Original Article
Decreased expression of *microRNA-20a* promotes tumor progression and predicts poor prognosis of cutaneous squamous cell carcinoma

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Abstract: Background: MicroRNA-20a (*miRNA-20a* or *miR-20a*) plays a key role in tumorigenesis and progression. But the prognostic value of *miR-20a* in cutaneous squamous cell carcinoma (CSCC) remains unclear. The aim of this study was to identify the association of *miR-20a* and the prognosis of CSCC patients. Methods: The *miR-20a* expression was detected using quantitative real-time polymerase chain reaction (qRT-PCR) in 152 CSCC tissues and matched adjacent normal tissues. Kaplan-Meier and Cox regression analysis were utilized to determine the association of *miR-20a* with overall survival as well as the prognosis of CSCC patients. Results: The expression of *miR-20a* was lower in CSCC tissues compared with adjacent normal tissues ($P=0.000$). Moreover, the expression of *miR-20a* was closely correlated with TNM stage ($P=0.013$). Kaplan-Meier analysis showed that patients with low *miR-20a* expression had significantly poorer overall survival than those with high *miR-20a* expression ($P<0.05$). Multivariate analysis revealed that *miR-20a* expression ($P=0.001$, HR=3.262, 95% CI: 1.635-6.520) could influence the prognosis and might be an independent prognostic predictor in CSCC. Conclusions: Our results indicated that low *miR-20a* expression was associated with tumor stage of CSCC and suggested that *miR-20a* expression would be a novel biomarker for predicting clinical outcomes in CSCC patients. The inhibition of *miR-20a* might even become a new therapeutic method for the treatment of CSCC.

Keywords: MicroRNA-20a, cutaneous squamous cell carcinoma, prognosis

Introduction

Cutaneous squamous cell carcinoma (CSCC) is the second most common non-melanoma skin cancer and a malignant tumor of keratinocytes which tends to metastasis and leads to mortality [1, 2]. The most important risk factor for the occurrence of this cancer is UV exposure [3]. Its morbidity is greater in males than females, and increases with age [4]. Moreover, patients with CSCC also have an elevated the mortality rate of lung cancer, colorectal cancer, rectum cancer, prostate cancer and breast cancer [5]. Metastasis is the main reason that causes cancer-related death in CSCC which can lead to the 5-year survival rate less than 30% [6]. Therefore, the prognosis is unfavorable after metastasis in CSCC and it is necessary to study the molecular mechanisms underlying metastasis in CSCC as well as look for a new effective prognostic marker to improve clinical outcome.

MicroRNAs (*miRNAs* or *miRs*) are a large group of non-coding, single-stranded RNA with approximately 22 nucleotides in length [7]. These small molecules have been found to regulate endogenous gene expression through translational repression and messenger RNA cleavage [8]. Recent studies showed that miRNAs play key roles in the biology of various human cancers, including cell differentiation, proliferation, apoptosis, invasion and angiogenesis [9, 10]. It also has been confirmed to be important diagnostic or prognostic markers in various cancers. *MiR-20a* (*microRNA-20a*) belongs to the *miR-17-92* cluster, which includes
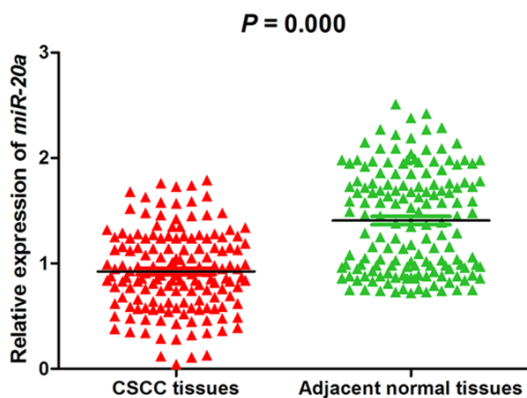
Figure 1. Expression level of *miR-20a* in the specimens of CSCC patients QRT-PCR Demonstrated that the expression level of *miR-20a* was lower in tumor tissues than in adjacent normal tissues (*P*<0.05).

six microRNAs: *miR-17-5p*, *miR-18a*, *miR-19a*, *miR-19b*, *miR-20a*, and *miR-92a-16* [11]. It plays different roles in many tumors including prostate cancer, anaplastic thyroid cancer, and gastric cancer [12-14]. Besides, *miR-20a* was also considered to be decreased and correlated with the recurrence and prognosis of hepatocellular carcinomas [15]. However, although *miR-20a* had been reported to be an inhibitor for the metastasis and proliferation of CSCC [16], its prognostic implication in CSCC had never been explored.

The purpose of this study was to investigate the expression of *miR-20a* and explored whether it was related to the prognosis of CSCC.

Methods and materials

Patients and tissue samples

A total of 152 patients with CSCC were collected at the department of plastic and reconstructive surgery, First Affiliated Hospital of Bengbu Medical College. These CSCC cases included 102 men (67.1%) and 50 women (32.9%), with a median age of 53.9 years. No previous local or systemic treatment had been conducted on these patients before the operation or biopsy. All protocols were approved by the Ethics Committee of the First Affiliated Hospital of Bengbu Medical College and all participators had signed written informed consent in advance.

