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Biological functions and diagnostic implications of microRNAs in *Mycobacterium tuberculosis* infection

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ABSTRACT

MicroRNAs (miRNAs), small non-coding RNAs, play important roles in regulating host defense against pathogenic infections. This review provides information on the role of miRNAs in the antimycobacterial immune response and summarizes their possible diagnostic utility. It was compiled using scientific literature retrieved from such databases as PubMed, Scopus, ScienceDirect, Google Scholar, and PubMed Central. Relevant articles published in the English language until December 2020 were taken into consideration. It has been revealed that specific host miRNAs induced by *Mycobacterium tuberculosis* can target diverse factors and pathways in immune signaling to ensure longer pathogen survival inside the phagocytes. The potential use of miRNAs in tuberculosis diagnosis or therapeutic strategies has been attracting increasing attention in recent years. However, despite considerable efforts devoted to miRNA profiling, further studies are needed to elucidate the full potential of miRNAs as novel tuberculosis biomarkers or therapeutic targets.

KEYWORDS: microRNA; *Mycobacterium tuberculosis*; Biomarker; Diagnostics; Tuberculosis

1. Introduction

Tuberculosis (TB) is a contagious and infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). It is estimated that about 2 billion people worldwide are infected with the pathogen, of which 5%-10% develop active TB, and the remaining 90% stay asymptomatically infected with *M. tuberculosis* within their lifetime[1]. A priority for TB control programs is to strengthen the capacity to diagnose TB and treat its multidrug-resistant (MDR-TB) forms. Standard TB diagnostic methods involving microbial growth in culture media or diverse immunological and genetic techniques are not sensitive enough to confirm *M. tuberculosis* infection in all TB patients[2]. Therefore, it has become necessary to develop new diagnostic tools, and many different classes of molecules have been studied for this purpose.

The discovery of the mechanism of post-transcriptional silencing of target messenger RNA (mRNA) by small RNA molecules called microRNAs (miRNAs) has altered the understanding of the control of the genetic information expression system. Micro RNAs were first discovered in 1993 in the nematode *Caenorhabditis elegans*[3], and in the following years, they were identified in plants, viruses, and human cells[4]. These small non-coding RNA molecules regulate many biological processes, mainly by inhibiting translation or inducing mRNA degradation[5,6]. According to the latest analyses, more than 2 600 miRNAs have been identified in the human genome, which together regulates 50% of genes in the genome[7,8]. It has been found that a single miRNA molecule can modulate the expression of multiple genes, while one mRNA can be targeted by several different miRNAs[9,10]. The potential use of miRNAs in diagnosis or therapeutic strategies has attracted increasing attention in recent years[11−13]. In this review, we focus on the role of several miRNAs in the antimycobacterial immune response and summarize their utility as potential biomarkers.

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2. Research methodology

We searched the following electronic, web-based databases to extract relevant studies from research articles, review articles, and book chapters in English language: PubMed, PubMed Central, Scopus, ScienceDirect, and Google Scholar. Non-English articles and letters to the editor were omitted. The following terms were used to generate the search: "microRNA," "miRNA" or "miRNA *vs.* tuberculosis" or "miRNA *vs.* mycobacteria" or "miRNA function" or "miRNA biomarker," or "miRNA diagnostics".

3. Involvement of miRNAs in the regulation of antimycobacterial immune response

M. tuberculosis has developed various strategies to avoid being killed and escape immune surveillance[14,15]. The inhibition of phagosomal maturation, down-regulation of major histocompatibility complex class Ⅱ antigen presentation, attenuation of apoptosis and autophagy processes, and reduction of reactive oxygen and reactive nitrogen intermediates function, are some of the best-characterized *M. tuberculosis* mechanisms. As essential modifiers of host-pathogen interactions, miRNAs influence both innate and acquired antimycobacterial immunity by regulating maturation, activation, and cell effector functions (Figure 1). They are also involved in cell apoptosis and play a role in *M. tuberculosis* pathogenesis by the modulation of the lipid metabolic pathway[16,17]. In the next subsections, we review the involvement of several host miRNAs in the regulation of the host immune response to *M. tuberculosis* infection.

3.1. miRNA-20

The specific effect of miRNA-20b on *M. tuberculosis* infection has been documented by Lou *et al.*[18]. They observed a reduced expression of miRNA-20b-5p accompanied by the activation of nod-like receptor (NLR) family pyrin domain containing 3 (NLRP3)-mediated immune response, in TB patient macrophages[18]. Downregulation of miR-20b-5p expression was also found in *M. tuberculosis*-infected mice. Targeting the NLRP3/caspase-1/IL-1β pathway *via* injection of mice with the miR-20b mimics inhibited the inflammatory response of murine alveolar epithelial cells co-cultured with macrophages[18].

