

miR-183 and miR-141 in lesion tissues are potential risk factors for poor prognosis in patients with infected abdominal aortic aneurysm

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Abstract. The expression levels of micro ribonucleic acid-183 (miR-183) and miR-141 in the lesion tissues of infected abdominal aortic aneurysm (IAAA) and their relationship with prognosis were investigated. Thirty-six patients with IAAA admitted and who underwent vascular surgery in People's Hospital of Shenzhen from June 2003 to June 2013 were selected. Reverse transcription polymerase chain reaction (RT-PCR) was utilized to detect the expression levels of miR-183 and miR-141 in lesion tissues and adjacent tissues 1 cm away from the aneurysm in 36 patients with IAAA. The relationship between the expression levels of miR-183 and miR-141 as well as the clinicopathological features of patients with IAAA were analyzed, and the factors influencing the prognosis of IAAA were analyzed by univariate and multivariate analysis. The expression levels of miR-183 and miR-141 were significantly downregulated in the lesions of patients with IAAA, and miR-183 and miR-141 levels in the lesion tissues of the IAAA patients were significantly lower than those in the adjacent tissues ($P < 0.05$). The expression levels of miR-183 and miR-141 were not related to sex, age, history of hypertension, and alcoholism ($P > 0.05$), but they were related to smoking history or aneurysm size ($P < 0.05$). The overall survival rate of patients with IAAA was 41.6% (15/36). The multivariate analysis found that aneurysm size, low expression of miR-183, and low expression of miR-141 were independent factors affecting the prognosis of patients with IAAA. In conclusion, the expression levels of miR-183 and miR-141 in the lesion tissues of IAAA are low, and the lower the expression level

is, the worse the prognosis gets. miR-183 and miR-141 can be used as predictors of prognosis in patients with IAAA.

Introduction

Infected abdominal aortic aneurysm (IAAA) is a special type of abdominal aortic aneurysm (AAA), accounting for 0.7-3.0% of all AAA (1), and approximately 1-3% of infected aneurysms (2). The disease is not easy to be found in the early stage, and the missed diagnosis and misdiagnosis rate is high. Once the rupture occurs, it risks the lives of patients directly and brings huge economic burden to the patients as well as their families. In some developed countries, the screening of high-risk population of IAAA may effectively increase the survival rate of patients (3-5). At present, the main molecular mechanism of IAAA has not yet been identified, and most scholars believe that the disease is caused by genetic, inflammation and other factors.

Micro ribonucleic acids (miRNAs) are a class of endogenous, non-coding small molecule RNA, which regulate the expression of target genes by inhibiting or promoting the lysis of messenger RNA (mRNA) through specific translation of sequences, resulting in the different impacts of biological behavior of tumors (6). Many studies have found that miRNA is expressed in other cancers and diseases. miRNA-141 (miR-141), a member of the miRNA-200 family, plays an important role in the occurrence, development and physiological status of diseases by regulating different signaling pathways (7). Research has shown that (8) miR-141 is closely related to tumor and angiogenesis. In the study of Liu *et al* (9), through the analysis of 7 GEO chips, it was found that miR-141 was differentially expressed in the tissues of liver cancer patients. miRNA-183 (miR-183), located on human 7q32.2 chromosome, is one of the most important members of the miR-183 family. In addition, research has shown that miR-183 is closely related to tumor occurrence and recurrence (10). The study of Fan *et al* (11) has shown that the differential expression of miR-183 in breast cancer patients was found through the analysis of GEO (GSE39093) chip. However, it is not clear whether miR-141, miR-183 and IAAA are related.

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Some studies have shown that (12,13) miR-183 and miR-141 are highly expressed in TB patients, while TB bacillus, as the main pathogenic microorganism of IAAA, has not been reported in relevant studies before. The relationship between miR-183, miR-141 and clinicopathological characteristics as well as follow-up data were studied by detecting the expression levels of miR-141 and miR-183 in IAAA to explore whether miR-183 and miR-141 were potential risk factors for the poor prognosis of patients with IAAA.

