

Original Article

High serum miR-183 level is associated with the bioactivity of macrophage derived from tuberculosis patients

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Abstract: As a major health threat, tuberculosis (TB) is resistant against the current therapeutic strategies. Increasing evidence indicates that miRNAs are implicated in various disorders by affecting specific target genes. Recently, the association of miRNAs with TB has also been established by several studies, and their potentials in the prognosis and treatment of TB have also been verified. miR-183 is shown to promote the activation of macrophage through NF- κ B pathway. However, it is still unclear if serum miR-183 can be used to assess the activity of TB-associated macrophage. This study was aimed to address this issue. We employed qPCR assay to detect the expression level of miR-183 in blood from TB patients and healthy individuals. miR-183 abundance was found to be increased in serum samples from TB patients, compared with healthy controls. Further analysis revealed that miR-183 level is positively associated with the activity of macrophages from TB patients, evidenced by their increased phagocytosis rates and enzyme activity in high serum miR-183 group. In conclusions, high level of serum miR-183 is associated with the activity of macrophage originating from TB patients.

Keywords: Tuberculosis, miR-183, macrophage

Introduction

Despite the medical advances in recent decades, tuberculosis (TB) is still a major challenge to human public health, and more than 30% of people are estimated to be infected with *Mycobacterium tuberculosis* [1]. Especially in developing countries such as China and India, pulmonary TB remains among the most common deadly diseases with high infectiveness.

miRNAs belong to evolutionarily conserved non-coding RNAs with a length of 20-22 nt, and can recognize and bind their 3'UTRs. This event can lead to the inhibition of mRNA translation or degradation [2]. Numerous publications have shown that miRNAs play critical roles in almost all of the biological processes including cell differentiation and proliferation, and organism development [3]. In addition to their physiological functions, more and more studies have pointed that miRNA are also implicated with various disorders such as infectious diseases.

For examples, *Salmonella* infection leads to the suppression of let-7 family in macrophages, and consequently elevates the level of secreted immunostimulatory cytokines [4]. These findings suggest that miRNAs are worth studying for better understanding of the biological processes by which a host resists against infection and developing new strategies for treatment.

Similar to many other infectious diseases, the progression and prognosis of TB is closely associated with its reaction with the host immunity [5]. Both of innate and adaptive immune responses participate in the defense against *M. tuberculosis*, the major TB pathogen. As the effector of innate immunity, macrophages engulf *M. tuberculosis* entering pulmonary alveolus, and subsequently, recruit other immune cells such as CD4⁺ and CD8⁺ T cells, dendritic cells (DC) and natural killer (NK) cells [5]. Reasonably, the activity of macrophage is important for the progression and prognosis of TB during patients are infected with *M. tuberculosis*. However, the biomarkers that can indi-

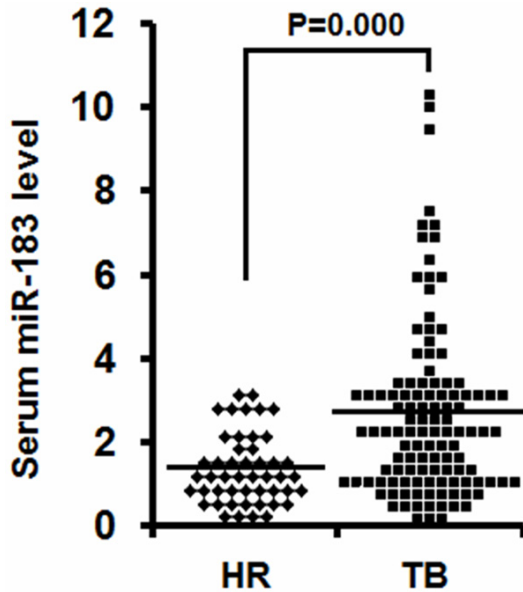


Figure 1. Serum miR-183 level is higher in TB patient than healthy individuals. A. qPCR assay was used to evaluate the levels of miR-183 from the blood samples of TB patients ($n = 110$) and healthy volunteers ($n = 48$). The relative value of each sample was shown as a dot. The lines represented the average of TB patients and healthy controls (HR). The difference between RCC and HR was analyzed using students' tests.

cate the active status of TB-associated macrophages are still lacked.

By recent studies, miRNA has also been verified as a potent regulator for the biology of macrophages [6]. Among the identified macrophage-associated miRNAs, *miR-183* attracts our attention because of its potential ability to promote the activation of macrophage by $\text{TNF}\alpha/\text{NF-}\kappa\text{B}$ signaling [7]. However, there has been no study focusing on the expression levels of *miR-183* in TB patient-derived macrophages and blood samples, or its ability to reflect the activity of macrophages yet.

