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Biomarker and therapeutic targets for tuberculosis: role of micro RNAs

Abstract

Effective biomarkers are utmost important for early diagnosis of tuberculosis (TB) infection and treatment for successful TB control. Current diagnostic tests are not rapid and sufficient for TB diagnosis. Therefore identification of potential biomarkers for TB will be highly beneficial. microRNA plays pivotal role in various biological processes and involves in shaping immunity by regulating the repertoire of genes expressed in immune cells among mycobacterium tuberculosis infected individuals. This review summarizes; role of miRNAs as immune modulator, recent studies on identification of surrogate biomarker for active and latent TB; finally usage of miRNAs as a novel class of therapeutic drug targets against TB.

Keywords: *Mycobacterium tuberculosis*, miRNA, biomarker, therapeutic drugs, cytokine regulation, autophagy, chemotaxis, phagocytosis, tumour necrosis factor, interferon-γ

Volume 3 Issue 3 - 2016

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Received: October 20, 2016 | **Published:** November 16, 2016

Abbreviations: *M. Tb, mycobacterium tuberculosis*; WHO, world health organization; LTBI, latent tuberculosis infection; TST, tuberculin skin test; IGRA, interferon-gamma release assay; miRNA, microrna; HCS, healthy control subjects; HHC, healthy household contacts; PTB, pulmonary tuberculosis; PPD, purified protein derivative; PBMC, peripheral blood mononuclear cells

Introduction

Tuberculosis is one of the deadliest infectious diseases caused by *Mycobacterium tuberculosis*. As per World Health Organization (WHO) global Tuberculosis (TB) report 2015, nearly 9.6 million new TB cases have emerged and 1.5million deaths taken place in 2014.[1] Over 95% of TB deaths occur in low- and middle-income countries. Targets of global End TB strategy include 95% and 90% reduction in TB deaths and incidence by 2035 respectively. In order to control the TB epidemic, an adequate TB diagnosis in resource limited settings is essential. Currently, there are two major gaps in the existing diagnostics pipeline: lack of point of care (POC) test for TB (rapid diagnosis at the primary care level) and lack of a biomarker for latently infected individuals (who will benefit most from preventive therapy). High global prevalence of latent TB infection (LTBI) is a key challenge in distinguishing patients with active TB from those with LTBI. Current diagnostic methods TST and IGRA are insufficiently accurate for differentiating LTBI and active TB. MicroRNA (miRNA) is short nucleotides (19-24nts), non coding RNAs and evolutionarily conserved from prokaryotes to eukaryotes. It regulates the specific target gene expression by binding messenger RNA (mRNA) and stimulates degradation of mRNA or inhibition of translation. Currently there are approximately 1,000 human miRNAs sequences were found in mammals and it targets about 60% of all genes. It plays a pivotal role in various biological processes including cell proliferation, cell cycle, cell growth, tissue organization, apoptosis and different metabolisms. It also involves in shaping immunity by regulating the repertoire of genes expressed in immune cells and the magnitude and duration of responses to particular pathogens.[2] It has been thought, that serum miRNAs were derived from the infected epithelial cells and other types of immune cells. The changes of several miRNA levels in plasma, serum, urine and saliva have been associated with various diseases.[3] Circulating miRNAs are stable which are protected from endogenous RNAse activity.

miRNAs in immune response

It has been reported that alterations in miRNA expression levels may lead to the changes in host immune response in active TB.[4] Macrophages are involved in the regulation of inflammatory diseases, or even infectious diseases, including tuberculosis. Many of the miRNAs regulate the gene expression in macrophages and these miRNAs are crucial for maintaining tissue homeostasis and also prevent the development of disease. The association between target genes and miRNA expression leads the differentiation and maturation of macrophage from monocytes. So, these miRNAs and their targets may be crucial in macrophage function by determining the polarization of macrophages. The miRNAs regulate the function of macrophages such as to diminish the phagocytosis process (miR-142-3p), inhibition of autophagy (miR-125a, miR-30a), induction of inflammation (miR-155), anti apoptosis (Let-7e, miR-29) and chemotaxis (miR-223).