Patients and methods

Sample source. Thirty-six patients with IAAA admitted and who received vascular surgery in People's Hospital of Shenzhen (Shenzhen, China) from June 2003 to June 2013 were collected and selected, the average age of the patients was 41 ± 16.1 years, and they were diagnosed with IAAA by computerized tomography (CT) scanning and abdominal magnetic resonance imaging (MRI) in the imaging department of the hospital. There were 20 male patients and 16 female patients. The samples and adjacent tissues 1 cm away from the aneurysm collected from surgical resection were stored in liquid nitrogen within 5 min. None of the patients received radiotherapy, chemotherapy or other anticancer therapies before operation. The study was approved by the Ethics Committee of People's Hospital of Shenzhen, and the family members and patients were informed and signed the informed consent form.

Reagents and main equipment. TRIzol reagent and miRNA reverse transcriptase kit were purchased from Invitrogen; Thermo Fisher Scientific, Inc. (Waltham, MA, USA); SYBR Green Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) and ABI Prism 7900PCR instrument was purchased from Applied Biosystems; Thermo Fisher Scientific, Inc., and the reverse transcription primers and internal reference primers were synthesized by Shanghai Biological Engineering Co. Ltd. (Shanghai, China).

RNA extraction. The extraction procedure of the total RNA from the collected aneurysm and adjacent tissues by TRIzol reagent was carried out according to the instructions. The concentration and purity of the extracted RNA were detected by ultraviolet spectrophotometer (Bio Rad, Hercules, CA, USA), and the integrity of RNA was detected by agarose gel electrophoresis.

Synthesis of complementary deoxyribonucleic acid (cDNA). The miRNAs of the total RNA were reversely transcribed to synthesize cDNA following the instructions of the miRNA reverse transcription kit. The reverse transcription conditions of miRNA-183 and miRNA-141 were as follows: 37°C for 30 min, and then 95°C for 3 min. The synthesized cDNA solution was stored at -20°C.

Detection of expression levels of miRNA-183 and miRNA-141 via reverse transcription polymerase chain reaction (RT-PCR). BI Prism 7900 PCR instrument was used for PCR. The reaction system was 25 μ l, including 12 μ l SYBR Green Master Mix, 3 μ l of 10X miRNA specific primers, 2.5 μ l 10X universal

primer, 2.5 μ l diluted cDNA, and RNase-free double distilled water was added up to 25 μ l. For miRNA-183, upstream primer: 5'-CGTTGGATTCCATGGCACTGGT-3' and downstream primer: 5'-TTCAAGCAGGGTCCGAGGTATTC-3'; for miRNA-141, upstream primer: 5'-TTCCGATGGCGTAA CACTGTCTG-3' and downstream primer: 5'-TTCAAGC AGGGTCCGAGGTATTC-3'; the reaction conditions were as follows: 95°C for 5 min, 95°C for 30 sec, 60°C for 45 sec, and 72°C for 45 sec; a total of 40 cycles. U6 was used as reaction internal reference. All samples were repeated in 3 wells, and the results were analyzed by $2^{-\Delta\Delta C_q}$ method (14).

Statistical analysis. These experimental data were analyzed by Statistical Product and Service Solutions (SPSS) 19.0 software package (IBM Corp., Armonk, NY, USA), measurement data were expressed as mean \pm standard deviation, enumeration data were tested by χ^2 test, data between groups were compared by the independent sample Student's t-test and survival data were analyzed by Cox regression analysis. $P < 0.05$ suggested that the difference was statistically significant.

Results

Expression levels of miRNA-183 and miRNA-141 in IAAA tissues and adjacent tissues. Detection of tissues in 36 patients with IAAA by RT-qPCR revealed that the relative expression levels of RNA-183 and miRNA-141 in IAAA tissues were significantly lower compared to those in the adjacent tissues, and the difference was statistically significant ($P < 0.05$) (Fig. 1).

Analysis of correlation between IAAA clinical factors and expression levels of miRNA-183 and miRNA-141. Through the expression levels of miRNA-183 and miRNA-141 and clinicopathological features, we found that there was no statistical significance ($P > 0.05$) between the expression levels of miR-183 and miR-141 and sex, age, history of hypertension or alcoholism, but there was statistical significance in patients with smoking history and aneurysm size ($P < 0.05$) (Table I).

