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ORIGINAL ARTICLE

Expression of miR-34a in basal cell carcinoma patients and its relationship with prognosis

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Summary

Purpose: *To investigate the relationship between the expression level of miR-34a in the serum of basal cell carcinoma patients and the clinical prognosis.*

Methods: *Eighty-six patients with basal cell carcinoma who underwent surgery from July 2011 to July 2013 were enrolled in the experimental group, and 85 healthy volunteers were selected from the physical examination department of Henan Province Luoyang Orthopedic Traumatological Hospital to serve as control group. Real-time PCR was used to detect the expression of miR-34a in the serum of the study subjects. Patients were divided into high- and low-expression groups according to the median expression levels of miR-34a. Survival analysis was performed using Kaplan-Meier method.*

Results: *Serum miR-34a levels were significantly lower in basal cell carcinoma patients than in healthy volunteers ($p<0.001$). The expression level of miR-34a was correlated with tumor cell diameter, lymph node metastasis and histological types of basal cell carcinoma ($p<0.001$). The expression of miR-34a was not associated with patients' age, primary site and the pathological type of tumors ($p>0.05$). Median progression-free survival of patients with high expression and low expression was 37 and 20 months, respectively ($p<0.05$). Median overall survival time was 44 and 31.5 months, respectively ($p<0.05$). Overall survival rate was 76.74% in the high expression group, significantly higher ($p<0.05$) compared with the low expression group. miR-34a was significantly underexpressed in basal cell carcinoma, and the prognosis of basal cell carcinoma patients with low expression levels of miR-34a was poor.*

Conclusion: *MiR-34a is expected to be an effective biomarker for basal cell carcinoma assessment and prognosis.*

Key words: *basal cell carcinoma, clinicopathological factors, miR-34a, prognosis*

Introduction

Basal cell carcinoma is a malignant tumor of the skin that is prone to occur in the elderly. Basal cell carcinoma is easy to occur in head, face, neck, etc. [1]. This malignancy affects 886,000 new cases every year, accounting for 70-80% of global skin cancer cases [2]. Previous studies have shown that the onset of basal cell carcinoma is associated with exposure to sunlight and ultraviolet light. The incidence of basal cell carcinoma is generally high among Caucasians who love outdoor activities and sunbathing [3]. Although basal cell carcinoma is not very malignant, tumor cells are destructive, and due to the limitations of clinical diagnostic techniques, most patients are diagnosed at advanced stages [4]. Surgical resection is still the first clinical therapeutic option, but scar formation after surgery may have great impact on the patient's appearance [5]. Advanced basal cell carcinoma can even appear to all parts of the body, endangering the patient's life [6]. All in all, basal cell carcinoma imposes a great burden on the patient's psychological and physical health.

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MiRNAs are a group of small RNAs that can regulate the expression of related proteins after transcription and regulate the development of tumors [7]. miR-34a acts as tumor suppressor [8] that can regulate cell cycle and inhibit the migration, spread and invasion of tumor cells [9]. miR-34a is closely related to the occurrence and development of many malignant tumors such as breast and esophageal cancer [10,11]. Studies have shown that permanent inactivation of miR-34a may be another protective mechanism of tumor cells [12], and low expression level of miR-34a is a prognostic risk factor for multiple malignant tumors such as lung adenocarcinoma and esophageal cancer [13,14]. However, studies on the involvement of miR-34a in basal cell carcinoma are rare.

In this study we assessed the expression of miR-34a in the sera of basal cell carcinoma patients and tested whether miR-34a can be used as a biomarker for this disease.

Table 1. Clinicopathological features of the experimental group

Factors	*Cases n*	*%*
Primary sites		
Face	68	79.07
Head and neck	10	11.63
Other	8	9.30
Tumor diameter, cm		
≤1.5	54	62.79
>1.5	32	37.21
Lymph node metastasis		
Yes	42	48.84
No	44	51.16
Histological type		
Invasive	60	69.77
Non-invasive	26	30.23
Pathological types		
Nodular	71	82.56
Non-nodular	15	17.44

Methods

Basic information

Eighty-six patients with basal cell carcinoma who had been operated from July 2011 to July 2013 were enrolled in the experimental group. The experimental group included 38 males and 48 females, and age ranged from 21-93 years (mean 64.36±12.37). At the same time, 85 healthy volunteers were selected from the physical examination department of Henan Province Luoyang Orthopedic Traumatological Hospital to serve as control group. The control group included 37 males and 48 females, and age ranged from 18-89 years (mean 63.84±12.95). The clinicopathological features of the experimental group are shown in Table 1.

