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Original Article

Differential Expression of miR-146 and miR-155 in Active and Latent Tuberculosis Infection

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Abstract

Background: Tuberculosis (TB) is one of the leading causes of death worldwide. Besides, one-third of the world population is infected with *Mycobacterium tuberculosis* (MTB) while staying clinically asymptomatic; the situation is called latent TB infection (LTBI). MiR-21, miR-31, miR-146a, and miR-155 play an important role in many immune and inflammatory pathways. In the present study the expression levels of MiR-21, miR-31, miR-146a, and miR-155 in peripheral blood mononuclear cells (PBMCs) from patients with active TB, latently infected individuals (LTBI), and healthy controls (HC) were investigated. Participants were recruited at the Bouali Hospital, Zahedan University of Medical Sciences, Zahedan, Iran from 2010 to 2011.

Methods: PBMCs were stimulated with PPD before RNA extraction. TaqMan RT-qPCR assay was used to analyze the expression levels of miRNAs.

Results: The results indicated no significant differences in the expression of miR-21 and miR-31 between different groups; however, in patients with active TB, the expression of miR-21 (P=0.03) and miR-31 (P=0.04) were significantly increased after stimulation with PPD compared to the unstimulated condition. The expression of miR-146 in response to PPD in both LTBI (P=0.02) and TB (P=0.03) groups compared to the HC group was increased. No significant differences were found in the expression level of miR-155 in response to PPD between LTBI and HC groups. However, the fold change was significantly higher in the TB group in comparison with the HC (P=0.03) and LTBI (P=0.05) groups.

Conclusion: The results confirm the main role of miR-146 and miR-155 in TB infection and suggest a role for miR-146 and miR-155 as infection and activation markers in tuberculosis infection, respectively.

Keywords: Latent TB infection; microRNA; Mycobacterium tuberculosis; PPD Purified protein derivative

Introduction

Tuberculosis (TB) is an ancient disease caused by *Mycobacterium tuberculosis* (MTB) and remains one of the most important infectious diseases, particularly in developing countries. In 2019 around 10 million new TB infections and 1.4 million deaths were reported worldwide. In addition to the disease, one-third of the world population is thought to be latently infected (LTBI) with MTB; however, only about 5%–10% of them progress to active disease (1,2).

Besides the role of bacillary virulence determinants, host genetic and immune backgrounds

play a major role in determining the clinical manifestations and the disease outcome. Recently, the role of microRNAs (miRNAs) in the immune system has been considered. MiRNAs are a class of endogenous, small, non-coding RNAs of approximately 20~25 nucleotides, and are potent modulators of gene expression by binding to the 3' untranslated region (UTR) of target mRNA sequences and post-transcriptionally down-regulate their expression. They regulate about one-third of the human genes (3). Growing evidence suggests that miRNAs are implicated in the development and differentiation of immune cells and their immunological functions including both innate and acquired immune systems (4). The expression levels of miRNAs have also been associated with different noninfectious and infectious. Altered expression of miRNAs has been reported in various bacterial infections correlated with an imbalance of the Th17/Treg ratio (5). MiRNAs are also involved in viral infections by inhibiting virus replication or promoting pathogenesis (6). Moreover, miRNAs perform an important role in the pathogenesis of human cancer; some miRNAs may act as oncogenes, and others can act as tumor suppressors based on the target gene character. Tumor-suppressive miRNAs by targeting cancer-inducing genes decrease their expression in cancer cells. Conversely, tumor-promoting miRNAs inhibit the expression of tumor inhibitor genes (7,8). Indeed, disease-associated miRNAs represent a new class of diagnostic markers or therapeutic targets (6,9,10).

Numerous studies have indicated the roles of miRNAs in the diagnosis, treatment, and disease pathogenesis of TB. Upon MTB infection, several miRNAs modulate the host mechanism, either favoring the host or the pathogen. Signaling pathways, cytokine production, and host-killing machinery are regulated by miRNAs during MTB infection (11). Altered expression of different miRNAs has been linked to the expression of IFN-□ and TNF-□; two critical cytokines in control of TB (6). Moreover, the levels of circulating miRNAs have been considered potential biomarkers for the diagnosis or prognosis of TB (11). Downregulated miR-31, mmu-miR-150, and mmu-miR-146a (12) in PBMCs, and miR-155 in plasma (13), and upregulation of miR-144 levels in sputum and serum (14) have been reported. The potential of miRNAs as biomarkers of LTBI has also been the subject of some studies. Wang et al identified 17 miRNAs that were differentially expressed in active TB and LTBI (15). The levels of miR-145 in serum have been suggested for distinguishing TB patients from healthy individuals and differentiating between active TB and latent TB cases. miR-889 expression was increased in patients with LTBI compared to uninfected individuals (16). Further studies are required to confirm the findings in a larger subset of patients and more defined conditions.

