The FEBS Journal

FEBS PRESS science publishing by scientists

STATE-OF-THE-ART REVIEW

Epigenetic code during mycobacterial infections: therapeutic implications for tuberculosis

Samreen Fatima[1], Anjna Kumari[1], Meetu Agarwal[2], Isha Pahuja[1], Vinod Yadav[3], Ved Prakash Dwivedi[1] and Ashima Bhaskar[1]

1 Immunobiology Group, International Centre for Genetic Engineering and Biotechnology, New Delhi, India
2 Department of Biosciences, Jamia Hamdard University, New Delhi, India
3 Department of Microbiology, Central University of Haryana, Mahendragarh, India

Keywords
drug-resistant tuberculosis; epigenetics; immunotherapy; *Mycobacterium tuberculosis*; vaccine

Correspondence
A. Bhaskar, Immunobiology Group, International Centre for Genetic Engineering and Biotechnology, New Delhi, India
Tel: +91-11-26741360
E-mail: ashimabhaskar23@gmail.com

Samreen Fatima and Anjna Kumari contributed equally to this work

(Received 11 June 2021, revised 23 July 2021, accepted 26 August 2021)

doi:10.1111/febs.16170

Epigenetics involves changing the gene function without any change in the sequence of the genes. In the case of tuberculosis (TB) infections, the bacilli, *Mycobacterium tuberculosis* (*M.tb*), uses epigenetics as a tool to protect itself from the host immune system. TB is a deadly disease-causing maximum death per year due to a single infectious agent. In the case of TB, there is an urgent need for novel host-directed therapies which can effectively target the survival and long-term persistence of the bacteria without developing drug resistance in the bacterial strains while also reducing the duration and toxicity associated with the mainstream anti-TB drugs. Recent studies have suggested that TB infection has a significant effect on the host epigenome thereby manipulating the host immune response in the favor of the pathogen. *M.tb* alters the activation status of key genes involved in the immune response against TB to promote its survival and subvert the antibacterial strategies of the host. These changes are reversible and can be exploited to design very efficient host-directed therapies to fight against TB. This review has been written with the purpose of discussing the role of epigenetic changes in TB pathogenesis and the therapeutic approaches involving epigenetics, which can be utilized for targeting the pathogen.

Introduction

Tuberculosis caused by the obligate intracellular pathogen, *M.tb*, leads to approximately 1.3 million deaths each year [1]. One-fourth of the global population is latently infected with *M.tb*. However, only 5–10% of infected people develop active TB during their lifetime [1]. The latently infected population poses a major threat globally, as it may turn into a pandemic if it gets activated. Despite almost a century of research, Bacillus–Calmette–Guerin (BCG) still remains the only licensed vaccine against TB [2]. BCG vaccine is efficacious against disseminated forms of TB in children, but is unsuccessful in protecting against adult pulmonary TB. Although many new vaccines are in clinical trial, none has been approved till date [2].

Abbreviations
APC, antigen-presenting cell; BCG, Bacillus–Calmette–Guerin; CIITA, class II transactivator gene; DC, dendritic cells; DOTS, directly observed treatment short-course; HAT, histone acetyltransferase; HDAC, histone deacetylase; IFN-γ, interferon-γ; *M.tb*, *Mycobacterium tuberculosis*; MMP, matrix metalloproteinases; PRMT, protein arginine N-methyltransferases; ROS, reactive oxygen species; SAM, S-adenosyl methionine; TB, tuberculosis; TNF-α, tumor necrosis factor-α; TWEAK, TNF-like weak inducer of apoptosis; UVRAG, UV radiation resistance-associated gene.

Current anti-TB therapy, directly observed treatment short-course (DOTS) consists of multiple antibiotics, is lengthy, and causes severe lung toxicity [3] leading to noncompliance among the patients and therefore to the emergence of drug resistance in *M.tb* strains.

Recently, there has been a major advance in research on host-directed therapies against various diseases including TB. Host-directed therapies work on the host system and work in complementarity to pathogen-targeted therapeutic approaches [4]. During the 70 000 years co-evolution of *M.tb* with humans, *M.tb* has evolved various mechanisms to survive within the host system [5]. Understanding these mechanisms can result in the development of new therapeutic approaches to eradicate this deadly organism.

Recent studies have reported the interaction between *M.tb* infection and changes in host epigenetic machinery; however, the molecular basis of the mechanism is yet to be fully explored [6]. Epigenetic changes, for example, histone modifications, DNA methylation, and miRNA-mediated up/downregulation of immune genes, play an important role in immunomodulation of the host at the post-TB infective phase. Notably, *M.tb* secretes virulence products that regulate the host cellular transcriptional machinery and induces a cascade of reactions that help in further pathogenesis and establishment of infection [6,7].

The area of epigenetics is new and needs intense high-throughput research in order to identify potential therapeutic targets which can be used as host-directed therapy against TB. Host-directed therapies can successfully ameliorate host responses to pathogens, attack virulence factors of the pathogens, and potentially initiate both innate and adaptive immune responses and develop long-living immunological memory response without the risk of development of drug resistance [8]. Therefore, in this paper we review all the host epigenetic changes reported in case of TB infection and their role which can be exploited for therapeutic advantage.

Pathogenesis of TB and role of immunity

Tuberculosis infection cycle starts with the inhalation of *M.tb* containing aerosol droplets in the alveoli of the lungs. Lung alveolar macrophages are the primary cells to encounter *M.tb* bacteria and hence provide the first line of defense against the invading pathogen. Alveolar macrophages are the preferred habitat of the *M.tb* bacteria in the human system [9]. After infection, *M.tb*-infected alveolar macrophages invade the underlying lung epithelium and secrete myriad cytokines and chemokines to allow other immune cells to invade the lung through nearby blood vessels such as dendritic cells (DCs), monocytes, and neutrophils [10]. Together, these cells form a compact structure in the lungs known as the granuloma. Formation of the granuloma containing fused macrophages, DCs, and various other cell types is an attempt of the host immune system to restrict the disease, followed by its eventual elimination. Granuloma formation is the hallmark of pulmonary TB infection [9]. In case of *M.tb* infection, the initial immune response in the lung plays a crucial role in the outcome of the infection. During maximum number of cases, the initial innate immune defense is sufficient to clear the infection. These individuals do not rely on the adaptive immune system to get rid of the disease. However, in cases of active TB disease, the natural resistance of the individual fails and this leads to active disease and therefore those affected need the anti-TB therapy and other immunogenic/immunomodulatory agents which help in clearing the bacteria [9]. The infected lung macrophages move through the lymphatic system to other organs such as lymph nodes, kidneys, and other extra-pulmonary tissues [10]. The alveolar macrophages often destroy the bacteria after ingesting it through autophagy, nitric oxide species, and reactive oxygen species (ROS) mediated killing mechanisms [11]. *M.tb*-primed macrophages and DCs secrete copious amounts of proinflammatory cytokines such as interferon-γ (*IFN*-γ) and tumor necrosis factor-α (*TNF*-α). Interleukin-12 and interleukin-18 are cytokines produced by macrophages that favor Th1 type of protective immune response. These cytokines help in granuloma formation as well as maintenance and also in bacterial elimination [10].

