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Review Article

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Expression and Regulation of microRNAs in Tuberculosis: A Review

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ABSTRACT

Several diagnostic tools and techniques are available for the diagnosis of tuberculosis (TB) but these techniques shown an inadequate response in early diagnosis of tuberculosis and hence they have low sensitivity and specificity. Also, these techniques have some inaccuracy in the diagnosis of different forms of tuberculosis such as pulmonary/extra-pulmonary tuberculosis and active/latent tuberculosis. From the previous studies in various diseases such as cancer and cardiac diseases, microRNAs (miRNAs) shows the remarkable growth due to their reliable and stable nature in the circulating fluid of the body. This review focused on the role of various miRNAs in the host during the Mycobacterium tuberculosis infection and the miRNA's efficiency to act as a potential biomarker for the diagnosis of tuberculosis in the cases such as Active/Latent Tuberculosis, Pulmonary/Extra-Pulmonary Tuberculosis. Immunological aspects and miRNA regulation during the tuberculosis infection were also discussed in this review.

Keywords: *miRNA, Tuberculosis, Biomarker, Diagnosis, Mycobacterium tuberculosis, Up-regulation, Down-regulation, Therapeutic target.*

INTRODUCTION

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* bacteria (*M.tb*) generally affects the lungs but can also affect other sites like in extra-pulmonary tuberculosis (such as lymph node, pleural, genital and urinal, etc.). The bacteria of tuberculosis spreads through the air medium such as by a cough, sneezing, etc. According to the World Health Organisation's (WHO) Global Tuberculosis Report 2018, tuberculosis makes its place in the top 10 as one of the most death causing disease worldwide. In 2017, 10 million people had tuberculosis, and 1.6 million died from the disease (including 0.3 million among people with HIV) (Global tuberculosis report, 2018). An uncontrolled epidemic of tuberculosis and multiple-drug resistant tuberculosis caused by the spreading of *Mycobacterium tuberculosis* requires an adequate and efficient diagnostic and treatment measures.

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For its own sake, manipulation of cellular environment is done by the bacteria**.** Under various environmental stresses such as nitric oxide, low pH, nutrient starvation and drug exposure, extensive studies have been conducted on bacteria's gene expression profiles (Rohde et al., 2012). On the other hand, there is very limited information on the level of RNA during host-pathogen interaction. Also, there are less number of studies which reveals the regulatory role of RNA during tuberculosis and the regulation of RNA affects the interaction between bacteria and macrophages (Guo et al., 2010).

microRNAs (miRNAs) are short, small, non-coding 21-25 nucleotides long and have an important role in Post-Transcriptional Gene Silencing (PTGS) (Melo & Melo, 2014). The genome of human may encode about more than two tousand miRNAs. Each miRNA have the ability to suppress multiple genes and also one messengerRNA (mRNA) by multiple miRNAs. Therefore, miRNAs associated with a disease can represent a new class diagnostic markers (Sabir et al., 2018). The first miRNA was discovered in 1993 was *lin-4* (Fazli Wahid et al., 2010). Entry of any pathogen or foreign particles into the human body followed by their activation in the bloodstream and several associated factors are released for their protection. In the cellular system, several changes occur in response to the pathogenic activity, simultaneously, the miRNA's production starts which are controlling the diseased condition through the cellular signaling.

In the miRNA development process, transcription of the non-coding part of the host genome is done by RNA Polymerase II to form the Primary miRNA (pri-miRNA) having a hairpin loop structure. Then, DROSHA (RNase III protein) cleaves the Primary miRNA (pri-miRNA) to convert it into precursor miRNA (pre-miRNA). To the cytoplasm, pre-miRNA is exported for the further maturation processing by DICER (RNase III Endonuclease) to form RNA duplex followed by their loading onto AGO1-4 (Argounate protein). After then, the second strand (the Passenger strand) is discarded from the complex which then bring the formation of fully mature miRNA complexes with AGO protein (Minju Ha & Narry Kim, 2014). Then this miRNA takes part in Post-Transcriptional Gene silencing where it inhibits the translation process either by blocking the mRNA or by degrading the mRNA (David P. Bartel, 2004, Leigh-Ann MacFarlane & Paul, 2010).

