Long non-coding RNA NEAT1 acts as oncogene in NSCLC by regulating the Wnt signaling pathway

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Abstract. – OBJECTIVE: The present study aimed to explore the role of long non-coding RNA NEAT1 (NEAT1) in mediating non-small cell lung cancer (NSCLC) cell migration and invasion, as well as the underlying regulatory mechanisms.

PATIENTS AND METHODS: The NEAT1 expression in NSCLC tissues and cell lines was measured using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The relationships between NEAT1 expression and clinicopathological parameters were examined by chi-square test. Overall survival curves were analyzed using the Kaplan-Meier method. Effects of NEAT1 on cell growth, invasion and migration were evaluated by cell counting kit-8 assay and transwell assay, respectively. Western blotting was used to address the impact of NEAT1 on Wnt/β-catenin signaling.

RESULTS: We observed that the expression of NEAT1 in NSCLC tissues and cell lines were much higher than that in normal control, respectively. High NEAT1 expression was statistcally associated with poor differentiation, Lymph node metastasis and advanced TMN stage (all \(p<0.05\)). According to the Kaplan-Meier survival analysis, NSCLC patients with high NEAT1 expression had a significantly shorter overall survival than those with high NEAT1 expression (\(p<0.001\)). Moreover, the downregulation of NEAT1 expression significantly inhibited the NSCLC cells proliferation, migration, and invasiveness. Finally, we found that decreased expression of NEAT1 inhibited the Wnt/β-catenin signaling pathway activity.

CONCLUSIONS: Our data for the first time showed that NEAT1 contribute to the tumorigenesis and development of NSCLC by activating Wnt/β-catenin signaling pathway, suggesting that NEAT1 may provide a therapeutic strategy for the treatment of NSCLC patients.

Key Words: Long non-coding RNA, NEAT1, NSCLC, Wnt/β-catenin, Prognosis.
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plays a critical role in cancer invasion and metastasis. NEAT1 is a novel long non-coding RNA (lncRNA) which served as a crucial regulator in several cancers\(^9,10\). In the present work, we explored the association between NEAT1 expression and clinicopathological significance. Subsequently, we completed a series of in vitro experiments to investigate the role of NEAT1 in NSCLC proliferation and metastasis. Finally, we explore whether NEAT1 could regulate Wnt/β-catenin signaling pathway.

**Patients and Methods**

**Specimens**

133 paired NSCLC and adjacent non-tumor specimens were collected from the Department of Oncology, General Hospital of People’s Liberation Army. All tissue samples were flash-frozen in liquid nitrogen immediately after collection and stored at -80°C until use. None of the patients had received preoperative adjuvant therapy. For the use of these clinical materials for research purposes, written informed consent was obtained from all the participants. This study was approved by the Ethics Committee of General Hospital of People’s Liberation Army.

**Cell Culture and Transfection**

The human normal lung cell line (BEAS-2B), and the human NSCLC cell lines (A549, H1299, SPCA1 and H358) were obtained from the Cell Bank of Chinese Academy of Science (Songjiang, Shanghai, China). The cells were maintained in F-12K Medium (Invitrogen, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA). The siRNA of NEAT1 and negative control siRNA (si-NC) were obtained from Ribonuclease (Shanghai, China). Cells were seeded in 6-well plates. After approaching almost 100% confluence, the cells were scratched with a 20 μl tips, followed by washing with PBS and treatment with 0, 15, 20 and 25 μM of PEITC for 24 h. The cell numbers were counted from five nonoverlapping fields of each membrane. Three independent assays were performed.

**Western Blot**

Collected cells were lysed immediately in RIPA buffer supplemented with a protease inhibitor cocktail (Calbiochem, San Diego, CA, USA). Samples were electrophoresed by using 10% SDS-PAGE. The protein was then transferred onto a PVDF (polyvinylidene fluoride) membrane (Bio-Rad, Shanghai, China). After blocking in skim milk, the membranes were incubated with specific antibodies. Autoradiograms were quantified by densitometry (Quantity One software; Bio-Rad, Shanghai, China). All the antibodies were brought from the Bioworld Company (St. Paul, MN, USA). Finally, the membrane was developed by enhanced ECL (Beyotime Biotechnology, Beijing, China).

**Statistical Analysis**

Statistical analyses were performed using SPSS 17.0 computer software (SPSS Inc., Chi-
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Expression Levels of NEAT1 in NSCLC Tissues and Cell lines

To explore whether NEAT1 was upregulated in NSCLC, we first performed RT-PCR to determine the levels of NEAT1 in NSCLC. Our results showed that the relative level of NEAT1 was significantly higher in NSCLC compared to the adjacent normal lung tissues ($p = 0.001$, Figure 1A). Next, we further explore the expression levels of NEAT1 in four NSCLC cell lines (A549, H1299, SPCA1, and H358). Our data showed that higher level of NEAT1 was observed in the NSCLC cell lines (A549, H1299, SPCA1, and H358) compared with the human normal lung cell line, BEAS-2B ($p < 0.01$, respectively, Figure 1B).

**Figure 1. Expression of NEAT1 in NSCLC tissues and cell lines. (A) Levels of NEAT1 that were detected in NSCLC tissues were significantly higher than the levels of NEAT1 detected in the corresponding adjacent, non-cancerous tissues. (B) Levels of NEAT1 in NSCLC cell lines (A549, H1299, SPCA1, and H358) were significantly higher than in normal lung cell line (BEAS-2B). "p < 0.01."**

**NEAT1 upregulation was Associated with Aggressive Progression in NSCLC and Predicted poor Prognosis in Patients with NSCLC**

To explore the clinical significance of NEAT1 in NSCLC patients, associations between NEAT1 expression and various clinicopathologic features of glioma patients were evaluated statistically. We found that high NEAT1 expression was statistically associated with poor differentiation, Lymph node metastasis and advanced TMN stage (all $p < 0.05$, Table I). Furthermore, we use the Kaplan–Meier method and log rank test to detect the overall survival of patients with NSCLC. Our results showed that NSCLC patients with high NEAT1 expression had a significantly shorter overall survival than those with high NEAT1 expression ($p < 0.001$, Figure 2).

