

Original Article

The expressions and a correlation analysis of miR-155 and miR-27b in mycobacterium brain abscess

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Abstract: miR-27b is associated with inflammation, and miR-155 is involved in the regulation of *Mycobacterium tuberculosis*. However, the expressions and correlation of miR-155 and miR-27b in mycobacterial brain abscess remain unclear. New Zealand white rabbits were randomly divided into a control group and a brain abscess group. 1×10^7 CFU/mL *Mycobacterium tuberculosis* suspension was injected intracranially through the skull followed by an analysis of the cerebrospinal fluid and the expressions of miR-155 and miR-27b by real time PCR. The correlations between miR-155 and miR-27b and cerebrospinal fluid pressure, cerebrospinal fluid cell number, protein, sugar, and chloride content were analyzed. In the brain abscess group, the cerebrospinal fluid pressure, the cerebrospinal fluid cell number, and the protein content significantly increased, and the sugar and chloride content decreased with an increased miR-155 level and decreased miR-27b level compared with the control group ($P < 0.05$). miR-155 was positively correlated with cerebrospinal fluid pressure, the cerebrospinal fluid cell number, the protein content and negatively correlated with the sugar and chloride content ($P < 0.05$). On the other hand, miR-27b had a negative correlation with the cerebrospinal fluid pressure, the cerebrospinal fluid cell number, and the protein content, and a positive correlation with sugar and chloride content ($P < 0.05$). In addition, miR-155 and miR-27b were negatively correlated. The miR-155 expression was increased and miR-27b was decreased in mycobacterial brain abscess. miR-155 and miR-27b were negatively correlated. The combined detection of miR-155 and miR-27b was beneficial to the diagnosis of mycobacterial brain abscess.

Keywords: Mir-155, Mir-27b, mycobacteria, brain abscess, correlation

Introduction

Mycobacteria are a type of actinomycete and contain mycolic acid. They are classified into three groups: *Mycobacterium tuberculosis* complex, nontuberculous mycobacteria, and *Mycobacterium leprae* with bacilli being more common [1]. Tuberculosis caused by *Mycobacterium tuberculosis* is one a worldwide health problem, and it is a serious threat to human life [2]. In recent years, the incidence of tubercular infectious diseases has risen, and with the wide application of antibiotic drugs, the number of patients with multi-drug resistant tuberculosis and extensive drug-resistant tuberculosis has increased [3, 4]. The emergence of drug-resistant strains and HIV co-infection leads to an increase in the incidence of tuberculosis and the difficulty in diagnosis and treatment [5]. According to statistics, about 9 million active tuberculosis patients are diagnosed each year, and more than one million patients die from tuberculosis [6]. *Mycobacterium tubercu-*

losis is most susceptible to infection in the lungs, and *M. tuberculosis* infection in the brain is an important site of extrapulmonary tuberculosis infection, because it leads to higher disability and mortality [7, 8]. The most common form of *M. tuberculosis* infection in the brain is tuberculous meningitis [9]. Mycobacterial brain abscesses are a rare manifestation of central nervous system tuberculosis. Although the incidence of mycobacterial brain abscess is low, it can cause serious complications such as meningitis, subdural effusion, sepsis, etc., and are increasingly attracting attention [10, 11]. Because mycobacterial brain abscess lacks the typical "megacytic and epithelioid cell granuloma" reaction associated with tuberculosis, it is only accompanied by focal neurological symptoms, which makes the diagnosis and treatment of mycobacterial brain abscess difficult, resulting in delayed diagnosis and treatment [12]. Therefore, the early detection of its molecular markers is conducive to the diagnosis and treatment of mycobacterial brain abscess.