Cox regression survival analysis. In this experiment, the 3-year overall survival rate after surgery of 36 patients was 41.6% (15/36). Univariate analysis of the collected clinicopathological features found that sex, age, history of hypertension, alcoholism, smoking history, and aneurysm location were not related to prognosis ($P > 0.05$), but low expression of miR-183, low expression of miR-141, and aneurysm size in patients were significantly related to prognosis (Table II). The results of subsequent Cox multivariate analysis showed that low expression of miR-183 [hazard ratio (HR)=3.587, 95% confidence interval (95% CI): 2.641-6.541, $P=0.014$] and low expression of miR-141 (HR=3.841 95% CI: 2.894-5.981, $P=0.016$) could be used as independent prognostic factors for survival rate (Table III).

Discussion

The current traditional therapy for IAAA is surgical operation, including local excision of aneurysm tissues, debridement at a large area, and then vascular reconstruction in the outside bypass of *in situ* disease (13). However, IAAA patients have

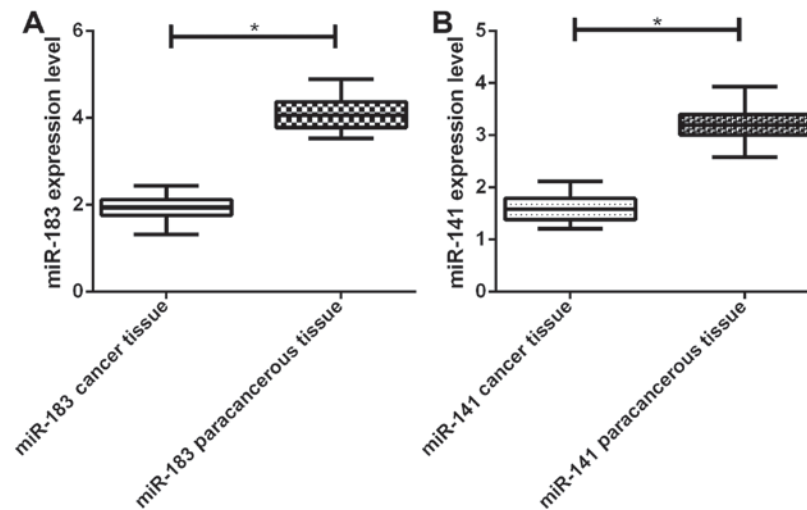


Figure 1. The expression levels of miRNA-183 and miRNA141 and their adjacent tissues. (A) The expression levels of miRNA-183 in aneurysm tissues was lower than that of adjacent tissues. *P<0.05. (B) The expression levels of miRNA-141 in aneurysm tissues was lower than that of adjacent tissues. *P<0.05.

Table I. Analysis of clinical factors of IAAA and expression levels of miRNA-183 and miRNA-141.

Groups	n	Expression level of miRNA-183	χ^2 value	P-value	Expression level of miRNA-141	χ^2 value	P-value
Sex			1.331	0.267		0.224	0.755
Male	20	1.825±0.417			1.247±0.389		
Female	16	1.617±0.674			1.384±0.247		
Age			0.354	0.651		0.574	0.511
≥55	17	1.687±0.644			1.174±0.217		
<55	19	1.715±0.347			1.089±0.373		
History of hypertension			2.219	0.212		0.667	0.493
Yes	21	1.574±0.734			1.294±0.241		
No	15	1.674±0.684			1.188±0.299		
Alcoholism			0.063	0.497		0.684	0.572
Yes	16	1.674±0.774			1.177±0.244		
No	20	1.578±0.627			1.341±0.274		
Smoking history			0.184	0.577		0.441	0.617
Yes	20	1.684±0.547			1.147±0.379		
No	16	1.841±0.454			1.214±0.214		
Tumor size			4.513	0.031		3.847	0.042
≥10 mm	22	1.324±0.241			0.841±0.102		
<10 mm	14	1.784±0.404			1.347±0.341		

a sudden onset of disease, and the majority of patients are admitted to hospital due to rupture of aneurysm and hemorrhage. As the condition is relatively serious and there are a variety of complications, the disease is difficult to treat with traditional surgery. The mortality rate during operation is high and the survival rate is not ideal (15).

