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Original Article

Down-regulated microRNA-625 is a prognostic biomarker in cutaneous melanoma

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Abstract: The deregulation of miR-625 and its clinical significance has been investigated in many types of cancer, however, until now, the link between miR-625 expression and the clinicopathological features of cutaneous melanoma have not yet been reported. In present study, we used real-time PCR to analyze the expression of miR-625 in tissue samples. The Chi-square test was used to assess miR-625 expression with respect to clinicopathological parameters. The overall survivals of patients were performed using Kaplan-Meier model with log-rank test. Hazard ratios and confidence intervals at 95% were analyzed using cox proportional hazard regression model. Compared with the adjacent normal tissues, the expression levels of miR-625 were significantly decreased in cutaneous melanoma tissues ($P<0.05$). Its expression level was significantly associated with tumor thickness ($P<0.05$) and stage ($P<0.05$). The overall survival rate was significantly lower in the patients with low miR-625 level than in those with high level ($P<0.05$). Furthermore, multivariate analysis of the prognosis factors confirmed that low miR-625 expression was an independent predictor of poor survival in cutaneous melanoma ($P = 0.021$). Our study suggested that down-regulation ofmiR-625 was significantly correlated with tumor progression and might be a potent prognostic marker of cutaneous melanoma.

Keywords: microRNA-625, cutaneous melanoma, survival, prognosis, biomarker

Introduction

Cutaneous melanoma is the most severe form of cutaneous malignancy [1]. The obvious characteristics are aggressive invasion, early metastasis and resistance to chemotherapy or radiotherapy, which result in the increased mortality worldwide [2, 3]. Thus, a better understanding of the genetic and molecular basis of cutaneous melanoma progression will contribute to exploit novel therapeutic targets as well as prognostic biomarkers for cutaneous melanoma.

MicroRNAs (miRNAs) are noncoding small RNAs (~18-25 nucleotides in length) that regulate various cellular or molecular mechanisms by binding the 3'-untranslated region (3'-UTR) of target genes to suppress gene expression or induce protein degradation [4]. It has been shown that miRNAs can regulate cell differentiation, proliferation, invasion, metastasis, and apoptosis [5, 6]. A large body of evidence has suggested that miRNA mutations or misexpression may correlate with various human cancers, and miRNAs may act as tumor suppressors or oncogenes depending on specific cancer types [7-9].

The deregulation of miR-625 has been detected in many types of cancer, including colorectal cancer, breast cancer, hepatocellular carcinoma (HCC), esophageal squamous cell carcinoma (ESCC), gastric cancer, and malignant pleural mesothelioma [10-19]. These studies show that miR-625 is frequently deregulated in tumor tissues, and the decreased expression of miR-625 correlated with the invasiveness and metastasis predicted high malignancy and poor prognosis. Previously, Fang et al found that miR-625 levels were frequently decreased in malignant melanoma, and ectopic expression of miR-625 suppressed proliferation, wound healing, migration, and tumorigenicity in malignant melanoma. Moreover, miR-625 has acted, at least in part, a role of suppressing potential target SOX2. These results suggested that miR-625 was a tumor suppressor inhibiting the

Table 1. The miR-625 levels and the clinicopathological factors of patients diagnosed with cutaneous melanomas

Clinical variables	Cases (n)	miR-625 level		*P* value
		Low (n = 43)	High (n = 44)	
Gender				
Male	48	26	22	0.391
Female	39	17	22	
Age				
≤60	51	24	27	0.666
>60	36	19	17	
Ulceration				
Absent	42	18	24	0.286
Present	45	25	20	
Thickness (mm)				
≤2.0	26	6	20	0.002
>2.0	61	37	24	
Histologic type				
SSM	34	16	18	0.799
LMM	27	14	13	
Other	26	13	13	
Anatomical site				
Axial (trunk/head & neck)	69	33	36	0.605
Peripheral (limbs)	18	10	8	
Stage				
I/II	30	3	27	<0.001
III/IV	57	40	17	

LMM = lentigomaligna melanoma; SSM = superficial spreading melanoma.