Table 1. Primer sequences

Gene	Forward 5'-3'	Reverse 5'-3'
GAPDH	ACCAGGTATCTTGGTTG	TAACCATGTCAGCGTGGT
Mir-155	AGCTTGACAGTCGCTCTCTTG	CAGCAGTTAACACCATGTGC
Mir-27b	TCGTCACCATGTCTGTAA	GTCCTTGGTAACACGTCT

MicroRNAs (miRNAs, also known as microRNAs) are widely found in animals, plants and even eukaryotes such as viruses, at 22-23 nucleotides in length [13]. MicroRNA plays a role in the endogenous negative regulation of gene expression mainly at the post-transcriptional level, which binds mRNA by targeting complementary pairing, thereby promoting mRNA degradation or translational inhibition [14]. MiRNAs can participate in the regulation of various diseases, and they also participate in the regulation of the occurrence and development of tuberculosis [15]. miR-155 has been shown to be involved in the regulation of tuberculosis, and miR-27b is involved in the inflammatory response [16, 17]. However, the expressions and correlation of miR-155 and miR-27b in mycobacterial brain abscesses have not been elucidated.

Materials and methods

Experimental animals

Healthy, male New Zealand white rabbits, aged 6-8 weeks, weighing $2.5 \text{ kg} \pm 0.5 \text{ kg}$, of SPF grade, were purchased from the experimental animal center of this unit and fed in in the SPF animal experiment center at a temperature of $21 \pm 1^\circ\text{C}$ and a relative humidity of 50-70% under constant temperature and constant humidity conditions, ensuring a 12-hour day/night cycle. Animal experiments were performed in strict accordance with the experimental design and performed by experienced technicians to minimize animal suffering. This study was approved by the Ethics Committee of our hospital.

Main reagents and instruments

Pentobarbital was purchased from Shanghai Medical Reagent Co., Ltd. The *Mycobacterium tuberculosis* suspension H37RV was supplied by the laboratory and stored in liquid nitrogen. A Medlab-U automatic biochemical analyzer was purchased from Nanjing Meiyi Technology Co., Ltd. Other commonly used reagents were purchased from Shanghai Shenggong Biological Co., Ltd. ABI7900 HT Real-time PCR was pur-

chased from ABI, USA. Surgical microscopy equipment was purchased from the Suzhou Medical Instrument Factory. The Labsystem Version 1.3.1 microplate reader was purchased from the Bio-Rad Corporation of the United States.

Grouping and processing of the experimental animals

Twenty male New Zealand white rabbits were randomly divided into two groups ($n=10$ in each group), a control group and a brain abscess group, in which the rabbits were anesthetized with pentobarbital and fixed on the operating table followed by an intracranial injection of 1×10^7 CFU/mL through the skull and subsequent infection with H37Rv standard strain *Mycobacterium tuberculosis*. The model was established successfully if the brain abscess cavity occurred.

Specimen collection

After 4 weeks of treatment, 5 ml blood was collected from the tail vein, centrifuged at 3000 rpm for 15 minutes, and then the serum was placed in a -80°C refrigerator. The white rabbits were sacrificed, and the cerebrospinal fluid was separated and stored in a -80°C refrigerator. The brain tissue was collected and quickly frozen in liquid nitrogen for 2 hours, and then stored in a -80°C refrigerator for use.

Cerebrospinal fluid routine and biochemical testing

The routine and biochemical tests of cerebrospinal fluid in each group of white rabbits were analyzed by automatic biochemical analyzer. The analysis included the cerebrospinal fluid pressure, the cerebrospinal fluid cell number, and the protein, sugar, and chloride content.

Real time PCR analysis of the expressions of miR-155 and miR-27b

Total RNA was extracted using Trizol reagent, and DNA reverse transcription synthesis was performed according to the kit's instructions. The primers were designed by Primerpremier 6.0 according to each gene sequence and synthesized by Shanghai Yingjun Biotechnology Co., Ltd. (Table 1). Real-time PCR was performed for detection of the gene of interest using the conditions as follows: 92°C 30 S, 58°C 45 S, 72°C 35 S, for a total of 35 cycles. GAPDH was selected as a reference. According to the fluorescence quantification, the starting

Table 2. Conventional and biochemical analysis of cerebrospinal fluid in a rabbit model of mycobacterial brain abscess

Group	Cerebrospinal fluid pressure (mmH ₂ O)	Cell number ($\times 10^5$ /L)	Protein content (g/L)	Sugar (mmol/L)	Chloride (mmol/L)
Control	121.25 \pm 17.05	87.61 \pm 9.05	0.41 \pm 0.06	3.32 \pm 0.98	145.31 \pm 2.98
Model	186.75 \pm 22.20*	132.55 \pm 0.09*	1.35 \pm 0.67*	2.12 \pm 0.17*	102.11 \pm 1.16*
P value	< 0.0001	< 0.0001	0.0003	0.0013	< 0.0001

Student's t test. Compared with control group, *P < 0.05.