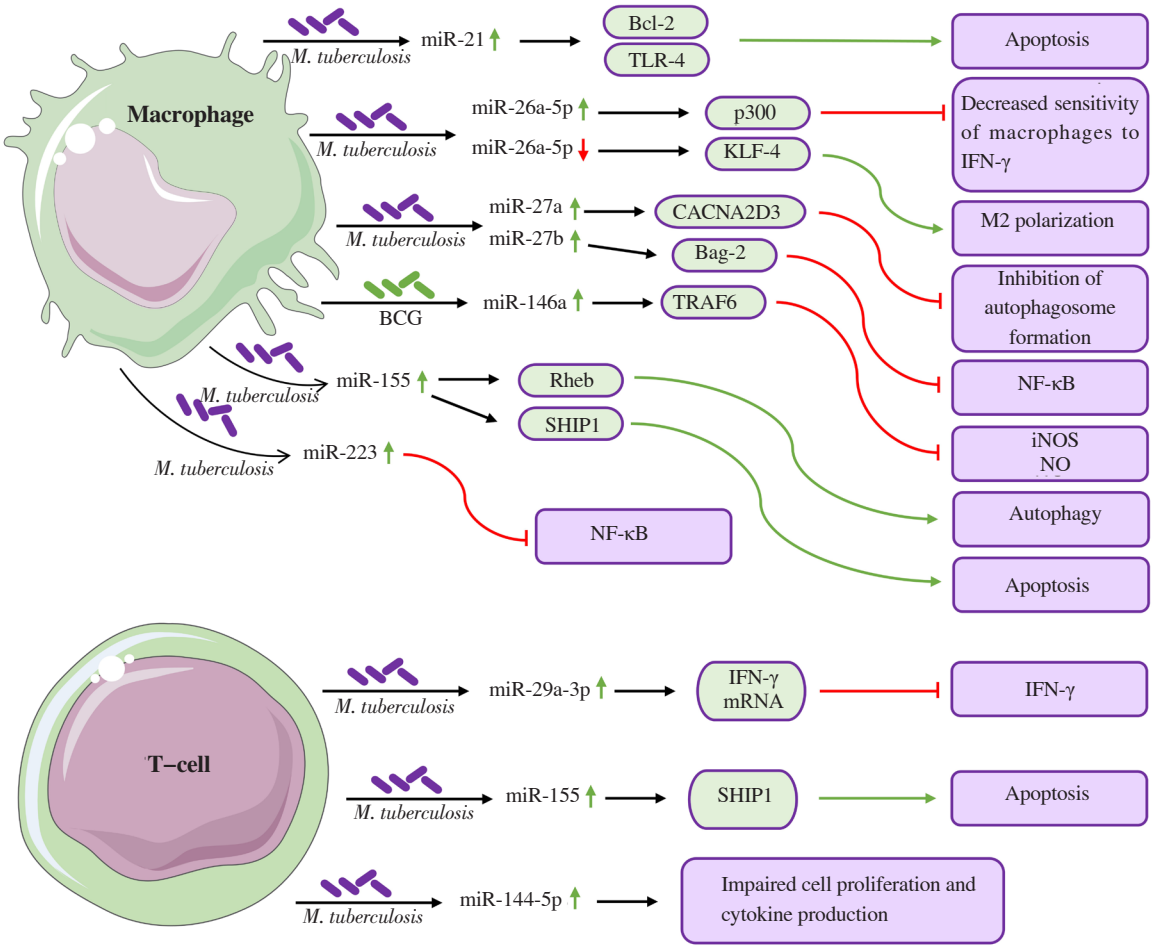

Figure 1. A schematic presentation of miRNA regulation of host immune responses against *Mycobacterium tuberculosis* (*M. tuberculosis*) infection. The depicted miRNAs promote or inhibit important pathways in the cellular response in macropages and T cells. BCG: *Mycobacterium bovis* bacillus Calmette-Guérin.

3.2. miRNA–21

miRNA-21 is one of the most commonly deregulated miRNAs in almost all cancer cell types and is therefore called an "onco-miRNA"[19]. In recent years, additional roles of miRNA-21 in pulmonary and cardiovascular diseases have been described. It has been suggested that increased miRNA-21 activity may contribute to the attenuation of the antimycobacterial immune response and longer pathogen survival. Wu *et al*. reported that miR-21 was upregulated in alveolar macrophages obtained from *Mycobacterium bovis* (*M. bovis*) bacillus Calmette-Guérin (BCG)-vaccinated mice, as well as in macrophages and dendritic cells experimentally infected with *M. bovis* BCG[20]. miRNA-21 impaired IL-12 production and promoted dendritic cell apoptosis by targeting IL-12p35 and B-cell lymphoma 2 (Bcl-2) proteins. In another study, it was found that in a RAW264.7 cell line, miRNA-21 expression, enhanced after the stimulation with *M. tuberculosis* protein, MPT64, might have participated in the inhibition of apoptosis due to an increased NF-κB expression and upregulation of Bcl-2[21]. Moreover, miRNA-21 was proved to boost *M. tuberculosis* survival and apoptosis and impair the production of inflammatory cytokines (IL-1β, IL-6, and TNF-α) by targeting the Bcl-2 and Toll-like receptor 4 in RAW264.7 and THP-1 cells[22]. Sheedy *et al*. observed that miRNA-21 impaired the development of a pro-inflammatory immune response by upregulating IL-10 production[23].

3.3. miRNA–26

The miRNA-26 family consists of miRNA-26a-1, miRNA-26a-2, and miRNA-26b members, which are involved in various biological processes including cell growth, development, and tumorigenesis[24]. Ni *et al*. found that *M. tuberculosis*-induced overexpression of miRNA-26a and miRNA-132 decreased the levels of the p300 transcriptional coactivator, thereby diminishing the transcription of IFN-γ–induced genes and macrophage response to this cytokine[25]. On the contrary, Sahu *et al*. showed downregulation of miRNA-26a-5p in macrophages infected with *M. tuberculosis* and in the lungs, spleen, and lymph nodes of mice infected with *M. tuberculosis*[26]. The decrease in miRNA-26a-5p expression was correlated with the upregulation of the Kruppel-like factor 4, which is involved in the polarization of macrophages towards the M2 phenotype[26]. Kleinsteuber *et al*. confirmed a significant reduction in the expression of miRNA-26a, along with miRNA-29a and miRNA-142-3p, in the peripheral blood of TB patients compared to individuals latently infected with *M. tuberculosis*[27]. An increase in miR-26a expression during treatment pointed to miR-26a as a potential biomarker not only indicating *M. tuberculosis* infection but also confirming the effectiveness of the applied therapy.

