Original Article

Expression of miR-203 is decreased and associated with the prognosis of melanoma patients

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Received April 17, 2015; Accepted May 29, 2015; Epub October 1, 2015; Published October 15, 2015

Abstract: MicroRNAs (miRNAs or miRs) are a class of small, non-coding RNAs that can regulate the gene expression in various diseases. MicroRNA-203 (miRNA-203 or miR-203) has previously shown significant alteration in a number of cancers. However, the clinical value of miR-203 in melanoma is rarely reported. The present study aimed to clarify the expression pattern and prognostic role of miR-203 in melanoma patients. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was used to characterize the expression level of miR-203 in 148 cases of melanoma tissues and adjacent non-cancerous tissues. Results showed that miR-203 expression was significantly decreased in melanoma tissues compared with that in adjacent non-cancerous tissues (P<0.05). Additionally, chi-square was performed to analyze the relationship between miR-203 and clinicopathological features and the down-regulation of miR-203 was significantly associated with tumor thickness and tumor stage (P<0.05). Moreover, Kaplan-Meier analysis showed that low miR-203 expression was associated with short overall survival time of patients. Multivariate analysis indicated that miR-203 could be an independent prognostic marker (P=0.003, HR=2.851, 95% CI=1.439-5.650) in melanoma. This study for the first time provided evidence that miR-203 could be an independent potential prognostic marker for patients with melanoma, and might even become a new therapeutic target for the treatment of melanoma.

Keywords: Melanoma, miR-203, prognosis

Introduction

Cutaneous malignant melanoma represents the primary cause of death among skin cancers and its incidence rate is increasing in recent years [1]. Most melanomas could detect at early stage but once it develops to the metastatic period it might expand to be an incurable skin disease and the median survival is very poor [2, 3]. The most relevant prognostic factors for primary melanoma without metastases are vertical tumor thickness (Breslow’s depth) and the presence or absence of histological ulceration while the influence of mitotic activity and invasion level (Clark’s level) have less effect [4]. However, some patients with thin neoplasms often face recurrence, metastases and death after surgical excision, while those with thick melanomas do not suffer this phenomenon according to clinical experience. New prognostic markers defined by gene expression profiling, have been established such as metallothionines or genetic subtypes, but additional reliable markers to identify the patients for early therapy are urgently needed [5, 6]. Besides, exploring the potential molecular mechanisms involved in melanoma progression and identifying the important molecular markers are of great meaning for the improvements of therapies for metastatic melanomas.

MicroRNAs (miRNAs or miRs) have recently been identified as a kind of short (18-25 nucleotides), noncoding, single stranded, small RNA molecules that can induce post-transcriptional silencing by binding to the complementary region of the 3′-untranslated region of their target mRNA [7-10]. Recently, a number of miRNAs, including miR-203, miR-204-5p, miR-205-5p, miR-211-5p, miR-23b-3p, miR-26a-5p and miR-26b-5p have been demonstrated to play important roles in melanoma [11-13]. miR-203 locates at chromosome 14, has been confirmed to be abnormally expressed and involved in many processes of various malignant diseases, such as breast cancer, lung cancer, and squa-
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Table 1. Relationship between miR-203 expression and clinicopathological characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n)</th>
<th>miR-203 expression</th>
<th>χ²</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n=92)</td>
<td>High (n=56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>80</td>
<td>53</td>
<td>1.237</td>
<td>0.266</td>
</tr>
<tr>
<td>Female</td>
<td>68</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>70</td>
<td>42</td>
<td>0.264</td>
<td>0.607</td>
</tr>
<tr>
<td>≥65</td>
<td>78</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1.0</td>
<td>63</td>
<td>33</td>
<td>4.462</td>
<td>0.035</td>
</tr>
<tr>
<td>≥1.0</td>
<td>85</td>
<td>59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulceration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>108</td>
<td>68</td>
<td>0.109</td>
<td>0.741</td>
</tr>
<tr>
<td>+</td>
<td>40</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>73</td>
<td>43</td>
<td>0.650</td>
<td>0.420</td>
</tr>
<tr>
<td>+</td>
<td>75</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>57</td>
<td>29</td>
<td>5.019</td>
<td>0.025</td>
</tr>
<tr>
<td>III</td>
<td>91</td>
<td>63</td>
<td></td>
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<tr>
<td>Tumor subtype</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALM</td>
<td>103</td>
<td>61</td>
<td>5.467</td>
<td>0.065</td>
</tr>
<tr>
<td>NM</td>
<td>32</td>
<td>20</td>
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<td></td>
</tr>
<tr>
<td>SSM</td>
<td>13</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ALM: Acral lentiginous melanoma; NM: Nodular melanoma; SSM: Superficial spreading melanoma; χ²: Chi-square distribution.