miR-21, miR-146a, and miR-155a have been central in much miRNA research due to their expression levels following TLR activation (17). We selected four miR-21, miR-31, miR-146a, and miR-155, which play an important role in many immune and inflammatory pathways and are among the most vastly studied miRNAs influencing the host-pathogen interaction in TB. To find the disease relevance of the four mentioned miRNAs, we attempted to compare their expression among peripheral blood mononuclear cells (PBMCs) from patients with active TB, LTBI, and healthy controls (HC) after stimulation with PPD. We believe that investigation of miRNAs in PBMCs after stimulation with specific antigens could better reflect their changes in the particular disease and less affected by several miRNA-affecting factors.

Materials and Methods

Study subjects

Twenty-one patients with untreated active pulmonary TB, 20 volunteers with LTBI, and 17 healthy controls (HC) were studied. Patients were recruited at the Bouali Hospital, Zahedan University of Medical Sciences, Zahedan, Iran (2010-2011). The diagnosis of active TB was based on clinical presentation, chest radiography, and acid-fast stain of sputum smear. Participants with LTBI and HC were recruited from the healthy

contacts of tuberculosis patients and hospital staff. The results of tuberculin skin testing (TST) and QuantiFERON TB gold test (Cellestis Limited, Victoria, Australia) were used to distinguish between LTBI and HC participants. TST of more than 10 mm was considered positive. The LTBI volunteers were TST- and IGRA-positive; while the healthy controls were TST- and IGRA- negative. All of the participants were HIV, hepatitis B virus (HBV), and hepatitis C virus-negative.

This study was approved by the Ethical Committee of Pasteur Institute of Iran and written informed consent was obtained from all participants.

PBMCs isolation and activation

PBMCs were isolated from heparinized-blood samples by Ficoll Hypaque density gradient centrifugation (Histopaque 1077, Sigma, Germany), as previously described by Ajdary et al (18). The cells were frozen and kept in liquid nitrogen. Before use the cells were rapidly thawed and washed with RPMI-1640. The viability was ascertained by Trypan blue dye exclusion. PBMCs (10^6/ml) were treated or untreated with 24 ug PPD of MTB for 4 hr before RNA extraction.

miRNA extraction and reverse transcription

Total RNA, including miRNAs, was extracted from PBMCs using the mirPremier™ microRNA Isolation Kit (Sigma, Germany) following the manufacturer's instructions. The RNA concentration and purity were quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Complementary DNA (cDNA) was synthesized by using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystem, USA) containing RNA-specific RT primer according to the manufacturer's instruction.

Real-time quantitative Real-time (RT-qPCR) analysis

For RT-qPCR-based detection of miRNA TaqMan™ MicroRNA Assays (Applied Biosystems, USA) were used. The amplification was performed in 10 µL PCR reactions according to the supplier's protocol in Corbet PCR System. The amplification curves were analyzed using the Rotor-Gene 6000 Series Software 1.7. Triplicates were performed for each sample and mean Ct values were calculated. The expression of miRNAs was normalized using the RNU24 as the endogenous control. dCt was determined using the following formula: Ct number of target gene - CT number of the reference gene. Fold change was calculated using the comparative threshold method ($2^{-\Delta\Delta CT}$).

Statistical analysis

Statistical comparisons between the groups were performed using Graphprism (version 8.3.0, GraphPad Software, Inc., San Diego, CA). The *P* values were calculated by Kruskal-Wallis test followed by Dunn's multiple comparisons test for analysis of fold changes and dCts. Paired t-test was used to compare dCt of PPD-stimulated and un-stimulated cells in each group. $P \leq 0.05$ was considered statistically significant. The results were expressed as a median.