The study was approved by the Ethics Committee of Henan Province Luoyang Orthopedic Traumatological Hospital, and all participants signed informed consent.

Inclusion criteria: 1) diagnosed by pathological examinations; 2) patients with complete medical record; 3) patients completed treatment in Henan Province Luoyang Orthopedic Traumatological Hospital.

Exclusion criteria: 1) patients with melanoma, hemangioma, squamous cell tumor, blackhead acne, etc.; 2) patients who had recently received other treatments (such as radiotherapy, chemotherapy).

Experimental instruments and reagents

Trizol was purchased from Wuhan Kehaojia Biotechnology Co., Ltd (Wuhan, China, 15596-026); ddH2O was purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd (Beijing, China, PER 018-1); Trace UV Spectrophotometer was purchased from Tomorgan Biotechnology Co., Ltd (Beijing, China, MD2000); High-speed large-capacity refrigerated centrifuge was purchased from Eppendorf Abbott (China Co., Ltd.); ABI PCR Amplifier was purchased from Shanghai Aolu Biotechnology Co., Ltd (Shanghai, China 2720); 2×SYBR Green PCR Mastermix was purchased from Beijing Solar Biotechnology Co., Ltd (Beijing, SR1110). Fluorescence Quantitative® RNA Reverse Transcription Kit was purchased from Shanghai Runingwell Industrial Co., Ltd. (AB-4366596, Shanghai, China).

Primers

Primers used in this study were designed using Primer Premier 5.0 (Premier, Palo Alto CA, USA) primer design software, and synthesized by Tianjin Saier Biotechnology Co (Tianjian, China Ltd). Sequences of primers are listed in Table 2.

Table 2. Primer sequences

		Sequences
hsa-miR-34a	Forward	5'CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACAACCA3'
	Reverse	5'ACACTCCAGCTGGGTGGCAGTGTCTTAGCTG3'
U6	Forward	5'CTCGCTTCGGCAGCACA3'
	Reverse	5'AACGCTTCACGAATTTGCGT3'

Experimental methods

Three mL of venous blood was extracted after the participants were fasted for 8-12 hrs. Blood samples were centrifuged at 10,000 rpm for 10 min at 4°C to collect serum. Serum samples were stored at -80°C until use. Total RNA was extracted using Trizol. Purity and concentration of extracted total RNA and protein samples were tested using an ultraviolet spectrophotometer. Integrity of the extracted RNA was detected by agarose gel electrophoresis. Total RNA was reversely transcribed according to the instruction of the reverse transcription kit. PCR reactions were carried out on ABI PCR amplification instrument. PCR reaction system consisted of 0.4 μL of upstream and downstream primers, 1 μL of cDNA, 0.4 μL of passive reference dye (50×), and 10 μL of 2×SYBR Green PCR Mastermix. ddH2O was added to make a 20μL volume. Reaction conditions were predenaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C for 30 s. This experiment was performed in triplicate. Ct values were processed using the following formula: $RQ=2^{-\Delta Ct}$, ΔCt=Ct(miR-34a)-Ct(U6).

Statistics

Experimental data were analyzed by SPSS19.0 (SPSSInc., Chicago, IL, USA) data analysis software. Count data (%) were compared using the chi-square test. Measurement data were expressed by mean ± standard deviation. t-test was used for comparisons between groups. Survival analysis was performed using Kaplan-Meier method and log-rank test. P<0.05 was considered as denoting statistically significant difference.

Results

Comparison of miR-34a expression levels between experimental group and control group

The expression of miR-34a in the serum of 86 basal cell carcinoma patients and 85 healthy volunteers was detected by real-time PCR and compared. The results showed that the expression levels of