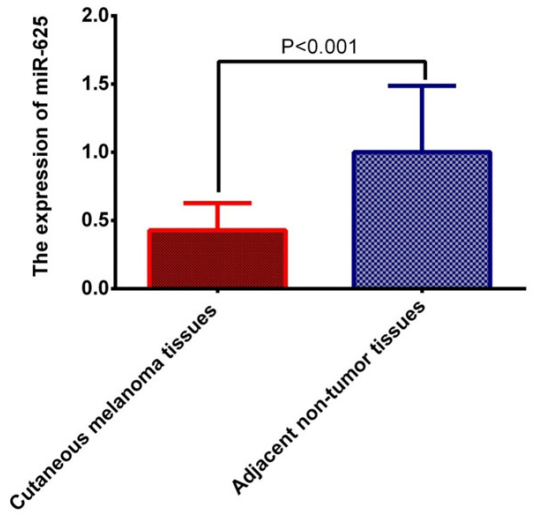

Figure 1. The expression levels of miR-625 in 87 pairs of cutaneous melanoma tissues and adjacent non-tumor tissues using qRT-PCR.

development and progression of malignant melanoma [20]. However, until now, the relationships between miR-625 expression and the clinicopathological features of cutaneous melanoma have not yet been reported.

Materials and methods

Patient and tissue samples

Informed written consent was obtained from all patients, and research protocols were approved by the ethical committee of Third Xiangya Hospital, Central South University. Case selection was performed by identifying patients with a diagnosis of cutaneous melanoma who underwent surgery for the primary tumor in the Departments of Dermatology, Third Xiangya Hospital, Central South University between January 2009 and August 2016. None patients had received any radiotherapy or chemotherapy prior to surgery. All tissues were snap frozen in liquid nitrogen, followed by storage at -80°C until use. The histological diagnosis, Breslow thickness and Clark level were re-examined from 1 to 5 original sections of the primary tumor by the same pathologist who was unaware of the clinical data. The characteristics of patients are shown in **Table 1**.

RNA extraction and qRT-PCR

Total RNA was extracted from samples with aTrizol reagent (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. The expression of miR-625 was determined by qRT-PCR assay. Briefly, total RNA was extracted from the tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. MiRNA expression was then quantitated using the TaqMan miRNA real-time RT-PCR kit (Applied Biosystems) according to the manufacturer's protocol. Data were analyzed using 7500 software v.2.0.1 (Applied Biosystems), with the automatic Ct setting for adapting baseline and threshold for Ct determination. The universal small nuclear RNA U6

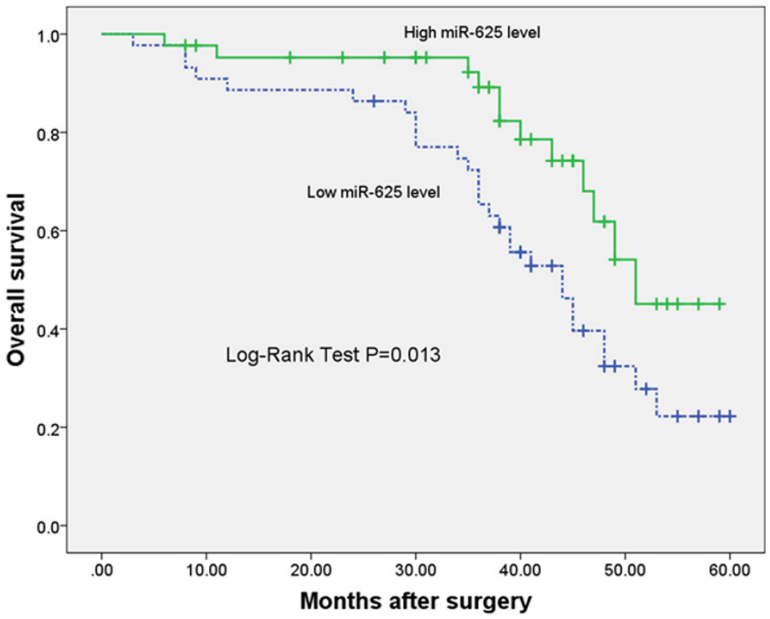

Figure 2. Kaplan-Meier curves for overall survival time in patients with cutaneous melanoma divided according to miR-625 level.