3.4. miRNA–27

miRNA-27a and miRNA-27b play a vital role in modulating tumorigenesis, proliferation, apoptosis, and angiogenesis[28,29]. Liu *et al*. demonstrated that miRNA-27a was upregulated in *M. tuberculosis*-infected mice and macrophages as well as in patients with active TB[30]. miRNA-27a-knockout mice had fewer histological lesions and inflammatory infiltrates after *M. tuberculosis* infection in the lungs than wild-type mice. The target of miRNA-27a was CACNA2D3, a component of a voltage-dependent calcium transporter, present in the endoplasmic reticulum. Targeting CACNA2D3 inhibited autophagosome formation and promoted intracellular survival of *M. tuberculosis*. The role of miRNA-27b in the macrophage response to *M. tuberculosis* infection was investigated by Liang *et al*.[31]. They showed that miRNA-27b expression targeted Bcl-2–associated athanogene 2 in macrophages and inhibited the production of pro-inflammatory factors *via* TLR-2/MyD88/NF-κB signaling cascade. Besides, miRNA-27b decreased the mycobacterial load and, at the same time, it increased cell apoptosis and the production of reactive oxygen intermediates[31].

3.5. miRNA–29

The miRNA-29 family members (miRNA-29a, miRNA-29b-1, miRNA-29b-2, and miRNA-29c) demonstrate anti-fibrotic activity and are involved in the regulation of many extracellular matrix proteins such as collagen, fibronectin, and laminin[32]. Recent studies showed alterations in miRNA-29 expression during mycobacterial infection[33,34]. Ma *et al*. noted that infection with the attenuated *M. bovis* (BCG) strain increased miRNA-29a-3p expression in natural killer cells, CD4(+), and CD8(+) T lymphocytes[33]. A direct target of miRNA-29a-3p was found to be IFN-γ mRNA, and miRNA-29a-3p expression inversely correlated with IFN-γ production. On the contrary, Afum-Adjei *et al*. did not observe any correlation between miRNA-29a and IFN-γ expression in active TB patients, suggesting that decreased IFN-γ expression had not been caused by differences in miRNA-29a expression[35]. A recent study by Ndzi *et al*. showed that miRNA-29a was significantly elevated in the blood of active TB patients compared to individuals latently infected with *M. tuberculosis* and healthy controls, and this miRNA exhibited the potential to be a diagnostic biomarker for TB. It was also upregulated in active TB patients who had not yet started the treatment compared to those who had completed anti-TB therapy, which demonstrated its potential as a biomarker for TB treatment follow-up[36].

3.6. miRNA–144

Many studies have documented the overexpression of miRNA-144-5p in the serum, peripheral blood mononuclear cells (PBMCs), and sputum from active TB patients, suggesting its involvement in the regulation of anti-TB immunity[37–39]. *M. tuberculosis*-induced upregulation of miRNA-144-5p was found to decrease phagosomal maturation in human monocytes[39], while miRNA-144-5p overexpressed in T cells impaired cell proliferation and production of cytokines[37]. Significantly lower levels of miRNA-144 observed

in the sputum and serum after a successful TB treatment suggest a potential of miRNA-144-5p as a new biomarker in the evaluation of the effects of antituberculous therapy[38].

3.7. miRNA-145

miRNA-145, commonly downregulated in many types of cancer cells, regulates many cellular processes including proliferation, apoptosis, and cell cycle[40]. Some studies reported downregulation of miRNA-145 expression during *M. tuberculosis* infection[41,42]. Significantly lower serum miRNA-145 levels were negatively correlated with serum levels of IL-1β and TNF-α, which were observed in patients with active TB compared with healthy subjects, including those latently infected with *M. tuberculosis*[42]. The decreased expression of miRNA-145 showed its relatively high potential in TB diagnosis, providing evidence that miRNA-145 could serve as a candidate diagnostic biomarker.

3.8. miRNA-146a

Some studies found elevated expression of miRNA-146a in *M. tuberculosis*-infected human dendritic cells and human monocyte-derived macrophages, whereas in PBMCs and mononuclear pleural fluid cells as well as plasma samples from TB patients, a decrease in miRNA-146a levels was noted[41,43–45]. In macrophages, BCG-induced miRNA-146a inhibited the expression of inducible nitric oxide (NO) synthase (iNOS) and the production of NO, which was due to the targeting of interleukin-1 receptor-associated kinase-1 and tumor necrosis factor receptor-associated factor 6 *via* miR-146a-5p[44,46]. Changes in mycobacteria-induced miRNA-146a expression depending on the infection dose and duration could modulate the inflammatory response and facilitate mycobacterial replication in macrophages[46].

3.9. miRNA-155

miRNA-155 is a major regulator of inflammation, and increased expression of miRNA-155 has been found in a variety of inflammatory diseases and various cancers. *In vivo* and *in vitro* studies have demonstrated that miRNA-155 also plays an important role in the regulation of host immune responses to mycobacteria[47]. Iwai *et al*. found that miRNA-155-/- knockout mice were more susceptible to *M. tuberculosis* infection and were characterized by higher bacterial loads in the lungs and decreased numbers of antigen-specific $CD4^+$ T cells as well as an impaired IFN-γ production compared to wild-type animals[47]. An increased expression of miRNA-155 has been observed in many types of *M. tuberculosis*-infected cells[47–50], but different effects induced by miRNA-155 in the early and chronic phases of *M. tuberculosis* infection suggest that miRNA-155 should be considered a pleiotropic immune regulator[49]. In macrophages, *M. tuberculosis*-induced miRNA-155-5p targeted a negative regulator of autophagy, Rheb (Ras homologue enriched in brain), leading to the inhibition of intracellular survival of the bacteria[48]. Upregulation of miRNA-155-5p in T cells and macrophages during *M. tuberculosis* infection was found to suppress the expression of Src homology-2 domain-containing inositol 5-phosphatase 1, a negative regulator of the PI3K/Akt pathway involved in TNF biosynthesis, which induces cell apoptosis[49,51]. In turn, in *M. tuberculosis*-infected human dendritic cells, miRNA-155 impaired the autophagy process *via* targeting ATG3, an E_2-ubiquitin-like conjugating enzyme involved in autophagosome formation[50], while targeting the forkhead box O3 by miRNA-155 was reported to inhibit apoptosis of monocytes[52]. Moreover, overexpression of miRNA-155 was negatively correlated with cytotoxic activity and the production of TNF-α by natural killer cells[53].

