Low Expression of Mir-137 Predicts Poor Prognosis in Cutaneous Melanoma Patients

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: The present study aimed to measure miR-137 expression in patients with cutaneous melanoma (CM) and to estimate the correlation of miR-137 expression and the prognosis of CM patients.

Material/Methods: The expression level of miR-137 was assayed by quantitative real-time PCR (qRT-PCR) and presented as mean ±SD. Chi-square was used to evaluate the relationship between miR-137 expression and clinical characteristics. We used a Kaplan-Meier survival curve to determine the overall survival rate of CM patients. Moreover, the correlation between miR-137 expression and the prognosis of CM patients was confirmed by Cox regression analysis.

Results: The relative expression of miR-137 in CM tissue was 1.59±0.43, while that in paired normal tissue was 2.41±0.54, which was significantly higher. Chi-square analysis showed statistical significance between miR-137 expression and clinical characteristics such as TNM stage, ulcer, and occurrence site ($P<0.05$). However, no association was found between miR-137 expression and age, sex, or family history ($P>0.05$). According to the survival curve outcome, patients with low miR-137 expression showed relatively higher mortality ($P=0.000$) and multivariate analysis verified that low expression of miR-137 predicted poor prognosis of CM patients (HR=8.531, 95% CI=2.950–24.668, $P=0.000$).

Conclusions: Compared with paired normal tissues, miR-137 expression was lower in CM tissues. Patients with low miR-137 expression had higher mortality than those with high miR-137 expression, suggesting that low miR-137 expression indicated poor prognosis for CM patients.

MeSH Keywords: Administration, Cutaneous • MicroRNAs • Prognosis

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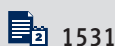
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Background

Skin cancer is one of the most common malignancies among people, whose incidence exceeds the sum of other malignant tumors [1–3]. Cutaneous melanoma (CM) is a member of skin cancers and is of high malignancy in clinical. It accounts for about 6.8–20% of skin cancers, with an increasing incidence of 3–8% every year [4,5]. CM is becoming the leading cause of skin cancer-related deaths. Therapy for CM is mainly surgery, and sometimes chemo- or radio- therapy is also used [6–8]. Because of its early metastasis and high mortality, it is important to diagnose and treat CM as early as possible [9]. In addition, most CM cases suffer from poor prognosis. Therefore, novel biomarkers are urgently needed for therapy and prognosis of CM patients.

MicroRNAs (miRNAs) are a kind of noncoding RNAs and consist of about 21–23 nucleotides, which can regulate the gene expression at post-transcriptional level by specific binding to the messenger ribonucleic acids (mRNAs) [10,11]. They also play important roles on some other aspects, such as differentiation and apoptosis of cells, biological development, and disease processes [12–14]. MiRNAs offer great potential as cancer biomarkers.

miR-137, located on chromosome 1p22 [15], is a brain-enriched micro-RNA with important roles during neurogenesis, including the proliferation and differentiation of neural stem cells, the regulation of dendritic morphogenesis, and synaptic plasticity [16]. Several existing reports confirmed that miR-137 participated in the development and processes of various cancers, such as gastric cancer, head and neck cancer, colorectal cancer, and brain tumors [17–20]. Previous studies have also demonstrated that miR-137 expression is associated with melanoma progression [21,22]. Bemis et al. first reported that miR-137 downregulated MITF, which is an important regulator in melanocyte development [23]. Recently, Chen et al. demonstrated that miR-137 inhibited melanoma cell proliferation by down-regulation of MITF and cyclin-dependent kinase 6 [24].

Although considerable evidence has confirmed that miR-137 can serve as a cancer suppressor or candidate site for cancer therapies, proof of a link between miR-137 and its clinical value in CM is still lacking. Therefore, additional studies are needed to elucidate the function of miR-137 in CM. In this study, we aimed to explore the miR-137 expression in CM tissues and paired normal tissues and evaluate its possibility as a prognostic biomarker for CM patients.

Material and Methods

Patients and samples

A total of 97 patients, who were diagnosed with malignant cutaneous melanoma at Guangdong General Hospital, were enrolled in this study. Among them, 55 were males and the others were females. Clinical characteristics of CM patients were collected based on the hospital records. Our study was approved by the Ethics Committee of Guangdong General Hospital. Patients selected in the study all signed consent forms. Fresh CM tissues and paired normal tissues were reserved to use immediately after resection.

A 5-year follow-up was performed with all CM patients. The data, including age, sex, family history, TNM stage, ulcer, and occurrence site, were recorded in a database. The information about follow-up was updated every 3 months by telephone or questionnaire.