M.tb-primed macrophages and dendritic cells present *M.tb* antigen to the T cells and encourage them to differentiate into specific lineages based on their cytokine milieu [12]. Following establishment of *M.tb* infection in the lungs, the migratory cells (monocytes) carry the phagocytosed bacteria to the draining lymph nodes through the action of cytokines (interleukins: IL) and chemokines involved in lymphocyte recruitment such as IL-12p40, CCR2, and CCR7 [13]. The mycobacterial antigens subsequently stimulate naïve T cells through antigen-presenting cells (APCs) via MHC class I and class II molecules. APCs secrete additional cytokines and chemokines to drive T-cell proliferation and also to determine their polarization depending on the antigen. Hereafter, the activated T cells move from their activation site in the lymph nodes to the site of infection through the vasculature. T cells which are CXCR3+ move to the lung parenchyma whereas CX3CR+ cells remain in the vasculature [14]. The

activated T cells, especially Th1 cells and Th17 cells that produce *IFN-γ*, *TNF-α*, and IL-17, are crucial for eliminating intracellular bacterial infection by recruiting and activating macrophages, natural killer (NK) cells, and granulocytes at the site of infection and initiating mycobactericidal activity [15]. *M.tb*-primed immune cells secrete cytokines such as *IL-1β, IL-6, IL-12, IL-18,* and *IL-23* that play important roles in the containment of TB infection and effective immune clearance [12,15]. Notably, cytotoxic $CD8^+$ T cells (CTLs) are also involved in the direct killing of *M.tb*-infected cells through cytotoxic granules like granzyme B and perforin [16]. However, T regulatory cells have also been shown to proliferate during *M.tb* infection and effectively suppress the antimycobacterial immunity through anti-inflammatory cytokine *IL-10* and *TGF-β* to maintain immune homeostasis and prevent tissue damage [16]. So, the availability of both pro- and anti-inflammatory cytokines influences the effective clearance of *M.tb* and is crucial in determining the disease outcome. Moreover, *M.tb* is known to secrete several virulence molecules that actively target both innate and adaptive immune cells. Therefore, the interplay between the host immune responses and bacterial virulence determines the pathogenesis and severity of TB infection. Host-directed therapies have evolved which target the immune system to effectively eliminate the pathogen and provide long-term protection against reinfection by induction of memory T cells [8].

The emerging role of epigenetics during infections

The concept of epigenetics was introduced in 1940s by CH Waddington [17]. He described epigenetics as the process which may influence genetic outcome without changing the gene sequences of the cell [17]. Lately, epigenetic changes have been identified as an important regulatory process which has critical contribution in various processes such as X chromosome inactivation, stem cell differentiation, chromosome condensation, and transcriptional activation or repression of the genes [18].

Recently, it has been reported that pathogenic organisms target epigenetic process to alter host protective immune response to favor its survival in the host system [18]. These processes include DNA methylation, histone modifications, and RNA-modifying regulatory processes which effectively modulates host immune response [17,18]. Since these modifications are reversible, they may be effectively targeted to develop a therapy against the pathogen.

Epigenetic modifications during TB infection

Epigenetic modifications are heritable changes in the DNA, which regulate the transcription of genes without affecting the nucleotide sequence of the DNA [19]. These changes are stable and heritable but reversible [19]. Epigenetic modifications which have been studied till date in the context of TB include DNA methylation, histone modification, and microRNA (miRNA) regulation (Fig. 1). We will discuss about each of these modifications in detail (Table 1).

DNA methylation

Alteration in the transcriptome is eventually related with the epigenome and therefore DNA methylation is majorly involved in changing the cellular response to external stimuli [20]. DNA methylation has a crucial role in various physiological and developmental processes. It is important in regulating epigenetics mechanism not only in eukaryotes but also in the bacterial system [21]. This is a highly specified chemical process that involves addition of methyl (CH_3) group derived from S-adenosyl methionine (SAM) to the cytosine (C) residue of CpG sequence present in the genome [22]. SAM is an intermediate metabolite of methionine and acts as a co-substrate in the transfer of methyl groups. CpG islands, 500-1500 base pairs (bp) long stretches of DNA found at promoters near the 5′ end of the transcript, are considered to be the methylation hot spots [22,23]. Various enzymes responsible for initiating and maintaining DNA methylation are DNMT, DNMT2, DNMT3a, and DNMT3b. Among all the DNMTs, DNMT1 is the most abundant and methylates the hemi-methylated CPG dinucleotides. DNMT3 shows preference for both hemi- and nonmethylated CPG [24,25]. The epigenetic control by DNA methylation is sometimes irreversible and may lead to long-term gene silencing [26]. Identification of 6-methyladenine motifs coupled methyltransferases (*MamA, MamB,* and *HsdM*) is correlated with epigenetic modifications conferred by *M.tb* [27].

During any bacterial attack on the host cells, multiple lineages of genes in different cells undergo acetylation and methylation at their promoter region [27]. The $CD8^+$ and $CD4^+$ T cells also provide the hub for epigenetic regulations.

The *in vitro* studies in *M.tb*-infected THP-1 macrophages [28] have uncovered the presence of 23 000 differentially methylated regions. Moreover, the global DNA methylation status also revealed the modifications occurring in the cytosine of non-CPG

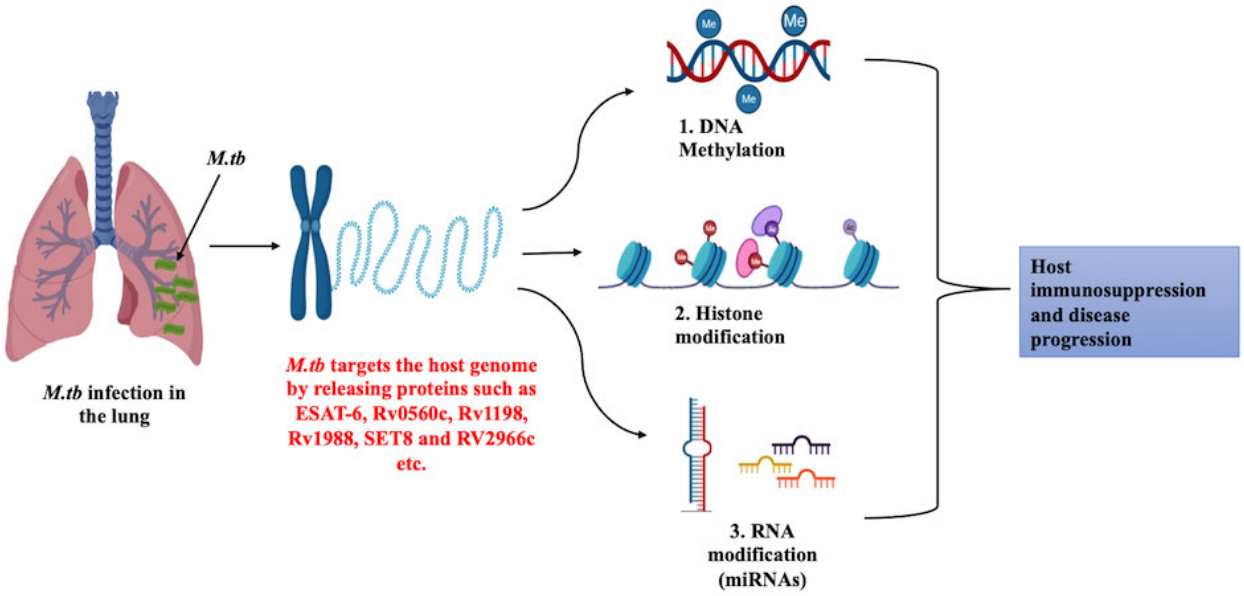

Fig. 1. Epigenetic modifications induced by *M.tb* in the host cell. Upon uptake by the alveolar macrophages, *M.tb* targets the host genome directly or indirectly to induce epigenetic modifications like DNA methylations, histone modifications, or miRNA expression for better survival and persistence.