3.10. miRNA-223

miRNA-223 plays an important role in promoting granulocytic differentiation[54]. The upregulation of miRNA-223 in blood and lung parenchyma has been confirmed in both human and murine TB[55]. miRNA-223 has been found to control lung recruitment of myeloid cells and neutrophil-driven inflammation by acting on the chemokines CXCL2 (C-X-C motif ligand 2), CCL3 (C-C motif ligand 3), and IL-6 in myeloid cells. Significantly increased expression of miR-223 in patients with TB was accompanied by the inhibition of NF-κB activation in monocyte-derived macrophages, suggesting a role for miRNA-223 in the control of excessive inflammation during *M. tuberculosis* infection[56].

3.11. miRNA-889

miRNA profiling in PBMCs from subjects with latent *M. tuberculosis* infection recognized miRNA-889 as one of the most overexpressed miRNAs[57]. The TNF-related weak inducer of apoptosis (TWEAK) was identified as the target of miRNA-889. *M. tuberculosis* infection upregulated TWEAK expression, which induced autophagy and promoted autophagosome maturation through the activation of AMP-activated protein kinase (AMPK). It has been suggested that the overexpression of miRNA-889 that inhibits autophagy *via* posttranscriptional suppression of TWEAK expression is responsible for maintaining the survival of mycobacteria in granulomas.

4. miRNAs as potential TB biomarkers

TB detection, along with prevention and treatment, remains an essential element of most of the world's disease control programs. Despite the availability of many different diagnostic techniques, the diagnosis of TB is still based on the presence of acid-fast bacilli in microscopic smears, observation of bacterial growth in 6-8-week-old cultures, and the result of a skin tuberculin test. However, this standard TB diagnostic procedure does not make it possible to diagnose all patients infected with *M. tuberculosis*. The microscopic examination is characterized by low sensitivity and

the inability to distinguish *M. tuberculosis* from non-tuberculous mycobacteria. The sensitivity and specificity of methods based on the amplification of mycobacterial nucleic acids are variable, and their use requires specialized equipment, which is often unavailable in routine mycobacterial laboratories. The lack of diagnosis has serious consequences, forcing clinicians to undertake empirical therapy, which, in the case of patients infected with drug-resistant mycobacteria undergoing standard treatment, is sometimes ineffective and may additionally exacerbate the phenomenon of *M. tuberculosis* drug resistance.

Several advantages that characterize miRNAs make them suitable biomarker candidates. These molecules are present in various body fluids with high stability despite repeated freezing and defrosting, and are not difficult to extract[58]. Calin *et al*. were the first to investigate the potential use of miRNAs as disease biomarkers and demonstrate that miRNA expression levels were altered in patients with chronic lymphocytic leukemia[59]. Shortly after this discovery, many studies revealed differentially expressed miRNA profiles in a variety of diseases, including TB, suggesting the use of miRNAs as potential diagnostic or prognostic biomarkers[59–66]. There is also a growing interest in using miRNAs as appropriate indicators of therapeutic efficacy[14,45]. Potential miRNA biomarkers in the diagnosis of *M. tuberculosis* infection are presented in Table 1.

Many studies have shown the alteration of miRNAs expression profiles in clinical samples (sputum, serum, pleural effusion, PBMC) in patients with active TB[14,41,67,68]. In PBMCs from active TB patients, Cao *et al*. identified a set of 26 differentially expressed miRNAs, among which miRNA-23a-5p, miR-NA-183-5p, miRNA-193a-5p, and miRNA-941 were overexpressed, and miRNA-16-1-3p was reduced compared to healthy subjects, suggesting their potential utility in the diagnosis of active TB[69]. Expression patterns of miRNA in pleural effusions from pulmonary TB patients showed upregulation of miRNA-378i compared to individuals with lung cancer or pneumonia[70]. An increase miRNA-625-3p level in the urine was demonstrated in *M. tuberculosis* smear-positive compared to *M. tuberculosis* smear-negative patients[71]. Profiling of miRNAs in the sputum of active TB patients showed overexpression of miRNA-3179 and miRNA-147 and underexpression of miRNA-19b-2[68]. A recent study has shown that miRNA-155 in the sputum may serve as another potential biomarker of active pulmonary TB[72].

The diagnosis of latent *M. tuberculosis* infection can also be challenging, due to the limitations of available tests – the tuberculin skin test and interferon-gamma release assays. In this context, several miRNA signatures distinguishing active TB from latent TB have been identified[73–75]. Lyu *et al*. observed unique sets of serum miRNAs for both latent TB (hsa-let-7e-5p, hsa-let-7d-5p, hsa-miR-450a-5p and hsa-miR-140-5p) and active TB (hsa-miR-1246, hsa-miR-2110, hsa-miR-370-3P, hsa-miR-28-3p and hsa-miR-193b-5p)[74]. Interestingly, using the expression profile of eight different miRNAs (hsa-miR-122-5p, miR-151a-3p, miR-451a, miR-486-5p, hsa-let-7i-5p, miR-148a-3p, miR-21-5p, and miR-423-5p), latent TB could be distinguished from active disease with an accuracy of 71.8%[75]. Moreover, Wang *et al*. identified a specific miRNA profile composed of hsa-miR223, hsa-miR-424, hsa-miR-451, and hsa-miR-144 that is capable of distinguishing between latent *M. tuberculosis* infection and active TB[73]. The lack of consistency in the miRNA signatures reported may be due to the differences in the

Table 1. Candidate miRNA biomarkers in *Mycobacterium tuberculosis* infection.

