

EDITORIAL

Emerging Role of MicroRNA Manipulation by Mycobacterium Tuberculosis for Its Survival in Humans

Pankaj Kumar Ahluwalia, Prabodh Kumar Sehajpal

Pankaj Kumar Ahluwalia, Prabodh Kumar Sehajpal, Department of Molecular Biology and Biochemistry, Guru Nanak Dev University, Amritsar, Punjab, 143005, India

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Correspondence to: Prabodh Kumar Sehajpal, Department of Molecular Biology and Biochemistry, Guru Nanak Dev University, Amritsar, Punjab 143005, India.

Email: sehajpalpk@yahoo.com

Telephone: +919216218220

Fax: +911832258820

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ABSTRACT

In 1884, *Mycobacterium tuberculosis* (*M. tuberculosis*) was discovered as the causative agent for tuberculosis (TB). Effective drugs for this disease are available since 1940's but TB still killed around 1.5 million people worldwide in 2015. The evolutionary adaptation of the pathogen to survive in lethal immune cells of the host during adverse situations and its ability to grow under unfavorable circumstances, hints at the extent of manipulation exercised by *M. tuberculosis*. The pathogen outmaneuvers host's immune mechanisms by modulating key defensive pathways like autophagy and phago-lysosomal fusion. MicroRNAs are emerging as a new class of molecules known to affect

gene regulation by degrading the mRNA or blocking its translation. Insights into interactions between microRNAs and immune cells are unraveling novel strategies adapted by *M. tuberculosis* to manage its survival. Classically, the pathogen down-modulate inflammatory pathways of the host as one of its strategy for survival. Recent studies have revealed the role of several microRNAs in inflammation modulation and metabolic regulation. Variation(s) of microRNAs in latency and active disease are also beginning to emerge. Further, the active role of microRNAs in granuloma and its temporal variations during transition from acute disease to chronic latency and vice-versa are fascinating areas that are anticipated to yield insightful findings. In this editorial, we present recent studies referring to multiple approaches adopted by *M. tuberculosis* to alter gene expression of critical targets by manipulating microRNAs for its survival. The information will be helpful in formulating future strategies for preventing TB.

Key words: micro RNA; Tuberculosis; TB; Macrophage; Gene regulation

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INTRODUCTION

Tuberculosis (TB) is one of the ancient diseases which has scourged humanity since its origin in East Africa ca. 40,000 years ago^[1]. The causative microorganism, *Mycobacterium tuberculosis* (*M. tuberculosis*), hitchhiked inside the bodies of ancient humans out of Africa to all the corners of the planet. Such a long mutual co-existence hints at the exquisite adaptation of this pathogen to its host. Throughout the pathogenic spectra, variety of microbes can potentially infect humans and cause disease. Humans have evolved variety of immunological measures to deal with these pathogenic

insults where most of the pathogens are killed. *M. tuberculosis* is unique as it has evolved a remarkable ability to harness the deadly niche inside the immune cells of the host for its survival and later, dissemination. If progression to active disease is hindered, *M. tuberculosis* can keep deadly and damaging immune confrontations at bay by maintaining a low profile inside the host. It can remain dormant or latent inside humans without causing symptoms of active disease for decades^[2]. According to WHO figures, 90 percent of infected individuals will never have active TB but will carry *M. tuberculosis* in dormant or latent form^[3]. Only 10 percent of infected individuals will continue to develop active disease. Progression of TB disease to active form involves caseation and liquefaction of granuloma in lungs which enables the bacterial spread to other individuals^[4]. In certain cases, these bacterial cells can break free and affect other organs leading to development of extra-pulmonary tuberculosis. Host's immune mechanisms fail in 10 percent of infected individuals as various factors of the host; pathogen and environment interact for the development of active disease. The cellular and molecular mechanisms behind this immune failure are of great interest as new players are continuously being discovered.

At cellular level, *M. tuberculosis* is detected initially by cells of the innate immune system like neutrophils and macrophages through specialized pattern recognition receptors (PRRs) including TLRs (Toll-like receptors) and CLRs (C-type lectin receptor)^[5]. In most of the encounters, the pathogen is neutralized and eliminated from the host. When this first line of defense is breached by the pathogen, a more calibrated response by the host is initiated through activation of cell mediated immunity. This is critical as it primes the antigen presenting cells (APCs) to finish off the recognized pathogen through phago-lysosomal fusion, apoptosis and autophagy^[6]. The cell mediated immunity is triggered once dendritic cells gets infected and then interact with specific CD4 and CD8 T cells^[7]. Intracellular pathogens like *M. tuberculosis* are predominantly cleared by the subsequent immune wave of adaptive immunity. Now in the face of potent cellular response against it, *M. tuberculosis* generates a niche in the potential environment which was supposed to kill it.