The tumor tissues and adjacent tissues were obtained with surgical removal and frozen in liq-

uid nitrogen immediately. Then the tissues were stored at -80°C until use. Clinicopathological characteristics for these patients, including age, gender, location, tumor size, tumor grade, pT classification, pN classification and stage were detailed in a database. Tumor differentiation was determined based on the World Health Organization tumor classification criteria. TNM (tumor, nodes, and metastasis) stage of cutaneous carcinoma was defined according to the Union for International Cancer Control. A 5-years' follow-up was conducted according to a telephone or questionnaire. The overall survival referred to the period of time from the date of diagnosis until death from any cause.

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

Total RNA was extracted and purified from all the 152 CSCC tissues and matched adjacent normal specimens using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). Only those total RNA samples with an OD A260/A280 ratio close to a value of 2.0, which indicates that the RNA was pure, were subsequently analyzed. The cDNAs were synthesized using gene-specific primers according to the TaqMan MicroRNA assays protocol (Applied Biosystems, Foster City, CA, USA). Then the PCR reaction was performed in the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). *U6* was taken as the internal control. Each sample was examined in triplicate, and the raw data were presented as the relative quantification of *miR-20a* expression evaluated by the comparative cycle threshold (CT) method using SDS 2.2.2 software (Applied Biosystems), normalized with respect to *U6*. The mean normalized *miR-20a* expression ± the standard deviation (SD) was calculated from triplicate analyses.

Statistical analysis

All statistical analysis was performed using SPSS version 18.0. Comparisons of *miR-20a* expression levels between CSCC tissues and adjacent normal tissues were estimated using T-test. The correlation between *miR-20a* expression and clinicopathological characteristics of patients with CSCC was evaluated by X^2-test. Association of *miR-20a* expression with overall survival was estimated by Kaplan-Meier

Table 1. Relationship between clinicopathological characteristics and *miR-20a* expression in patients with CSCC

| Parameters | Cases (n) | *miR-20a* expression level | | X² | P-value |
		Low (n=98)	High (n=54)		
Age (yeas)					
≤55	80	53	27	0.233	0.630
>55	72	45	27		
Gender					
Male	102	72	50	0.712	0.399
Female	50	26	24		
Location					
Neck	32	18	14	1.083	0.406
Face	48	30	18		
Head	72	50	22		
Tumor size (cm)					
≤5	67	40	27	1.191	0.275
>5	85	58	27		
Grade of differentiation					
Well to moderate	141	89	52	1.557	0.212
Poor	11	9	2		
pT classification					
T1-T2	62	41	21	0.125	0.723
T3-T4	90	57	33		
pN classification					
N0	115	72	43	0.717	0.397
N1	37	26	11		
TNM Stage					
I-II	108	63	45	6.141	0.013
III	46	35	9		

analysis, and the resulting curves were compared using the log-rank test. The multivariate analysis was used to evaluate the prognostic factors to patient's survival via Cox regression analysis. $P<0.05$ was considered to be statistically significant.

Results

MiR-20a expression was significantly lower in CSCC tissues compared with adjacent normal tissues

With the purpose of revealing the expression and significance of *miR-20a* in CSCC, we first detected the expression of *miR-20a* in 152 cases of CSCC and adjacent normal tissues by qRT-PCR. The expression of *miR-20a* was significantly down-regulated in CSCC tissues compared with adjacent normal skin tissues

(P=0.000; **Figure 1**). This result might reveal that *miR-20a* was a tumor suppressor in CSCC.

Relationship between the expression of miR-20a and clinicopathological characteristics

The relationships between *miR-20a* expression and clinicopathological characteristics were analyzed by chi-square test. As shown in **Table 1**, the expression of *miR-20a* in CSCC was significantly associated with tumor stage (P=0.013). However, there were no correlation with other clinical features, such as age, gender, tumor size, grade of differentiation, pT classification, pN classification (P>0.05).

Decrease expression of miR-20a in CSCC was associated with poor prognosis

To further explore the clinical relevance of *miR-20a*, overall survival curves were plotted according to *miR-20a* expression level by the Kaplan-Meier analysis. As shown in **Figure 2**, patients with low *miR-20a* expression had a significantly shorter overall survival than those with high *miR-20a* expression which was tested by the log-rank test (P=0.001, **Figure 2**). To get insight into the survival prediction potential of *miR-20a*, we performed multivariate analysis with Cox regression analysis. The results showed that *miR-20a* expression (HR=3.262, 95% CI=1.632-6.520, P=0.001) was an independent prognostic factor for patients with CSCC (**Table 2**).