miRNAs have been found closely related to the occurrence, development, invasion and metastasis of tumors. They are a kind of molecular biological target. miRNAs match the target by the principle of complementary base pairing to degrade the target mRNA and inhibit the translation process to achieve the target gene differential expression (6,16,17). Therefore, to the

best of our knowledge, we studied the expression of miRNA in IAAA patients for the first time, hoping to explore the expression of miRNAs and adverse prognosis in IAAA patients.

miRNA-183 is a member of the miRNA-183 family (mainly including miRNA-183, miRNA-182 and miRNA-96), located on the human 7q32.3 chromosome, involved in the occurrence and development of a variety of tumors and closely related to their physiological processes (18,19). miRNA-141 is a member of the miRNA-200 family, which plays a major role in the stable direction of epithelial cells. When the expression disorders occur, it leads to cell epithelial-mesenchymal transition increase, resulting in proliferation and metastasis

Table II. Univariate analysis.

Factors	Single factor	
	HR (95% CI)	P-value
Age	1.541 (1.122-3.895)	0.062
Sex	1.714 (1.399-1.654)	0.788
History of hypertension	0.813 (0.375-1.727)	0.174
Alcoholism	2.143 (1.288-2.755)	0.034
Smoking history	1.241 (0.355-2.943)	0.237
Tumor size	2.064 (1.282-5.723)	0.064
Low expression of miR-183	2.124 (1.557-4.479)	0.002
Low expression of miR-141	2.315 (1.231-3.524)	0.004

Table III. Multivariate analysis.

Factors	Multiple factor	
	HR (95% CI)	P-value
Tumor size	0.974 (0.384-1.894)	0.074
Low expression of miR-183	3.587 (2.641-6.541)	0.014
Low expression of miR-141	3.841 (2.894-5.981)	0.016

of epithelial tumors (20,21). This study on the detection of the expression levels of miRNA-183 and miRNA-141 in aneurysm and adjacent tissues in 36 cases of IAAA patients revealed that the expression of miRNA-183 and miRNA-141 in IAAA patients was low, and compared with the expression levels in the adjacent tissues, there was statistical significance ($P < 0.05$). This may indicate that these two kinds of miRNA are associated with the clinicobiological behavior of IAAA patients. The downregulation of miRNA-183 expression in hepatocellular carcinoma is mentioned in the study of Li *et al* (22), and the downregulation of miRNA-141 in gastric cancer is also noted by Du *et al* (23), which may indicate that different miRNAs have the same expression in different cancers. The correlation analysis of the clinical factors of IAAA patients showed that there was no statistical significance between the expression of miR-183 and miR-141 and sex, age, history of hypertension, alcoholism or aneurysm location ($P > 0.05$), but there was statistical significance in patients with smoking history, and the aneurysm size ($P < 0.05$). Finally, Cox regression analysis showed the low expression levels of miRNA-183 and miRNA-141 could be used as independent prognostic factors for IAAA patients. The main treatments of IAAA are antibiotic therapy and thorough debridement for *in situ* vascular reconstruction. However, as the course and the causes of IAAA are not clear at present, the location of the lesions is deep and the adjacent organs are difficult to handle when they are affected, resulting in the rupture of the aneurysm, which is mostly fatal (24). Therefore, we detected miRNA in IAAA patients and found better detection methods through this study to prevent the occurrence of the disease.

However, this study has certain limitations. As samples were difficult to collect and the number of samples was small, there was bias on the results, and there were also differences among different races. The current clinical trials are not sufficient on this subject. We hope to increase the collection of samples and increase animal experiments to support our results in future studies.

Collectively, miRNA is related to the occurrence and development of IAAA, but specific impact needs to be proven by test and study in the future. The results of this study indicate that downregulation of miRNA-183 and miRNA-141 is one of the pathogenesis of IAAA patients, and the lower the expression level is, the worse the prognosis gets, which can be used as a marker of prognosis in patients with IAAA. Thus, a new method is provided for the detection of IAAA, laying the foundation for future study of IAAA patients.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

CM and ZG conceived and designed the study. CM, DL, HL and JZ were responsible for the collection and analysis of the data. CM, DW and BL interpreted the data and drafted the manuscript. ZG and DL revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of People's Hospital of Shenzhen (Shenzhen, China). Signed informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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