test the differential expression of lincRNA-ROR between cancer tissues and adjacent normal tissues. The chi-square test was used to evaluate the relationship between lincRNA-ROR expression levels and clinicopathologic characteristics. Overall survival was analyzed using the Kaplan-Meier method and compared with the log-rank test. The independent prognostic factor was analyzed by performing the Cox multivariate proportional hazards model. A *P* value

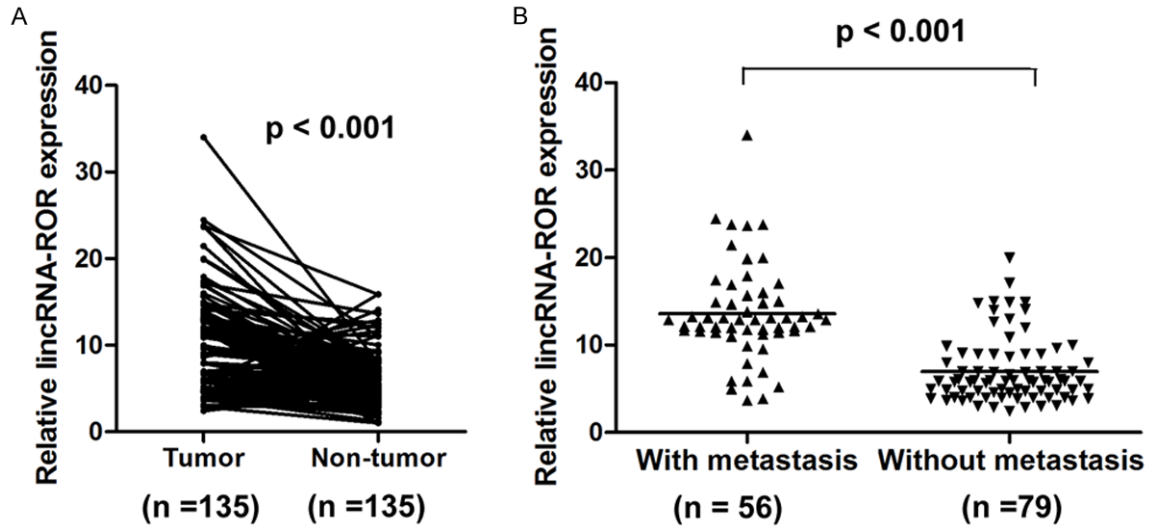

Figure 1. lincRNA-ROR is significantly up-regulated in gastric cancer tissues. A. Real-time PCR analysis showed increased expression of lincRNA-ROR in cancer tissues than adjacent non-tumor tissues ($P < 0.001$); B. Real-time PCR analysis showed higher expression of lincRNA-ROR in tissues with distant metastasis than that without distant metastasis ($P < 0.001$).

of less than 0.05 was considered statistically significant.

Results

lincRNA-ROR was significantly up-regulated in gastric cancer

lincRNA-ROR expression level was determined in 137 gastric cancer tissues and adjacent non-tumor tissues by real-time PCR. As shown in **Figure 1**. The expression level of lincRNA-ROR was significantly higher in tumor tissues as compared with normal tissues ($P < 0.001$). Moreover, expression of lincRNA-ROR was higher in patients with distant metastasis than those without metastasis ($P < 0.001$).

lincRNA-ROR associates with aggressive tumor phenotypes in gastric cancer patients

We next analyzed the association between the expression of lincRNA-ROR and clinicopathological parameters of gastric cancer. Based on the median expression level of lincRNA-ROR, Patients were divided into the low lincRNA-ROR expression group (n = 67) and the high lincRNA-ROR expression group (n = 68). As shown in **Table 1**, lincRNA-ROR expression was significantly associated with tumor size (< 5 cm vs. T ≥ 5 cm; $P < 0.001$), tumor depth ($P < 0.001$), Differentiation ($P < 0.001$), lymph node invasion ($P < 0.001$) and TNM stage ($P = 0.007$). However, there was no correlation between lincRNA-ROR expression level and age (< 60 vs. ≥ 60, $P = 0.139$), gender (female vs. male, P = 0.143) and Peritoneal dissemination (P = 0.170).

Up-regulation of lincRNA-ROR is correlated with poor prognosis in gastric cancer

To further determine the prognostic value of lincRNA-ROR in gastric cancer patients, Kaplan-Meier analysis with the log-rank test was performed to evaluate the expression of lincRNA-ROR on prognosis of gastric cancer patients. The results revealed that high-level expression of lincRNA-ROR was significantly correlated with overall survival of gastric cancer patients ($P < 0.001$, **Figure 2**). High expression of lincRNA-ROR was associated with shorter survival time. Moreover, To determine whether the expression of lincRNA-ROR was an independent prognostic factor for gastric cancer patients, univariate and multivariate analyses were carried out. Univariate analysis indicated that lymph node invasion ($P = 0.002$), TNM stage ($P = 0.013$) and lincRNA-ROR expression level ($P < 0.001$) were associated with overall survival. However, multivariate analyses revealed that only lincRNA-ROR expression level was an inde-

Table 1. The correlation between clinicopathological characteristics and lincRNA-ROR expression in 135 gastric cancer patients

Characteristics	n	High expression	Low expression	*P* value
Age				0.139
< 60	74	33	41	
≥ 60	61	35	26	
Gender				0.143
Male	68	30	38	
Female	67	38	29	
Tumor size				< 0.001
< 5 cm	64	13	51	
≥ 5 cm	71	55	16	
Tumor depth[a]				< 0.001
m/sm/mp	78	21	57	
ss/se/si	57	47	10	
Differentiation				< 0.001
Well	19	7	12	
Moderate	65	21	44	
Poor and others	51	40	11	
Lymph node invasion				< 0.001
Absent	65	19	48	
Present	70	49	21	
Peritoneal dissemination				0.025
Absent	84	36	48	
Present	51	32	19	
TNM stage				0.007
I-II	59	22	37	
III-IV	76	46	30	

[a]m: tumor invasion of mucosa, sm: submocosa, mp: muscularis propria, ss: subserosa, se: serosa penetration, si: invasion to adjacent structures.

