Decreased expression of microRNA-124 is an independent prognostic factor in patients with cervical cancer

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Abstract: Purpose: Recently, microRNA-124 (miRNA-124 or miR-124) has been demonstrated as a potential tumor suppressor in several types of cancers. However, the role of miR-124 in cervical cancer remains unclear. This study was aimed at investigating the prognostic significance of miR-124 in cervical cancer. Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to analyze miR-124 expression in 127 cervical cancers tissues and matched adjacent normal tissues. The expression of miR-124 was assessed for the correlation with clinicopathologic characteristics and matched adjacent normal tissues. The expression of miR-124 was assessed for the correlation with clinicopathologic characteristics and matched adjacent normal tissues. The overall survival of patients with different level of miR-124 expression was analyzed by the Kaplan-Meier analysis. The influences of clinicopathologic characteristics and miR-124 in the prognosis of cervical cancer were estimated via Cox regression analysis. Results: The expression of miR-124 was lower in cervical cancer tissues compared with adjacent normal tissues according to qRT-PCR (P<0.001). Low expression of miR-124 was closely correlated with FIGO stage (P=0.041), vascular invasion (P=0.021) and lymph node metastasis (P=0.020). Patients with low miR-124 expression had a significantly shorter overall survival than those with high miR-124 expression (P<0.05). Multivariate analysis revealed that low miR-124 expression could be an independent bio-marker in the prognosis of cervical cancer (P=0.044, HR=2.759, 95% CI=1.027-7.413). Conclusions: MiR-124 was decreased and might play a certain role in the development of cervical cancer. The down-regulation expression of miR-124 may be an independent prognostic factor in patients with cervical cancer.

Keywords: microRNA-124, prognosis, cervical cancer

Introduction

According to WHO reports, cervical cancer is the third most common cancer in the women all over the world [1]. The main cause of cervical cancer is a persistent infection with high-risk (hr)-human papillomavirus (HPV) [2]. And HPV-based strategies may be a promising therapeutic methods [3]. Meanwhile, the radiotherapy, chemotherapy and surgery were used as standard treatment modalities for patients with cervical cancer recently. However, as there are many factors such as hormonal contraceptive, smoking, parity, number of sexual partners, and molecular alterations seem to be promoter to this cancer [4-7]. Therefore, it is necessary to find a new effective prognostic markers and therapeutic strategies to improve the treatment of cervical cancer.

MicroRNAs (miRNAs) are a class of small non-coding RNAs with 18-23 nucleotides in length. It can regulate the stability and expression efficiency of mRNAs at the post-transcription level mainly by binding to 3’-UTR of target mRNAs, leading to mRNA degradation or translation inhibition [8, 9]. miRNAs can not only act as an oncogene but also can serve as tumor suppressor in various cancers. And depending on the targets of the miRNAs, which may provide insights into the functional detection of human malignancies [10]. MicroRNA-124 (miR-124) is one of the most abundant miRNAs in the central nervous system and has been shown to play a key role in the pathogenesis of various cancers [11-17]. In previous studies, miR-124 had been confirmed to repress vasculogenic mimicry, migration and invasion in cervical cancer by targeting amotL1 in vitro and its down-
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Regulation had also been observed cervical which may be caused by DNA methylation-based silencing [13, 18]. Nevertheless, the clinical and prognostic significance of miR-124 expression in cervical cancer has not been determined yet.

In this study, we aimed to investigate the expression of miR-124 in cervical cancer and further explore the prognostic significance of miR-124 in cervical cancer.

Methods and materials

Samples collection

The tumor tissues and adjacent normal tissues were obtained from 127 patients who were diagnosed as cervical cancer. All patients recruited in this study were not subjected to preoperative radiotherapy and/or chemotherapy. This study was approved by the Ethical Committee at the affiliations, and the written consents were obtained from all these participants in advance.

Tumor specimens and corresponding adjacent normal tissues were collected and frozen by liquid nitrogen immediately, then stored at -80°C for RNA extraction. Clinicopathologic characteristics including age, tumor size, histology type, FIGO stage, differentiation, lymph node metastases and vascular invasion were recorded in a database. A 5-years’ follow-up was conducted and the information was updated through a telephone or questionnaire. The overall survival of patients was defined as the day of surgery to the day of death. Patients died from unexpected events or other disease was excluded in our study.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tumor tissues and adjacent normal tissues using the TRIzol reagent (Invitrogen, CA, USA). The expression of miR-124 was determined by RT-PCR with SYBR Premix Ex Taq II kit (Takara, Dalian City, Liaoning Province, China) according to the manufacturer’s instructions. RNU6B was taken as the endogenous control. The PCR primers for miR-124 or RNU6B were designed as follows: miR-124 forward, 5’-GATACTCATAAGGCACGCGG-3’ and reverse, 5’-GTGCAGGGTCCGAGGT-3’. RNU-6B forward, 5’-TGCGGGTGCTCGCTTCGGCAGC-3’ and reverse 5’-CCAGTGCAGGGTCCGAGGT-3’. The relative expression level of miR-124 was calculated by the 2^−ΔΔCt method. The experiment was operated in triplicate.