Clinical sample	miRNA candidates	Regulation	Study groups	Reference
Serum	miRNA-21-5p	↑	ATB *vs*. HC; ATB before and after therapy, ATB *vs*. LTBI	[74]
	miRNA-92a-3p	↑	ATB before and after therapy	[75]
	miRNA-151a-3p	↓	ATB *vs*. LTBI	[74]
	miRNA-155	↓	ATB *vs*. HC; ATB before and after therapy	[2]
	hsa-let-7i-5p	↑	ATB *vs*. LTBI	[74]
Sputum	miRNA-19b-2*	↓	ATB *vs*. HC	[67]
	miRNA-144	↑	ATB *vs*. HC	[37]
	miRNA-155	↑	ATB *vs*. HC	[71]
	miRNA-3179	↑	ATB *vs*. HC	[67]
Whole blood	miRNA-26a	↓	ATB *vs*. LTBI	[27]
	miRNA-142-3p	↓	ATB *vs*. LTBI	[27]
$CD4^+$ cells	miRNA-21-5p	↓	ATB *vs*. LTBI	[27]
	miRNA-26a-5p	↓	ATB *vs*. LTBI	[27]
	miRNA-142-3p	↓	ATB *vs*. LTBI	[27]
Plasma	miRNA-26a-5p	↑	ATB before and after therapy	[44]
	miRNA-29a	↑	ATB *vs*. HC; ATB before and after therapy	[44]
	miRNA-99b	↑	ATB *vs*. HC; ATB before and after therapy	[44]
	miRNA-652	↓	ATB *vs*. HC	[44]
PBMC	miRNA-16-1-3p	↓	ATB *vs*. HC	[68]
	miRNA-144*	↑	ATB *vs*. HC	[36]
	miRNA-424	↑	ATB *vs*. LTBI	[72]
	miRNA-451	↑	ATB *vs*. HC	[72]
	miRNA-941	↑	ATB *vs*. HC	[68]

ATB: active tuberculosis; HC: healthy controls; LTBI: latent *Mycobacterium tuberculosis* infection; PBMC: peripheral blood mononuclear cells; *: passenger strand or star strand.

characteristics of the study population (age, sex, comorbidities), type of samples taken for miRNA isolation (sputum, serum, urine) as well as the applied study protocols.

There is also growing evidence on using miRNA expression profiles as indicators of antituberculous treatment efficacy[2,38,76]. The upregulation of the serum miR-125a-5p level accompanied by downregulation of the miR-NA-21-5p, miRNA-92a-3p, and miRNA-148b-3p levels was found in TB patients, who responded properly to the applied therapy[76]. Moreover, it was noticed that miRNA-144, miRNA-16, and miRNA-155 expression that was altered during TB returned to the levels observed in healthy volunteers after the end of treatment, suggesting the possibility of using these proteins as markers of therapy success[2,38].

In recent years, much effort has been devoted to the use of miRNAs in novel therapeutic approaches including the delivery of exogenous miRNAs to host cells[77-79]. However, since miRNAs target the expression of many genes, miRNA therapy can have various unpredictable systemic consequences. Another problem is the efficient delivery of miRNAs, as miRNA molecules can be rapidly degraded. Accurate characterization of the individual roles of miRNAs is needed to assess their therapeutic potential in the future.

5. Conclusions

The recognition of the role of miRNAs as gene silencers and their active participation in the regulation of the immune response against *M. tuberculosis* has opened a new chapter in TB diagnosis, treatment, and prevention. However, despite the growing number of studies, the high variability of miRNA expression profiles that depend on the experimental conditions and protocol variations, makes it difficult to apply them in practice. Therefore, although some miRNAs are proposed as potential diagnostic biomarkers for TB, further extensive studies in this field are urgently needed.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

MD developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations. Both MD and MG contributed to the final version of the manuscript. MD supervised the project.

References

[1] Barry CE, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, et al. The spectrum of latent tuberculosis: Rethinking the biology and intervention strategies. *Nat Rev Microbiol* 2009; **7**(12): 845-855.

[2] Wagh V, Urhekar A, Modi D. Levels of microRNA miR-16 and miR-155 are altered in serum of patients with tuberculosis and associate with responses to therapy. *Tuberculosis (Edinb)* 2017; **102**: 24-30.

[3] Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993; **75**(5): 843-854.

[4] Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**(2): 281-297.

[5] Sabir N, Hussain T, Shah SZA, Peramo A, Zhao D, Zhou X. miRNAs in tuberculosis: New avenues for diagnosis and host-directed therapy. *Front Microbiol* 2018; **9**: 602.

[6] Behrouzi A, Alimohammadi M, Nafari AH, Yousefi MH, Riazi Rad F, Vaziri F, et al. The role of host miRNAs on *Mycobacterium tuberculosis*. *ExRNA* 2019; **1**(40): 1-10.

[7] Hammond SM. An overview of microRNAs. *Adv Drug Deliv Rev* 2015; **87**: 3-14.

[8] Juzenas S, Venkatesh G, Hubenthal M, Hoeppner MP, Du ZG, Paulsen M, et al. A comprehensive, cell specific microRNA catalogue of human peripheral blood. *Nucleic Acids Res* 2017; **45**(16): 9290-9301.

[9] Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: MicroRNA biogenesis pathways and their regulation. *Nat Cell Biol* 2009; **11**(3): 228-234.