The immune mechanism is rendered functional due to cross talk between immune cells through various cytokines. All these effector molecules are essentially protein(s) that mediate their effect either by protein-protein, protein-DNA or protein-RNA interactions. The expression of protein can be regulated at several levels. In TB disease, as there is a shift from homeostatic state to diseased one, the expression profile of various cells changes. Inflammatory transcription factors like IRF5, STATs, NF- κ B among many others, are induced upon infection^[8-9]. Gene expression can be controlled by various mechanisms in the cell. One of the exciting areas of research is control of gene expression through microRNA. microRNAs are small ~ 22nt RNA molecules which bind complementarily to 3'UTR of genes and can translationally repress or degrade the molecule. The microRNAs vary during developmental processes and its abundance in various tissues^[10]. Its role in disease is increasingly being appreciated. New observations are pouring in which suggests the role of microRNA in regulation of disease progression^[11]. *M. tuberculosis* specifically has been shown to not only modulate critical mechanisms of killing but also selectively modulate microRNA which later on affects its survivability.

It would be interesting to point out that microRNAs are dynamically produced in the cell. In a granulomatous condition, where the *milieu* is constantly in flux the microRNA profile is subjected to change. Although, microRNAs are beginning to emerge as promising circulating biomarkers in cancer, their role in

TB pathophysiology is under evaluated^[12]. Although, there is clear evidence that microRNAs interfere with different limbs of pathogen identification and critical steps of immune surveillance (Table 1) thus, allowing *M. tuberculosis* to micromanage its survival within the host.

M. TUBERCULOSIS BASED MODULATION OF MICRORNA FOR ITS SURVIVAL

Macrophage polarization

Macrophages are one of the primary immune cells which play an instrumental role in defense against *M. tuberculosis*. These are highly plastic cells which can show a range of phenotypes, depending on the *milieu* of its tissue. The observed extremes of this polarization spectrum are M1 and M2 type macrophages^[13]. The former is pro-inflammatory in their properties and are lethal for *M. tuberculosis* whereas the later ones are anti-inflammatory cells that favour intracellular survival for *M. tuberculosis*. Evolutionary arms race between humans and *M. tuberculosis* has endowed this pathogen with several adaptations which allows its survival in the macrophages. The mechanisms by which *M. tuberculosis* modifies intracellular environment for its advantage and the role of M1 to M2 transition in it is an intense area of research^[14,15]. Increasing observations are pointing at the role of microRNAs in macrophage polarization^[16,17]. MicroRNAs are one of the many players which could be targeted by *M. tuberculosis* for its intracellular survival in one of the most toxic cells in human body.

Neutrophil recruitment

Neutrophil recruitment is one of the early signs of host innate immune response. During *M. tuberculosis* infection this initial phase has been shown to be compromised. Higher expression of miR-223 was observed in lung parenchyma and blood of TB patients. Interestingly, miR-223 dampens the expression of critical pro-inflammatory chemokines CXCL2, CCL3 and IL-6, reducing the neutrophil recruitment to site of infection^[18].

TLR signaling cascade

TLRs (Toll like receptors) are one of the PRRs which recognize the invasion of *M. tuberculosis*. miR-124 has been shown to be up-regulated in tuberculosis patients and alveolar macrophages. miR-124 negatively regulates TLR signaling cascade by reducing the expression of TLR-6 and downstream signaling molecules, Myd-88 and Traf-6^[19]. *M. tuberculosis* up-regulates miR-124 to counter its identification by innate immune mechanisms of the host.

Table 1 microRNA modulated by *M. tuberculosis* for its intracellular survival.

Pathways/effector molecules involved	Modulated microRNAs
Macrophage Polarization	miR-302c[15], miR-454[15], miR-29b[16], miR-125a-5p[16]
Neutrophil recruitment	miR-223[18]
TLR signaling cascade	miR-124[19]
Autophagy	miR-33[20], miR-125a[21], miR-17-5p[22]
Apoptosis	miR-223[24]
Nitric oxide	let-7f[25], let-146a[26]
Tumor necrosis factor	miR-125b[27]
IFN- γ signaling cascade	miR-132[28], miR-26a[28]
Other cytokines	miR-99b[29]
Metabolism	miR-33[20]

Autophagy

Autophagy acts as a defense mechanism by sequestering intracellular bacteria through autophagosome formation and later delivering it to lysosome for degradation (xenophagy). *M. tuberculosis* induces miR-33 expression in macrophages to modulate autophagy by repressing key players like ATG5, ATG12, LAMP1^[20]. Macrophages infected with *M. tuberculosis* have also shown increased expression of miR-125a which in turn reduces the expression of UV response associated gene (UVRAG)^[21]. UVRAG is known to inhibit autophagy. In another study, miR-17-5p has been shown to regulate autophagy in *M. tuberculosis* infected macrophages^[22]. Manipulation of autophagy is critical for *M. tuberculosis* survival and maintenance of latency in the host.