RNA extraction and qRT-PCR

Total RNA was isolated and purified from tissues by Trizol Reagent (Ambion, Foster City). Then the RNA was used to synthesize the cDNAs of miR-137 with M-MLV reverse transcriptase (Promega, Madison, WI) according to the manufacturer's directions. The reverse transcriptive products were amplified and assayed by qRT-PCR. Each sample was determined in triplicate under optimal conditions. U6 was used as an endogenous standard. The relative expression of miR-137 was calculated with cycle threshold (CT) method and was normalized to U6. The data are presented as mean ± standard deviation (SD).

Statistical analysis

All computations were carried out with SPSS 18.0 software. The relationship of miR-137 expression and clinical characteristics was confirmed by chi-square testing. Kaplan-Meier survival analysis was performed to estimate the overall survival rate of CM patients, and the differences between the survival curves were tested by using the log-rank test. Correlation between miR-137 expression and prognosis of CM patients was evaluated by Cox regression analysis. Statistical difference was considered to be significant when P value was less than 0.05.

Results

Down-regulation of miR-137 in CM tissues

We used qRT-PCR to determine the expression of miR-137. The relative expression of miR-137 in CM tissues was 1.59±0.43 (mean ±SD), while that in normal tissues was 2.41±0.54,

indicating that miR-137 expression was lower in CM tissues (Figure 1, *P*<0.05).

Relationship between miR-137 expression and clinical characteristics

Further detection was conducted to explore the potential relationship of the miR-137 expression and clinical characteristics. We manually divided the CM tissues into 2 groups: the low expression group had a miR-137 expression level of less than 1.35, and others belonged to the high expression group. miR-137 expression was tightly associated with clinical characteristics, such as TNM stage, ulcer, and occurrence site (*P*<0.05). However, no significant relation was found between miR-137 expression and age, sex, and family history (Table 1, *P*>0.05).

Low expression of miR-137 presented poor prognosis in CM patients

A follow-up was conducted to estimate the overall survival rate of CM patients. The follow-up lasted for about 60 months and 53 patients died. Among the dead patients, 49 were in the low miR-137 expression group and the rest were in the high
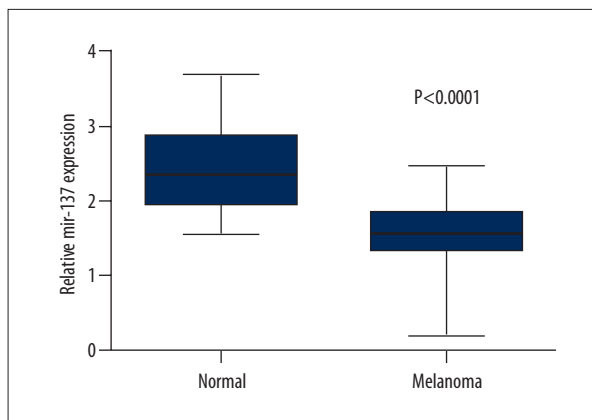

Figure 1. The expression levels of miR-137 both in CM tissues and paired normal tissues were measured by qRT-PCR. The expression levels of miR-137 were presented as mean ±SD. The result showed that miR-137 expression was lower in CM tissues by comparison with normal tissues.

miR-137 expression group. Kaplan-Meier survival curve showed that the CM patients with low miR-137 expression presented significantly shorter survival time compared to those with high

Table 1. The association between clinical characteristics and the *miR-137* expression.

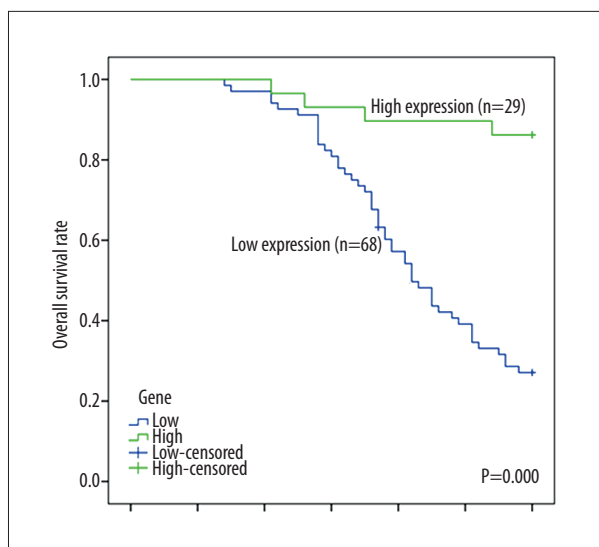| Clinical characteristics | Case (n) | miR-137 expression | | χ^2 | *P* value |
		Low	High		
Age (years)				0.001	0.997
≤20	60	42	18		
>20	37	26	11		
Gender				0.039	0.843
Male	55	39	16		
Female	42	29	13		
Family history				1.712	0.191
Yes	50	38	12		
No	47	30	17		
TNM stage				5.882	0.015
I, II	52	31	21		
III	45	37	8		
Ulcer				3.923	0.048
Yes	45	36	9		
No	52	32	20		
Occurrence site				5.432	0.020
Trunk + head	51	41	10		
Extremities	46	27	19		

