Reduced miR-300 expression predicts poor prognosis in patients with laryngeal squamous cell carcinoma

Abstract. - OBJECTIVE: miR-300 has been demonstrated to play an important role in the progression of several tumors, but its role in tumorigenesis of laryngeal squamous cell carcinoma (LSCC) is still unclear. The purpose of this study was to explore miR-300 expression in LSCC patients and analyze its association with clinicopathological factors and prognosis.

PATIENTS AND METHODS: In the present study, we measured the expression level of miR-300 in LSCC tissues by RT-PCR. Associations between miRNA-300 expressions and various clinicopathological characteristics were analyzed. Patient survival and their differences were determined by Kaplan-Meier method and log-rank test. The univariate and multivariate analysis were performed using the Cox proportional hazard analysis.

RESULTS: miR-300 expression was significantly increased in LSCC tissues compared with that in adjacent non-cancerous tissues \((p < 0.01)\). In addition, lymph node metastasis \((p = 0.004)\) and TNM stage \((p = 0.001)\) were obvious influence factors for the expression of miR-300. More importantly, Kaplan-Meier analysis showed that LSCC patients with low miR-300 expression tended to have shorter overall survival \((p < 0.001)\). Finally, multivariate analysis revealed that miR-300 expression was an independent prognostic factor for LSCC patients.

CONCLUSIONS: Our results pointed to miR-300 as a powerful prognostic marker in LSCC and as a novel target for tumor-suppressive therapy.

Key Words: miR-300, LSCC, Prognosis, Overall survival.

Introduction

As a common head and neck malignancy, laryngeal carcinoma has a high incidence of approximately 2.4% of new cases around the world every year1. In 2008, laryngeal carcinoma was responsible for 82,000 deaths worldwide1. Laryngeal squamous cell carcinoma (LSCC) is the most common type of laryngeal carcinoma1. Despite the great progress that has been made in the treatment of the disease, including surgery or radiotherapy, the overall 5-year survival rates for laryngeal carcinoma were less than 50%, which are mainly due to the metastasis and recurrence. Therefore, it is necessary to explore effective biomarkers for early-stage diagnosis and potential targets for therapy.

MicroRNAs (miRNAs) are small endogenous non-coding RNAs with 20-22 nucleotides4. Mature miRNAs can be generated from sequential processing of primary miRNA transcripts by Drosha and Dicer, and they can silence their cognate target genes by specifically binding and cleaving mRNAs or inhibiting their translation5,6. Previous evidence revealed that the expression patterns of miRNAs played an important role in cancer progression7,8. Emerging studies have reported that some of miRNAs are commonly dysregulated in LSCC. For example, miR-2069, microRNA-34a/c10 and miR-14411 were reported to be down-regulated always, while miR-15512, miR-744-3p13, and miR-14514 were overexpressed in LSCC. It has been shown that miRNAs play an important role in all type of cancers. However, the expression of miRNAs in cervical cancer has not been studied in-depth.

In the present study, we focused on miR-300. We firstly determine the expression levels of miR-300 in LSCC tissues and match normal non-tumor tissues. Finally, Kaplan-Meier analysis showed that LSCC patients with low miR-300 expression tended to have shorter overall survival \((p < 0.001)\). Multivariate analysis revealed that miR-300 expression was an independent prognostic factor for LSCC patients. Our results pointed to miR-300 as a powerful prognostic marker in LSCC and as a novel target for tumor-suppressive therapy.
miR-300 expression in LSCC

Patients and Methods

Patients and Clinical Specimens
Seventy-one cases of human LSCC tissue specimens were obtained at the time of surgical resection from Yidu Central Hospital of Weifang. Tissue samples were snap-frozen in liquid nitrogen at the time of total thyroidectomy and subsequently stored at -80°C. These patients were pathologically diagnosed with LSCC. Of the 133 patients, 87 were males and 46 were females, with a mean age of 61.33 ± 7.86 years. The characteristics of the patients are described in Table I. All patients provided written informed consent for the use of their tissues. The study approved by the Human Research Ethics Committee of Yidu Central Hospital of Weifang.

Quantitative Real-time Polymerase Chain Reaction (qPCR)
Total RNA was isolated from fresh tissues and cells with TRizol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was reverse transcribed with a miRNA-specific primer, followed by real-time PCR using TaqMan probes. The expression level of miR-300 was measured by quantitative real-time PCR (qRT-PCR), which was performed using the Applied Biosystems 7900HT (Applied Biosystems, Foster City, CA, USA). The PCR results were analyzed using the Mastercycler ep Realplex Program and reported as relative quantities with respect on a calibrator sample using the 2-ΔΔCt method. The expression levels of miR-300 were normalized to U6. The specific primers were shown in Table I.

Statistical Analysis
Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The unpaired t-test was applied to test the differential expression of miR-300 in cancer tissues compared to adjacent non-malignant tissues. The chi-square and t-tests were performed to assess the relationship between miR-300 expression levels and clinicopathological features. The Kaplan-Meier survival curves were plotted, and the log-rank test was done. The univariate analysis was used in multivariate analysis on the basis of Cox proportional hazards model. In all cases, a p < 0.05 was considered statistically significant.