dinucleotides [29]. MamA, one of the mycobacterial DNA methyl transferase necessary for the replication of *M.tb in vitro*, has the capability to introduce N^6-methyladenine in a recognition sequence which has 2000 copies in the *M.tb* genome [30]. Their presence marks the survival of *M.tb* even under hypoxic conditions [31,32]. These methylation changes play crucial role both in regulating *M.tb* survival function and in the host cells where it infects. In a study by Looney *et al.* [33], it has been stated that upon *M.tb* infection, host miRNAs and methylation changes together govern the critical macrophage responses such as immune cell activation, metabolism, and signaling pathways (AMPK signaling). The mRNAs dysregulated are the targets of the altered miRNAs. *M.tb* infection is studied to alter the miRNA expression pattern as well as DNA methylation patterns in infected macrophages [33] and these changes aid in changing the transcriptional response of the host to eventually alter the host *M.tb* clearance capacity and may lead to immune suppression, to effect the outcome of the TB disease.

It is well-known fact that host susceptibility to infection by *M.tb* is increased by VDR gene polymorphisms. Therefore, hypermethylation in the region of CpG islands in the VDR gene increases the pathogenicity of TB [34]. According to a recent study, region-specific strain of *M.tb* has the ability to cause drug resistance and DNA methylation thus depicting lineage specificity. Due to the effect of *M.tb* on the host chromatin, there are many pathways that are critically examined in response to the infection within host. NFκB is crucial during proinflammatory response and DNA methylation of NFκB is evident during tuberculosis along with IFN-γ, TNF, IL-6, and IL-1β [35]. Hypermethylation of nuclear factor-kappa B (NF-κB) implicates the downregulation of various cytokines and chemokines [35]. Methylation in signaling components of pathways like NF-κb, MAPK, and AKT signaling affects the outcome of immune responses and leads to the secretion of cytokines or initiates processes which assist in bacterial clearance. We have summarized the role of histone methylation in regulation of key pathways involved in *M.tb* pathogenesis [18,36]. Specific methylation-inducing agents add methyl group to explicit moieties in the DNA and change the expression of genes. These methylation regulators also change the expression of nonhistone proteins such as components of cellular signaling pathways. Methylation occurs on two abundant histone residues lysine (K) and arginine (R), although other amino acids such as histidine, aspartic acid, and glutamic acid also get methylated [37]. Methylation of proteins function as docking sites for specific binding proteins called histone readers [38]. Histone methylation modifiers are proteins which modify a lysine or arginine residue in the protein. The protein arginine N-methyltransferases (PRMTs) facilitate the adding of the methyl groups to the arginine residues in a cellular

Table 1. An overview of the epigenetic changes induced by *M.tb*.

Epigenetic change	Target	Effect of modification	Nature of experiments	References
DNA methylation	Hypermethylation in CpG islands of VDR gene of *M.tb*	Increase in pathogenicity	Human study	32
	Hypermethylation of Nuclear factor-kappa B (NF-κB) of host	Decreases the cytokine production in host	Human study	33
	Hypomethylation of CD82 of the host	Decrease in phagosomal lysosomal fusion	*In vitro* and human studies	34–36
	M.tb genome is targeted by Rv0560c, a methyltransferase of *M.tb*	Increase in drug resistance	*In vitro* study	40
	Methylation of Kca3.1, potassium calcium-activated channel upon BCG immunization	Effect on cytokine production in the host	Human studies	42,43
Histone modification	ESAT-6 induces methylation of histone at H3K4 site and acetylation at CIITA of host	Regulating the expression of IFN-γ	*In vitro* study	49
	Dimethylation at the arginine residue on the histone H3 (H3R42me2 modification) of host cells	Downregulates production of ROS and type I IFN	*In vivo* studies	52–55
	Rv2966c and Rv1988 modify the key histones of the host and hijack the host immune system	Downregulates phagolysosomal fusion and secretion of proinflammatory cytokines	*In vitro* and *in vivo* studies	51,52
	In TB patients H3K14Ac is decreased and H3K27me2 increased	Increase in active TB cases	Human study	56
	Increase in H3K27me3 in host	Leads to high pathogenicity and bacterial burden in patients	Human study	56
	H3K14 hypoacetylation in host	Leads to development of active pulmonary TB disease	Human study	56
	Deacetylation of CIITA (class II transactivator) at its promoter site	Reduced antigen presentation and *M.tb* elimination	*In vitro* study	58
	Acetylation of free histones by Rv2416c in the macrophages and T cells	Inhibits the activation of the T cells	*In silico* and *in vitro* study	59
	Acetylation of matrix metalloproteinases (MMP-1 and MMP-3)	Enhanced bacterial survival by degradation of the extracellular matrix of the lung	*In vitro* and *in vivo* studies	60–63
	Rv3423.1, a novel histone acetyltransferase acetylates histone H3 at the K9/K14 (H3K9/K14) positions	Responsible for the increase in virulence of the bacterial strain	*In silico* and *in vitro* study	64
	Class I HDAC inhibitors reduce the expression of IL-6 and TNF and increase the level of proinflammatory cytokines	Promote reduced bacterial survival and less level of host evasion in the host	*In vivo* study	66
	M.tb Eis protein acetylates Lys55 of DUSP16/MKP-7	Leads to inhibition of JNK-dependent autophagy pathway, phagosome maturation, and ROS synthesis, enabling better bacterial survival	*In silico* and *in vitro* studies	57–59,70
	miR-155 targets SHIP1, an inositol phosphatase that promotes apoptosis in T cells and macrophages during M.tb infection	It increases the level of IFN-γ, IL-4, TLR-2, and TLR-4 and provides better protection	*Ex vivo*, *in vitro*, and *in vivo* studies	85–89
RNA modification	miR-21 inhibits IL-12 expression by targeting 3′UTR of genes such as IL-10, IL-12, TLR-4	It promotes bacterial survival and host evasion in the host	*In silico*, *in vitro*, and *in vivo* studies	90–92
	miR-125a targets the UV radiation resistance-associated gene (UVRAG) in the macrophages	Inhibits the autophagy process and promotes survival of the bacteria	*In vitro* study	94

Table 1. (Continued).