**Knockdown of NEAT1 Suppressed NSCLC Cell Proliferation, Migration, and Invasion**

To validate the transfection efficiency, the relative expression levels of NEAT1 in NSCLC cells after transfection of si-NEAT1 were detected by qRT-PCR. As shown in Figure 3A-B, the NEAT1 expression level was successfully downregulated in A549 and H1299 ($p < 0.001$, respectively). Next, CCK-8 assays were carried out to explore the functional role of NEAT1 in NSCLC.

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Table 1. Correlation Between NEAT1 Expression and clinicopathological parameters of NSCLC.

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>No. of cases</th>
<th>Relative NEAT1 expression</th>
<th>p</th>
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<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤65</td>
<td>55</td>
<td>34</td>
<td>21</td>
</tr>
<tr>
<td>&gt;65</td>
<td>78</td>
<td>47</td>
<td>31</td>
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<tr>
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</tr>
<tr>
<td>Female</td>
<td>59</td>
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<tr>
<td>Differentiation</td>
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<tr>
<td>Well, moderate</td>
<td>67</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>Poor</td>
<td>67</td>
<td>49</td>
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</tr>
<tr>
<td>Tumor size</td>
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<td></td>
</tr>
<tr>
<td>≤5 cm</td>
<td>66</td>
<td>36</td>
<td>31</td>
</tr>
<tr>
<td>&gt;5 cm</td>
<td>67</td>
<td>45</td>
<td>21</td>
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<tr>
<td>Lymph node metastasis</td>
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</tr>
<tr>
<td>Positive</td>
<td>71</td>
<td>51</td>
<td>20</td>
</tr>
<tr>
<td>Negative</td>
<td>62</td>
<td>30</td>
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<tr>
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Discussion

Although many studies showed IncRNA expression signatures in a variety of malignant human cancers for decades\(^1\), elucidation of the role of the dysregulation of specific IncRNAs in carcinogenesis remains in the initial stages of development. In the present study, we found that the expression of NEAT1 in NSCLC tissues and cell lines were much higher than that in normal control, respectively, which is consistent with previous studies focused on other human malignancies\(^12,13\). We also found that increased NEAT1 expression in NSCLC tissues was significantly correlated with aggressive clinicopathological features. Also, based on the

![Figure 2. Kaplan-Meier survival curves showed that patients with high NEAT1 expression demonstrated poorer clinical outcome (p < 0.001).](image-url)
Figure 3. NEAT1 inhibits NSCLC cell proliferation, migration, and invasion. (A,B) NEAT1 knockdown efficiency was confirmed by RT-qPCR in NSCLC cells (A549, H1299). (C,D) CCK-8 analysis of the effects of NEAT1 on the proliferation of A549 and H1299. (E,F) Transwell assay was conducted to analyze the migration of A549 and H1299. (G,H) Transwell assay was conducted to analyze the invasion of A549 and H1299. **p < 0.01.
Kaplan-Meier method, we observed that NEAT1 overexpression was associated with lower overall survival rates. Further in vitro experiment showed that knockdown of NEAT1 could suppress NSCLC cells proliferation, migration, and invasion. Those results revealed that NEAT1 may play an important role in development, tumorigenesis, and progression of NSCLC.

NEAT1 has been extensively demonstrated to be involved in several cancers. For instance, Chen et al\textsuperscript{14} found that NEAT1 contributes to the malignant characters of ESCC through involvement in proliferation, migration, and invasion, and over-expression of NEAT was an independent risk factor of overall survival. Li et al\textsuperscript{15} reported that NEAT1 promoted endometrial endometrioid adenocarcinoma invasion and migration via regulating c-myc, IFG1, MMP-2, and MMP-7. Zhen et al showed that NEAT1 promoted glioma cell proliferation, invasion, and migration by regulating miR-449b-5p/c-Met axis\textsuperscript{16}. Most recently, Sun et al\textsuperscript{17} reported that NEAT1 was highly expressed in patients with NSCLC. Moreover, they identified that NEAT1 could be a crucial oncogenic regulator through acting as a ceRNA for miR-377-3p. Those results revealed that NEAT1 served as a tumor predictor in several tumors, including NSCLC.

To elucidate the molecular mechanism by which NEAT1 promotes NSCLC migration and invasion, we focused on the association between NEAT1 and Wnt/\(\beta\)-catenin signaling pathway. The effect of Wnt/\(\beta\)-catenin signaling pathway has been reported in many studies\textsuperscript{18,19}. We performed the western blot to determine the expression levels of a gene related to Wnt/\(\beta\)-catenin signaling pathway. Our results showed that these protein expressions were also down-regulated in si-NEAT1 transfected NSCLC cells. In the current study, we found that the effect of NEAT1 on the Wnt/\(\beta\)-catenin pathway, which may be a potential therapeutic target to prevent NEAT1 metastasis and progression.

**Conclusions**

To our best knowledge, this was the first study to report that NEAT1 promoted the proliferation and metastasis of NSCLC cells and the activity of the Wnt/\(\beta\)-catenin signaling pathway. These results provided new evidence of NEAT1 as a promising tumor gene therapeutic target for NSCLC patients.

**Conflict of interest**

The authors declare no conflicts of interest.

**References**


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