The traditional techniques for the diagnosis of tuberculosis are nowadays inadequate or inefficient (Chong Wang, 2018). So we need a reliable diagnostic method for tuberculosis diagnosis. miRNAs are regulated at different levels and different conditions of the disease. So, there is a need to identify them in the particular disease along with their expression during the condition. Various studies show that miRNAs are not only useful in cancer diagnosis but can be useful as a diagnostic tool in the tuberculosis (Wagh et al., 2016). Biomarkers can either be host or pathogen specific. A biomarker may provide information about the pathological processes including the current health status and the long-term prognosis of the disease state. miRNAs have been reported in their relation with altered gene expression profiles in the macrophages and the Natural Killer (NK) cells reported from Active and Latent tuberculosis, tuberculosis infected and healthy controls. Innate immunity functions of macrophages and NK cells have been found that have some important role of miRNAs in their regulation (Harapan Harapan, 2013).

IMMUNOPATHOLOGY OF TUBERCULOSIS:

Upon the entry into the host, only the sum of 10% bacteria reaches the respiratory bronchioles and alveoli, settlement of most of the bacteria occurs in the upper respiratory epithelium, where the mucociliary escalator expelled the bacteria (Nardell, 1993). Alveolar macrophages phagocytosed the bacteria that make their way to the deep lung (Dannenberg, 1993). Bacteria which survives the phagocytosis, will multiply and kill their macrophages after the 2-3 weeks (Spencer, 1985). Recognition of *M.tb* is primarily done

by Toll-Like receptors (TLRs) in macrophages upon which their signals can activate Nuclease factor κ beta (NF-κβ) that induces pro-inflammatory cytokines (Kleinnijenhuis et al., 2011, Moynagh, 2005). Against tuberculosis infection, the main pro-inflammatory cytokines are Interferon-γ (IFN-γ) and Tumor necrosis factor-α (TNF-α) and having an important role in inhibiting *Mycobacterium* growth (Marín et al., 2013, Bruns et al., 2009, Serbina et al., 2008).

Various studies shows that Interleukin-6 (IL-6) and IL-β in host provides resistance against the tuberculosis infection (Cooper et al., 2011, Mayer-Barber et al., 2010). Where, primary suppression of anti-mycobacterial responses is through IL-10 (Redford et al., 2011). Successful invasion and parasitization of macrophages are done by *M.tb* via inhibiting phagolysosome fusion followed by neutralizing the acidic environment of the phagolysosomal compartment, T-cells do not respond to antigens in a neutralized acidic environment (Rohde et al., 2007, Behar et al., 2011).

USING microRNAs AS A POTENTIAL BIOMARKER FOR TUBERCULOSIS DIAGNOSIS:

From various studies**,** it is a fact that the expression of thousands of mRNAs are regulated by microRNAs. For a number of infectious diseases, extensive investigation have been conducted on dysregulation of microRNAs. Although, the role of microRNAs during parasitic and viral infections is the major focused work in the early days (Ding & Voinnet, 2007, Cullen, 2011, Hakimi & Cannella, 2011). However in recent times, studies on microRNAs role during the interaction between bacteria and the host has been conducted (Eulalio et al., 2012). By diagnosing the tuberculosis at an early stage, we can effectively control the spread of tuberculosis infection. The systems which are currently in use are not up to the mark in the diagnosis of tuberculosis and fails to discriminate between active and latent tuberculosis. To differentiate between active tuberculosis and latent tuberculosis, altered expressions of miRNA in the host upon tuberculosis infection may help and can also work as a reliable biomarker for the diagnosis of tuberculosis. In recent years, this characteristic of miRNAs has been explored by various researchers as a potential biomarkers but no suitable and specific miRNA have not been set up yet (Sabir et al 2018).

In different pathogenic processes, dysregulation of miRNAs plays a crucial role. In the body circulation, release of various miRNAs have been demonstrated. In clinical samples, isolation and quantification of miRNAs showed a good stability property. Also, the abundance of miRNAs in the body's circulating fluid are due to their independency on age and gender (Amal et al., 2013). Recent discoveries of new miRNAs revealed their roles in regulation whether they were up regulated or down regulated during various disease conditions and cell physiology. Regulation of various miRNAs were reported in communicable and non-communicable disease condition in the host. Also, the host miRNAs were have the potential of using them as a biomarker in the diagnosis of a disease condition (Harapan Harapan et al., 2013).

