Clin. Lab. 2020;66:XXX-XXX
©Copyright

ORIGINAL ARTICLE

MiR-424 Functions as Potential Diagnostic and Prognostic Biomarker in Melanoma

Sheng-Jing Xu [1, *], Wen-Jing Xu [1, *], Zhi Zeng [2], Min Zhang [1], Dong-Yan Zhang [1]

[*] *These two authors contributed equally to this work*
[1] *Department of Dermatology, The Affiliated Jiangning Hospital of Nanjing Medical University, Nanjing, China*
[2] *Department of Pathology, Renmin Hospital of Wuhan University, Wuhan, China*

SUMMARY

Background: **Melanoma is one of the most aggressive and lethal skin cancers worldwide. To our knowledge, no specific or sensitive biomarkers have been clinically used to diagnose or predict melanoma prognosis. MicroRNAs (miRNAs) have been shown to regulate oncogenesis and tumor development in various cancers including melanoma. The aim of present study was to determine the clinical value of miR-424 in melanoma.**
Methods: **First, we examined the expression levels of miR-424 in tissue and serum samples of melanoma patients using real-time quantitative polymerase chain reaction (RT-qPCR) analysis. Then, receiver operating characteristic curves were used to determine the diagnostic value of miR-424. Furthermore, the chi-square test was used to analyze the relationship between the expression of miR-424 and the clinical characteristics of the patients. Finally, the Kaplan-Meier survival analysis was applied to validate the prognostic value of miR-424 in melanoma.**
Results: **The results demonstrated that miR-424 expression was remarkably increased in tissues and serum of patients with melanoma. Moreover, results of ROC analysis showed both tissue and serum expression of miR-424 can serve as diagnostic biomarker for melanoma. Meanwhile, miR-424 expression was significantly associated with tumor thickness ($p = 0.031$), metastasis ($p = 0.010$) and tumor stage ($p = 0.005$) and ulceration ($p < 0.001$). Finally, patients with higher miR-424 expression have shown decreased overall survival and disease-free survival than those with low miR-424 expression, implying that high miR-424 expression will contribute to poor prognosis of melanoma.**
Conclusions: **MiR-424 may function as a diagnostic and prognostic biomarker for melanoma.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.190917)

Correspondence:
Sheng-Jing Xu
Department of Dermatology
The Affiliated Jiangning Hospital of
Nanjing Medical University
Nanjing 211100
China
Phone: +86 15952909219
Email: Shengjingxu@Yandex.com

Manuscript accepted October 22, 2019

KEY WORDS

miR-424, melanoma, diagnosis, prognosis, biomarker

INTRODUCTION

Melanoma is one of the most aggressive and lethal malignancies with an increasing incidence of 3 - 8% annually [1,2]. It arises from melanocytes and accounts for more than 80% of skin cancer deaths worldwide [3]. Most melanoma patients could be detected at early stage; however, the five-year survival rate for patients with advanced disease is still low despite improvements [4]. Therefore, it is urgent to explore therapeutic agents and prognostic markers in the treatment of melanoma patients.

MicroRNAs (miRNAs) are a group of small, non-coding RNAs, consisting of 22 nucleotides on average which could regulate gene expressions by binding to the complementary region of the 3'-untranslated region of specific target mRNA. Numerous studies have pointed out that aberrant expression of specific miRNAs may be related to oncogenesis and tumor development [5,6]. Accumulating evidence has elucidated that miRNAs play important roles in melanoma development and prognosis [7,8]. Recently, a study pointed out that miR-424 was up-regulated in melanoma [9], indicating that miR-424 may be related to melanoma development. However, whether miR-424 may exert diagnostic and prognostic value in melanoma remains unknown.

Therefore, in the present study, the expressions of miR-424 in tumor samples and serum samples of patients with melanoma will be compared with the adjacent normal tissues and serum of healthy controls by RT-qPCR method. The diagnostic and prognostic value of miR-424 in melanoma will be investigated.