Figure 2. The overall survival rate of CM patients was evaluated by Kaplan-Meier survival analysis. Patients with low miR-137 expression showed higher mortality than those with high miR-137 expression.

miR-137 expression (Figure 2, *P*=0.000). In addition, the correlation of miR-137 expression and prognosis of CM patients was evaluated by Cox regression analysis and the results revealed that low expression of miR-137 was an independent prognostic marker of CM patients (Table 2, HR=8.531, 95% CI=2.950–24.668, *P*=0.000).

Discussion

CM is a common skin tumor that derives from excessive hyperplasia of abnormal melanocytes. Clinical manifestations include bleeding, itching, tenderness, and ulcer [25]. Because of its high malignancy, incidence, and mortality, CM has drawn more and more attention. Previous studies have demonstrated that malignant melanoma patients have a very poor prognosis, with median survival of 6–10 months and a 5-year survival rate of <5% [26]. It is urgent to find new strategies and novel indicators to improve the diagnosis, treatment, and prognosis of CM patients. In recent years, miRNAs have been recognized as important factors in various cancers, including CM.

miRNAs are single-stranded fragments with about 21 nucleotides and they participate in many kinds of cell regulation. They have no open reading frame and encode no protein. Each miRNA may have several target genes or several miRNAs that co-regulate the same gene. These agents make miRNA tightly related to metabolism, cell cycle, cell differentiation, cell apoptosis, and development of individuals. The misregulation of miRNAs has been verified to be related with cancer initiation, promotion, and progression [27,28]. Functionally, miRNAs can serve as diagnostic and prognostic biomarkers for cancers. Since miR-137 was first found as a regulator via targeting MITF [23], there has been increasing interest in the role of miR-137 in the development and progression of different tumors. Many studies have reported that miR-137 presented significant down-regulation in various cancers [29,30].

In the present study, we investigated the miR-137 expression in CM tissues and evaluated the relationship between miR-137 expression and the prognosis of CM patients. The results showed that the miR-137 expression level in CM tissues was lower than in paired normal tissues, which was in accord with existing reports. The association of miR-137 expression and clinical characteristics was evaluated by chi-square. The result revealed that miR-137 expression was closely related with TNM stage, ulcer and occurrence site. miR-137 has been proved to be a tumor suppressor in several diseases. Zhang et al. verified that miR-137 acted as a tumor suppressor in non-small cell lung cancer [31]. Zheng et al. showed that miR-137 serves as a suppressor for gastric cancer [32]. Based on previous evidence, further investigations were conducted in this study. The survival curve showed that patients with low miR-137 expression had shorter survival time than those with high miR-137 expression. Moreover, the Cox regression analysis showed statistical significance between miR-137 expression and prognosis of CM patients, suggesting miR-137 was a target biomarker for CM. Thus, we presumed that miR-137 might act as a prognostic factor for CM patients.

Although several targets of miR-137 have been identified in various cancers, the mechanism of miR-137 on CM remains unclear. Cheng et al. demonstrated that miR-137 functioned on gastric carcinoma by targeting PI3K/AKT signaling pathway [17]. Shin et al. [33] illustrated that miR-137 could regulate the p53 level in human keratinocytes. Besides, some research

Table 2. The multivariate analysis of prognostic factors in CM.

Clinical characteristics	P value	HR	95% CI
TNM stage	0.486	1.313	0.610–2.823
Occurrence site	0.889	1.052	0.518–2.133
miR-137 expression	0.000	8.531	2.950–24.668

outcomes indicated that c-Met and YB1 were candidate targets of miR-137 in malignancy melanoma [34]. Interestingly, a recent study has shown that c-Met expression was decreased by transfection of miR-137 in the melanoma cells [24]. Therefore, we conjectured that miR-137 might affect CM by regulating the expression of its candidate target genes like MITF, c-Met and YB1, or by regulating some signaling pathways. This hypothesis needs to be validated by more studies, which might provide directions for future research.

Conclusions

There was a statistically significant relationship between miR-137 expression and clinical characteristics. Low expression of miR-137 predicted poor prognosis for CM patients. Thus, miR-137 could be regarded as a prognostic biomarker for CM patients.

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