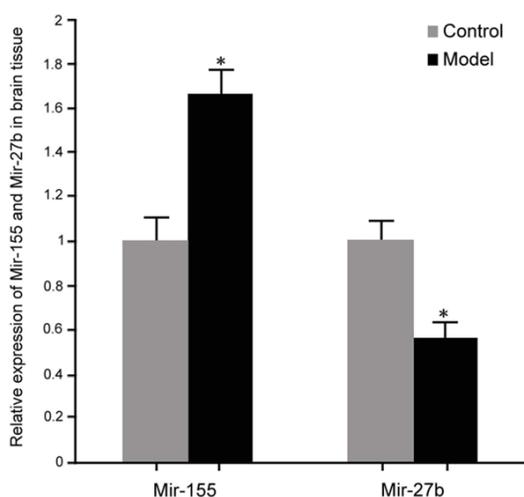


Figure 1. Expression of miR-155 and miR-27b in brain tissue of rabbit model of mycobacterial brain abscess. Compared with the control group, *P < 0.05.

cycle number (CT) of all the samples and standards was calculated. Based on the standard CT value, a standard curve was drawn and then a semi-quantitative analysis was carried out using the 2- $\Delta\Delta$ Ct method.

Statistical analysis

The data were analyzed using SPSS 19.0 software. The measurement data were expressed as the mean \pm standard deviation, and the comparisons among multiple groups was performed using a one-way analysis of variance. The correlation analysis was performed using the Pearson correlation analysis. P < 0.05 indicates a significant statistical difference.

Results

Conventional and biochemical analysis of the cerebrospinal fluid

In the rabbit model of mycobacterial brain abscess, the cerebrospinal fluid pressure, the

cerebrospinal fluid cell number, and the protein content increased, but the sugar and chloride content decreased. Compared with the control group, the differences were statistically significant (P < 0.05) (Table 2).

The expressions of miR-155 and miR-27b in brain tissue

A real-time PCR analysis of miR-155 and miR-27b expressions in the brain tissue of rabbit models of mycobacterial brain abscess confirmed that the expression of miR-155 in the brain tissue of the *Mycobacterium tuberculosis* rabbit model was significantly increased, and the expression of miR-27b was significantly decreased, compared with the rabbits in the control group (P < 0.05) (Figure 1).

miR-155 and miR-27b expressions in the cerebrospinal fluid

A real-time PCR analysis of miR-155 and miR-27b expressions in the cerebrospinal fluid of the rabbit model of mycobacterial brain abscess showed that the expression of miR-155 in the cerebrospinal fluid of the rabbit model of mycobacterial brain abscess was significantly increased, and the expression of miR-27b was decreased compared with the rabbits in the control group (P < 0.05) (Figure 2).

miR-155 and miR-27b expressions in the blood

A real-time PCR analysis of miR-155 and miR-27b expressions in the blood of the rabbit model of mycobacterial brain abscess revealed that the expression of miR-155 was significantly increased in the rabbit model of mycobacterial brain abscess, and the expression of miR-27b was decreased. Compared with the control group, the differences were statistically significant (P < 0.05) (Figure 3).