Patients and specimens

Fresh melanoma specimens and adjacent normal tissues were collected from 148 patients who underwent surgery between in Henan Provincial People’s Hospital. The present study was approved by the Ethics Committee of the hospital. The written informed consents had been signed by all patients in advance. None patients had received any radiotherapy or chemotherapy prior to surgery. The tissue samples were frozen in liquid nitrogen and stored at -80°C for RNA isolation. The clinicopathological characteristics of the patients were presented in Table 1. A complete follow-up was conducted for at least 5 years. Overall survival was defined as the interval from the end of treatment to the death date of the patients with melanoma.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the tumor tissues and paired adjacent tissues of 148 melanoma patients using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instruction. The miR-203 and U6 internal control-specific cDNAs were synthesized from the total RNA using gene-specific primers according to the TaqMan MicroRNA assays protocol (Invitrogen, Carlsbad, CA, USA). The primers were as follows: for miR-203 F: 5’-ACA CTC CAG CTG GCG TGA ATT TAG GAC CA-3’; R: 5’-CTC AAC TGG TGT CGT GGA-3’; for U6 F: 5’-CTC GCT TCG GCA GCA CA-3’; R: 5’-AAC GCT TCA CGA ATT TGC GT-3’. The reverse transcrip-
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Figure 2. Kaplan-Meier survival analysis in patients with melanoma. The result indicated that patients with low miR-203 expression lived shorter than those with high miR-203 expression (P=0.001).

Table 2. Multivariate analysis for prognostic factors in melanoma

<table>
<thead>
<tr>
<th>Variables</th>
<th>P values</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness</td>
<td>0.073</td>
<td>0.639</td>
<td>0.392-1.043</td>
</tr>
<tr>
<td>Tumor stage</td>
<td>0.995</td>
<td>1.002</td>
<td>0.604-1.660</td>
</tr>
<tr>
<td>Low-MiR-203-expression</td>
<td>0.003</td>
<td>2.851</td>
<td>1.439-5.650</td>
</tr>
<tr>
<td>High-MiR-203-expression</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Statistical analysis

The difference of miR-203 expression between melanoma tissues and adjacent tissues was compared with students’t test. The association between miR-203 expression and clinicopathological characteristics was analyzed via chi-square test. Kaplan-Meier analysis was used to estimate the relationship between miR-203 and overall survival of melanoma patients, and difference of the survival time of patients was evaluated by log-rank test. The prognostic values of miR-203 and clinicopathological characteristics were identified through Cox regression analysis. The difference was considered to be statistically significant when the P value was less than 0.05.

Results

miR-203 expression was decreased in melanoma tissues

The expression of miR-203 in melanoma tissues and adjacent tissues was analyzed by qRT-PCR. The relative expression of miR-203 in the melanoma tissues normalized to U6 was 1.36 ± 0.32 (mean ± SD), while the relative expression of miR-203 in adjacent tissues was 1.59 ± 0.27. The statistical analysis showed that the expression level of miR-203 was significantly lower in melanoma tissues than in adjacent tissues (P<0.05, Figure 1). The result suggested that miR-203 might play a role as a tumor suppressor in melanoma.

Relationship between miR-203 and clinicopathological characteristics of melanoma patients

To facilitate further analysis of the association of miR-203 expression with clinicopathological characteristics and prognosis, we manually divided the melanoma patients into two groups. The patients with a miR-203 expression lower level than 1.50 were attributed into low miR-203 expression group, while those with miR-203 expression level higher than 1.50 were defined as high miR-203 expression group. 92 cases were classified into low miR-203 expression group, while 56 cases were belonged to the high expression group.