To answer this question, we collected a panel of patients with TB to investigate the expression profile of *miR-183* in their serum, and to study the association of serum *miR-183* levels with the activity of TB-associated macrophage.

Materials and methods

Cell culture

U937 macrophage cell line was purchased from Shanghai Cell Culture Center (Shanghai,

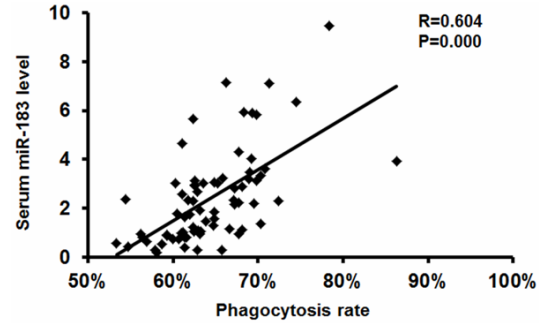


Figure 2. Serum miR-183 level was positively correlated with the phagocytosis of TB-related macrophages. The phagocytosis rates of primary macrophages ($n = 73$), which were derived from TB patients, were determined by phagocytosis assays after co-culturing with chicken red blood cells. The dots indicated means of three independent experiments. The association between miR-183 level and phagocytosis rates was calculated with Pearson analysis.

China). This cell line was cultured with RPMI 1640 media containing 10% fetal bovine serum at 37°C in a 5% CO_2 humidified condition.

Ethics statement

In our study, peripheral blood was collected from TB patients and healthy volunteers with written informed consent. The samples were collected following the procedures approved by the Ethics Committee of The First Affiliated Hospital of Xinxiang Medical University.

Study design and patients

110 confirmed TB patients and 48 healthy individuals were recruited for this study. TB was diagnosed by clinical manifestation, chest X-ray examination, and sputum test. The blood samples were collected from these TB patients prior to any treatments at the Departments of Tuberculosis medicine, the First Affiliated Hospital of Xinxiang Medical University,

Serum collection

We collected serum samples following the procedures previously described. Briefly, blood were collected from vena and transferred into serum collection tubes. The samples were centrifuged at 820 g for 10 min at 4°C , after 1 h coagulation at room temperature. Then, the serum samples were transferred into new tubes for centrifugation at 16000 g for 10 min at 4°C .

High miR-183 indicates active macrophages in TB

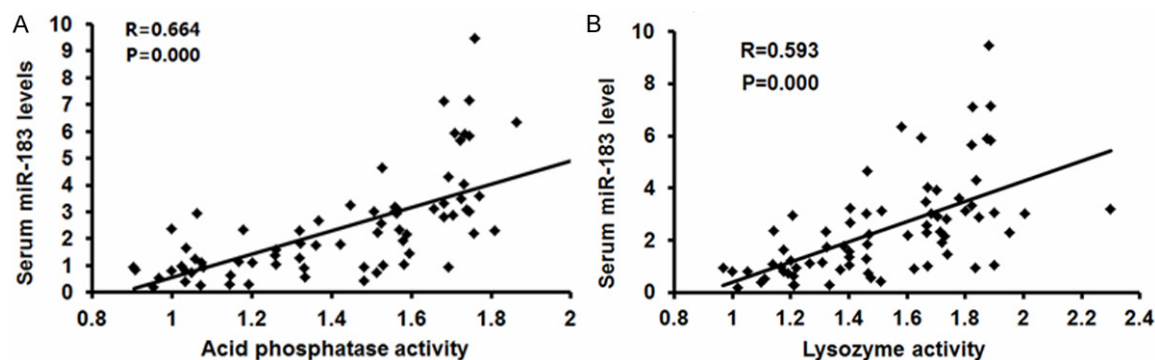


Figure 3. Serum miR-183 level was positively correlated with the activities of acid phosphatase and lysozyme in TB-related macrophages. The activities of acid phosphatase (A) and lysozyme (B) in primary macrophages ($n = 73$), which were derived from TB patients, were determined using Acid Phosphatase Assay Kit and Lysozyme Assay Kit, respectively. The dots indicated means of three independent experiments. The association between miR-183 level and enzyme activities was calculated with Pearson analysis.

Quantitative PCR (qPCR) assay

Total RNA was extracted the serum samples with the Trizol Solution (Invitrogen, Carlsbad, CA, USA) according to the routine protocols. The obtained RNA was subjected to reverse transcription with TaKaRa microRNA transcription kit (Takara, Japan), in order to produce cDNA. The resulting cDNA was subjected to quantitative PCR (qPCR) assays using SYBR Premix Ex Taq II kit (Takara, Japan) on an ABI-7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) supplied with analytic software. U6 was used as endogenous references in this study.