miRNA in cytokine regulation

miRNAs can potentially regulate cytokine production by directly binding to target sites of mRNA. It has been demonstrated that, the major protective cytokines against tuberculosis disease are Interferon-g (IFN-g)[5] and Tumour Necrosis Factor (TNF)-α.[6] Many of the miRNAs were directly or indirectly linked to these cytokines production and subsequently play a role in disease outcome. Ma et al.[7] have reported, the mouse miRNA, miR-29, has been shown to target IFN-g and suppress immune responses against *M. Tuberculosis*.[7] The miR-125b directly inhibits the mRNA of TNF-α, while miR-155 enhances TNF-α expression by increasing the half life of TNF-α Mrna.[8] In another study, it was found that, both TNF-α and IFN-g expression was inhibited by miR-144* among tuberculosis patients.[9] Singh and his co-workers reported that, by inhibiting expression of miR-99b in dendritic cells there was enhanced production of pro inflammatory cytokines and resulted significantly reduced bacterial burden.[10] Thus these observations suggest that there is a direct or

indirect relation between miRNA expression and cytokine regulation during *M. tuberculosis* infection. Though the role of miRNAs in mycobacterial infections has been addressed recently, most of the studies concluded, miRNAs are explored in host pathogen interaction and promising targets in biomarker and therapeutic fields.[11]

miRNAs as biomarker in TB infection

Early diagnosis of TB and treatment is utmost important in effective tuberculosis control. Current diagnostic tests are not sufficient and rapid for TB diagnosis. Therefore identification of potential biomarkers for TB diagnosis will be highly beneficial. Although proteins are more diverse and potentially more informative, complexity of protein composition in blood, the diversity of post-translational modifications, the abundance of many protein of interest and difficulties in developing suitable protein detection agents making us hard to develop protein based biomarkers. On the other hand, miRNA expression profiling has provided abundant clues in understanding the pathophysiology of active TB, besides being served as biomarker and therapeutic targets. Most miRNAs are widely expressed in a variety of organs, body fluids and tissues. Subtle differences in miRNA expression profiles are likely to have a significant influence on the outcome of disease. Furthermore, miRNAs are emerging as highly specific biomarkers with potential clinical applicability. Recently, these miRNAs have been extensively studied as new biomarkers for diagnosis and prognosis in various diseases such as cancers,[12] heart disease,[13] diabetes,[14] and infectious diseases.[15] After elucidating the role of miRNA as biomarker for cancer, several investigators extended studies on the role of miRNAs in various infectious diseases such as tuberculosis. Finding new biomarkers for tuberculosis is not only necessary for diagnosing patients with TB, but also for the classification of TB depend on disease stage, TB prognosis, and TB drug and vaccine trials.[16] Reports are showing that many of miRNAs differentially expressing at specific disease stage. There are distinct miRNA levels have been found in PBMCs,[9,17] serum[18] and sputum[19] between tuberculosis patients and healthy controls. Identification of biomarkers which can discriminate between latent TB infection and active TB disease is one of the most important issues in TB prevention and control. Several miRNAs that were differentially expressed among active TB, latent TB and healthy controls could serve as biomarkers at specific disease stage. Studies have shown that specific miRNAs expressed differentially in active TB both in serum and after stimulation of PBMCs with PPD.[4] Based on this, mi-RNAs may potentially used as biomarkers for different stages of TB infection. It has been demonstrated that several miRNAs are highly expressing in blood (PBMC & serum) and sputum of active TB patients than healthy controls and these miRNAs can be used as a biomarker for active TB.[17–19] It is well known that compared to measuring single miRNA, signature of miRNAs enhances the disease detection rate.[20] The combination of eight miRNAs (miR-150, miR-146a, miR-125b, miR-31, miR-10a, miR-1, miR-155, miR-29) showed increased discrimination of childhood active TB from uninfected children.[21] Wang reported that miRNAs hsa-miR-223, hsa-miR-424, and hsa-miR-451 and hsa-miR-144 that were differentially expressed between active TB and LTBI.[4] A previous study that whole genome expression profile differences between latent and active TB found that 407 genes were up regulated and 364 genes were down regulated in active TB.[22] Most of these genes were regulating by specific mi-RNAs which in turn regulates the cytokines levels in different stages of TB infection. Further, Stern et al.[23] found that antigen specific miR-155 and miR-155* are distinguished active TB from latent TB upon stimulation of PBMC with PPD.[23] Thus, identification of disease specific miRNAs will led to development of algorithm for screening TB patients. Based on the existing literature, we have listed the differentially regulated miRNA among different stages of TB infection in Table 1. Further, few of the miRNAs were identified as biomarkers for TB prognosis. miR-142-3p, miR-21, miR-26a and miR-29a, were down-regulated in TB patients but increased during recovery stage.[24] This indicates that, miRNAs not only considered as potential biomarkers for TB, but also help to assess the post-treatment recovery. Further differential miRNA profiles associated with the transition from latent to active TB [reactivation] and suggest that miRNAs expression may play an important role in the pathogenesis of *M. tuberculosis* disease by controlling reactivation related gene expression.[4] In this direction, very recent study has been reported that hsa-let-7b and hsa-miR-30b might be associated with TB disease development by regulating target genes.[25] Nevertheless, number of miRNAs have been identified as biomarkers to differentiate healthy controls, latent TB and active TB[4,18] still there is no clear-cut evidence or association of those miRNAs, in the pathophysiology of the disease, whether these miRNAs are specific for TB or any other diseases.[10] Therefore, further research is needed to elucidate the expression of these biomarkers in TB and its closely related diseases. In addition, so far there is no common miRNAs has been identified in *M. Tuberculosis* infected individuals. So detailed understanding of the TB specific miRNA abnormalities could contribute to novel approaches in early diagnosis of tuberculosis and can serve as a biomarker. The other ambiguous question is whether the up and down regulated miRNA expressions will drive the disease occurrence or consequence of the disease.

