Long non-coding RNA ROR is a novel prognosis factor associated with non-small-cell lung cancer progression

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Abstract. - OBJECTIVE: The aim of the present study was to determine the expression levels of long intergenic non-protein coding RNA, regulator of reprogramming (linc-ROR) in non-small-cell lung cancer (NSCLC) patients and to further explore the prognostic value of this IncRNA.

PATIENTS AND METHODS: In our investigation, we determined the expression of linc-ROR in human NSCLC tissues and matched normal lung tissues by quantitative Real-time-PCR analysis. Also, correlations between linc-ROR expression and the clinicopathological features were evaluated. Survival curves were plotted using the Kaplan-Meier method and differences in survival rates were analyzed using the log-rank test. Cox regression analyses were performed to explore the effect of linc-ROR as an independent predictor of survival.

RESULTS: We found that linc-ROR had high expression in NSCLC specimens than that in matched adjacent normal lung tissues (p < 0.01). In addition, higher linc-ROR expression levels were positively correlated with advanced TNM stage (p = 0.007), positive distant metastasis (p = 0.001) and LN metastasis (p = 0.011). Furthermore, significantly shorter 5-year overall survival (OS) and disease-free survival (DFS) were observed in patients with higher expression of linc-ROR (both p < 0.001). In a multivariate Cox model, it was found that linc-ROR expression was an independent prognostic factor for both 5-years OS (p = 0.001) and 5-year DFS (p = 0.001) in NSCLC.

CONCLUSIONS: Our findings indicate that linc-ROR plays an oncogenic role in NSCLC development and may function as a prognostic and predictive biomarker for NSCLC.

Key Words: Long non-coding RNA ROR, Non-small-cell lung cancer, Prognosis.

Introduction

Lung cancer, contributed to 29% of male and 26% of female cancer estimated deaths, is widely considered as the leading cause of cancer-related death around the world1,2. Approximately 80-85% of all lung cancers are non-small-cell lung cancer (NSCLC), which are classified to adenocarcinoma, squamous cell carcinoma, and large cell carcinoma3. In spite of recent advances in surgical techniques and computed tomography-based screening programs, the long-term outcome remains poor4. Thus, it is imperative to search novel biomarkers for NSCLC, which can provide new strategies for the diagnosis and treatment of this disease.

Long noncoding RNA (IncRNA) is a class of RNA over 200 nucleotides in length with no protein-coding potential5. The quick development of tumor genomics has highlighted the function of IncRNAs in human tumors6,7. Scholars have suggested that IncRNAs participate in many biological processes, such as chromatin remodeling, posttranscriptional regulation, and intercellular signaling8. Notably, some IncRNAs are involved in both oncogenic and tumor-suppressive pathways9. To date, a series of IncRNAs had been identified to be dysregulated in certain types of human tumor and contributing to tumorigenesis10-12. However, the role of most IncRNAs remains unclear. Pan et al13 reported that linc-ROR was involved in chemoresistance in docetaxel resistant lung adenocarcinoma cells. They found that linc-ROR may play a tumor promoter in progression of lung adenocarcinoma. Given the importance of this IncRNA, further research on the function of linc-ROR in NSCLC is required.
In this work we aimed to explore the clinical significance of linc-ROR in NSCLC patients. To our best knowledge, this is the first report about the prognostic value of linc-ROR in NSCLC patients.

**Patients and Methods**

**Patients**

We screened 229 patients diagnosed with NSCLC at Linyi Rehabilitation Hospital between August 2007 and August 2011. The mean age of patients at the time of surgery was 58.5 years (range = 31 years to 79 years). The histopathological diagnosis of all samples was respectively diagnosed by two pathologists. All patients did not receive radiotherapy and/or chemotherapy before surgery. Tissues were snap frozen in liquid nitrogen after surgical resection until use. Follow-up information obtained from medical records was available for all of the selected patients. Samples were used only after written consent was obtained from the patients. This study was approved by the Research Ethics Committee of Linyi Rehabilitation Hospital, Linyi, Shandong, China.

**RNA Extraction and qRT-PCR Analyses**

Total RNA was extracted using TRIzol reagents (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. RNA concentration and purity were determined by Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). Quantitative RT-PCR reactions were performed on the Step One Plus Real-time PCR system (Applied Biosystems, Foster City, CA, USA) using the standard SYBR-Green PCR Kit protocol. GAPDH was used as an internal control for miRNA. The primer sequences used were as follows: linc-ROR forward: 5'-GAATCAGAGTGCTGGGCAGT-3', and reverse: 5'-TCAGCAGTGCTCATGCCCTAAC-3'; GAPDH forward: 5'-CGGAGTCAACGGATTTGGTCGTAT-3', and reverse: 5'-AGCCTTCTCCATGGTGGTAGAC-3'. All experiments were performed using the 2-ΔΔCt method. Each experiment was performed in triplicate.

**Statistical Analysis**

SPSS version 20 (IBM, Armonk, NY, USA) and GraphPad Prism 6 software were used to analyze data. Data were expressed as means ± standard deviation. The difference between patients and control samples was determined by Student’s t-test. The χ²-test was used to analyze the associations between linc-ROR expression and clinicopathological feature. Kaplan-Meier method was used for the survival analysis. Multivariate analysis of the prognostic factors was performed with Cox regression model. p-values < 0.05 were considered statistically significant.