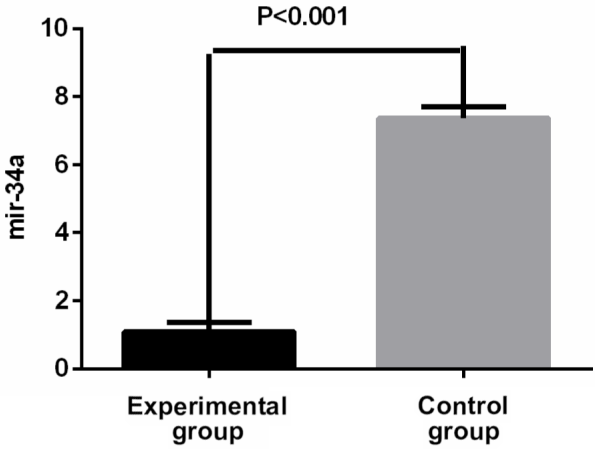

Figure 1. Comparison of miR-34a expression levels. The expression of miR-34a in the serum of experimental and control group was detected by real-time PCR. The expression levels of miR-34a in the serum were significantly lower in basal cell carcinoma serum patients than in healthy volunteers (t=129.400, p<0.001).

Table 3. Relationship between miR-34a expression level and clinicopathological features of basal cell carcinoma patients

Clinicopathological features	*Cases n*	*miR-34a expression levels mean±SD*	*t*	*p*
Age, years			1.747	0.084
<60	29	1.23±0.12		
≥60	57	1.02±0.64		
Primary sites			1.298	0.198
Head and neck	78	1.21±0.23		
other	8	1.08±0.54		
Tumor diameter, cm			6.926	<0.001
≤1.5	54	4.89±1.44		
>1.5	32	2.88±1.02		
Lymph node metastasis			7.158	<0.001
Yes	42	3.26±1.24		
No	44	5.28±1.37		
Histopathological types			18.37	<0.001
Invasive	60	6.32±1.02		
Non-invasive	26	2.31±0.67		
Pathological types			1.578	0.118
Nodular	71	1.13±0.25		
Non-nodular	15	1.02±0.22		

miR-34a in the serum of the experimental group (1.09±0.28) were significantly lower than those in the control group (7.36 ± 0.35) (p<0.001; Figure 1).

Relationship between miR-34a expression level and clinicopathological features of basal cell carcinoma patients

The expression levels of miR-34a were correlated with basal cell carcinoma tumor diameter, lymph node metastasis and histological types. The expression level of miR-34a with larger tumor diameter was lower than that in patients with smaller tumor diameter. The expression levels of miR-34a were higher in patients without lymph node metastasis than in patients with lymph node metastasis. The expression level of miR-34a was higher in patients with non-invasive disease than in patients with invasive disease (p<0.001). The expression of miR-34a was not related to the patient's age, primary site and pathologic tumor type (p>0.05; Table 3).

Survival analysis

Patients were divided into high (n=43) and low (n=43) expression groups according to the median expression level of miR-34a. Median progression-free survival of patients with high and low expression was 37 and 20 months, respectively (p<0.05). Median overall survival was 44 and 31.5 months, respectively (p<0.05). Overall survival was 76.74% in the high expression group, significantly higher than that in the low expression group (51.16%, p<0.05; Figures 2 and 3).

Discussion

Basal cell carcinoma is a malignant tumor of basal cells of the skin or epidermis and is prone to occur in exposed areas of the skin [15]. Studies have shown that basal cell carcinoma is closely related to ultraviolet and solar radiation [16]. In recent years, the incidence of basal cell carcinoma is in a constant increase [17]. Basal cell carcinoma is highly destructive [18], seriously violating the patient's life and health. Early and complete resection of the lesion is still the first treatment option of this disease [19]. However, the scar after operation brings psychological problems to the patient and affects his/her social communication activities [20].

At the same time, diagnosis of basal cell carcinoma patients still lacks effective biomarkers, and the gold standard is still histological biopsy [21], leading to delayed diagnosis. It has been reported that miR-34a is underexpressed in malignant tumors, such as ovarian cancer, pancreatic cancer and lymphoma [22-24]. Studies have shown that downregulation of miR-34a is closely related to the development of many malignant tumors [25]. However, studies on the involvement of miR-34a in basal cell carcinoma are rare.