Epigenetic change	Target	Effect of modification	Nature of experiments	References
	miR-27b inhibits the TLR-2/MyD88 signaling pathway	Reduces the production of proinflammatory cytokines and prevents host inflammation	*In vitro*, *in vivo*, and human studies	95–97
	miR-146a targets IRAK1 and TRAF6 genes of the host	Leads to reduction in the level of nitric oxide production and supports bacterial survival in the host	*In vitro*, *ex vivo*, and Human studies	99-100
	miR-223 inhibits the genes of CCl3, CXCL2, and IL-6 cytokines	Promotes myloid cell entry in the lungs and protection against the bacteria	*Ex vivo* and *in vivo* study	101
	miR-99b targets TNF-α and TNFRSF-4 receptor genes	Promotes bacterial survival in the host macrophages	Human study	103
	miR-33 targets autophagy genes and increases host lipid generation	It leads to better intracellular survival and pathogen stability in the host	*Ex vivo* and *in vivo* study	104
	miR-144 targets autophagy by directly binding to the 3′UTR region of DRAM2 mRNA	Upregulation of miR-144 in macrophages and T cells upon M.tb infection inhibits phagosome maturation and depreciates T-cell function	*In silico*, *in vitro*, and human study	108

protein [39]. p65 methylation leads to the binding of p65 to the promoters of genes regulated by NF-KB such as IKBA, IP-10, and TNF. Other pathways are also initiated and regulated by these histone modifiers such as the MAPK pathway in which the lysine demethylase KDM2A activates ERK signaling by downregulating the expression level of DUSP3, an ERK phosphatase [39]. Also, PRMT1 enhances AKT signaling by methylating Er-alpha [40]. These pathways play a protective role in *M.tb* infection and are targeted by the pathogen to evade the host immune system by modulating the host defense mechanisms (Fig. 2).

Another example of DNA methylation playing essentially important role in TB pathogenesis is the tumor suppressor protein, CD82. Diversified role of CD82 has gathered a lot of attention during tuberculosis infection [41]. Phagosome formation and fusion with lysosomes are associated with MHC-II and CD82 upregulation, suggesting a vital role of CD82 in antigen presentation during *M.tb* infection [42]. According to a study, CD82 is reprogrammed epigenetically during *M.tb* infection by RUNX1–Rab5/22 transcription factor. The hypomethylation of CD82 is necessary for its interaction with this transcription factor and so the elevated level of both (CD82 and RUNX1-Rab5/22) is preferentially desired and studied in granuloma formation [43].

Although isoniazid and rifampicin are the first-line treatment regimen for TB but extensive resistance to these drugs is a major concern [44]. Drug inactivation and modification can be due to many underlying reasons, with DNA methylation being one of the major cause [45]. For instance, negative association of methylated genes involved in nitrogen metabolism pathway has been observed in rifampicin and isoniazid resistance [46]. Furthermore, Rv0560c, a methyltransferase of *M.tb* nullifies the activity of pyrido-benizimidazole and TPSA [47].

Bacillus–Calmette–Guerin is the only available vaccine against *M.tb* but its unpredictable efficiency is a global concern. Epigenetic reprogramming of various immune cells can be seen in BCG-vaccinated models [48]. In South African infants, methylation of Kca3.1, potassium calcium-activated channel was observed during a study in groups with high and low IFN-γ and IL-2, respectively [49]. Kca 3.1 is a potassium channel that hyperpolarizes the membrane due to increase in intracellular calcium levels [49]. Phagocytosis is an important event during *M.tb* infection that is strongly correlated with differential methylated genes. Many different methylated genes have been identified that are involved in actin modification which in turn affects the phagocytosis of mycobacteria [50]. These studies on the role of methylation changes although few are very significant in hinting at the relatively unstudied role these changes may play in *M.tb* internalization, disease establishment, and clearance mechanisms in the host. Therefore, understanding the host and bacterial methylation upon infection to restore the host balance may have far reached implications for altering outcome of *M.tb* infection in the host and may be

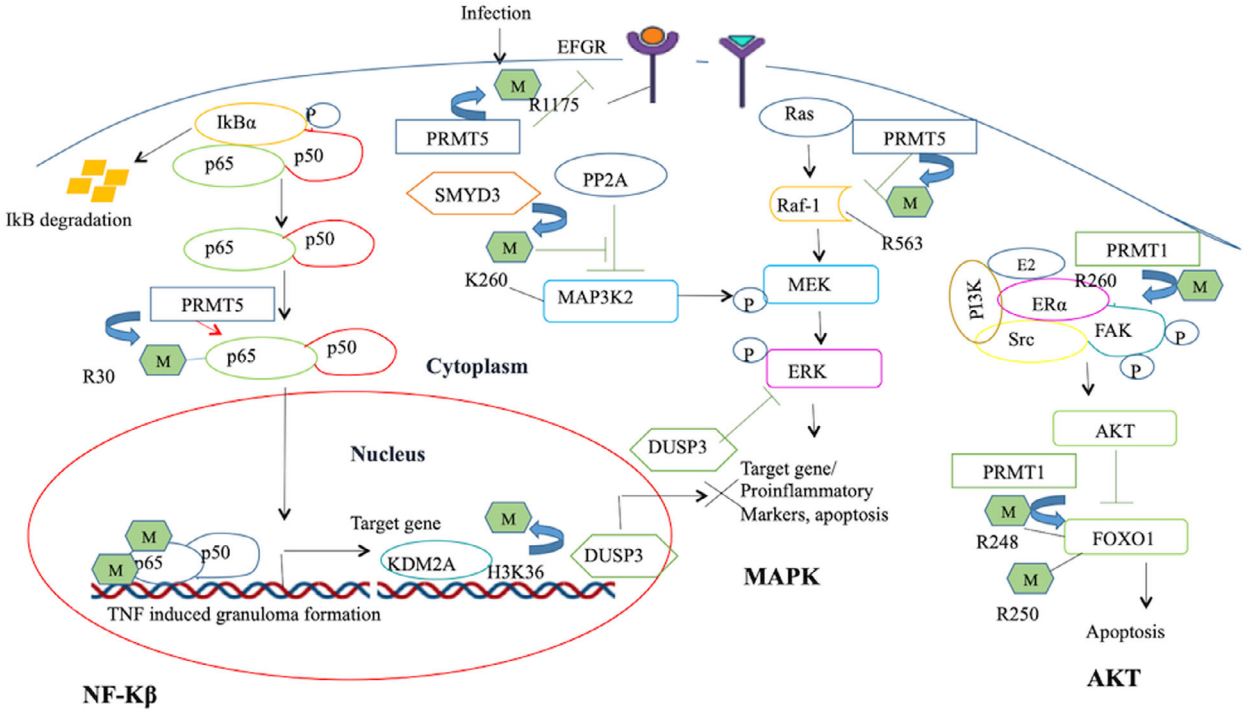

Fig. 2. Role of methylation in effecting different signaling pathways involved in infection and in inflammation. Methylation-inducing agents change the activation of genes involved in signaling pathways involved in infection which govern the host immune response.

used as a target for developing host-directed therapies targeting key regulatory pathways.

Histone modifications

Histone modifications are reversible epigenetic modifications which are responsible for maintaining the structure of the chromatin which decides the accessibility of the transcription factors to the DNA [51]. Histone proteins also known as chromatin remodeling proteins are basic proteins which form an octamer which binds the DNA to form a nucleosome [51]. During the formation of nucleosomes, the four core histone proteins are responsible for forming the octamer which binds through 1.7 turns of DNA (approximately 147 bp). These histone proteins are H2A, H2B, H3, and H4. The nucleosome consisting of DNA and histone proteins is the elementary unit of chromatin organization which has a repeat length of approximately 200 bp. The core histone octamer is secured by the linker histone H1 which induces condensation of the DNA helix and prevents its transcriptional activity [52]. The chromatin is an extremely dynamic structure which keeps changing from highly condensed heterochromatin to an actively transcribed euchromatin structure [52]. The chromatin remodeling depends upon the function of various histone and chromatin remodeling enzymes and proteins such as ISWI (Imitation SWItch), SWI/SNF (switching defective/ sucrose nonfermenting), Ino 80 (inosine requiring 80), and Mi-2 [53]. These enzymes introduce various post-translational modifications on the chromatin such as ubiquitylation, sumoylation, phosphorylation, ADP-ribosylation, methylation, and acetylation on the N termini of the histone core [6]. Due to these covalent modifications, the positive charge of the histone proteins which interacts with negatively charged DNA is neutralized and it effects the association and therefore the association of histone with DNA. Their binding loosens up and destabilizes to expose the DNA promoters for transcription factors to bind on them and promote gene expression [6].