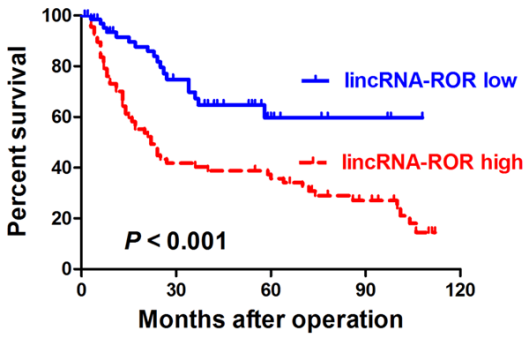

Figure 2. lincRNA-ROR expression is associated with overall survival of gastric cancer patients, patients with high lincRNA-ROR expression is presented with worse overall survival ($P < 0.001$).

pendent prognostic factor of prognosis in patients with gastric cancer ($P = 0.011$, **Table 2**).

Discussion

Long non-coding RNAs (lncRNAs) are generally defined as transcribed RNA molecules longer than 200 nt and lacking an open reading frame of significant length (less than 100 amino acids) [5]. More and more lncRNAs that involved in the progression of gastric cancer have been found which could serve as novel biomarkers for early diagnosis, prognosis prediction, and therapeutic targets in gastric cancer patients [14]. Recent study indicated that lncRNA expression in gastric cancer tissue was significantly altered through screening lncRNA expression profile [15, 16]. For instance, lncRNA GAPLINC (gastric adenocarcinoma predictive long intergenic noncoding RNA) has been reported to be significantly up-regulated in gastric cancer samples compared with paired normal adjacent brain samples. Furthermore, lncRNA GAPLINC overexpression significantly promotes gastric cancer cell proliferation and invasion [17]. Moreover, Xu et al. found that lncRNA FENDRR expression was associated with deeper tumor invasion, higher tumor stage, and lymphatic metastasis of gastric cancer. In addition, up-regulation of FENDRR inhibits tumor cell migration and invasion by downregulating FN1 and MMP2/MMP9 expression [18]. However, no study has elucidated the clinical significance and biological functions of lincRNA-ROR in gastric cancer.

Previously, It has been found that lincRNA-ROR can modulate the reprogramming of human induced pluripotent stem cells [19]. Hou et al. reported that lincRNA-ROR induces an epithelial-to-mesenchymal transition (EMT) program and promotes tomoregenesis and metastasis in breast cancer [20]. More recently, Zhan et al. demonstrated that lincRNA-ROR up-regulates ZEB1 and then induces epithelial-mesenchymal transition (EMT), which promotes the aggressive biological behaviors of Pancreatic cancer [21]. These studies indicated that lincRNA-ROR plays important role in cancer development and metastasis. However, the status of lincRNA-ROR expression in gastric cancer and its prognostic significance are still unclear.

Table 2. Univariate and Multivariate analysis of various potential prognostic factors in 135 gastric cancer patients

Characteristics	Univariate analysis		Multivariate analysis	
	HR[b] (95% CI[c])	*P*	HR[b] (95% CI[c])	*P*
Age	0.93 (0.80-1.23)	0.168	-	-
Gender	1.13 (0.87-1.53)	0.436	-	-
Differentiation	1.02 (0.71-1.46)	0.073	-	-
Treatment	1.08 (0.89-1.52)	0.543	-	-
Tumor depth	1.11 (0.89-1.72)	0.075	-	-
Lymph node invasion	1.42 (1.21-2.11)	0.002[a]	0.97 (0.68-1.35)	0.672
TNM stage	1.39 (1.12-2.05)	0.013[a]	1.36 (0.89-1.93)	0.123
LincRNA-ROR level	2.02 (1.52-2.75)	< 0.001[a]	1.44 (1.21-2.32)	0.011[a]

[a]*P* < 0.05, [b]HR: hazard ratio, [c]CI: confidence interval.

Thus, the aim of this study was to investigate the correlations of lincRNA-ROR expression with clinicopathologic features and prognosis of gastric cancer patients.

In this study, we explored the clinical role of lincRNA-ROR in gastric cancer patients. Our results indicated that lincRNA-ROR expression was significantly higher in gastric cancer tissues compared with that of adjacent non-tumor tissues. In addition, higher lincRNA-ROR expression was associated with tumor size, tumor depth, Differentiation, lymph node invasion and TNM stage, suggesting that up-regulation of lincRNA-ROR plays an important role in gastric cancer progression. This is in accordance with that reported in other tumor entities [20, 22, 23]. Moreover, we found that patients with high lincRNA-ROR expression showed shorter overall survival than those with low lincRNA-ROR expression. More importantly, both the univariate and multivariate survival analyses revealed that high lincRNA-ROR expression was correlated with shorter overall survival in gastric cancer patients, which indicated that lincRNA-ROR was an independent prognostic marker for patients with gastric cancer. Our data proved that lincRNA-ROR might be an important modulator involved in gastric cancer progression.

In conclusion, our results offer the first evidence that lincRNA-ROR plays important role in the progression of gastric cancer and that the overexpression of lincRNA-ROR could independently predict shorter overall survival of patients, implying that lincRNA-ROR might be a potential marker for further risk stratification in the treatment of gastric cancer. However, further studies are needed to explore the molecular mechanisms of lincRNA-ROR in gastric cancer.

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Disclosure of conflict of interest

None.

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