Potential therapeutic role of mi-RNA

The emerging research directing is usage of miRNAs as a novel class of drug targets for various diseases. The expression of miRNA could be manipulated for therapeutic purpose either through positive and negative regulation. There are many modes for the modulation of miRNA expression. Modified antisense oligo nucleotides complementary to miRNA, or anti-miRNA have been used by many groups to inhibit the target mature miRNA activity.[34,35] Conversely, miRNA-mimics are duplex oligonucleotides with a sequence identical to a mature miRNA of interest. Once inside the cell, the miRNA-mimics associate with the miRISC complex and post-transcriptionally inhibit the target mRNAs. An alternative approach is to target miRNAs therapeutically by inhibiting its processing [Targeting miRNA processing] which leads to preventing the formation of mature miRNA production.[36] Another alternative strategy is specific miRNA replacement by gene therapy with the help of viral vectors.[37] Few reports have shown some mi-RNAs can be used as therapeutic targets in cardio vascular diseases,[38] cancer[39] and Hepatitis C virus infection.[40] Some miRNAs [miR-99b] play an important role in the pathogenesis of *M. tuberculosis*. It inhibits the secretion of pro-inflammatory cytokines which were involved in the control of *M. Tuberculosis*.[10] By targeting this miR-99b may open up new approach for designing miRNA-based vaccines and therapy. Spinelli et al.[28] reported that, at the beginning of anti-tuberculosis treatment, miR-424 was up regulated and miR-164a was down regulated and became normal levels after 2months treatment.[28] However, these differentially expressed miRNAs have the potentiality to use as future therapeutics, so far, there is no clinical trials has progress in this line.[10] The main limitation factor is most of the miRNAs are not entirely gene specific. One miRNA may target more than one mRNA and suggests exogenous miRNA may display off-target effects. More animal studies are advocated to study exclusively the role of these miRNAs as therapeutic targets.