**Results**

**Increased Expression of Linc-ROR in Human NSCLC Tissues**

The expression levels of linc-ROR between NSCLC tissues and paired adjacent non-tumor tissues were determined by qRT-PCR. As shown in Figure 1, linc-ROR was frequently up-regulated in NSCLC tissues compared to matched non-tumor tissues (p < 0.01).

**Association Between linc-ROR Expression and Clinicopathological Factors**

229 NSCLC patients were divided into low-linc-ROR group (n=116) and high-linc-ROR group (n=113) by using the median level of linc-ROR as the cutoff. Table I presented association between linc-ROR expression and clinicopathological parameters in NSCLC. Overexpression of linc-ROR in NSCLC tissues was significantly associated with advanced TNM stage (p = 0.007) and positive distant metastasis (p = 0.001) and LN metastasis (p = 0.011). However, there was no association between linc-ROR expression and other clinical features, such as sex, age, tumor size, and surgery margins (p > 0.05).

**Figure 1.** Linc-ROR expression was significantly higher in NSCLC tissues than in the corresponding non-tumorous samples (p < 0.01).
Correlation Between linc-ROR Expression and Clinical Outcome of NSCLC

To further evaluate the prognostic value of linc-ROR in NSCLC patients, we performed Kaplan-Meier curve analysis. As shown in Figure 2 and 3, NSCLC patients with high levels of linc-ROR expression had shorter OS ($p < 0.001$) and DFS ($p < 0.001$) time than those with low levels of linc-ROR expression. Subsequently, we performed Cox proportional hazards regression analysis. The results of multivariate analyses showed that linc-ROR expression was an inde-

Table I. Correlation between linc-ROR expression and clinicopathological features of patients with NSCLC.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. of cases</th>
<th>High</th>
<th>Low</th>
<th>$p$-value</th>
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<tr>
<td>Sex</td>
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<td></td>
</tr>
<tr>
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<td>102</td>
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<td>53</td>
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<tr>
<td>Female</td>
<td>127</td>
<td>64</td>
<td>63</td>
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<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60</td>
<td>95</td>
<td>41</td>
<td>54</td>
<td>0.115</td>
</tr>
<tr>
<td>≥ 60</td>
<td>134</td>
<td>72</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
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<tr>
<td>&lt; 3</td>
<td>90</td>
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<td>40</td>
<td>0.130</td>
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<tr>
<td>≥ 3</td>
<td>139</td>
<td>63</td>
<td>76</td>
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<td>Surgery margins</td>
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<td>39</td>
<td>45</td>
<td>0.502</td>
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<tr>
<td>Not free</td>
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<td>74</td>
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</tr>
<tr>
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<td>157</td>
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<td>89</td>
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<tr>
<td>III/IV</td>
<td>72</td>
<td>45</td>
<td>27</td>
<td></td>
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<tr>
<td>Distant metastasis</td>
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<td>Positive</td>
<td>61</td>
<td>41</td>
<td>20</td>
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</tr>
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<td>Negative</td>
<td>168</td>
<td>72</td>
<td>96</td>
<td>0.011</td>
</tr>
<tr>
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<tr>
<td>No</td>
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<td>76</td>
<td>95</td>
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</table>
dependent prognostic indicator for OS (HR = 2.983; \( p = 0.001 \)) and DFS (HR = 3.421; \( p = 0.001 \)) in patients with NSCLC (Table II).

**Discussion**

NSCLC is becoming one of the most lethal threats to human health and life. Many efforts have been made to explore tools in predicting outcome of tumor patients during the past several decades\(^1\). Although the TNM staging is used for predicting the prognosis and treatment of patients with NSCLC, this classification system is an imprecise predictor of the outcome of an individual patient\(^2\). Therefore, it is necessary to develop new prognostic tools that may be beneficial for improving the clinical management of NSCLC.

Recently, thousands of lncRNAs have been identified and strong evidence reveals the great role in regulating tumor development and progression\(^3\). Some lncRNAs have been well studied, such as lncRNA MALAT1\(^4\), lncRNA HOTAIR\(^5\) and lncRNA BANCR\(^6\). Those lncRNAs were considered to have potential to serve as prognostic biomarker in several tumors. In the present investigation, our attention focused on linc-ROR. As a newly identified lncRNA, its role has been reported in several tumors. For instance, Li et al found\(^7\) that over-expression of linc-ROR significantly promoted colorectal cancer cell proliferation and viability by affecting P53. Fu et al\(^8\) reported that linc-RoR promotes pancreatic cancer cell proliferation and invasiveness by sponging some different miRNAs. Arun Kumar et al\(^9\) showed that linc-RoR overexpression may be associated with poor prognosis of patients with oral cancer. Importantly, Shi et al\(^10\) found that linc-ROR may play a negative role in drug treatment of NSCLC cells by regulating PI3K/Akt/mTOR signaling pathway. All of these findings revealed that linc-RoR served as a tumor promoter in tumor progression, including NSCLC. Thus, we wondered whether the expression levels of linc-RoR were associated with the prognosis of NSCLC patients.

In the present work, by RT-PCT, we observed that linc-RoR expression might be an independent prognostic factor and a therapeutic target for NSCLC. Further study should focus on elucidating the exact molecular mechanisms of linc-ROR acting on NSCLC.

**Conclusions**

We observed that linc-ROR expression might be an independent prognostic factor and a therapeutic target for NSCLC. Further study should focus on elucidating the exact molecular mechanisms of linc-ROR acting on NSCLC.

**Conflict of interest**

The authors declare no conflicts of interest.

**References**