Results

The best time for analysis was 4 h after stimulation (data not shown). The RT-qPCR analysis revealed no significant differences in the expression of miR-21 and miR-31 between different groups (Fig. 1a, 1d). Besides, the expression levels of miRNAs without PPD stimulation were analyzed using dCt. There were no significant differences in the expression of miR-21 in un-stimulated PBMCs (Fig. 1b). However, the expression of miR-31 in un-stimulated PBMCs from the TB group was significantly lower than those from HC (P=0.02) and LTBI (P=0.02) groups (Fig. 1e). Interestingly, in patients with active TB, the expression of miR-21 (P=0.03) and miR-31 (P=0.04) were significantly increased after stimulation with PPD compared to the un-stimulated condition (Fig. 1c, 1f).

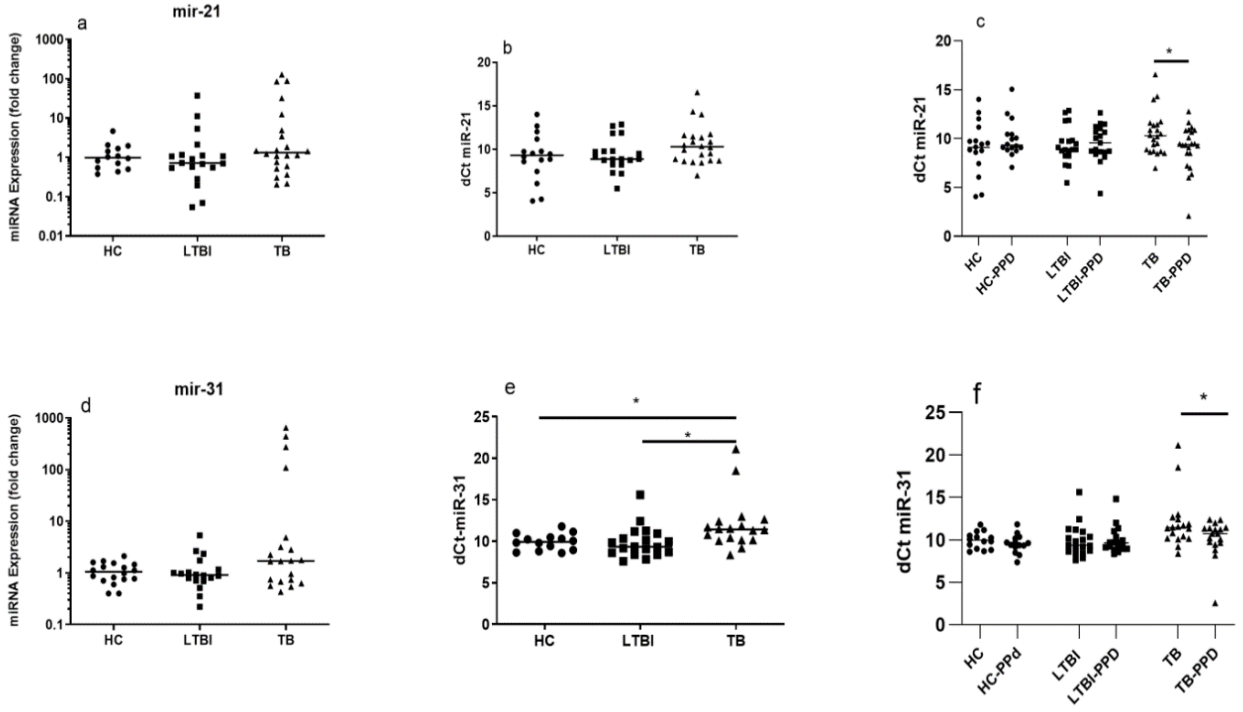

Fig. 1: PPD-induced miRNA-21, and miRNA-31 expression in qRT-PCR analysis. Fold change (a, d); miRNA expression without PPD stimulation (b, e); comparison of dCt of PPD-stimulated and un-stimulated cells (c, f). * $P \leq 0.05$

Considering the expression of miR-146a, the results indicated an increased expression of miR-146a in response to PPD in both LTBI (P=0.02) and TB (P=0.03) groups compared to the HC group (Fig. 2a). There were not any differences in miR-146a expression in un-stimulated PBMCs among study groups (Fig. 2b). Likewise, the expression of miR-146a was not increased after stimulation with PPD compared to the unstimulated condition (Fig. 2c).

There was not any significant difference between LTBI and HC groups in the expression level of miR-155 in response to PPD; however, the fold change was significantly higher in the TB group in comparison with the HC group (P=0.03); likewise, the fold change of miR-155 in the TB group was significantly higher than that of LTBI (P=0.05; Fig. 2d). Moreover, in patients with active TB, the expression of miR-155 significantly increased after stimulation with PPD compared to the unstimulated condition (P=0.03). No change was detected in the expression of miR-155 after stimulation with PPD compared to the unstimulated condition (Fig. 2e).