Histone methylation is associated with both gene activation and repression depending on the amino acid which is methylated whereas deacetylation usually leads to condensation of the chromatin and suppression of the gene expression [54]. Recently, within the past decade several new studies have reported the role of chromatin remodeling in the host during the mycobacterial TB infection. Many studies have

reported histone modifications and chromatin changes in the host by the bacteria and their metabolic products in order to establish their long-term survival by modulating the host immune machinery [18,54,55]. Few studies have reported chromatin remodeling in the host during TB infections [56,57]. The *M.tb* secretory protein, ESAT-6, has been reported to induce methylation of histone at H3K4 site and acetylation at CIITA (class II trans activator gene) promoter, thereby regulating the expression of primary Th1 cytokine, *IFN-γ* [56]. CIITA is responsible for expression of various genes involved in the process of antigen presentation and bacterial killing [56]. This and many other studies before and post this study have given major insight on the role of histone modifications in establishment of infection as well as in host evasion mechanism. In the next paragraphs, we would be discussing the effect of both histone methylation and acetylation/deacetylation on *M.tb* infection establishment, survival, and clearance as reported by different studies.

Histone methylation

Histone methylation occurs majorly on lysine and/or arginine residue of the histone and may, respectively, activate or suppress the expression of genes which are involved in the immune response against TB [57]. Methylation can be mono, di, or trimethylation depending upon the number of methyl groups added on the lysine residues. Each of the number of methyl groups changes the chromatin differently. For instance, trimethylation at H3K9 (K: lysine residue) leads to suppression of gene expression by stabilizing the histone–DNA complex and preventing the transcription factor binding [57]. Several *M.tb*-specific proteins have been identified and studied for their role in methylation of histones during TB infection. Few of these proteins with role clearly defined are, Rv1198, Rv1988, SET8, and RV2966c, to name a few [58]. Rv1988 is a methyltransferase which dimethylates the arginine residue at the 42 position on the histone H3 (described as H3R42me2 modification) [59]. This secretory protein is present only in pathogenic *M.tb* and hijacks the host immune system to support bacterial survival by altering the expression of genes important for the ROS production, viz., NOX1, NOX4, NOS2, and TRAF3, which is responsible for type I IFN production [60–62]. Sharma *et al.* [58] reported that Rv2966c, another secretory protein of *M.tb* and a methyltransferase, moves to the host cell nucleus from the cytoplasm, and binds to specific DNA sequences to methylate at non-CpG islands. Rv2966c methylates cytosines preferentially in non-CpG fashion and its methylation function is supported by its phosphorylation status. Rv2966c together with Rv1988 modifies host histone proteins and plays a key role in hijacking the host immune system and altering its action [59]. Moreover, Rv2966c interacts with histone H3 and H4 and has similarity in action to the mammalian DNA methyltransferases, DNMT3L in promoting bacterial survival [58].

A modification within the host histone by *M.tb* can lead to multiple outcomes. For instance, methylation at histone 3 lysine 9 residue trimethylation (H3K9me3) is associated with repression and histone 3 lysine 4 residue trimethylation (H3K4me3) with activation of DNA complex [63,64]. These modifications which include global methylation within the genome, control cell signaling, alter the host histones, and even acts upon the BCG vaccine response [65].

Global H3K14Ac is decreased and H3K27me2 increased in TB patients as compared to that in healthy humans [66]. This study suggested that H3K14 hypoacetylation and H3K27 hypermethylation play a role in the development of active pulmonary TB disease by upregulation of histone deacetylase 1 (HDAC1) and downregulation of KDM6B gene [66]. These studies ardently demonstrate that bacterial methylases modulate the host environment and aid in the long-term bacterial survival in the host. However, this area has very little information available and needs more research input.

Histone acetylation

Alteration of the histone proteins by acetylation on lysine residues of core histones has also been very well documented in case of TB. This modification leads to the addition of acetyl group to the chromatin binding the DNA which leads to the activation of the genes [52]. Histone acetyltransferases (HATs) are enzymes which add acetyl groups onto lysine residues of host proteins such as histones and transcription factors. *M.tb* proteins and enzymes act as HATs and induce the acetylation of the lysine residue on both histone and nonhistone proteins for their better survival during infection [67–69]. An example of acetylation on nonhistone proteins is NF-κB p65 acetylation. p65 is responsible for activation of NF-κB [67]. TLR signaling is modulated at NF-κB transcription factor level by DC-SIGN, a C-type lectin. *M.tb* infection activates DC-SIGN on DCs and activates Raf-1 (a serine and threonine kinase), leading to acetylation of p65. Acetylation of p65 augmented IL-10 transcription and increased its expression level to promote anti-

inflammatory immune response [67]. Mycobacterial lipoprotein, LpqH, facilitates the internalization of *M.tb* by interaction with mannose receptors. LpqH is a 19-KDa mycobacterial lipoprotein. LpqH triggers TLR-2 activation in host macrophages, leading to increase in expression of death receptors and ligands, leading to activation of death receptor signaling cascade by upregulation of both caspase 8 and caspase 3 [67]. This leads to apoptosis in macrophages via both extrinsic and intrinsic pathways. This reduces antigen presentation by macrophages to T cells via deacetylating the CIITA gene (class II transactivator) at its promoter site [68]. Furthermore, mycobacterial Eis (enhanced intracellular survival) protein (Rv2416c) acts as an acetyltransferase and is reported to add acetyl groups to free histone proteins [69]. Eis activates DUSP16/MKP-7 (dual-specificity protein phosphatase 16 and mitogen-activated protein kinase phosphatase-7 via acetylation of Lys55, inactivating JNK protein through phosphorylation) [69]. Acetylation of free histones by Eis inhibits the induction of the ERK1/2-JAK pathway and thus leads to compromised function of the T cells [69]. Acetylation of matrix metalloproteinases (MMP-1 and MMP-3) regulates intracellular survival of the bacteria in the host by degrading the extracellular matrix of the lung. Acetylation not only regulates host factors but also targets bacterial histone-like proteins [70–73]. Nucleoid-associated protein HU is conserved across eubacterial species and is essential for nucleoid organization and regulation of gene expression in bacteria [60]. MtHU is mycobacterial histone-like protein. Eis acts as an acetyl transferases which modifies MtHU at lysine residues. Acetylation of MtHU alters its function and may help in the bacterial entry and replication in the host cell [68]. Another study demonstrated that Rv1151c deacetylates the acetylated HU (MtHUAc) which alters the DNA conformation and function [60–63]. However, our review mainly focuses on the role of epigenetic changes in the host.

Many histone acetyltransferases of the bacteria help in the survival of the *M.tb* bacteria in the host. Rv3423.1 is one such acetyltransferase which acetylates histone H3 at the K9/K14 (H3K9/K14) positions while residing in the nucleus of infected macrophages [74]. The researchers reported that Rv3423.1 protein was detected in the culture filtrate of only virulent and not avirulent *M. tuberculosis* which indicates at its potentially crucial role in the virulence of the bacterium as well as its survival.