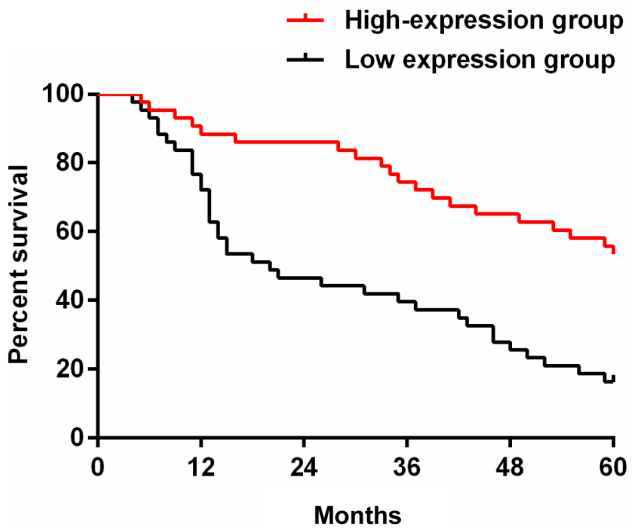

Figure 2. Comparison of progression-free survival between experimental group and control group. Kaplan-Meier method showed that the median progression-free survival of patients with high expression and low expression was 37 (range 5-60) and 20 (range 4-60) months, respectively. Progression-free survival of high-expression group was significantly better than that of patients in low expression group (p<0.05).

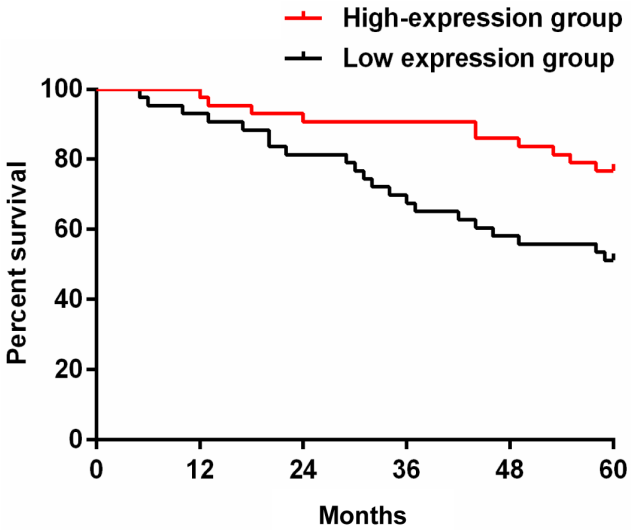

Figure 3. Comparison of overall survival between the experimental and control group. Kaplan-Meier method showed that the median survival time was 44 and 31.5 months for the high expression and the low expression group, respectively. Overall survival rate was 76.74% and 51.16%, respectively. Median overall survival and overall survival of patients in the high expression group were significantly better than of patients in low expression group (p<0.05).

In this study, the expression levels of miR-34a in the serum of 86 basal cell carcinoma patients and 85 healthy volunteers were quantified by real-time quantitative PCR. The results showed that the expression of miR-34a in patients was significantly lower than in healthy volunteers. The expression levels of miR-34a in basal cell carcinoma patients were closely related to the tumor diameter, lymph node metastasis and histological types. Survival of patients in the miR-34a high expression group was significantly better than that of patients in the low expression group. We therefore hypothesized that miR-34a may be involved in the development of basal cell carcinoma and the expression of miR-34a is associated with the prognosis of patients with basal cell carcinoma. MiR-34a is expected to be a new marker for basal cell carcinoma.

Studies have shown that miR-34a can cooperate with other tumor suppressors to regulate cell cycle in non-small cell lung cancer and induce arrest in G1/S phase, thereby inhibiting the abnormal proliferation of tumor cells [26]. miR-34a can also directly influence downstream apoptosis-related genes such as Bcl-2 and Survivin through signaling pathways such as Notch and c-met, thereby regulating tumor cell invasion and apoptosis [27,28]. In addition, studies have shown that miR-34a can inhibit the function of various cancer stem cells by targeting CD44 or regulating the length of telomeres [29,30]. Inhibited expression of miR-34a is closely related to poor prognosis of various malignant tumors such as hepatocellular carcinoma and cervical cancer [31,32]. Therefore, miR-34a may also regulate the apoptosis of basal carcinoma cells, and inhibit abnormal cell differentiation, proliferation, invasion and migration.

This study is limited by its small sample size. Our future studies will include more participants to further confirm the present conclusions. Data in the present study suggest that miR-34a may play a pivotal role in the development of basal cell carcinoma, while the mechanism is still not clear. More analyses are surely needed.

In summary, miR-34a may be involved in the development of basal cell carcinoma, and is closely related to multiple factors, such as lymph node metastasis and prognosis. MiR-34a is expected to be a new indicator for assessing the condition and prognosis of patients with basal cell carcinoma.

Conflict of interests

The authors declare no conflict of interests.

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