Apoptosis

Forkhead box (FOXO) is a family of evolutionary conserved transcriptional factors and FOXO3 is a member of this family that participates in apoptosis^[23]. *M. tuberculosis* enhances the expression of miR-223 to target FOXO3 transcription factor for its survival by inhibiting apoptosis^[24].

Nitric Oxide

NF- κ B (Nuclear factor kappa B) is one of the inflammatory transcription factors which regulate the expression of inflammatory genes. One of its downstream molecules is Nitric oxide (NO) which is detrimental for the pathogen. During *M. tuberculosis* infection, expression of microRNA let-7f is significantly reduced which enhances the production of its target A20 (TNFAIP3). With increased amount of A20, NF- κ B signaling is compromised leading to poor inflammatory response which in-turn promotes *M. tuberculosis* survival^[25].

In another study, when infected with BCG strain, macrophages produced higher levels of miR-146a which significantly reduced the expression of iNOS (inducible Nitric Oxide Synthase) expression. This in turn lowered NO generation thus, enhancing the survivability of *M. tuberculosis*^[26].

Tumor necrosis factor-alpha (TNF- α)

TNF- α is one of the inflammatory cytokines which is essential for *M. tuberculosis* killing. Lipomannan, a glycoconjugate component of the cell envelope of *M. tuberculosis*, has been shown to inhibit TNF biosynthesis through microRNA perturbation. Infection of macrophage with live *M. tuberculosis* led to the over-expression of miR-125b which interacts with 3'UTR of the TNF mRNA transcript. Higher expression of miR-125b compromised TNF biosynthesis^[27]. This study showed that active cell wall components of *M. tuberculosis* can potentially modulate microRNAs in the cell thus, disrupting host's immune functions.

IFN- γ signaling cascade

IFN- γ is a central cytokine of cell mediated immunity which is necessary for killing *M. tuberculosis*. Nano-string analysis was employed to identify 31 differentially expressed microRNA in *M. tuberculosis*-infected macrophages^[28]. Among them miR-132 and miR-26a were shown to be up-regulated during the infection. These microRNAs were shown to negatively regulate p300, a co-activator of IFN- γ signaling cascade. This interruption abrogates the downstream signaling through IFN- γ resulting in compromised host's response.

Other cytokines

M. tuberculosis up regulated miR-99b in macrophages and dendritic

cells after infection and down-regulates the expression of cytokines such as IL-6, IL-12, IL-1 β ^[29]. By quantitatively modulating the expression of pro inflammatory cytokines, *M. tuberculosis* creates an environment that favors its survival.

Metabolism

Most of the studies target intracellular survival of *M. tuberculosis* by studying evasion strategies of the pathogen from host's immune mechanisms. Despite evading the host immune mechanisms, *M. tuberculosis* will still fail to survive for long in the absence of a steady source of nutrients. Autophagy is one of the strategies used to fulfill the nutritional requirement of cell by delivering lipid droplets to lysosomes (lipophagy)^[30]. Interestingly, *M. tuberculosis* induces miR-33 expression to disrupt expression of key players involved in these degradation pathways, consequentially, leaving lipid bodies as a source of nutrient for the pathogen^[20]. These lipid bodies act as a storehouse for dormant bacteria causing latent infection in an individual^[31].

Circulation

There is a lot of data which shows microRNA variation in circulation of TB patients, but a coherent picture is still lacking^[32-34]. MicroRNAs which play a role in inflammation regulation at the site of infection are under-represented in blood. Though, circulating microRNAs might reveal variable immune status of the persons but they reveal little insights as to what is transpiring at the site of infection. We believe that circulatory microRNA data could provide clinically relevant information in extra-pulmonary tuberculosis patients.

CONCLUSION

The understanding of host-pathogen interaction has tremendously improved in the recent past which is beginning to unravel multi layered complexities in the pathogenesis of TB. microRNAs provide a fine tune handle to host and the pathogen to compete for their survival. Their dynamic presence inside the cell and involvement in host processes, like cell recruitment to inflammation, present an attractive area to unravel novel insights in understanding TB pathophysiology. Besides, it presents an unprecedented opportunity to develop new generation of molecules effective against *M. tuberculosis*.

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