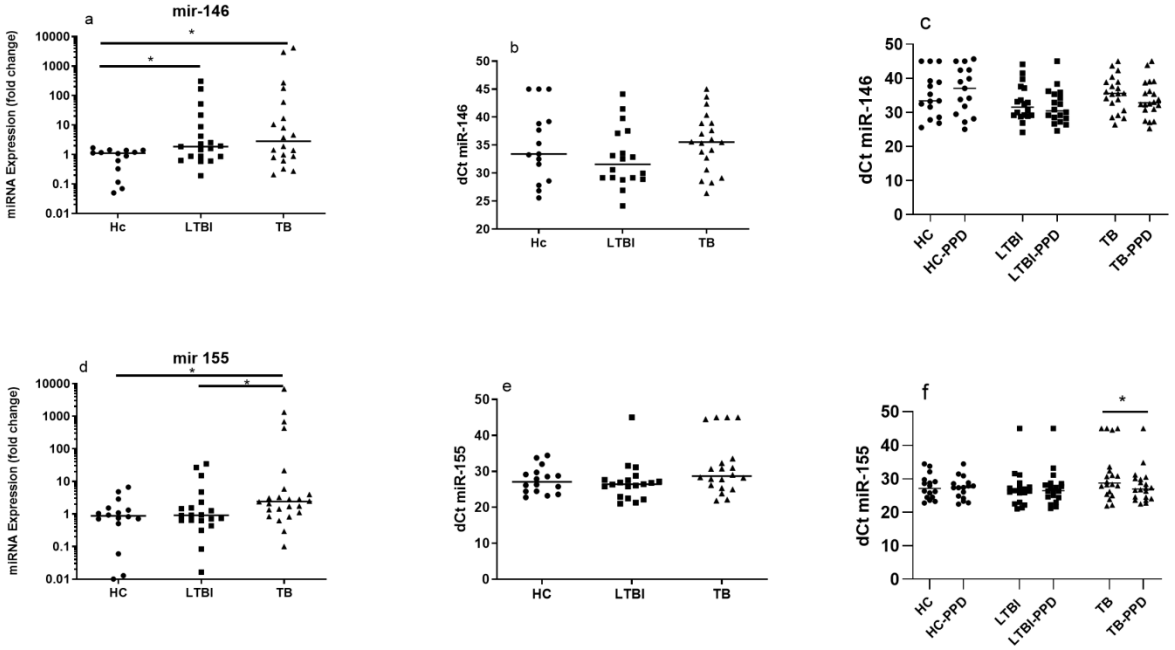

Fig. 2: PPD-induced miRNA-146a, and miRNA-155 expression in qRT-PCR analysis. Fold change (a, d); miRNA expression without PPD stimulation (b, e); comparison of dCt of PPD-stimulated and un-stimulated cells (c, f). * $P \leq 0.05$

Discussion

During the last few years, several studies have reported the important role of various miRNAs in regulating innate and acquired immune responses. miRNAs influence the differentiation of various immune cell subsets as well as their immunological functions (4).

In addition, pathogens modulate host immune responses through miRNAs (6). Several recent studies indicated differential miRNA expression in TB infection. For example, upregulation of miR-144 levels in sputum and serum from TB patients (14), upregulation of miR-21, miR-29a, miR-31, miR-155, and downregulation of miR-146a in children with active-TB (19), downregulation of miR-1, miR-155, miR-31, miR-146a, miR-10a, miR-125b, and miR-150, and upregulation of miR-29 in plasma from children with TB have been reported (20). These differences may reflect differences in ethnicity, the severity of MTB infection, latent TB status, and different experimental conditions.