MATERIALS AND METHODS

Patients and samples

In this study, the serum samples were collected from 93 patients with melanoma and 93 cancer-free healthy volunteers between June 2012 and April 2014 in Department of Dermatology, The Affiliated Jiangning Hospital of Nanjing Medical University without having received any chemotherapy, radiotherapy or any adjuvant therapy prior to surgery. A 5-mL sample of peripheral venous blood was drawn from all participants after overnight fast and centrifuged at 10,000 g at 4°C for 10 minutes. Moreover, 93 paired melanoma tissue samples and the adjacent tissues were also collected from the patients with melanoma. Before RT-qPCR assays, all blood samples and tissue samples were stored at -80°C. The present study was approved by the Ethics Committee of The Affiliated Jiangning Hospital of Nanjing Medical University and written consent was obtained from each patient. A five-year follow-up was conducted accordingly and detailed clinicopathological characteristics of melanoma patients were presented in Table 1.

RT-qPCR analysis

Total RNA was extracted from the tissue samples and blood samples of 93 patients with melanoma and healthy volunteers using RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Then, cDNA was synthesized from RNA using M-MLV reverse transcriptase (Promega, Madison, WI, USA) according to the protocol. The cDNA and primers were mixed with SYBR Premix for PCR with Takara Thermal Cycler Dice TP800 (Takara, Dalian, China). The PCR amplification was performed by 40 cycles of denaturation at 94°C for 5 seconds and annealing at 62°C for 10 seconds. U6 was used as an endogenous control and the expression level of miR-424 was analyzed using $2^{-\Delta\Delta Ct}$ method. All the procedures were conducted in triplicate. The primer sequences were described as follows: miR-424 forward, 5'-GCTCGAGATATGGAGGGCGCC-3' and reverse, 5'-GGAACGGCAGACACGTATCC-3'.

Statistical analysis

All data were analyzed with Graphpad software (Graphpad Prism 7.0, San Diego, CA, USA). All data were presented as means ± standard deviation (SD). The differences of miR-424 expression in serum samples of melanoma patients and healthy participants was analyzed using Student's *t*-test. The relationship of miR-424 expression and clinicopathological characteristics was confirmed by chi-square testing and receiver operating characteristic curve (ROC) was used to detect the diagnostic value of miR-424 in melanoma. The overall survival rate (OS) and disease-free survival rate (DFS) of melanoma patients was examined by Kaplan-Meier survival analysis. A p-value less than 0.05 was considered statistically significant.

RESULTS

miR-424 expression was significantly increased in tumor tissue and serum of patients with melanoma

First, the expressions of miR-424 in tumor tissue and the adjacent normal tissue from 93 melanoma patients were determined by RT-qPCR. As demonstrated in Figure 1, the expression of miR-424 was significantly increased in melanoma tissues compared with the adjacent normal skin (Figure 1A, $p < 0.001$); moreover, the expression of miR-424 in serum and tissue samples from 93 melanoma patients and healthy volunteers were also compared. We found that miR-424 was also up-regulated in serum of the melanoma patients compared with the healthy controls (Figure 1B, $p < 0.001$), and results of correlation analyses showed that the expression of miR-424 in tumor tissue and serum of patients with melanoma was strongly positively correlated (Figure 1C, 0.4010, $p < 0.001$).

The diagnostic value of miR-424 in melanoma

Next, the capacity of the expression level of miR-424 to discriminate melanoma patients from healthy volunteers was determined by a ROC curve. We found the AUC of tissue miR-424 was 0.8099 (Figure 2A, 95% confidence interval 0.7436 to 0.8762), and the AUC of serum miR-424 was 0.8028 (Figure 2B, 95% confidence interval 0.7345 to 0.8710) indicating both tissue and serum expression of miR-424 can serve as a diagnostic biomarker for melanoma.

Relationship between the serum expression of miR-424 and clinicopathological characteristics of patients with melanoma

The relationship between the serum expression of miR-424 and the clinicopathological characteristics of pa-

Table 1. Relationship between serum miR-424 expression and clinicopathological characteristics.