Moreover, certain class I HDAC inhibitors also effect the host immune response by modulating the level of proinflammatory cytokines after *M.tb* infection. It has been reported that upon treating peripheral blood monocytes with class I HDAC inhibitors, depsipeptide and sodium butyrate, there was reduction in expression of both IL-6 and TNF-α accompanied by elevation in IL1β levels [75] Another HDAC inhibitor, Tubastatin A, which is specifically a HDAC 6 inhibitor, prevents the growth of avirulent *M.tb* (H37Ra) in mice. Treatment with tubastatin A leads to augmentation in the levels of the host supportive proinflammatory cytokines such as TNF-α, IL-12p40, and IFN-γ and a significant reduction in levels of the anti-inflammatory cytokine, IL-10 [76]. The HDAC 6 inhibitor while initiating successful adaptive immune response also controls the pathogen by exaggerated innate immune responses. It has been reported that tubastatin A treatment led to huge recruitment of innate cells such as macrophages, dendritic cells, and neutrophils into the lungs. The result of the finding was also significant in case of the virulent H37Rv strain infection as shown by increase in the proinflammatory immune response and reduced growth of the bacterium [76]. In another important study on the role of HDACs, it was reported that HDAC3 inhibitor, RGFP966, is effective in reducing bacterial survival in case of both virulent and avirulent strains of *M.tb* while not being significantly useful in control of other intracellular and extracellular bacteria. This inhibitor has proven to increase proinflammatory cytokine secretion in response to *M.tb* infection [77].

In yet another study in zebrafish, it was observed that HDACs play a critical role in the differentiation of the macrophages [78]. It was shown that inhibition of HDACs enhanced the protective capacity of both proinflammatory macrophages (M1) and anti-inflammatory macrophages (M2). Moreover, inhibition of HDAC3 activity polarizes the macrophages into more bactericidal population. Also, there was a significant increase in levels of inflammatory cytokines and increase in the phagocytic ability of the macrophages. *In vivo* results demonstrated that inhibition of HDACs led to significant reduction in bacterial burden [78].

A report from Kleinnijenhuis *et al.* had shown that BCG vaccination causes the reprogramming of monocytes to exhibit enhanced innate immune response. It was demonstrated through both *in vitro* and *in vivo* studies that NOD2 mediated epigenetic modification at the level of histone methylation at H3K4me3 [79]. ManLAM, a cell wall component of *M.tb* binds to DC-SIGN, a C-type lectin expressed by DCs that modulates TLR-dependent responses in human DCs. Binding of ManLAM to DC-SIGN activate the serine and threonine kinase Raf-1 that led to the acetylation of p65, one of the key activating subunits of NF-κB.

Acetylation of p65 prolonged transcriptional activity of NF-κB and enhanced the transcription rate of immunosuppressive cytokine *IL10*. It was demonstrated that Raf-1-acetylation-dependent pathway is central to modulation of TLR-specific immune responses elicited by DCs in response to mycobacteria, fungi, and viruses [67]. *M.tb* Eis protein plays a critical role in mycobacterial survival in the host macrophages by suppressing host immune system [80]. It is an efficient N-acetyltransferase that acetylate Lys55 of DUSP16/MKP-7 (dual-specificity protein phosphatase 16 (DUSP16)/mitogen-activated protein kinase phosphatase-7) and initiates inhibition of JNK-dependent autophagy, phagosome maturation, and ROS [69]. Addition to this, recent microarray study of human CpG island showed DNA hypermethylation in IL-17 gene family members, promoter region of their receptors, and macrophage receptors in active and latent patients of TB [7]. The inflammatory immune response against *M.tb* is associated with tissue degradation and cavitation during pulmonary TB. MMPs are host enzymes required for cavitation. MMP-1 and MMP-3 increased expression drive immunopathology after *M.tb* infection is regulated by HDAC and HAT activity has been shown using inhibitors [81]. The chromatin remodeling and post-translational modification patterns regulating gene expression are pronounced target sites for pathogens for manipulation during the course of infection. The host immune system is a prominent target for manipulation by these pathogens required for the establishment of the disease, its transmission, and survival in the host system [82]. Mainly, the effector mechanism of adaptive immune system is epigenetically altered under pathogenic pressure leading to loss of capability to eliminate pathogens, for example, $CD8^+$ T-cell differentiation, T-cell polarization, FOXP3 expression to name a few [83,84]. In the case of *M.tb* infections, histone alterations in both innate and adaptive immune cells of the host system have been shown.

These studies establish that histone modification is a very important process through which the mycobacteria establishes itself in the host environment and targeting the enzymes responsible for histone modification is very promising area of future therapies.

RNA modifications

The host immune response against *M.tb* can eliminate mycobacteria through various mechanisms, such as induction of phagocytosis, apoptosis, and immune-inflammatory responses. However, *M.tb* has the ability to modulate the host immune response to establish its intracellular survival and persistence inside the host [85]. In addition to histone modifications, mechanisms of immune evasion also include dysregulation of the host miRNAs that are involved in apoptosis, autophagy, inflammation, and other immune processes [86]. miRNAs are small, noncoding RNAs that regulate gene expression at the post-transcriptional level [87]. miRNAs bind to their complementary sequences in the 3'untranslated region (3'UTR) of their respective protein-coding mRNA targets to either degrade the transcript or inhibit translation [88]. It is estimated that half of all protein-coding transcripts are regulated by miRNAs [89,90]. Therefore, disease-associated miRNAs have potential as diagnostic markers or therapeutic targets [91,92]. Different miRNAs are distinctively expressed in the diverse stages of TB infection which might disclose the outcome of the infection. miRNAs can be exploited as host-directed therapy against TB due to their active involvement in regulation of immune response against *M.tb* [93]. The following section focuses on the miRNAs involved in TB pathogenesis and on the mechanisms through which miRNAs induced during TB, modulate cellular antimicrobial responses.

Many studies reported altered expression profiles of circulating and cellular miRNAs in patients with active TB vs. those with LTBI or healthy controls [94]. Differentially expressed miRNAs were further investigated to identify their role within the innate immune response during *M.tb* infection. Recent studies confirmed the involvement of miRNAs in modulating gene expression in the major target cells of *M.tb*, like macrophages, DCs, natural killer (NK), and T cells [87]. *M.tb* can induce or inhibit miRNA expression in order to escape the immune response (Fig. 3).

In the following section, we have discussed all the host miRNAs which have been found to be significant during TB infection.

miR-155

miR-155 is one of the most studied miRNA during *M.tb* infection. miR-155 is found upregulated in *M.tb*-infected macrophages, DCs and T cells [95]. It inhibits innate immunity during the early phases of infection but plays a protective role in chronic *M.tb* infection. miR-$155^{-/-}$ mice were able to control *M.tb* infection during the early stage of infection, when macrophage function is critical, but during the chronic phase of infection, miR-$155^{-/-}$ mice showed a higher bacterial load and increased cellular apoptosis in the lungs.