In the present study, the expression levels of miR-21, miR-31, miR-146a, and miR-155 were evaluated in the PPD-stimulated PBMCs from TB, LTBI, and HC individuals due to their important role in immune responses and host–MTB interaction. Our results indicated no significant difference in the expression of miR-21, and miR-31 after stimulation with PPD among different groups. This unchanged miR-21 expression is in line with the finding that reported no induction of miR-21 by MTB (21). Both suppressed and increased expressions of miR-21 and miR-31 have also been reported in active TB. MTB infection of macrophages increases the expression of miR-21 which enhances MTB survival and attenuates pro-inflammatory cytokine production (22). Suppressed expression of miR-31 by leukocytes of pediatric TB patients (15,20,23,24), and by PBMCs of TB patients without any re-stimulation of the cells (25) are in line with lower expression of this miRNA in unstimulated PBMCs from TB patients of our study. miRNA-31 expression in leukocytes of children with TB has been negatively correlated with serum levels of IL-6, TNF-α, NF-κB, and IFN-γ (23). On the other hand, a significant increase in the plasma level of miR-21 and miR-31 has been reported in children with active-TB

(19). miRNA profiling of CD4+ T cells from patients with TB, and LTBI indicated lower levels of miR-21 expression in TB patients compared to LTBI; however, whole blood miRNA analyses revealed an opposite result (26). Our results indicated a significantly higher expression of miR-146 in TB-infected individuals in response to PPD stimulation. This increased expression of miR-146 was significantly more in both LTBI and TB groups compared to the HC group; which suggests miR-146 as an MTB infection marker. In addition, an increased expression of miR-155 in response to PPD was present in TB patients compared to HC individuals. Likewise, miR-155 expression in the TB group was higher than that of the LTBI group; while, no significant difference in expression of mir-155 was found between LTBI and HC individuals. The miR-155 finding is consistent with Wu et al. who reported miR-155 and miR-155* induction in PBMCs from TB patients after 4 hr stimulation with PPD (27). Higher expression of mir-155 in the TB group compared to LTBI and HC groups suggests a role for miR-155 as an activation marker for active TB. MTB causes high levels of miR-155 and lower levels of miR-125b, while avirulent *M. smegmatis* is an inducer of low levels of miR-155 and high levels of miR-125b (28). There are some inconsistencies in the reported expression pattern of miR-146 and miR-155 in TB. For example, upregulated expression of miR-155 and downregulated expression of miR-146a has been reported in PBMCs and plasma from TB patients (19), while in pediatric TB patients both miR-155, and miR-146a were downregulated (20).

miR-155 and miR-146 are the two most well-studied miRNAs, with characterized roles in immunity and inflammation regulation during MTB infections (29,30). The expression of both miR-146 and miR-155 has been reported to be increased in human macrophages after infection with MTB (31). They represent a reciprocal role in the macrophage inflammatory response by enhancement of inflammation towards the eradication of pathogens, meanwhile, avoiding excessive damage through a negative feedback loop (32). MiR-146 and miR-155 are complementary miRNAs with strongly entangled inflammatory-anti-inflammatory functions. MiR-146a represents an anti-inflammatory and miR-155 a pro-inflammatory role miRNAs (33). miR-146a through a feedback system down-regulates TLR signaling and thereby controls the inflammatory response (34). Moreover, this negative regulation of inflammation by miR-146 has been shown in human lung alveolar epithelial cells (35).

miR-155 plays a dual role during different stages of MTB infection. After MTB infection, miR-155 increases in macrophages which leads to increased macrophage viability and increased bacterial growth during the innate response to MTB. Thus, miR-155 deficient macrophages control MTB growth better than wild-type macrophages during the innate stage of infection. However, experiments with miR-155-deficient-mice indicated earlier death of MTB-infected animals with more bacilli in their lungs compared to wild-type mice (36). miR-155 promotes the survival and function of MTB-specific T cells, which are critical for an effective adaptive immune response during MTB infection. Therefore, miR-155-deficient mice control infection at the initial stages but fail to do so after the onset of adaptive immunity (37).

Micro RNAs have also been shown to affect MTB growth. miR-146a promotes mycobacterial survival in RAW264.7 macrophages (38). Likewise, MiR-155 promotes MTB survival in macrophages (37). On the other hand, miR-155 represses the expression of factors that support long-lasting activation of the MTB dormancy regulon (29); in other words, miR-155 represses MTB to enter the latent phase. This is consistent with our finding that the expression of miR-155 was higher in TB patients compared to that of the LTBI group.