In clinical specimens, current diagnostic approaches rely on the detection of the pathogen. Relying on the detection of the pathogen for the diagnosis of tuberculosis have some clinical problems due to heterogeneous clinical presentations of *Mycobacterium tuberculosis* infection such as Active TB/Latent TB, Pulmonary TB/Extra-Pulmonary. The attention has been raised to miRNAs as tuberculosis biomarker. miRNAs are available in sputum, serum, plasma (due to its availability in different samples like sputum serum plasma). Circulating miRNAs are novel and specific diagnostic biomarkers for several diseases (Harapan Harapan et al., 2013)**.** The group of 8 miRNAs (miR-150, miR-146a, miR-125b, miR-31, miR-10a, miR-1, miR-144, miR-29) showed an increased level in active TB children as compared to in uninfected children (Mengyao Zhou et al., 2016). A study found that the miR-155 which

is antigen-specific, is able to discriminable Active TB from Latent Tb (Stern et al., 2009). hsa-miR-223, hsa-miR-424, hsa-miR-451, and hsa-miR-144 were reported that were expressed in Active TB and Latent TB (Wang et al., 2011).

RELATIONSHIP BETWEEN IMMUNOLOGICAL MOLECULES AND VARIOUS micr0RNAs DURING TUBERCULOSIS:

Production of cytokines are regulated by miRNAs by silencing their messengerRNA (mRNA) via binding to these mRNA directly. Against tuberculosis, (Interferon-γ) IFN-γ and (Tumor Necrosis Factor- α) TNF-α also with major Interleukins (IL) are the major protective cytokines (Cooper et al.,1993, Flynn et al., 1995). Various miRNAs are stated following that regulates the cytokines and other immunological molecules production.

miRNA-21:

miRNA-21 suppresses the pro-inflammatory cytokines expression and promotes the expression of an anti-inflammatory cytokine, IL-10 (Sheedy et al., 2011). Studies found that the down-regulation of the gene of protective cytokines (TNF-α & IL-6) during *M.tb* infection by miRNA-21 have been reported. However, production of IL-12 is induced by miRNA-21 inhibitors which trigger more anti-mycobacterial responses (Riendeau & Kornfeld, 2003). The inhibition of IL-12 is also reported by miRNA-21 which targets 3'UTR of IL-12p35 and leads to the suppression of anti-mycobacterial response (Zhongwen et al., 2012). During *Mycobacterium tuberculosis* infection, an anti-apoptotic response was generated in the RAW264.7 macrophages via the associative inhibition by MPT64 protein along with B-cell Lymphoma-2 (Bcl-2) protein leaded by the miRNA-21. NF-κB is the main transcription factor behind the up-regulation of miRNA-21 in tuberculosis (Wang et al., 2014).

miRNA-29:

Upon the infection of *M.tb* to the human, miRNA-29 was overexpressed in the several human cell types. miRNA-29 down-regulates the IFN-γ which results in immune response suppression. The dissociation of IFN-γ mRNA with Argonaute-2 protein which forms a RISC (RNA Induced Silencing Complex) promoted by miRNA-29 that initiates the gene silencing and resulting in the suppression of the IFN-γ expression post-transcriptionally (Ma et al., 2011). Various anti-apoptotic proteins such as B-cell lymphoma 2 (Bcl-2), Myeloid cell leukemia-1 (Mcl-1), the kinase p85α and the Cdc42, all are targeted by miRNA-29 and hence having a major role in immune cell's apoptotic pathway. Therefore, miRNA-29 having some role in the inhibition of IFN-γ and increased apoptosis of cells involving anti-Tb responses (Park et al., 2009, Xiong et al., 2010).

`miRNA-125b and miRNA-147:

High expression of miRNA-125b occur when macrophages are incubated with M.tb with low TNF production. TNF mRNA's 3'UTR is targeted directly by miRNA-215b resulting in the inhibition of TNF mRNA translation which contributes in the down-regulation of TNF production (Rajaram et al., 2011). miRNA-125b also improves the stability of κB-Ras2, an inhibitor of NF-κB in macrophages, hence decreasing the inflammatory response in TB (Murphy et al., 2010). Previous studies shown that TLR/NFκβ signaling pathway in macrophages induces miRNA-147 which suppresses the expression of TNF-α and IL-6 (Liu et al., 2009). The concentration of TNF-α and IL-6 were found higher in serum or Peripheral Blood Mononuclear Cells (PBMCs) in Tuberculosis as compared to the healthy individuals (Bongiovanni et al., 2012, Santucci et al., 2011, Spinelli et al., 2013).