[10] Kittelmann S, McGregor AP. Modulation and evolution of animal development through microRNA regulation of gene expression. *Genes (Basel)* 2019; **10**(4): 321. Doi: 10.3390/GENES10040321.

[11] Miotto P, Mwangoka G, Valente IC, Norbis L, Sotgiu G, Bosu R, et al. MiRNA signatures in sera of patients with active pulmonary tuberculosis. *PLoS One* 2013; **8**(11): e80149.

[12] Weiner J, Maertzorf J, Kaudmann SH. The dual role of biomarkers for understanding basic principles and devising novel intervention strategies in tuberculosis. *Ann N Y Acad Sci* 2013; **1283**(1): 22-29.

[13] Tribolet L, Kerr E, Cowled C, Bean AGD, Stewart CR, Dearnley M, et al. MicroRNA biomarkers for infectious diseases: From basic research to biosensing. *Front Microbiol* 2020; **11**: 1197.

[14] Ehrt S, Schnappinger D. Mycobacterial survival strategies in the phagosome: Defence against host stresses. *Cell Microbiol* 2009; **11**(8): 1170-1178.

[15] Stanley SA, Cox JS. Host-pathogen interactions during *Mycobacterium tuberculosis* infections. *Curr Top Microbiol Immunol* 2013; **374**: 211-241.

[16] Zhou X, Li X, Wu M. miRNAs reshape immunity and inflammatory responses in bacterial infection. *Signal Transduct Target Ther* 2018; **3**(14): 1-13.

[17] Chandan K, Gupta M, Sarwat M. Role of host and pathogen-derived MicroRNAs in immune regulation during infectious and inflammatory diseases. *Front Immunol* 2019; **10**: 3081.

[18] Lou J, Wang Y, Zhang Z, Qiu W. MiR-20b inhibits *Mycobacterium tuberculosis* induced inflammation in the lung of mice through targeting NLRP3. *Exp Cell Res* 2017; **358**(2): 120-128.

[19] Kumarswamy R, Volkmann I, Thum T. Regulation and function of miRNA-21 in health and disease. *RNA Biol* 2011; **8**(5): 706-713.

[20]Wu Z, Lu H, Sheng J, Li L. Inductive microRNA-21 impairs anti-mycobacterial responses by targeting IL-12 and Bcl-2. *FEBS Lett* 2012; **586**(16): 2459-2467.

[21]Wang Q, Liu S, Tang Y, Liu Q, Yao Y. MPT64 protein from *Mycobacterium tuberculosis* inhibits apoptosis of macrophages through NF-κB-miRNA21-Bcl-2 pathway. *PLoS One* 2014; **9**(7): e100949.

[22]Zhao Z, Hao J, Li X, Chen Y, Qi X. MiR-21-5p regulates mycobacterial survival and inflammatory responses by targeting Bcl-2 and TLR4 in *Mycobacterium tuberculosis*-infected macrophages. *FEBS Lett* 2019; **593**(12): 1326-1335.

[23]Sheedy FJ, Palsson-McdermottE, Hennessy EJ, Martin C, O'Leary JJ, Ruan Q, et al. Negative regulation of TLR4 *via* targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. *Nat Immunol* 2010; **11**(2): 141-147.

[24]Gao J, Liu QG. The role of miR-26 in tumors and normal tissues (Review). *Oncol Lett* 2011; **2**(6): 1019.

[25]Ni B, Rajaram MV, Lafuse WP, Landes MB, Schlesinger LS. *Mycobacterium tuberculosis* decreases human macrophage IFN-γ responsiveness through miR-132 and miR-26a. *J Immunol* 2014; **193**(9): 4537-4547.

[26]Sahu SK, Kumar M, Chakraborty S, Banerjee SK, Kumar R, Gupta P, et al. MicroRNA 26a (miR-26a)/KLF4 and CREB-C/EBPβ regulate innate immune signaling, the polarization of macrophages and the trafficking of *Mycobacterium tuberculosis* to lysosomes during infection. *PLoS Pathog* 2017; **13**(5): e1006410.

[27]Kleinsteuber K, Heesch K, Schattling S, Kohns M, Sander-Julch C, Walzl G, et al. 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* 2013; **8**(4): e61609.

[28]Li X, Xu M, Ding L, Tang J. MiR-27a: A novel biomarker and potential therapeutic target in tumors. *J Cancer* 2019; **10**(12): 2836.

[29]Zhang J, Cao Z, Yang G, You L, Zhang T, Zhao Y. MicroRNA-27a (miR-27a) in solid tumors: A review based on mechanisms and clinical observations. *Front Oncol* 2019; **9**: 893.

[30]Liu F, Chen J, Wang P, Li H, Zhou Y, Liu H, et al. MicroRNA-27a controls the intracellular survival of *Mycobacterium tuberculosis* by regulating calcium-associated autophagy. *Nat Commun* 2018; **9**(1): 4295.

[31]Liang S, Song Z, Wu Y, Gao Y, Gao M, Liu F, et al. MicroRNA-27b modulates inflammatory response and apoptosis during *Mycobacterium tuberculosis* infection. *J Immunol* 2018; **200**(10): 3506-3518.

[32]Harmanci D, Erkan EP, Kocak A, Akdogan GG. Role of the microRNA-29 family in fibrotic skin diseases. *Biomed Rep* 2017; **6**(6): 599-604.

[33]Ma F, Xu S, Liu X, Zhang Q, Xu X, Liu M, et al. The microRNA miR-29 controls innate and adaptive immune responses to intracellular bacterial infection by targeting interferon-γ. *Nat Immunol* 2011; **12**(9): 861-869.