Conclusion

Altogether, less miR-155 in LTBI individuals- in comparison with active TB- in our study might support the dormant state of mycobacterial infection; while high miR-146 levels control the in-

flammation. Moreover, higher miR-155 in TB patients may support the survival of active bacilli; while high miR-146, and miR-155 regulate inflammatory response to reduce tissue damage, a major cause of pulmonary morbidity and mortality in tuberculosis. Although the results of this study along with the other published studies indicate that miRNA profiles within immune cells are influenced by MTB infection, further research still is required to expand our knowledge of miRNAs linked to the immune system in MTB infection. Likewise, the potential of miRNAs as biomarkers in MTB infection and activity requires further extensive studies in this field.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Chakaya J, Khan M, Ntoumi F, et al (2021). Global Tuberculosis Report 2020 - Reflections on the Global TB burden, treatment and prevention efforts. *Int J Infect Dis,* 113 (Suppl 1): S7–S12.
2. Cohen A, Mathiasen V D, Schön T & Wejse, C (2019). The global prevalence of latent tuberculosis: a systematic review and meta-analysis. *Eur Respir J,* 54 (3): 1900655.
3. Treiber T, Treiber N & Meister G (2019). Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. *Nat Rev Mol Cell Biol,* 20 (1): 5–20.
4. Mehta A, Baltimore D (2016). MicroRNAs as regulatory elements in immune system logic. *Nat Rev Immunol,* 16 (5): 279–294.
5. Adibzadeh Sereshgi MM. Abdollahpour-Alitappeh M, Mahdavi M, et al (2019). Immunologic balance of regulatory T cell/T helper 17 responses in gastrointestinal infectious diseases: Role of miRNAs. *Microb Pathog,* 131, 135–143.
6. Chandan K, Gupta M, Sarwat M (2019). Role of Host and Pathogen-Derived MicroRNAs in Immune Regulation During Infectious and Inflammatory Diseases. *Front Immunol,* 10: 3081.
7. Ranjbar R, Hesari Ar, Ghasemi F, et al (2018). Expression of microRNAs and IRAK1 pathway genes are altered in gastric cancer patients with Helicobacter pylori infection. *J Cell Biochem,* 119 (9): 7570-7576.
8. Peng Y, Croce CM (2016). The role of MicroRNAs in human cancer. *Signal Transduct Target Ther,* 28 (1): 15004.
9. Fu Y, Yang X, Chen H, Lu Y (2020). Diagnostic value of miR-145 and its regulatory role in macrophage immune response in tuberculosis. *Genet Mol Biol,* 43 (2): e20190238.
10. Sabir N, Hussain T, Shah SZA, et al (2018). miRNAs in Tuberculosis: New Avenues for Diagnosis and Host-Directed Therapy. *Front Microbiol,* 29 (9): 602.
11. Sampath P, Periyasamy K M, Ranganathan UD, Bethunaickan R (2021). Monocyte and Macrophage miRNA: Potent Biomarker and Target for Host-Directed Therapy for Tuberculosis. *Front Immunol,* 25 (12): 667206.
12. Ghorpade DS, Leyland R, Kurowska-Stolarska M, et al (2012). N. MicroRNA-155 is required for Mycobacterium bovis BCG-mediated apoptosis of macrophages. *Mol Cell Biol,* 32 (12): 2239–2253.
13. Wagh V, Urhekar A, Modi D (2017). Levels of microRNA miR-16 and miR-155 are altered in serum of patients with tuberculosis and associate with responses to therapy. *Tuberculosis (Edinb)* 102: 24–30.
14. Lv Y, Guo Sh, Li X, et al (2016). Sputum and serum microRNA-144 levels in patients with