Variable	Cases (n)	Serum miR-424 expression		χ^2	p
		High (n = 48)	Low (n = 45)		
Gender					
Female	40	23	17	0.974	0.324
Male	53	25	28		
Age (years)					
≥ 60	47	22	25	0.878	0.349
< 60	46	26	20		
Tumor thickness (mm)					
≥ 2.0	52	32	20	4.653	**0.031**
< 2.0	41	16	25		
Metastasis					
Yes	50	32	18	6.644	**0.010**
No	43	16	27		
Tumor stage					
I/II	34	11	23	7.960	**0.005**
III	59	37	22		
Family history					
Yes	49	24	25	0.288	0.592
No	44	24	20		
Ulceration					
Yes	56	35	21	13.756	**< 0.001**
No	47	13	34		

tients were analyzed. As shown in Table 1, increased miR-424 expression was significantly associated with tumor thickness ($p = 0.031$), metastasis ($p = 0.010$), tumor stage ($p = 0.005$), and ulceration ($p < 0.001$). However, there was no association found between miR-424 expression and other characteristics, such as gender, ages and family history.

The relationship between the serum expression of miR-424 and the prognosis of patients with melanoma

Finally, we performed Kaplan-Meier survival analysis to determine the potential prognostic value of serum miR-424 in melanoma. As demonstrated in Figure 3, melanoma patients with higher miR-424 level showed decreased OS (Figure 4A, $p < 0.01$) and DFS (Figure 3B, $p < 0.05$) than those with low miR-424 expression.

DISCUSSION

In this study, we identified miR-424 as a potential biomarker for melanoma diagnosis and prognosis. We found that miR-424 level was significantly higher in melanoma patients compared with healthy controls. Furthermore, high expression of miR-424 may predict poor prognosis of melanoma patients.

Accumulating evidence has revealed that miR-424 is aberrantly expressed in various cancers and related to cancer development and prognosis. For example, aberrant expression was observed in colorectal [10], breast [11], and prostate cancer [12]. Recently, miR-424 was reported to be up-regulated in melanoma [13], and in the present study, we found that miR-424 was dramatically over-expressed in melanoma tissue compared with the adjacent normal tissue, which was consistent with the previous reports, suggesting that miR-424 may participate in the pathogenesis of melanoma.

Indeed, the detection of miRNAs in frozen tissue samples or formalin fixed and paraffin embedded tissue samples has been demonstrated as an accurate and effective method for the diagnosis of different types of cancers; however, this method has two major limitations. First, the detection of miRNAs in tissue samples is an inconvenient, invasive, and inexpensive method [14]; more importantly, the early diagnosis of the disease cannot be achieved, because most of the cancers did not have clinical symptoms at the early stage [15]. Therefore, the above limitations may impede the potential clinical application. Nevertheless, these problems

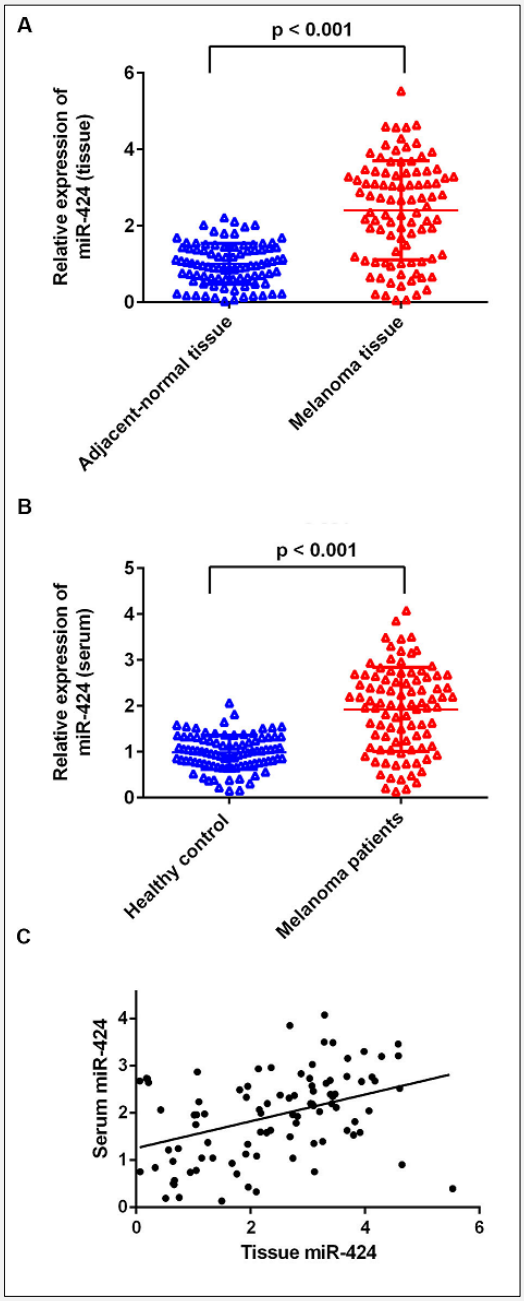

Figure 1. Increased expressions of miR-424 in tissue and serum samples of patients with melanoma.