[34]Sharbati J, Lewin A, Kutz-Lohroff B, Kamal E, Einspanier R, Sharbati S. Integrated microRNA-mRNA-analysis of human monocyte derived macrophages upon *Mycobacterium avium* subsp. hominissuis infection. *PLoS One* 2011; **6**(5): e20258.

[35]Afum-Adjei A, Ueberberg B, Owusu-Dabo E, Frempong M, Jacobsen M. Dynamics of T-cell IFN-γ and miR-29a expression during active pulmonary tuberculosis. *Int Immunol* 2014; **26**(10): 579-582.

[36]Ndzi EN, Nkenfou CN, Mekue LM, Zentilin L, Tamgue O, Pefura EWY, et al. MicroRNA hsa-miR-29a-3p is a plasma biomarker for the differential diagnosis and monitoring of tuberculosis. *Tuberculosis* 2019; **114**: 69-76.

[37]Liu Y, Wang X, Jiang J, Cao Z, Yang B, Cheng X. Modulation of T cell cytokine production by miR-144* with elevated expression in patients with pulmonary tuberculosis. *Mol Immunol* 2011; **48**(9-10): 1084-1090.

[38]Lv Y, Gou S, Li XG, Chi JY, Qu YQ , Zhong HL. Sputum and serum microRNA-144 levels in patients with tuberculosis before and after treatment. *Int J Infect Dis* 2016; **43**: 68-73.

[39]Kim JK, Lee HM, Park KS, Shin DM, Kim TS, Kim YS, et al. MIR144* inhibits antimicrobial responses against *Mycobacterium tuberculosis* in human monocytes and macrophages by targeting the autophagy protein DRAM2. *Autophagy* 2017; **13**(2): 423-441.

[40]Ciu SY, Wang R, Chen LB. MicroRNA-145: A potent tumour suppressor that regulates multiple cellular pathways. *J Cell Mol Med* 2014; **18**(10): 1913-1926.

[41]Furci L, Schena E, Miotto P, Cirillo DM. Alteration of human macrophages microRNA expression profile upon infection with *Mycobacterium tuberculosis*. *Int J mycobacteriol* 2013; **2**(3): 128-134.

[42]Fu Y, Yang X, Chen H, Lu Y. Diagnostic value of miR-145 and its regulatory role in macrophage immune response in tuberculosis. *Genet Mol Biol* 2020; **43**(2): e20190238.

[43]Spinelli SV, Diaz A, D'Attilo L, Marchesini MM, Bogue C, Bay ML, et al. 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* 2013; **53**(3): 265-269.

[44]Li M, Wang J, Fang Y, Gong S, Li M, Wu M, et al. MicroRNA-146a promotes mycobacterial survival in macrophages through suppressing nitric oxide production. *Sci Rep* 2016; **6**: 23351.

[45]Barry SE, Ellis M, Yang Y, Guan G, Wang X, Britton WJ, et al. Identification of a plasma microRNA profile in untreated pulmonary tuberculosis patients that is modulated by anti-mycobacterial therapy. *J Infect* 2018; **77**(4): 341-348.

[46]Li S, Yue Y, Xu W, Xiong S. MicroRNA-146a represses mycobacteria-induced inflammatory response and facilitates bacterial replication *via* targeting IRAK-1 and TRAF-6. *PLoS One* 2013; **8**(12): e81438.

[47]Iwai H, Funatogawa K, Matsumura K, Kato-Miyazawa M, Kirikae F, Kiga K, et al. MicroRNA-155 knockout mice are susceptible to *Mycobacterium tuberculosis* infection. *Tuberculosis (Edinb)* 2015; **95**(3): 246-250.

[48]Wang J, Yang K, Zhou L, Wu M, Wu Y, Zhu M, et al. MicroRNA-155 promotes autophagy to eliminate intracellular mycobacteria by targeting Rheb. *PLoS Pathog* 2013; **9**(10): e1003697.

[49]Rothchild AC, Sissons JR, Shafiani S, Plaisier C, Min D, Mai D, et al. MiR-155-regulated molecular network orchestrates cell fate in the innate and adaptive immune response to *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* 2016; **113**(41): E6172-E6181.

[50]Etna MP, Sinigaglia A, Grassi A, Giacomini E, Romagnoli A, Pardini M, et al. *Mycobacterium tuberculosis*-induced miR-155 subverts autophagy by targeting ATG3 in human dendritic cells. *PLoS Pathog* 2018; **14**(1): e1006790.

[51]Rajaram MV, Ni B, Morris JD, Brooks MN, Carlson TK, Bakthavachalu B, et al. *Mycobacterium tuberculosis* lipomannan blocks TNF biosynthesis by regulating macrophage MAPK-activated protein kinase 2 (MK2) and microRNA miR-125b. *Proc Natl Acad Sci USA* 2011; **108**(42): 17408-17413.

[52]Huang J, Jiao J, Xu W, Zhao H, Zhang C, Shi Y, et al. MiR-155 is upregulated in patients with active tuberculosis and inhibits apoptosis of monocytes by targeting FOXO3. *Mol Med Rep* 2015; **12**(5): 7102-7108.

[53]Zhang C, Xi X, Wang Q, Jiao J, Zhang L, Zhao H, et al. The association between serum miR-155 and natural killer cells from tuberculosis patients. *Int J Clin Exp Med* 2015; **8**(6): 9168.

[54]Yuan X, Berg N, Lee JW, Le TT, Neudecker V, Jing N, et al. MicroRNA miR-223 as regulator of innate immunity. *J Leukoc Biol* 2018; **104**(3): 515-524.

[55]Dorhoi A, Iannaccone M, Farinacci M, Fae KC, Schreiber J, Moura-Alves P, et al. MicroRNA-223 controls susceptibility to tuberculosis by regulating lung neutrophil recruitment. *J Clin Invest* 2013; **123**(11): 4836-4848.