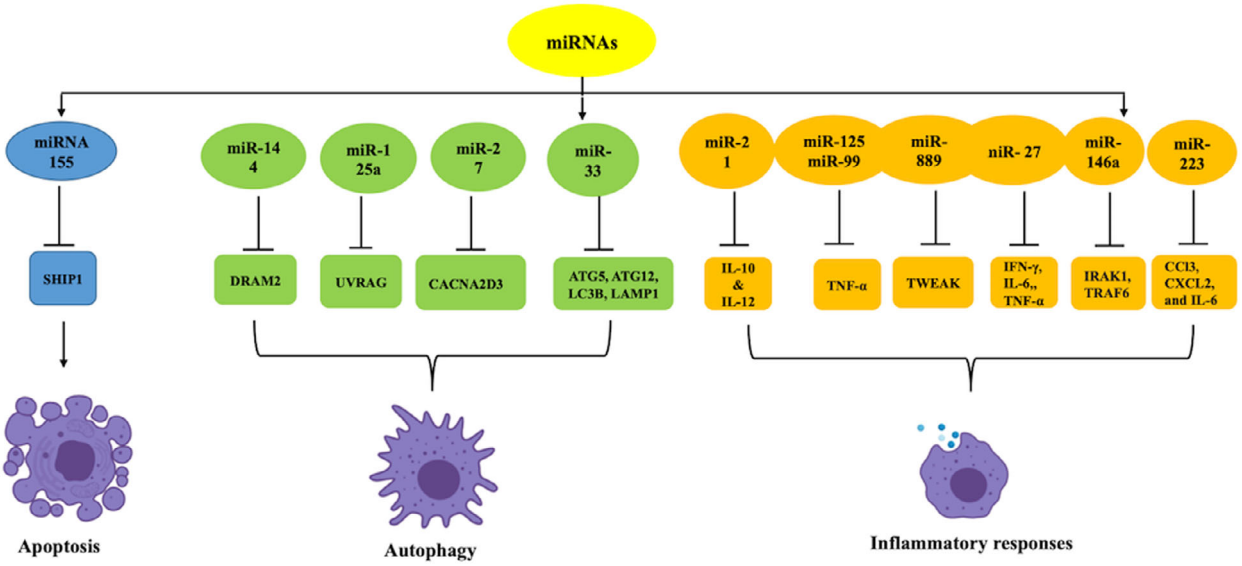

Fig. 3. Schematic representation of miRNA-mediated regulation of host immune responses during *M.tb* infection. Inside the host, *M.tb* is known to hijack various antimycobacterial pathways by modulating the expression of different miRNAs.

Additionally, miR-155$^{-/-}$ mice have less number of antigen-specific CD4^{+} and CD8^{+} T cells during chronic infection phase and have decreased production of protective cytokines, such as IFN-γ and TNF-α by T cells [95,96]. miR-155 targets SHIP1 (SH2 domain-containing inositol 5'-phosphatase 1), an inositol phosphatase that promotes cell apoptosis in T cells and macrophages during *M.tb* infection [97]. *M. bovis* also induces miR-155 expression in BCG-infected macrophages via TLR-2, NF-kB, and JNK signaling pathways [98]. In a nutshell, miR-155 exerts a protective role against TB infection by inhibiting IFN-γ, IL-4, TLR-2, and TLR-4 and regulating T-cell-mediated immune responses while promoting the survival of innate immune cells [99].

miR-21

miR-21 is an anti-inflammatory miRNA and highly expressed in myeloid cells [100]. In *Mycobacterium leprae* lesions, expression of miR-21 is associated with dormancy through negative regulation of multiple proinflammatory processes [100]. During *M.tb* infection, increased expression of miR-21 results in poor activation of both the macrophages and Th1-dependent immunity [101]. Inhibition of miR-21 induces antimycobacterial responses through IL-12 production [102]. miR-21 is upregulated in lung macrophages of BCG-vaccinated mice and in macrophages and DCs infected *ex vivo* with *M. bovis*, BCG [90]. In this infection model, miR-21-5p promotes DC apoptosis by targeting Bcl-2 and inhibiting host Th1 response by targeting IL-12 production [101]. A recent study reported that *M.tb* alters macrophage immune-metabolic programming via miR-21 to ensure survival and replication inside the host [103]. miR-21 targets phosphofructokinase muscle isoform and inhibits glycolysis in macrophages during *M.tb* infection [103]. In miR-21-deficient macrophages, intracellular growth of bacteria is reduced via increased production of proinflammatory mediators [103]. Collectively, miR-21 helps *M.tb* in intracellular survival and persistence by inhibiting IL-12 expression by directly targeting 3′UTR of target genes such as IL-10, IL-12, TLR-4 and reducing their expression level.

miR-125

M.tb is also an inducer of miR-125b which directly targets mRNA of TNF-α and results in its destabilization [88]. *M.tb* cell wall component lipomannan blocks TNF biosynthesis in human macrophages by upregulating miR-125b, thereby allowing *M.tb* to subvert host immunity and potentially increases its virulence [104]. *M.tb* infection of macrophages leads to increased expression of miRNA-125a-3p (miR-125a) which inhibits autophagy by targeting UV radiation

resistance-associated gene (UVRAG). miR-125a regulates the innate host defense by inhibiting the activation of autophagy and antimicrobial effects against *M.tb* through targeting UVRAG [105].

miR-27

A recent study reported the role of miR-27a in tuberculosis [106]. miR-27a targets ER-located Ca2+ transporter CACNA2D3 (calcium channel, voltage-dependent, alpha2/delta subunit 3) to downregulate Ca2+-mediated signaling which results in inhibition of autophagosome formation and intracellular survival of *M.tb* [106]. Following transfection of miR-27a mimics, miR-27a restrains immune response against *M.tb* by decreasing IFN-γ, IL-1b, IL-6, and TNF-α expression levels [107]. *M.tb* also induces the expression of miR-27b-3p by the TLR-2/MyD88/NF-kB signaling pathway and targets the production of proinflammatory factors and NF-kB activity. miR-27b-3p provides a negative feedback loop to inhibit excessive inflammation during *M.tb* infection [108].

miR-146a

M.tb infections in macrophages significantly induce the miR-146a expression in a dose-dependent manner. miR-146a modulates inflammatory response by targeting IRAK1 and TRAF6 and supports mycobacterial replication in macrophages [109]. *Mycobacterium bovis*-BCG-induced *miR-146a inhibits iNOS expression and NO production in macrophages to facilitate mycobacterial survival. Moreover, miR-146a suppresses NF-κB and MAPKs pathways by targeting TRAF6, thus impeding iNOS expression [110]. miR-146a might regulate mycobacterial growth or fitness; as a lower bacterial burden was observed in THP-1 differentiated macrophages transfected with miR-146a mimic [111].*

miR-223

miR-223 is found upregulated in blood and lung parenchyma of TB patients and during murine TB [112]. miR-223 controls neutrophil-driven lethal inflammation and lung recruitment of myeloid cells. Upregulated miR-223 is the inhibitor of CCl3, CXCL2, and IL-6 cytokines. miR-223$^{-/-}$ mice are found to be hypersensitive to TB infection due to high amount of aberrant neutrophils migration and exacerbated inflammation [112]. miR-223 also regulates macrophage function by inhibition of cytokine production and NF-κB activation [113].

miR-99b

miR-99b expression is significantly upregulated in *M.tb*-infected DCs and macrophages. Blockade of miR-99b expression reduces bacterial growth in DCs by upregulating proinflammatory cytokines such as IL-6, IL-12, and IL-1β. miR-99b targets TNF-α and TNFRSF-4 receptor genes [114]. MiR-99b is another miRNA which is upregulated by *M.tb* to modulate host immunity by controlling TNF-α production [114].