Statistical analysis

The software of SPSS version 18.0 for Windows was used for statistical analysis. Comparisons of miR-124 levels between cervical cancer tissues and adjacent normal tissues were performed using T-test. The correlation between miR-124 expression and clinicopathologic characteristics of patients with cervical cancer was evaluated by X^2-test. Association of miR-124 expression with overall survival was estimated by Kaplan-Meier analysis, and the resulting curves were compared using the log-rank test. The multivariate Cox proportional hazard regression analysis was used to evaluate the prognostic factors including miR-124 and clinicopathologic characteristics of patients with cervical cancer.
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Table 1. Correlation between miR-124 expression and clinicopathologic characteristics in cervical cancer patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case (n)</th>
<th>High miR-124 expression (n)</th>
<th>Low miR-124 expression (n)</th>
<th>X²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>127</td>
<td>57</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
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</tr>
<tr>
<td>&lt;50</td>
<td>52</td>
<td>23</td>
<td>29</td>
<td>0.036</td>
<td>0.849</td>
</tr>
<tr>
<td>≥50</td>
<td>75</td>
<td>34</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4</td>
<td>49</td>
<td>22</td>
<td>27</td>
<td>0.000</td>
<td>0.998</td>
</tr>
<tr>
<td>&gt;4</td>
<td>78</td>
<td>35</td>
<td>43</td>
<td></td>
<td></td>
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<tr>
<td>Histology</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous</td>
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<td>44</td>
<td>47</td>
<td>1.562</td>
<td>0.211</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>36</td>
<td>13</td>
<td>23</td>
<td></td>
<td></td>
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<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ib-Illa</td>
<td>63</td>
<td>34</td>
<td>29</td>
<td>4.172</td>
<td>0.041</td>
</tr>
<tr>
<td>IIb-Illa</td>
<td>64</td>
<td>23</td>
<td>41</td>
<td></td>
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<td>Differentiation</td>
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<td></td>
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<td></td>
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<tr>
<td>Well + moderate</td>
<td>82</td>
<td>35</td>
<td>47</td>
<td>0.452</td>
<td>0.501</td>
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<tr>
<td>Poor</td>
<td>45</td>
<td>22</td>
<td>23</td>
<td></td>
<td></td>
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<td>Lymph nodes metastasis</td>
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</tr>
<tr>
<td>Yes</td>
<td>85</td>
<td>32</td>
<td>53</td>
<td>5.438</td>
<td>0.020</td>
</tr>
<tr>
<td>No</td>
<td>42</td>
<td>25</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>52</td>
<td>17</td>
<td>35</td>
<td>5.289</td>
<td>0.021</td>
</tr>
<tr>
<td>No</td>
<td>75</td>
<td>40</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

cervical cancer. P<0.05 was considered that the difference was statistically significant.

Results

The expression of miR-124 in the cervical cancer tissues and adjacent normal tissues

We firstly examined miR-124 expression level in 127 cervical cancer tissues and matched adjacent normal tissues by qRT-PCR. As shown in Figure 1, after normalization to RNU6B expression levels, the expression level of miR-124 in cervical cancer tissues was significantly lower than that in adjacent normal tissues (0.5816 ± 0.3252 vs. 0.8046 ± 0.4625; P<0.001). The result indicated that miR-124 might be a tumor suppressor in cervical cancer.

Correlations between miR-124 and clinicopathologic characteristics

The relationships between miR-124 expression and clinicopathologic characteristics were analyzed by Chi-square test. As shown in Table 1, the expression of miR-124 in cervical cancer was significantly associated with FIGO stage (P=0.041), vascular invasion (P=0.021) and lymph node metastasis (P=0.020). However, there were no correlation with other clinicopathologic characteristics such as age (P=0.849), tumor size (P=0.998), histology type (P=0.211) and differentiation (P=0.501). This might demonstrated that miR-124 was related to the development of cervical cancer.