[56]Liu Y, Wang R, Jiang J, Yang B, Cao Z, Cheng X. miR-223 is upregulated in monocytes from patients with tuberculosis and regulates function of monocyte-derived macrophages. *Mol Immunol* 2015; **67**(2 Pt B): 475-481.

[57]Chen DY, Chen YM, Lin CF, Lo CM, Liu HJ, Liao TL. MicroRNA-889 inhibits autophagy to maintain mycobacterial survival in patients with latent tuberculosis infection by targeting TWEAK. *MBio* 2020; **11**(1): e03045-19.

[58]Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; **18**: 997-1006.

[59]Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2002; **99**(24): 15524-15529.

[60]Cortez MA, Welsh JW, Calin GA. Circulating microRNAs as noninvasive biomarkers in breast cancer. *Recent Results Cancer Res* 2012; **195**: 151-161.

[61]Nana-Sinkam SP, Croce CM. Clinical applications for microRNAs in cancer. *Clin Pharmacol Ther* 2013; **93**(1): 98-104.

[62]Wen Y, Han J, Chen J, Dong J, Xia Y, Liu J, et al. Plasma miRNAs as early biomarkers for detecting hepatocellular carcinoma. *Int J Cancer* 2015; **137**(7): 1679-1690.

[63]Agrawal S, Tapmeier T, Rahmioglu N, Kirtley S, Zondervan K, Becker C. The miRNA mirage: How close are we to finding a non-invasive diagnostic biomarker in endometriosis? A systematic review. *Int J Mol Sci* 2018; **19**(2): 599.

[64]Derkow K, Rössling R, Schipke C, Krüger C, Bauer J, Fähling M, et al. Distinct expression of the neurotoxic microRNA family let-7 in the cerebrospinal fluid of patients with Alzheimer's disease. *PLoS One* 2018; **13**(7): e0200602. Doi: 10.1371/JOURNAL.PONE.0200602.

[65]Rashad NM, El-Shal AS, Shalaby SM, Mohamed SY. Serum miRNA-27a and miRNA-18b as potential predictive biomarkers of hepatitis C virus-associated hepatocellular carcinoma. *Mol Cell Biochem* 2018; **447**(1-2): 125-136.

[66]Biswas S, Haleyurgirisetty M, Lee S, Hewlett I, Devadas K. Development and validation of plasma miRNA biomarker signature panel for the detection of early HIV-1 infection. *Ebio Med* 2019; **43**: 307-316.

[67]Qi Y, Cui L, Ge Y, Shi Z, Zhao K, Guo X, et al. Altered serum microRNAs as biomarkers for the early diagnosis of pulmonary tuberculosis infection. *BMC Infect Dis* 2012; **12**: 384.

[68]Yi Z, Fu Y, Ji R, Li R, Guan Z. Altered microRNA signatures in sputum of patients with active pulmonary tuberculosis. *PLoS One* 2012; **7**(8): e43184.

[69]Cao D, Wang J, Ji Z, Shangguan Y, Guo W, Feng X, et al. Profiling the mRNA and miRNA in peripheral blood mononuclear cells in subjects with active tuberculosis. *Infect Drug Resist* 2020; **13**: 4223.

[70]Lin J, Wang Y, Zou YQ, Chen X, Huang B, Liu J, et al. Differential miRNA expression in pleural effusions derived from extracellular vesicles of patients with lung cancer, pulmonary tuberculosis, or pneumonia. *Tumour Biol* 2016; **37**: 15835-15845.

[71]Wang J, Zhu X, Xiong X, Ge P, Liu H, Ren N, et al. Identification of potential urine proteins and microRNA biomarkers for the diagnosis of pulmonary tuberculosis patients. *Emerg Microbes Infect* 2018; **7**(1): 63.

[72]Hua Y, Sun FY, Wu YH, Huang YM, Zhou FY, Zhang HX, et al. MicroRNA-155 from sputum as noninvasive biomarker for diagnosis of active pulmonary tuberculosis. *Iran J Basic Med Sci* 2020; **23**(11): 1419-1425.

[73]Wang C, Yang S, Sun G, Tang X, Lu S, Neyrolles O, et al. Comparative miRNA expression profiles in individuals with latent and active tuberculosis. *PLoS One* 2011; **6**(10): e25832.

[74]Lyu L, Zhang X, Li C, Yang T, Wang J, Pan L, et al. Small RNA profiles of serum exosomes derived from individuals with latent and active tuberculosis. *Front Microbiol* 2019; **10**: 1174.

[75]Hashimoto S, Zhao H, Hayakawa M, Nakajima K, Taguchi Y, Murakami Y. Developing a diagnostic method for latent tuberculosis infection using circulating miRNA. *Transl Med Commun* 2020; **5**: 25.

[76]Wang C, Yang S, Liu CM, Jiang TT, Chen ZL, Tu HH, et al. Screening and identification of four serum miRNAs as novel potential biomarkers for cured pulmonary tuberculosis. *Tuberculosis (Edinb)* 2018; **108**: 26-34.

[77]Zhou Y, Zhang L, Zhao W, Wu Y, Zhu C, Yang Y. Nanoparticle-mediated delivery of TGF-α1 miRNA plasmid for preventing flexor tendon adhesion formation. *Biomaterials* 2013; **34**(33): 8269-8278.

[78]Gebert LFR, Rebhan MAE, Crivelli SEM, Denzler R, Stoffel M, Hall J. Miravirsen (SPC3649) can inhibit the biogenesis of miR-122. *Nucleic Acids Res* 2014; **42**(1): 609.

[79]Sethupathy P. The promise and challenge of therapeutic MicroRNA silencing in diabetes and metabolic diseases. *Curr Diab Rep* 2016; **16**(6): 52.