Results

Up-regulation of miR-300 in LSCC
qRT-PCR was performed to detect the expression levels of miR-300 in 133 pairs of LSCC and adjacent non-tumor tissues. Our results showed that miR-300 expression in LSCC tissues was significantly higher than in paired nontumor tissues (p < 0.05, Figure 1), suggesting that miR-300 may play an anti-oncogenic role in LSCC.

Correlation between miR-300 Expression and Clinical Features
To determine the correlation of miR-300 expression levels with the clinical features of LSCC, we divided patients into two groups based on the levels of miR-300 expression. As shown in Table II, no significant correlations were identified between miR-300 expression and clinicopathological parameters such as age, gender, thyroid cartilage invasion, and T classification. However, lymph node metastasis (p = 0.004) and TNM stage (p = 0.001) were obvious influence factors for the expression of miR-300.

miR-300 Expression and Patients’ Survival
Since miR-300 was associated with lymph node metastasis and TNM stage of LSCC pa-

Table I. RT-PCR primers.

<table>
<thead>
<tr>
<th>The specific primers</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>MiR-300</td>
<td>F:5’-TATACAAGGGCAGACTCTCT-3’</td>
</tr>
<tr>
<td></td>
<td>R:5’-GTGCAGGTTCCGAGGT-3’</td>
</tr>
<tr>
<td>U6</td>
<td>F:5’-CTCGTTCCGACAGCAATTACAT-3’</td>
</tr>
<tr>
<td></td>
<td>R:5’-ACGCTTCACGAATTTGCGTGC-3’</td>
</tr>
</tbody>
</table>

Figure 1. miR-300 was detected in LSCC tissues and matched non-cancerous tissues using qRT-PCR.
patients, we wonder whether miR-300 was correlated with survival of the patients. As expected, Kaplan-Meier survival analysis showed that high miR-300 expression predicted significantly better OS ($p < 0.001$, Figure 2).

Univariate analysis showed that lymph node metastasis, TNM stage, and miR-300 expression levels were significantly related to overall survival ($p = 0.011$, $p = 0.008$, and $p = 0.003$, resp., Table III). Multivariate analysis showed lymph node metastasis, TNM stage, and miR-300 expression levels were independent prognostic factors ($p < 0.014$, $p = 0.011$, and $p = 0.007$, resp., Table III).

**Discussion**

miRNAs do not encode any proteins. However, over the past decades, it has known to us that they participate in the regulation of cellular differentiation, proliferation, apoptosis, and development\(^{15,16}\). Novel prognostic markers are very important in the diagnosis and treatment of LSCC, and miRNAs are currently one of the promising candidates. Previous studies have shown the role of miR-300 in different types of tumors. For instance, Xue et al\(^{17}\) showed that the expression of miR-300 was upregulated in osteosarcoma tissues, and overexpression of miR-300 promoted cell proliferation and invasion and induced EMT. Furthermore, They also identified BRD7 as a target of miR-300. Another study by Liu et al\(^{18}\) revealed that serum miR-300 was an independent prognostic marker for osteosarcoma. Shen et al\(^{19}\) found that over-expression of miR-300 could promote cell proliferation and cell cycle progression by targeting p53. Zhou et al\(^{20}\) reported that miR-300 expression was downregulated in glioblastoma tissues, and...
miR-300 expression in LSCC

Table III. Univariate and multivariate analysis of overall survival in LSCC patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>Univariate 95% CI</th>
<th>p-value</th>
<th>HR</th>
<th>Multivariate 95% CI</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>1.41</td>
<td>0.55-2.63</td>
<td>0.461</td>
<td></td>
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<tr>
<td>Gender</td>
<td>1.24</td>
<td>0.83-2.11</td>
<td>0.335</td>
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<tr>
<td>Thyroid cartilage invasion</td>
<td>1.17</td>
<td>0.58-1.77</td>
<td>0.148</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T classification</td>
<td>1.63</td>
<td>0.79-2.26</td>
<td>0.231</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lymph node metastasis</td>
<td>3.23</td>
<td>1.13-3.89</td>
<td>0.011</td>
<td>3.12</td>
<td>0.98-3.16</td>
<td>0.014</td>
</tr>
<tr>
<td>TNM stage</td>
<td>2.66</td>
<td>1.21-3.15</td>
<td>0.008</td>
<td>2.16</td>
<td>0.77-2.47</td>
<td>0.011</td>
</tr>
<tr>
<td>miR-300 expression</td>
<td>2.32</td>
<td>0.78-2.67</td>
<td>0.003</td>
<td>1.89</td>
<td>0.66-2.33</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Conclusions

The expression of miR-300 was decreased in LSCC tissues, suggesting that miR-300 may be a negative prognostic factor for LSCC patients. We provided the evidence that miR-300 may have a diagnostic and therapeutic potential in LSCC.

Conflict of interest

The authors declare no conflicts of interest.

References

12. Zhao XD, Zhang W, Liang HJ, Ji WY. Overexpression of miR-155 promotes proliferation and inva-