tuberculosis before and after treatment. *Int J Infect Dis,* 43: 68–73.
15. Wang C., Yang Sh, Sun G, et al (2011). Comparative miRNA expression profiles in individuals with latent and active tuberculosis. *PloS One,* 6 (10): e25832 .
16. Chen DY, Chen YM, Lin Ch, et al (2020). MicroRNA-889 Inhibits Autophagy To Maintain Mycobacterial Survival in Patients with Latent Tuberculosis Infection by Targeting TWEAK. *mBio* 11 (1): e03045-19.
17. Quinn SR, O'Neill LA (2011). A trio of microRNAs that control Toll-like receptor signalling. *Int Immunol,* 23 (7): 421–425.
18. Ajdary S, Riazi-Rad F, Alimohammadian MH (2009). Immune response to Leishmania antigen in anthroponotic cutaneous leishmaniasis. *J Infect,* 59 (2): 139–143.
19. M K, S S, S M (2020). Expression levels of candidate circulating microRNAs in pediatric tuberculosis. *Pathog Glob Health,* 114 (5): 262–270.
20. Zhou M, Yu G, Yang X, et al (2016). Circulating microRNAs as biomarkers for the early diagnosis of childhood tuberculosis infection. *Mol Med Rep,* 13 (6): 4620–4626 .
21. Liu PT, Wheelwrigh M, Teles R, et al (2012). MicroRNA-21 targets the vitamin D-dependent antimicrobial pathway in leprosy. *Nat Med,* 18 (2): 267–273.
22. Zhao Z, Hao J, Li X, Chen Y, et al (2019). MiR-21-5p regulates mycobacterial survival and inflammatory responses by targeting Bcl-2 and TLR4 in Mycobacterium tuberculosis-infected macrophages. *FEBS Lett,* 593 (12): 1326–1335.
23. Wang JX, Xu J, Han YF, et al (2015). Diagnostic values of microRNA-31 in peripheral blood mononuclear cells for pediatric pulmonary tuberculosis in Chinese patients. *Genet Mol Res,* 14 (4): 17235–17243.
24. Wang Ch, Yang S, Liu Ch, et al (2018). Screening and identification of four serum miRNAs as novel potential biomarkers for cured pulmonary tuberculosis. *Tuberclosis (Edinb),* 108: 26–34.
25. Ghorpade DS, Holla S, Kaveri S, et al (2013). Sonic hedgehog-dependent induction of microRNA 31 and microRNA 150 regulates Mycobacterium bovis BCG-driven toll-like receptor 2 signaling. *Mol Cell Biol,* 33 (3): 543–556.
26. Kleinsteuber K, Heesch K, Schattling S, et al (2013). Decreased expression of miR-21, miR-26a, miR-29a, and miR-142-3p in $CD4^+$ T cells and peripheral blood from tuberculosis patients. *PloS One,* 8(4): e61609.
27. Wu J, Lu Ch, Diao N, et al (2012). Analysis of microRNA expression profiling identifies miR-155 and miR-155* as potential diagnostic markers for active tuberculosis: a preliminary study. *Hum Immunol,* 73 (1): 31–37.
28. Rajaram MVS, Ni B, Morris JD, et al (2011). Mycobacterium tuberculosis lipomannan blocks TNF biosynthesis by regulating macrophage MAPK-activated protein kinase 2 (MK2) and microRNA miR-125b. *Proc. Natl Acad Sci USA,* 108 (42): 17408–17413.
29. Liu Zh, Zhou G, Deng X, et al (2014). Analysis of miRNA expression profiling in human macrophages responding to Mycobacterium infection: induction of the immune regulator miR-146a. *J Infect,* 68 (6): 553–561.
30. Spinelli SV, Diaz A, D'Attilio L, et al (2013). Altered microRNA expression levels in mononuclear cells of patients with pulmonary and pleural tuberculosis and their relation with components of the immune response. *Mol Immunol,* 53 (3): 265–269.
31. Furci L, Schena E, Miotto P, et al (2013) Alteration of human macrophages microRNA expression profile upon infection with Mycobacterium tuberculosis. *Int J Mycobacteriology,* 2 (3): 128–134.
32. O'Connell RM, Rao DS, Chaudhuri AA, et al (2010). Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol,* 10 (2): 111–122.
33. Hirschberger S, Hinske LC, Kreth S (2018). MiRNAs: dynamic regulators of immune cell functions in inflammation and cancer. *Cancer Lett,* 431: 11–21.
34. Taganov KD, Boldin MP, Chang KJ, et al (2006). NF-kappa B-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci USA,* 103 (33): 12481–12486.
35. Perry MM, Moscho SA, Williams AE, et al (2008). Rapid changes in microRNA-146a expression negatively regulate the IL-1beta-

induced inflammatory response in human lung alveolar epithelial cells. *J Immunol,* 180 (8): 5689–5698.
36. Iwai H, Funatogawa K, Matsumura K, et al (2015). MicroRNA-155 knockout mice are susceptible to Mycobacterium tuberculosis infection. *Tuberculosis (Edinb),* 95 (1): 246–250.
37. Rothchild AC, Sissons JR, Shafiani Sh, et al (2016). MiR-155-regulated molecular network orchestrates cell fate in the innate and adaptive immune response to Mycobacterium tuberculosis. *Proc Natl Acad Sci USA,* 113 (41): E6172–E6181.
38. Li M, Wang J, Fang Y, et al (2016). microRNA-146a promotes mycobacterial survival in macrophages through suppressing nitric oxide production. *Sci Rep,* 6: 23351.