Citation: Pathakumari B, Bethunaickan R. Biomarker and therapeutic targets for tuberculosis: role of micro RNAs. *J Investig Genomics*. 2016;3(3):44–47.
DOI: 10.15406/jig.2016.03.00050

Table 1 Potential biomarkers for differentiating healthy controls, latent and active TB infections based on miRNA differential expression levels

Group	Sample	miRNA	References
Active TB and HCS	PBMC	miR-155, miR-144*	Wu et al.[17], Liu et al.[9]
Active TB and HCS	Serum	miR-29a, miR-93*	Fu et al.[18]
Active TB and HCS	Sputum	miR-29a	Fu et al.[18]
Active TB and HCS	Sputum	miR-19b-2*, miR-3179, miR-147	Yi et al.[19]
Active TB and HCS	Serum	miR-378, miR-483-5p, miR-22, miR-29c, miR-101 hsa-miR-320b	Zhang et al.[20]
Active TB and HCS	Serum	miR-361-5p miR-889 miR-576-3p	Qi et al.[26]
Active TB and HCS	Serum	miR-182 miR-197	Abd-El et al.[27]
Active TB and HCS	PBMC	miR-424 and miR-365	Wang et al.[4]
Active TB and HCS	PBM & PFMC	miR-146a	Spinelli et al.[28]
Active TB and HCS	Serum	miR-148a miR-16 miR-192 miR-193a-5p miR-25 miR-365 miR-451 miR-532-5p miR-590-5p miR-660 miR-885-5p let-7e miR-146	Miotto et al.[29]
Active TB and HCS	PBMC	miR-150 miR-146a miR-125b miR-31 miR-10a miR-1 miR-155 miR-29	Zhou et al.[21]
PTB and other viral infections	Serum	miR-361-5p, miR-889, miR-576-3p	Qi et al.[26]
Active TB and LTBI	Macrophages	hsa-miR-16, miR-95, miR-101, miR-137, miR-193	Zheng et al.[30]
Active TB and LTBI	Serum	hsa-miR-196b and hsa-miR-376c	Zhang et al.[31]
Active TB and LTBI	PBMC	has-miR-21*	Xu et al.[32]
Active TB and LTBI	PBMC	miR-130b*, miR-223, miR-424, miR-302a, miR-21*	Wang et al.[4]
Active TB and LTBI	PBMC	miR-146a, miR-150, miR-16 miR-221	Wu[33]
Active TB and LTBI	PBMC	miR-155 and miR-155*	Stern et al.[23]
Active TB and controls	Plasma	hsa-let-7b and hsa-miR-30b	Xin et al.[25]

Summary

We have summarized the role of miRNAs in modulating the host immunity against mycobacterial infection. The altered miRNAs in various patho-physiological stages of tuberculosis may be informative and valuable in discovering specific biomarkers for the diagnosis and prognosis for tuberculosis disease. Further the potential utilization of miRNAs in treatment could lead to development of novel therapeutic interventions for tuberculosis. More attention will be required to investigate on miRNAs and also to understand or correlate the association of these miRNAs with cytokine regulation. The challenge against understanding the mechanism behind the transition of latent TB to active TB has to be explored. Future work is also need to discover miRNA based biomarkers for the prediction of relapse and resistance of tuberculosis. The rapid advancement in miRNA research strengthens our hope in facing challenges and unsolved problems in the areas of TB diagnostics, biomarker and new therapeutics for the future.

Acknowledgements

None.

Conflict of interest

Author declares that there is no conflict of interest.

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Citation: Pathakumari B, Bethunaickan R. Biomarker and therapeutic targets for tuberculosis: role of micro RNAs. *J Investig Genomics*. 2016;3(3):44–47. DOI: 10.15406/jig.2016.03.00050

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Citation: Pathakumari B, Bethunaickan R. Biomarker and therapeutic targets for tuberculosis: role of micro RNAs. *J Investig Genomics*. 2016;3(3):44–47.
DOI: 10.15406/jig.2016.03.00050