Discussion

MicroRNAs play an important role in tumor development and occurrence according to act as a tumor suppressor or oncogene. This poten-
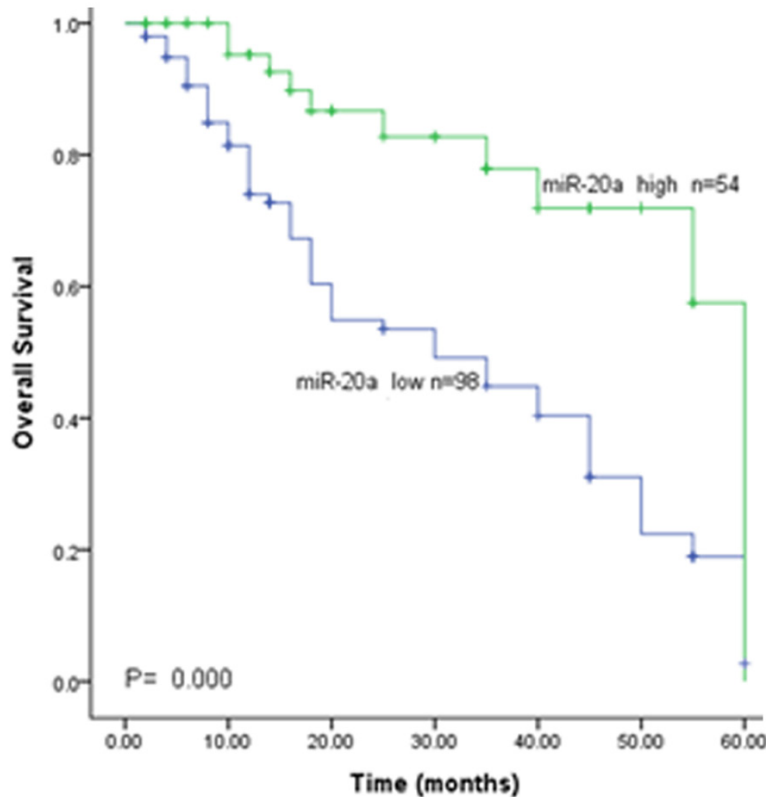
Figure 2. Kaplan-Meier analysis of with the *miR-20a* expression indicated that patients with low *miR-20a* expression lived shorter than those with high *miR-20a* expression.

Table 2. Multivariate analysis of different prognostic factors in the patients with CSCC

Parameters	HR	95% CIs	P-value
High *MiR-20a* expression Low *MiR-20a* expression	3.262	1.632-6.520	0.001
Tumor stage	1.398	0.785-2.490	0.255

HR: hazard ratio; CI: confidence interval.

tial effect of miRNAs is expected to make it to be molecular markers in the diagnosis and prognosis of cancers so that provide new therapy strategies.

In CSCC, there were also many microRNAs that proved to be participated in such as *miR-361-5p, miR-125b, miR-199a, miR-31, miR-365, and so* on [6, 17-20]. *miR-20a* has been found to have different roles in various tumors and acts as oncogene or tumor suppressor in previous studies. Indeed, *miR-20a* promoted gastric cancer [14], gallbladder carcinoma [21], and

prostate cancer [22]. On the contrary, it could inhibit in hepatic cancer [15] and oral squamous cell cancer [23]. It was also verified to be enhanced in the circulation and the tissue as well as associated with a higher risk of tumor metastasis in squamous cancer of the cervix, squamous cell cancer of the esophagus and CSCC [16, 24, 25]. In the present study, we investigated the *miR-20a* expression by qRT-PCR in 152 cases of CSCC and adjacent normal tissues. Results showed that the expression level of *miR-20a* was decreased in CSCC compared with that in adjacent normal specimens. This might indicate that *miR-20a* was a tumor suppressor in CSCC.

Based on the relative expression analysis, we investigated the association of *miR-20a* with clinicopathological features. We found that *miR-20a* expression was closely associated with the TNM stage of CSCC, as results revealed that low level of *miR-20a* expression was more frequently to be detected in tumors with advanced TNM stage. These findings reveal that the abnormal expression of *miR-20a* might play important roles in the tumorigenesis and progression of CSCC patients.

Furthermore, we assessed the prognostic role of *miR-20a* in CSCC. Kaplan-Meier analysis and log-rank test showed that patients with low *miR-20a* expression had worse overall survival compared with those with high levels of *miR-20a* expression. The statistically significant association indicated that *miR-20a* could be related to the prognosis of CSCC. To further evaluate the prognostic value of *miR-20a* in CSCC, we performed a Cox regression analysis adjusted for gender, age and other parameters related to survival of CSCC. Results proved that decreased *miR-20a* expression could be an independent prognostic marker in CSCC.

In summary, our investigation provides the effective evidence for the first time that the down-regulation of *miR-20a* may serve as a novel molecular marker to predict the tumor progression and inferior prognosis of CSCC patients. However, the sample size is small and the current study has not elucidated the exact molecular mechanisms of *miR-20a* acting on CSCC which make this study has some limitation. Hence, some further verified experiments need to be conducted.

Disclosure of conflict of interest

None.

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