Associations between miR-124 expression and overall survival of patients with cervical cancer

The association between miR-124 expression and overall survival of cervical cancer patients was investigated by Kaplan-Meier analysis and log-rank test. As shown in Figure 2, cervical cancer patients with low miR-124 expression tend to have a shorter overall survival than those with high miR-124 expression (log-rank test, P<0.05). Multivariate analysis using the Cox proportional hazards model for all variables showed that low miR-124 expression was an important prognostic factor and might be an independent prognostic indicator for patients with cervical cancer (P=0.044, HR=2.759, 95% CI: 1.027-7.413, Table 2).

Discussion

Cervical cancer remains to be one of the leading causes of mortality among women [19]. The incidence of this disease is mostly high in developing countries in spite of the presence of screening programs [1]. Despite the early stage diagnosis and treatment over high-risk population is an effective method that can reduce the
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incidence and mortality of cervical cancer, the prognosis of cervical cancer is still poor [20-22]. Therefore, finding new molecular targets for its prognosis has the potential to improve the clinical strategies and outcomes of this disease.

More and more studies indicated that the molecular mechanisms of carcinogenesis are not only relevant to protein coding genes but also to miRNAs which are non-protein coding. Due to its various regulations on gene expression, miRNAs participate in multiple cellular functions, such as proliferation, apoptosis, differentiation, cancer carcinogenesis and progression [23-25]. Many reports had indicated that various miRNAs were related to the occurrence, development and prognosis of cervical cancer. For instance, miR-335 was found to be decreased and a useful prognostic marker in cervical cancer in the study of Wang et al [26]. Yang et al., confirmed that miR-494 was an essential role in the carcinogenesis and progression of cervical cancer as it could promote cell proliferation by targeting with PTEN [27]. According to the study of Wang et al, the down-regulation of miR-145 act as an important prognostic indicator in cervical cancer [28]. MiR-124 was abnormal expressed in several cancers such as colorectal cancer, breast cancer, osteosarcoma, lung cancer, gastric cancer and so on [29-33]. Besides, miR-124 family is hypermethylated to a high degree in high-grade cervical lesions and could repress the EMT process in cervical cancer [13, 18]. However, its prognostic value is unclear in cervical cancer. Therefore, identifying the function of miR-124 may help to find a new bio-marker for the prognosis of this cancer which is enable deeper insight into the regulation of gene expression and the complexity of cancer progression. In this study, we investigated the expression of miR-124 in cervical cancer tissues and adjacent normal tissues by qRT-PCR. The result demonstrated the down-regulation of miR-124 in cervical cancer which was consistent with the previous studies [34].

Based on the relative expression level analysis, the association of miR-124 with clinicopathologic characteristics was analyzed to see whether it was involved in the development of cervical cancer. As a result, the expression of miR-124 was proved to be influenced by FIGO

Table 2. Multivariate analysis for factors influencing the overall survival rate of cervical patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-124 (low/high)</td>
<td>2.759</td>
<td>1.027-7.413</td>
<td>0.044</td>
</tr>
<tr>
<td>FIGO stage</td>
<td>1.037</td>
<td>0.624-1.725</td>
<td>0.888</td>
</tr>
<tr>
<td>Lymph nodes metastasis</td>
<td>1.003</td>
<td>0.564-1.783</td>
<td>0.993</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>1.405</td>
<td>0.819-2.409</td>
<td>0.217</td>
</tr>
</tbody>
</table>

Figure 2. Kaplan-Meier analysis of miR-124 expression which was used to estimate the association between it and overall survival of patients. Patients with low miR-124 expression had a shorter overall survival than those with high miR-124 expression (Log-rank test, \( P = 0.025 \)).
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stage, vascular invasion, and lymph node metastasis significantly. However, miR-124 expression was not associated with patient’s age, tumor size, histology and differentiation. Combing with present results, it is thus speculated that miR-124 may play a tumor suppressor role in cervical cancer progression.

We also estimated the prognostic value of miR-124 for its specific expression in cervical cancer. Kaplan-Meier analysis and log-rank test manifested the overall survival was strengthened by high miR-124 expression as patients with low miR-124 expression lived much shorter than those with high miR-124 expression. Then, Cox regression analysis adjusted for clinicopathologic characteristics showed that the miR-124 expression could be an independent prognostic marker in patients with cervical cancer. These data indicated that miR-124 expression play a crucial role in tumorigenesis, and progression of cervical cancer.

In conclusion, we discover that miR-124 is down-regulated in cervical cancer tissues and closely correlation with tumor progression. Furthermore, miR-124 is identified as an independent factor for predicting the clinical outcome of cervical cancer patients. The down-regulation of miR-124 plays an important role in cervical cancer progression and a novel prognostic biomarker.

Disclosure of conflict of interest

None.

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