miRNA-99b and miRNA-155:

Up-regulation of pro-inflammatory cytokines such as TNF-α, IL-6, IL-12, and IL-1β was reported upon the blocking of miRNA-99b which results in reduced *M.tb* growth. mRNAs of TNFRSF-4 & TNF-α are directly targeted by miRNA-99b (Singh et al., 2013). Upon the *M.tb* infection, the miR-155 reduce translation by inhibiting initiation of TNF mRNA (O'Connell et al., 2009). Studies found that the production of IL-4 is more and IFN-γ is less when miRNA-155 is knockdown in a mouse

model which indicates that a major role of miRNA-155 in regulating T-cell responses (Tsitsiou & Lindsay, 2009).

miRNA-144* and miRNA-146a:

In the patients of Active TB, miRNA-144* was overexpressed. Production of IFN-γ and TNF-α was inhibited by miRNA-144*, upon the transfection of T-cells with miRNA-144* (Liu et al., 2011). Upon tuberculosis, in alveolar macrophages negative regulation of TNF-α production was found by miRNA-146a (Liu et al., 2014).

miRNA-27a and miRNA-365:

In a recent study, miRNA-27a down-regulates the expression levels of IFN-γ, IL-β, IL-6 and TNF-α (Wang et al., 2017). Binding of miRNA-365 on 3'-UTR of IL-6 mRNA inhibits IL-6 protein however there is no direct and significant evidence was found but the study reported that miRNA-365 and IL-6 are indirectly proportional to each other (Song et al., 2015).

VARIOUS microRNAs REPORTED IN TUBERCULOSIS:

microRNAs reported by various authors are summarized in the following tables. Table 1 contains the miRNAs reported in different forms of tuberculosis such as active or latent tuberculosis. Table 2 comprises of regulation of miRNAs whether they up-regulated or down-regulated during tuberculosis.

Table 1: Expression of miRNAs in different forms of TB. Various miRNAs expressed in active and latent tuberculosis in the host

S.NO.	miRNA	Form of TB	Reference
1.	miR-155, miR-144*	Active TB	[56, 52]
2.	miR-29a, miR-93*, miR-29a	Active TB	[57]
3.	miR-19b-2*, miR-3179, miR-147	Active TB	[58]
4.	miR-378, miR-483-5p, miR-22, miR-29c, miR-101, has-miR-320b	Active TB	[59]
5.	miR-361-5p, miR-889, miR-576-3p	Active TB	[60]
6.	miR-182, miR-197	Active TB	[30]
7.	miR-124, miR-365	Active TB	[33]
8.	miR-146a	Active TB	[48]
9.	miR-148a, miR-16, miR192, miR-193a-5p, miR-365, miR-451, miR-532-5p, miR-590-5p, miR-660, miR-885-5p	Active TB	[61]
10.	miR-150, miR-146a, miR-125b, miR-31, miR-10a, miR-1, miR-29	Active TB	[31]
11.	hsa-miR-16, hsa-miR-137, hsa-miR-140-3p, has miR-193a-3p, hsa-miR-501-5p, has miR-598	Active TB	[62]
12.	hsa-miR-101 and hsa-miR-150	Latent TB	[62]
13.	hsa-miR-223-3p, hsa-miR-142-3p, hsa-miR-21-5p	Latent TB	[56]
14.	miR-361-5p, miR-29a	Active TB	[63]

Table 2: Regulation of host miRNAs in response to Mycobacterial pathogens. miRNAs were isolated from different-different sources with their regulation in the host upon tuberculosis