(A) Expression levels of miR-424 in melanoma tissue and the adjacent normal skin of 93 patients with melanoma. (B) Expression levels of miR-424 in serum of 93 melanoma patients and 93 healthy volunteers. (C) Correlation between the tissue and serum expression of miR-424 in patients with melanoma.

may be solved by the discovery of circulating miRNAs, which are the miRNAs that are released into the body fluid by various mechanisms. Increasing evidence suggested that circulating miRNAs may fulfill the criteria of ideal biomarkers for the early diagnosis of different type of diseases [15], including melanoma [16]. In the present study, we found the expression of miR-424 was also significantly increased in serum of the melanoma

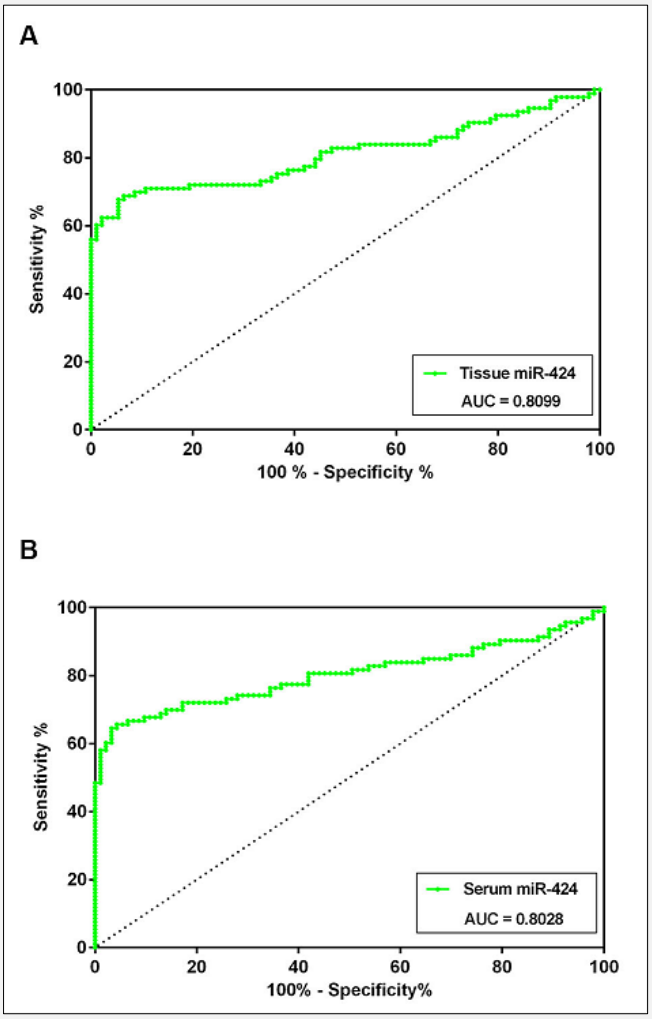

Figure 2. MiR-424 may serve as diagnostic marker for melanoma.

(A) Receiver operating characteristic curve of tissue miR-424 for the diagnosis of melanoma. (B) Receiver operating characteristic curve of serum miR-424 for the diagnosis of melanoma.

patients, which was consistent with its expression pattern in the melanoma tissue, and interestingly, the expression of miR-424 in melanoma tissues and serum samples were positively correlated, suggesting that the increased serum expression of miR-424 were mainly from the tumor cells. Moreover, results of ROC analysis showed that the AUC of tissue and serum miR-424 for the diagnosis of melanoma were similar (0.8028 vs. 0.8099), suggesting that both tissue and serum expression of miR-424 can serve as diagnostic biomarker for melanoma with high sensitivity and specificity. Therefore, to sum up, the results of our study confirmed the potential diagnostic value of miR-424, especially serum miR-424, for the early diagnosis of melanoma.