To explore the potential role of miR-203 in tumor progression, we further investigated the relationship of miR-203 expression with clinico-
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pathological characteristics of these patients. Results showed that miR-203 expression was significantly associated with tumor thickness ($P=0.035$) and tumor stage of melanoma ($P=0.025$). However, no association was found between miR-203 expression and other characteristics, including sex, age, ulceration, lymph node metastasis, and tumor subtype. It suggested that miR-203 might be closely associated with the progression of melanoma.

The relationship between miR-203 and the overall survival of patients with melanoma

During the entire follow-up period, 67 patients with melanoma had died and the follow-up rate was 54.7%. Overall survival curves were plotted according to miR-203 expression level by the Kaplan-Meier method. As shown in Figure 2, patients with low miR-203 expression had a significantly shorter overall survival time than those with high miR-203 expression (log-rank test, $P=0.001$). Moreover, as seen in Table 2, Cox regression analysis indicated that miR-203 expression (HR=2.851, 95% CI=1.439-5.650, $P=0.003$) was an independent prognostic factor for melanoma patients.

Discussion

Cutaneous malignant melanoma is a highly aggressive disease which arises from melanocytes and has the characteristics of aggressive invasion, early metastasis, and resistance to chemotherapy or radiotherapy [19, 20]. It can affect melanocytes, behavioral modifications such as skin self-examination and its development may be avoided through the use of sunscreen and protective clothing which can prevent the harm of UV light [21]. Although the diagnosis of melanoma has been improved a lot, the prognosis is still poor [22]. Therefore, it is necessary to find new and effective prognostic markers for melanoma.

miRNAs may offer a new regulatory model of gene expression, and the expression pattern of miRNAs is closely correlated with cancers’ specific clinical characteristics, so that they can be used to classify normal and cancerous samples, as well as to estimate the prognosis of diseases [23]. MiR-203 has been found to exhibit abnormal expression in various cancers such as pancreatic adenocarcinoma, epithelial ovarian cancer, esophageal squamous cell carcinoma, cervical cancer, bladder cancer, prostate cancer [24-30]. In our study, the miR-203 expression was detected in 148 melanoma specimens by qRT-PCR. The finding indicated that miR-203 expression was down-regulated in melanoma tissues compared with the adjacent non-cancerous tissues which were consistent with previous studies. To date, the associations between the expression of miR-203 and prognosis in melanoma have never been reported. This is the first study to investigate the impact of miR-203 on melanoma prognosis using a large number of clinical samples.

In addition, we also found that miR-203 expression was closely associated with melanoma tumor thickness and tumor stage as the study revealed that low expression of miR-203 was more frequently to be detected in tumors with larger tumor thickness or advanced tumor stage. These results indicated the possible participation of miR-203 in progression of melanoma. This association is consistent with previous findings in melanoma cells and cervical cancers [28, 31]. Based on the present results, together with the evidences above, it is thus proposed that miR-203 may play a tumor suppressor role in melanoma progression.

As miR-203 was found to be associated with tumor thickness and tumor stage of melanoma, considering that thickness and stage might be crucial factors affecting prognosis, we further evaluated the prognostic role of miR-203 in melanoma. According to the analysis between the relationship of miR-203 and overall survival by Kaplan-Meier, the overall survival time of patients with high miR-203 expression was longer than those with low miR-203 expression. In addition, we performed Cox regression analysis to evaluate the prognostic value of miR-203, sex, age and other clinical factors relating with survival of melanoma. Results proved that decreased miR-203 expression contributed as a marker for the prognosis of melanoma, and it might be utilized to identify high-risk individual patients with melanoma who were good candidates to receive more aggressive treatment. Based on available evidences, the positive association of miR-203 with progression and prognosis of melanoma may be at least partly caused by its targets, such as kinesin superfamily protein 5b (kif5b), which can reduce melanosome transport and promote melanosome transport and promote melanosome transport and promote melanosome transport.
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genesis [31]. However, the detailed investigation about this inference need to be further explored.

In conclusion, our investigation provides the convincing evidence for the first time that miR-203 expression is decreased in melanoma and associated with tumor progression, and it can serve as an independent prognostic factor for melanoma patients. However, there are some limitations. Firstly, the sample size is small which may lead to the accuracy is low. Secondly, the current study has not elucidated the exact molecular mechanisms of miR-203 acting on melanoma. To solve these problems, further studies with large-scale samples should be conducted.

Disclosure of conflict of interest

None.

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References

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