Table 2. Multivariate analysis of prognostic parameters in 87 patients with cutaneous melanomas

Clinical variables	Hazard ratio	95% confidence interval	*P*-value
Gender	0.572	0.355-2.018	0.891
Age	1.387	0.794-3.271	0.175
Ulceration	2.182	0.795-5.928	0.067
Thickness	2.991	1.283-7.364	0.038
Histologic type	0.683	0.372-2.934	0.725
Anatomical site	1.382	0.544-3.002	0.799
Stage	4.293	2.034-10.993	<0.001
miR-625 level	2.177	1.375-8.927	0.021

(RNU6B) was used as an endogenous control for miRNAs. Each sample was examined in triplicate, and the amounts of PCR products produced were non-neoplasticized to RNU6B. The primer sequences were 5'-CCAGGGGGAAAGTTCTATAGTCC-3' (miR-625) and 5'-CGCTTCACGAATTTGCGTGTCAT-3' (U6).

Statistical analysis

Data were expressed as means ± standard deviation. Differences between two groups were analyzed using the two-tailed Student's t-test for two groups. The Chi-square test was used to assess miR-625 expression with respect to clinicopathological parameters. The Kaplan-Meier method and log-rank test were used to analysis on patients' overall survival. The multivariate Cox regression analysis was used to assess the effect of multiple independent prognostic factors on survival outcome. Differences were considered statically significant when $P< 0.05$. All data were analyzed using SPSS version 17.0 for Windows (SPSSInc., IL, USA).

Results

Low expression of miR-625 in cutaneous melanoma tissues

We have used real-time PCR to evaluate miR-625 expression in 87 paired cutaneous melanoma tissues and adjacent non-cancerous tissues. The results showed that the expression of miR-625 were significantly decreased in cutaneous melanoma tissues, compared with the adjacent normal tissues ($P<0.0001$; shown in **Figure 1**).

Relationship between miR-625 expression levels and clinical factors

To investigate the clinical role of miR-625 during cutaneous melanoma carcinogenesis, we compared its expression level with clinicopathological features of the patients with cutaneous melanoma. As shown in **Table 1**, the expression levels of miR-625 in cutaneous melanoma were significantly associated with tumor thickness ($P = 0.002$) and stage ($P<0.001$), indicating that low expression of miR-625 was associated with cutaneous melanoma clinical progression and may play a negative role in cutaneous melanoma.

Correlation between miR-625 expressions with survival in cutaneous melanoma patients

Overall survival curves were plotted according to miR-625 expression level using the Kaplan-Meier method. In the study group (87 patients with cutaneous melanoma), the overall survival rate was significantly lower in the patients with

low miR-625 expression level than in those with high expression level (P = 0.013, shown in **Figure 2**). These results suggested that low expression levels of miR-625 were associated with poorer survival in cutaneous melanoma patients. Furthermore, multivariate analysis of the prognosis factors with a Cox proportional hazards model confirmed that low miR-625 expression was a significant independent predictor of poor survival in cutaneous melanoma (P = 0.021, shown in **Table 2**).

Discussion

Cutaneous melanoma is a malignant tumor originating from melanocytes and is a dangerous disease entity [1]. It is characterized by a rapid progression and lymph node metastasis. Although efforts have been made to develop an understanding of the causes of melanoma progression and more effective therapies, they have met with limited success [3]. Therefore, better understanding of the molecular mechanisms about malignant melanoma tumorigenesis and progression will be helpful to explore novel therapeutic agents and prognostic markers in the treatment of patients with cutaneous melanoma.

MiRNAs, as small non-coding RNA molecules, are involved in a variety of biological processes, such as cell development, differentiation, proliferation, apoptosis, metabolism, and cell-cycle control [4]. Many studies have shown that aberrant miRNA expression was often associated with the occurrence and development of various human cancers. In addition, deregulated miRNAs can be used as biomarkers for cancer diagnosis and prognosis [7-9].