According to previous studies, aberrant expressions of miRNAs were revealed to be related with the development of the disease, for example, tumor thickness, tumor stage, and ulceration as well [17-19]. In the present study, miR-424 was found to be strongly associated with tumor thickness, tumor stage, and ulceration, suggesting the expression level of miR-424 may be associated with the progress of the disease. On the other hand, miRNAs have also been proven to be related with the long-term prognosis of melanoma [20]. Results of the present study also demonstrated that high expression of miR-424 may result in decreases in both OS and DFS of the melanoma patients, suggesting that miR-424 was a prognostic factor in melanoma.

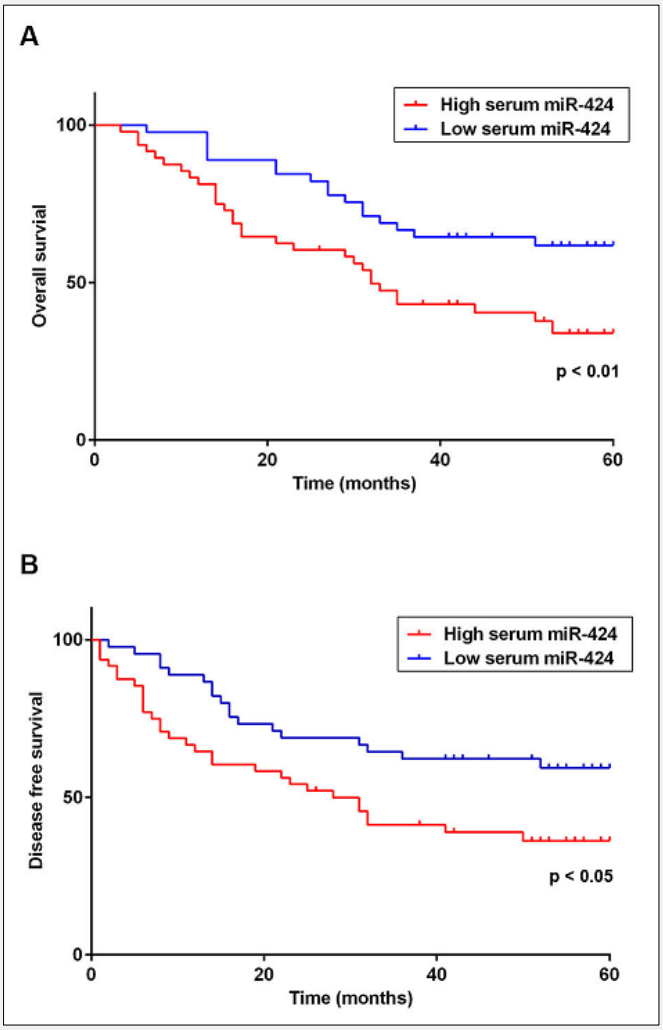

Figure 3. MiR-424 may serve as prognostic marker for melanoma.

(A) Kaplan-Meier survival analysis for the overall survival of melanoma patients with low miR-424 expression and miR-424 high expression. (B) Kaplan-Meier survival analysis for the disease-free survival of melanoma patients with low miR-424 expression and miR-424 high expression.

In our study, there are still some limitations. Firstly, the number of specimens is small. Secondly, our study has not validated the exact molecular mechanisms of miR-424 on regulating melanoma development. To solve the limitations, large-scale samples and molecular experiments should be carried out in the future.

CONCLUSION

In conclusion, this study provided evidence that miR-424 may function as diagnostic and prognostic biomarker for patients with melanoma and might contribute to clinical treatment of melanoma.

Acknowledgment:
This study was supported by National Natural Science Foundation of China (Grant No. 81602535) and Natural Science Foundation of Hubei Province of China (Grant No. 2016CFB249).

Declaration of Interest:
None.

References:

1. Garay T, Kenessey I, Molnar E, et al. Prenylation inhibition-induced cell death in melanoma: reduced sensitivity in BRAF mutant/PTEN wild-type melanoma cells. PLoS One. 2015;10: e0117021 (PMID: 25646931).

2. Junco JJ, Mancha-Ramirez A, Malik G, et al. Ursolic acid and resveratrol synergize with chloroquine to reduce melanoma cell viability. Melanoma Res. 2015;25(2):103-12 (PMID: 25647735).