Primary macrophage culture

Blood samples were collected from vena, followed by elimination of erythrocytes with Red Blood Cell Lysis Buffer (Beyotime Institute of Biotechnology, China). The resulting cells were washed with PBS twice, and then cultured in RPMI 1640 media containing 10% fetal bovine serum for 2 h. PBS was then used to wash the cells again. The attached cells were cultured with fresh RPMI 1640 media containing 10% fetal bovine serum.

Macrophage phagocytosis test

To evaluate the phagocytosis rate of macrophages of TB patients and healthy volunteers, we detected phagocytosis of macrophage to chicken erythrocyte in this study. Briefly, Blood was collected from the vein of chicken, and then, 20 μ l chicken blood sample was added to

2 ml culture of macrophages. After 2 h incubation, the cells were harvested and stained with Wright's stain. The macrophages swallowing chicken red blood cells were counted under microscope. The phagocytosis rate (%) = the number of macrophages engulfing erythrocytes/the number of total macrophages.

Enzyme activity examination

The activities of enzymes that reside in the lysosome of macrophages, such as acid phosphatase and lysozyme, were estimated using Acid Phosphatase Assay Kit (Beyotime Institute of Biotechnology, China) and Lysozyme Assay Kit (Shanghai Elisa Biotechnology Company). The experiments were performed for three times.

Statistical analysis

Most of the statistical analyses in this manuscript were two-tailed student's test. The association between serum miR-183 level and the activities of macrophage were determined by Pearson analysis. Differences were considered as statistically significant (*) when $P < 0.05$ and statistically very significant (**) when $P < 0.01$.

Results

Level of miR-183 is elevated in the blood of TB patients

First of all, we investigated the expression profile of miR-183 in the serum derived from patients with TB ($n = 110$) and healthy individuals ($n = 48$). qPCR assays were performed to

estimate the abundance of miR-183 in these samples. The results showed that miR-183 level was significantly increased in the serum collected from TB patients, compared to healthy controls ($P < 0.01$) (**Figure 1**).

Serum level of miR-183 is associated with the activity of macrophage derived from TB patients

Considering the role of miR-183 expression in the activation of macrophage, we subsequently studied the relationship between serum miR-183 level and the bioactivity of macrophage isolated from the blood sample of TB patients ($n = 73$). Phagocytosis assays revealed that the macrophages from TB patients with high serum miR-183 levels have elevated phagocytosis rates, compared with low miR-183 group (**Figure 2**). The phagocytosis rates are positively associated with the levels of serum miR-183 ($R = 0.604$, $P < 0.01$) (**Figure 2**).

We also examined the activity of enzymes, such as acid phosphatase and lysozyme, in macrophages from TB patients. The results indicated that there was a positive correlation between serum miR-183 level and the activity of macrophage-derived acid phosphatase in TB patients ($R = 0.664$, $P < 0.01$) (**Figure 3A**). The similar association was also observed in the activity of lysozyme. The macrophages from TB patients with high miR-183 expression levels have increased lysozyme activity, compared with low miR-183 group (**Figure 3B**).

The above data demonstrated that miR-183 level in blood is positively associated with the activity of macrophage from TB patients.

Discussion

miRNAs have been well documented to be associated with TB [8]. In fact, miRNAs have been verified as biomarkers for the discrimination between TB patients and healthy individuals [9-11]. In our study, miR-183 has been verified to be overexpressed in TB patients, and the difference is statistically significant. Therefore, miR-183 may be a novel biomarker that can be used for TB diagnosis.

Interestingly, the altered miRNA expression is also related to the immune response of host to TB infection. For example, miR-125b expres-

sion can be induced by bacterial cell-wall components in *Mycobacterium tuberculosis* [12]. Recently, miR-183 has been shown to be associated with immunity. miR-183 is found to contribute to the activation of macrophage through TNF α /NF- κ B pathway [7]. Our data revealed that serum miR-183 level is positively associated with the activity of macrophage, evidenced by increased phagocytosis rates and enzyme activity in macrophages derived from TB patients with high miR-183 abundance. To our knowledge, this is the first time to provide evidence that miR-183 is implicated with the biology of TB-associated macrophages.

Although the association of miR-183 with TB has been established in our study, its role in this infectious disease is still not clarified. Further studies should focus on the relationship between the activation of TNF α /NF- κ B pathway and serum miR-183 in TB patients. These studies can facilitate our understanding of the molecular mechanism of host immune response against TB.

Collectively, we verified that serum miR-183, which is overexpressed in TB patients, is associated with the activity of macrophages from TB patients. The above findings showed that miR-183 is linked to the biology of TB-associated macrophages. There is also a possibility that targeting miR-183 may regulate the immunity of patients against TB.

Disclosure of conflict of interest

None.

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