miR-155 and miR-27b in MBA

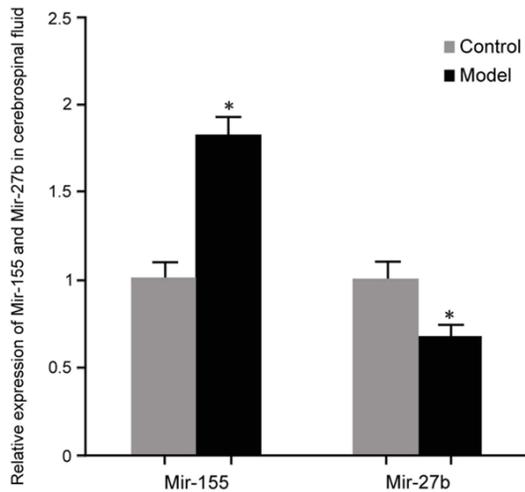


Figure 2. miR-155 and miR-27b expressions in the cerebrospinal fluid of the rabbit model of mycobacterial brain abscess. Compared with the control group, *P < 0.05.

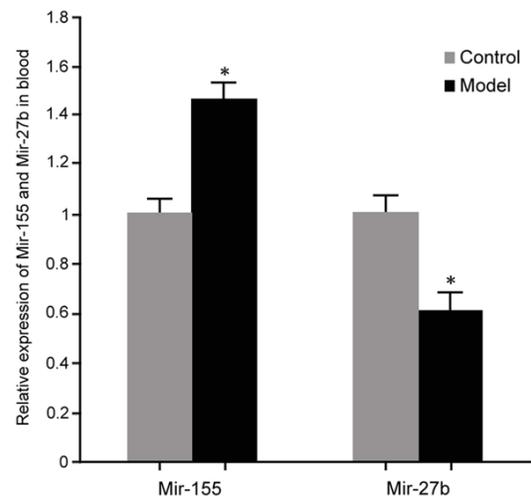


Figure 3. miR-155 and miR-27b expressions in the blood of the rabbit model of mycobacterial brain abscess. Compared with the control group, *P < 0.05.

Correlation analysis of routine and biochemical indicators with miR-155 and miR-27b

miR-155 was positively correlated with cerebrospinal fluid pressure, the cerebrospinal fluid cell number, and protein content, and negatively correlated with sugar and chloride content ($P < 0.05$). However, miR-27b was negatively correlated with the cerebrospinal fluid pressure, the cerebrospinal fluid cell number, and protein content, and sugar and chloride content was positively correlated ($P < 0.05$) (Table 3).

Correlation analysis between miR-155 and miR-27b in cerebrospinal fluid, brain tissue, and blood of the rabbit model of mycobacterial brain abscess

miR-155 and miR-27b were negatively correlated in the cerebrospinal fluid, brain tissue, and blood of the rabbit model of mycobacterial brain abscess ($P < 0.05$) (Table 4; Figure 4).

Discussion

Among the central nervous system infectious diseases, mycobacterial brain abscess is an important disease sub-type. Due to the blood-borne dissemination of the lungs, the cellular immune function of the patient decreases, leading to a mycobacterial infection of the brain tissue [11]. The relevant response to mycobacteria depends mainly on the immune status of

the individual to the mycobacteria, the location and number of infections of the infected bacteria, as well as the state of the patient's treatment [18]. Infecting the body, the inoculation of a small amount of mycobacteria can lead to the formation of infectious diseases such as tuberculosis. For example, the administration of a large number of bacilli may lead to an excessive exudation of a large amount of cheese, accompanied by the infiltration of inflammatory cells, resulting in the formation of pus, which may be accompanied by tuberculous necrosis, tuberculosis. Bacteria and their products need to be present in brain tissue to form brain abscesses [19, 20]. Mycobacterial brain abscess lacks typical giant cell and epidermoid granulomatous reactions and is often difficult to distinguish from purulent brain abscess. Patients may have focal neurological deficits corresponding to the degree of peripheral edema, the onset of the process is slow, and the appearance of brain signs depends on the location of the abscess [21]. The diagnosis of mycobacterial brain abscess mainly depends on clinical symptoms and signs, bacterial tests, and imaging analysis such as MRI or CT. However, because of other causes of brain abscess, the bacterial detection process takes a long time, leading to misdiagnosis, and the delayed treatment of patients [22]. Therefore, it is necessary to find a rapid and effective molecular target to assist in the diagnosis of mycobacterial brain

Table 3. Correlation analysis of routine and biochemical indicators with miR-155 and miR-27b

	Cerebrospinal fluid pressure	Cell number	Protein content	Sugar	Chloride
miR-155	0.62*	0.78*	0.69*	-0.58*	-0.88*
P values	0.031	0.023	0.032	0.043	0.021
miR-27b	-0.61*	-0.79*	-0.59*	0.77*	0.51*
P values	0.026	0.041	0.036	0.015	0.032

Pearson correlation analysis. *P < 0.05.