S.NO.	Derived Source	Altered miRNAs	Regulation	Reference
1.	Macrophages	miR-125b	Up	[43]
2.	Macrophages	miR-155	Down	[43]
3.	PBMCs	miR-144*, miR-155*	Up	[52, 56]
4.	Serum	miR-29a	Up	[57]
5.	Serum	miR-3179, miR-147,	Up	[58]
6.	Sputum	miR-19b-2*	Down	[57]
7.	Serum	miR-16	Up	[11]
8.	Serum	miR-21-5p, miR-92a-3p, miR-148b-3p	Up	[10]
9.	Serum	miR-144	Up	[64]
10.	Plasma	miR-99b	Up	[65]
11.	Plasma	miR-21, miR-146a, miR-652	Down	[65]
14.	Serum	miR-29a	Up	[63]
15.	Serum	hsa-miR-140-3p, 21 hsa-miR-3184-5p, hsa-miR-423-3p	Up	[66]
16.	Serum	miR-361-5p, miR-889, miR-576-3p, miR-210, miR-26a, miR-432, miR-134	Up	[60]
17.	Serum	hsa-miR-744, hsa-miR-574-5p, hsa-miR-576-5p, hsa-miR-218, hsa-miR-3911, hsa-miR-4308, hsa-miR-1184	Up	[67]
18.	Serum	hsa-miR-518d-5p, hsa-miR-520c-5p, hsa-miR-526a, hsa-miR-3125, hsa-miR-380*, hsa-miR-765	Down	[67]
19.	Serum	miR-197	Up	[30]
20.	Macrophages, PBMCs, Serum	miR-365	Down	[55]
21.	Serum	miR-361-5p	Up	[63]
22.	Plasma	miR-320, miR-22-3p	Up	[68]
23.	Serum	hsa-miR-140-3p, hsa-miR-3184-5p and hsa-miR-423-3p	Up	[66]

CHALLENGES COMING IN THE WAY OF microRNA AS A BIOMARKER:

Currently, there is availability of few standardized procedures for the isolation, purification and characterization of miRNAs. During the process of isolation, interference of small interfering RNA, premature miRNAs and transfer RNAs has occurred in experiments and observations and hence false positive results were the outcome of this interference. There is a need of experienced and well trained researcher for the isolation and characterization of miRNA and also the knowledge of molecular biology with bioinformatics is must. Not everyone use the tools for characterization of miRNA because they are highly expensive like real time reverse transcription PCR which requires a high expenditure. There is also chances of contamination that alters the interpretation of levels of miRNA.

microRNA's POTENTIAL AS A THERAPEUTIC TARGET:

Ongoing with emerging studies and research giving the direct evidences that miRNAs can be used as a novel and highly specific class of targets in various diseases for drugs. By manipulating or altering the expressions of miRNAs through positive or negative regulation. By using synthetic oligo nucleotides, possibilities of increasing the activity of down-regulated anti-mycobacterial miRNAs are there and also through antisense oligo nucleotides or anti-miRNA which is complimentary to target miRNA, effects of overexpressed pro-mycobacterial miRNAs can be reduce (Meister et al., 2004, Grimm et al., 2006, Baumann & Winkler, 2014). In the pathogenesis of *Mycobacterium tuberculosis*, miRNA-99b plays an important role by inhibiting the pro-inflammatory cytokines secretion (Singh et al., 2013). Researchers can target this miRNA and will leads this miRNA-99b as a target for lowering the symptoms of tuberculosis. Like miR-99b, there are several microRNAs which can be used as target for treating tuberculosis. So, there is a need of experimental models which can reveals the new drugs or molecules that targets these microRNAs. By targeting the 3'UTR of NF-κB, miRNA-21 is inhibited that leads to the down-regulation of Bcl-2 which in turn leads to the inhibition of anti-apoptotic activity of macrophages (Wang et al., 2014).

CONCLUSION

We studied that different-different miRNAs regulated during the tuberculosis infection and this regulation of miRNAs influences the regulation of various immunological molecules such as Tumour Necrosis Factors (TNFs), Inter-leukins (ILs), Interferons (IFNs) and other pro-inflammatory and inflammatory cytokines involved in the tuberculosis infection. Sometimes, these miRNAs are Up-regulated (such as miR-125b, miR-155*, miR-144, miR-29a, miR-99b, etc.) (Rajaram et al., 2011, Wu et al., 2012, Yan et al., 2016, Fu 2011, Barry Simone et al., 2018). and Down-regulated (such as miR-155, miR-19b-2*, miR-21, miR-652, miR-146a, etc.) (Rajaram et al., 2011, Fu et al., 2011, Barry Simone et al., 2018). The up-regulation and down-regulation of these miRNAs during tuberculosis infection is highly specific and can play a significant role in disease diagnosis as a biomarker for the diagnosis of tuberculosis in infected patient's serum. microRNAs are have advantage of their longer stability and abundance in the circulating fluid in host. From the above studies, it can be concluded that the miRNAs regulated during the tuberculosis infection are potential biomarkers for the diagnosis of tuberculosis.

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