3. Miller AJ, Mihm MC. Melanoma. N Engl J Med. 2006;355:51-65 (PMID: 16822996).

4. Garbe C, Eigentler TK. Diagnosis and treatment of cutaneous melanoma: state of the art 2006. Melanoma Res. 2013;14:253-7 (PMID: 17496787).

5. Zhao ZJ, Shen J. Circular RNA participates in the carcinogenesis and the malignant behavior of cancer. RNA Biol. 2017;14(5): 514-21 (PMID: 26649774).

6. Quattrochi B, Gulvady A, Driscoll DR, et al. MicroRNAs of the mir-17~92 cluster regulate multiple aspects of pancreatic tumor development and progression. Oncotarget. 2017;8(22):35902-18 (PMID: 28415794).

7. Zhang X, Lin D, Lin Y, et al. Proteasome beta-4 subunit contributes to the development of melanoma and is regulated by miR-148b. Tumour Biol. 2017;39(6):101010428317705767 (PMID: 28656878).

8. Bai M, Zhang H, Si L, Yu N, Zeng A, Zhao R. Upregulation of serum miR-10b is associated with poor prognosis in patients with melanoma. J Cancer. 2017 8(13):2487-91 (PMID: 28900486).

9. Varamo C, Occelli M, Vivenza D, Merlano M, Lo Nigro C. MicroRNAs role as potential biomarkers and key regulators in melanoma. Genes Chromosomes Cancer. 2017;56(1):3-10 (PMID: 27561079).

10. Fang Y, Liang X, Xu J, Cai X. miR-424 targets AKT3 and PSAT1 and has a tumor-suppressive role in human colorectal cancer. Cancer Manag Res. 2018;10:6537-47 (PMID: 30555259).

11. Wang J, Wang S, Zhou J, Qian Q. miR-424-5p regulates cell proliferation, migration and invasion by targeting doublecortin-like kinase 1 in basal-like breast cancer. Biomed Pharmacother. 2018; 102:147-52 (PMID: 29550638).

12. Suer I, Guzel E, Karats OF, Creighton CJ, Ittmann M, Ozen M. MicroRNAs as prognostic markers in prostate cancer. Prostate. 2019;79(3):265-71 (PMID: 30345533).

13. Mizaei H, Gholamin S, Shahidsales S, et al. MicroRNAs as potential diagnostic and prognostic biomarkers in melanoma. Eur J Cancer. 2016;53:25-32 (PMID: 26693896).

14. Ghanbari R, Rezasoltani S, Hashemi J, et al. Expression Analysis of Previously Verified Fecal and Plasma Dow-regulated MicroRNAs (miR-4478, 1295-3p, 142-3p and 26a-5p), in FFPE Tissue Samples of CRC Patients. Arch Iran Med. 2017;20(2):92-5 (PMID: 28193082).

15. Ganepola GA, Rutledge JR, Suman P, Yiengpruksawan A, Chang DH1. Novel blood-based microRNA biomarker panel for early diagnosis of pancreatic cancer. World J Gastrointest Oncol. 2014; 6(1):22-33 (PMID: 24578785).

16. Li J, Liu X, Li C, Wang W. miR-224-5p inhibits proliferation, migration, and invasion by targeting PIK3R3/AKT3 in uveal melanoma. J Cell Biochem. 2019;120(8):12412-21 (PMID: 308252 22).

17. Wang K, Zhang ZW. Expression of miR-203 is decreased and associated with the prognosis of melanoma patients. Int J Clin Exp Pathol. 2015;8(10):13249-54 (PMID: 26722525).

18. Li P, He QY, Luo CQ, Qian LY. Circulating miR-221 expression level and prognosis of cutaneous malignant melanoma. Med Sci Monit. 2014;20:2472-7 (PMID: 25430553).

19. Li N. Low expression of miR-137 predicts poor prognosis in cutaneous melanoma patients. Med Sci Monit. 2016;22:140-4 (PMID: 26763596).

20. Guo S, Guo W, Li S, et al. Serum miR-16: a potential biomarker for predicting melanoma prognosis. J Invest Dermatol. 2016; 136(5):985-93 (PMID: 26829037).