Table 4. Correlation analysis between miR-155 and miR-27b

	Cerebrospinal fluid	Brain tissue	Blood
miR-155	-0.73*	-0.85*	-0.64*
miR-27b			

Pearson correlation analysis. *P < 0.05.

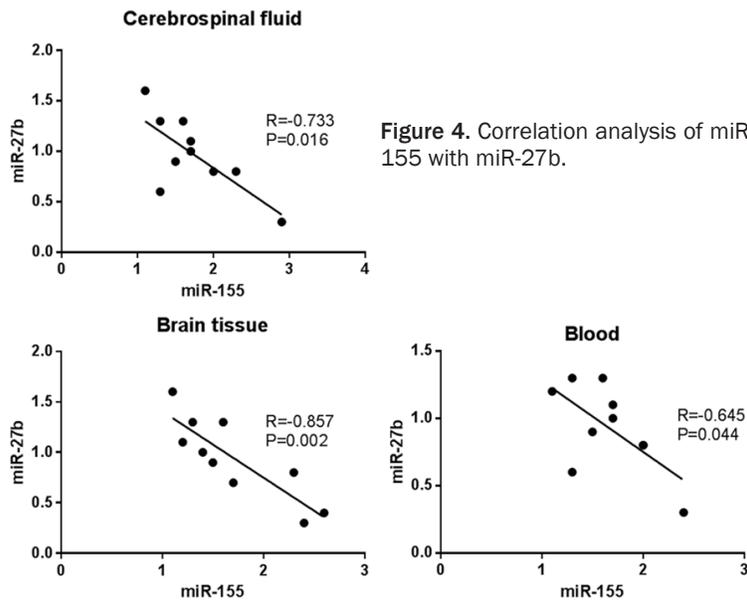


Figure 4. Correlation analysis of miR-155 with miR-27b.

[26]. It has been reported that microRNA levels change following mycobacterial infections [27]. A previous study confirmed that miR-155 is increased in tuberculosis, and miR-27b is involved in immune status and autoimmune diseases [16]. Therefore, this study analyzed the expression of both in mycobacterial brain abscess and their correlation with disease. The results showed that the expression of miR-155 in brain tissue, cerebrospinal fluid, and the blood of mycobacterial brain abscess was increased, but the expression of miR-27b was decreased. miR-155 and miR-27b were positively or negatively correlated with the cerebrospinal fluid pressure, the cerebrospinal fluid cell number and protein content, respectively, but negatively or positively correlated with the sugar and chloride content. In addition, miR-27b and miR-155 were negatively correlated. This result suggests that miR-27b and miR-155 are abnormally expressed in mycobacterial brain abscess and may be a promising molecular marker for mycobacterial brain abscess.

Conclusion

miR-155 is increased and miR-27b is decreased in mycobacterial brain abscess. miR-27b and miR-155 are negatively correlated. The detection of miR-27b is beneficial to mycobacterial brain abscess diagnosis.

Acknowledgements

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Disclosure of conflict of interest

None.

abscess, which is conducive to the diagnosis and treatment of the disease.

MicroRNAs are a class of highly conserved single-stranded 18-25 nt small RNA molecules that regulate the expression of target mRNAs [23]. Recent studies have shown that serum miRNAs are stable to repeated freeze-thaw cycles and thermal, acidic and alkaline conditions, among other extremes. It may be a useful biomarker for disease diagnosis, treatment outcome, and prognosis [24, 25]. Previous studies have suggested that microRNAs are involved in several diseases, including cancer and heart, immune and infectious diseases

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