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Original Article

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

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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-

Table 1. Relationship between *miR-203* expression and clinicopathological characteristics

Variable	Cases (n)	*miR-203* expression		χ^2	*P* values
		Low (n=92)	High (n=56)		
Sex					
Male	80	53	27	1.237	0.266
Female	68	39	29		
Age (years)					
<65	70	42	28	0.264	0.607
≥65	78	50	28		
Tumor thickness (mm)					
<1.0	63	33	30	4.462	0.035
≥1.0	85	59	26		
Ulceration					
-	108	68	40	0.109	0.741
+	40	24	16		
Lymph node metastasis					
-	73	43	30	0.650	0.420
+	75	49	26		
Tumor stage					
I/II	57	29	28	5.019	0.025
III	91	63	28		
Tumor subtype					
ALM	103	61	42	5.467	0.065
NM	32	20	12		
SSM	13	11	2		

Abbreviations: ALM: Acral lentiginous melanoma; NM: Nodular melanoma; SSM: Superficial spreading melanoma; χ^2: Chi-square distribution.

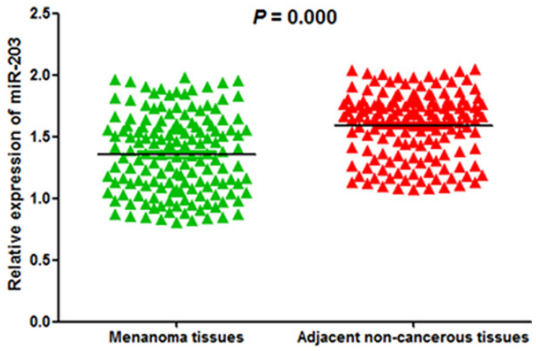

Figure 1. The expression level of *miR-203* in the tumor tissues and adjacent tissues of patients with melanoma. QRT-PCR demonstrated that the expression of *miR-203* was lower in melanoma tissues than in adjacent tissues ($P<0.05$). *U6* was used as an internal control.

mous cell carcinoma [14-16]. For example, Yu et al. reported that *miR-203* suppressed the proliferation and self-renewal of esophageal cancer stem-like cells by targeting BMI1 [17]. Sonkoly et al. detected that *miR-203* could serve as a tumor suppressor in basal cell carcinoma [18]. However, the clinical significance of *miR-203* in melanoma is still unclear.

The purpose of this study was to investigate the expression level of *miR-203* in melanoma tissues and explore the potential prognostic value of *miR-203* in melanoma patients.

Materials and methods

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 AAT GTT 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-

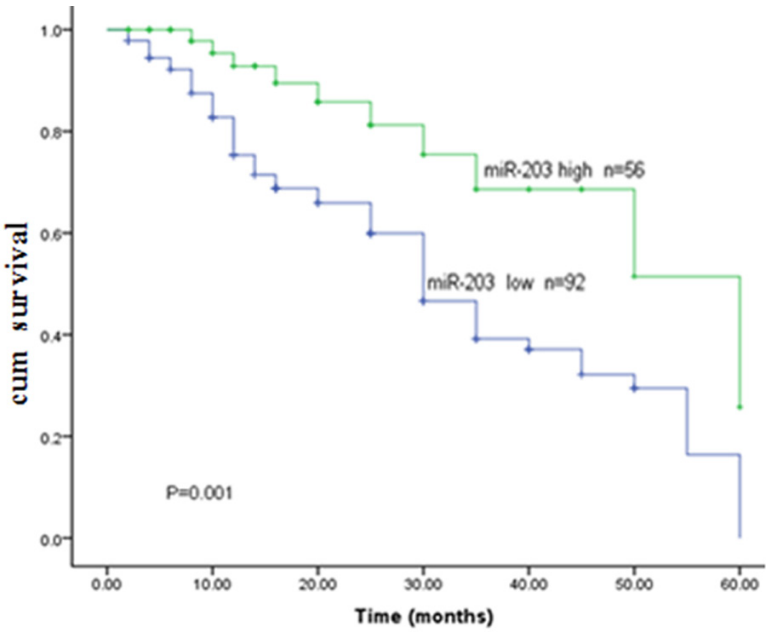

Figure 2. Kaplan-Meier survival analysis in patients with melanoma. The result indicated that patients with low *miR-203* expression lived shorter than those with high *miR-203* expression (*P*=0.001).

Table 2. Multivariate analysis for prognostic factors in melanoma

Variables	*P* values	HR	95% CI
Thickness	0.073	0.639	0.392-1.043
Tumor stage	0.995	1.002	0.604-1.660
Low-*MiR-203*-expresssion	0.003	2.851	1.439-5.650
High-*MiR-203*-expresssion	-	-	-

tion products were then amplified and detected by real-time PCR using a Taqman MicroRNA Assay (Applied Biosystems) specific for *hsa-miR-203* in an ABI 7500 system (Applied Biosystems). Each sample was run in triplicate, and the relative quantification of *miR-203* expression was evaluated by the comparative cycle threshold (CT) method.

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-

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 melano-

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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