The deregulation of miR-625 and its clinical significance has been investigated in many types of cancer. For example, Lou et al found that miR-625 was significantly downregulated in CRC tissues and cell lines. In addition, the decreased expression of miR-625 was positively associated with advanced lymph node metastasis, liver metastasis, poor overall survival, and an unfavorable prognosis for CRC patients, as determined through a multivariate analysis. Moreover, functional assays demonstrated that ectopic miR-625 expression inhibited the invasion and migration of HCT116 CRC cells both in vitro and in vivo, suggesting that miR-625 may serve as an efficient clinical biomarker and a therapeutic tool for the inhibition of metastasis in CRC [16]. Zheng et al found that miR-625 markedly inhibited suppressor of cancer cell invasion (SCAI) expression and subsequently suppressed E-cadherin and upregulated MMP-9 expression, leading to enhanced cell invasion in CRC [12]. Rasmussen et al identified miR-625 as a non-coding RNA related to the response to oxaliplatin based treatment both in patient CRC samples and in in vitro grown colon cancer cell lines [17]. Li et al found that the level of miR-625 in ESCC tissues was significantly lower than that in adjacent non-tumor tissues. Low miR-625 expression was observed to be closely correlated with lymph node metastasis, distant metastasis, tumor differentiation, and advanced TNM stage. The 5-year overall survival rate in the low expression group was 38.1%, compared with 68.8% in the high expression group. Multivariate Cox regression analysis showed that miR-625 expression was an independent factor in predicting the overall survival of ESCC patients [13]. Zhou et al found that miR-625 was frequently down-regulated in breast cancer. Decrease of miR-625 was closely associated with estrogen receptor, human epidermal growth factor receptor 2 (EGFR-2) and clinical stage. Kaplan-Meier and multivariate analyses indicated miR-625 as an independent factor for unfavorable prognosis, suggesting miR-625 as a promising prognostic biomarker for breast cancer [11]. Wang et al found that the expression of miR-625 was significantly down-regulated and negatively correlated with lymph node metastasis in gastric cancer. miR-625 significantly inhibited the invasion and metastasis of gastric cancer cells both in vitro and in vivo. Moreover, they identified that ILK was a direct target gene for miR-625 and knockdown of ILK had a phenocopy of overexpression of miR-625. Taken together, their findings suggested that miR-625 played an important role in gastric cancer [18].

Previously, Fang et al found that miR-625 levels were frequently decreased in malignant melanoma, and ectopic expression of miR-625 suppressed proliferation, wound healing, migration, and tumor gentility in malignant melanoma. Moreover, miR-625 acted, at least in part, a role of suppressing potential target SOX2. These results suggested that miR-625 was a tumor suppressor with inhibiting the development and progression of malignant melanoma

[20]. In the present study, we found that the expression of miR-625 was significantly decreased in cutaneous melanoma tissues compared with the adjacent normal tissues. The level of miR-625 in cutaneous melanoma was significantly associated with tumor thickness and stage, indicating that low expression of miR-625 was associated with cutaneous melanoma clinical progression and may play a negative role in cutaneous melanoma. Then overall survival curves were plotted according to miR-625 expression level using the Kaplan-Meier method, and we found that the overall survival rate was significantly lower in the patients with low miR-625 expression than in those with high expression. These results suggested that low expression of miR-625 were associated with poorer survival in cutaneous melanoma patients. Furthermore, multivariate analysis of the prognosis factors with a Cox proportional hazards model confirmed that low miR-625 expression was a significant independent predictor of poor survival in cutaneous melanoma.

In conclusion, our results showed that miR-625 may play a role in the development of cutaneous melanoma and have potential as biomarkers in the prognosis of cutaneous melanoma. However, the molecular mechanisms underlying the role of this miRNA in cutaneous melanoma need to be further investigation in future studies.

Disclosure of conflict of interest

None.

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