miR-33

miR-33 is upregulated in macrophages upon *M.tb* infection [115]. miR-33 targets genes (ATG5, ATG12, LC3B, and LAMP1) involved in autophagy and also reprograms host lipid metabolism for intracellular bacterial survival and pathogen stability [115].

miR-144

miR-144 is found overexpressed in the sputum and PBMCs of active TB patients. However, its level decreases after anti-TB treatment [116,117]. The expression of miR-144 is upregulated in human monocyte-derived macrophages (THP-1) after *M.tb* infection *in vitro* [118]. MiR-144 targets autophagy by directly binding to the 3′UTR region of DRAM2 mRNA (DNA damage-regulated autophagy modulator 2), which encodes a transmembrane lysosomal protein [119]. Transfection of T cells with miR-144 inhibits cell proliferation and decrease in IFN-γ and TNF-α secretion upon TCR stimulation [117]. Taken together, miR-144 upregulation in macrophages and T cells upon *M.tb* infection is a mechanism to inhibit phagosome maturation in the monocytes and diminishing the T-cell functions.

miR-889

miR-889 level is found to be elevated in patients with LTBI as compared to patients without infection. miR-889 targets TNF-like weak inducer of apoptosis (TWEAK) whose expression is upregulated in macrophages and PBMCs upon infection with *M.tb* or exposure to heat-killed *M.tb* [120]. In LTBI, increased miR-889 expression is associated with TNF-α and granuloma formation. MiR-889 targets autophagy via post-transcriptional inhibition of TWEAK expression to facilitate mycobacterial survival in granulomas. Treatment with adalimumab, an anti-TNF-α monoclonal antibody reduces levels of both TNF-α and miR-889 and causes granuloma destruction and LTBI reactivation to active disease [120].

These studies suggest that miRNAs are very significantly involved in pathogenesis of *M.tb* infection and may provide a promising therapeutic target for TB. Identifying differentially expressed miRNAs and targeting them for host-directed therapy can be a good approach to target MDR and XDR tuberculosis. Advantage of targeting miRNAs over other protein inhibitors is because of the ability of single miRNA to regulate multiple genes and pathways. Inhibiting and restoring the expression level and function of a dysregulated miRNA have more advantage over targeting multiple dysregulated proteins. The immense potential that this therapy can have highlights the importance of the need of better understanding of these noncoding RNAs in disease contexts. Several researchers are focusing to develop screening assays based on miRNAs, which are capable of detecting high-risk conditions of TB and active vs latent condition of TB, especially in low-income countries. However, more efforts are required to understand the role of the miRNA in modulating the epigenetic signatures of the host immune response genes in order to develop better therapeutic strategies.

Host targeted therapies targeting epigenetic modifications in TB

The intracellular pathogen, *M.tb,* has developed very smart strategies to evade the host immune response [121]. Among all the host evasion strategies employed by *M.tb*, changes in the epigenetic signatures of key protective genes play a very prominent role [122]. During the last decade, alteration in histone modifications and acetylation in host cells have emerged as a very interesting and clever strategy employed by the bacteria to undergo immune evasion [123]. Metabolic products and secretory proteins of the bacteria modulate the expression and transcriptional activity of histone-modifying enzymes, HATs, HDACs, and DNMTs in order to reduce the transcriptional activation of host protective genes through epigenetic changes [123]. This has created new avenues for using HDAC inhibitors as therapeutics to treat microbial infections. However, the mechanism(s) behind the therapeutic use of HAT, DNMT, and HDAC inhibition is not fully deciphered, ongoing studies using high-throughput genome sequencing and analysis of key targets and genes using bioinformatics tool may provide a better understanding of the mechanism(s) which can be unraveled to study epigenetics as therapy of the future.

Although there is lot of information regarding use of epigenetic modifications as therapy in cancer, studies involved in its role in TB are very few. Studies conducted till date establish that HATs acetylate histone proteins on their N-terminal lysine residues [124]. This leads to reduction in compactness of chromatin structure leading to increase in employment of transcriptional factors and their binding to the promoter which is associated with increased transcription of the downstream gene [124]. HDACs, however, remove the acetyl groups from histone proteins inhibiting the transcriptional machinery of the downstream gene [125]. This makes these enzymes as attractive targets to achieve immunomodulatory response during TB infection. HDAC inhibitors have been reported to regulate the activation of several cell types involved in antimicrobial responses such as macrophages and T cells. It has been reported that *M.tb* down-regulates sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD) dependent deacetylase, after infection; in macrophages, in mice model, and in TB patients infected with active TB [125]. Activation of SIRT1 leads to reduced viability of both drug-susceptible and drug-resistant strains of *M.tb* in mice model of TB [125]. Activation of SIRT1 leads to reduced inflammatory responses mainly by deacetylation of RelA/p65, followed by compromised attachment of RelA/p65 to the promoter of genes responsible for host defense [126]. Recently, our group has further reported the immunotherapeutic role of another deacetylase Sirtuin2 (SIRT2) where they have shown that after *M.tb* infection this deacetylase moves to the nucleus of the macrophage to deacetylate the histone H3K18 and leads to greater macrophage activation [127]. Also, in T cells, SIRT2 has been shown to deacetylate NFκB-p65 at K310 position to modulate T helper cell differentiation and promote bacterial survival. Inhibition of SIRT2 using pharmacological inhibitors has shown to reduce the bacterial burden both *in vitro* and in mice model infected with both drug-sensitive and resistant strains of *M.tb* [127]. Treatment with inhibitor of SIRT2 has also been reported to effectively augment the potential and duration of treatment by isoniazid [127]. Another study related to function of HDAC inhibitors revealed their role in epigenetic modification of MMP proteins, MMP-1 and MMP-3 which drives disease outcome [81]. MMPs are calcium and zinc-dependent endopeptidases, which are responsible for degradation of extracellular matrix and cavitation in TB progression. It has been shown that *M.tb* infection alters HDAC expression in macrophages and the level of MMP-1 depends on HDAC/HAT inhibition (108). In addition, a significant increase in the level of histone acetylation at MMP-1 and MMP-3 promoter regions was observed in infected cells compared with control. That further confirms that epigenetic modification via

histone acetylation by HDAC and HAT has a significantly considerable regulatory role in *M.tb* induced gene expression in macrophages and in the secretion of enzymes responsible for disease progression [81]. Targeting these enzymes by inhibitors has been proposed as a therapy. In yet another significant study, it was reported that inhibition of HDACs by chemical inhibitors reduces mycobacterial survival in macrophages while polarizing the macrophages toward a more bactericidal form (M1) while increasing the level of proinflammatory cytokines [78]. These studies offer that DNA-modifying enzymes and their inhibitors can be used as therapeutics in combination with the DOTs therapy in order to prevent formation of drug resistance and in reducing the treatment duration. To sum up, these host-directed therapies involving epigenetic changes in the host can be considered crucial in achieving the 2035 World Health Organization (WHO) "End TB goal" when administered along with the standard anti-TB treatments.

Conclusion

Epigenetic modifications play an important role in the pathogenesis of Tuberculosis. Epigenetic modifications regulate transcriptional profiles of genes related to the immune system that contribute to the host–pathogen interaction and stimulus specificity of the transcriptional response. We discussed the evidence that mycobacteria can modulate epigenetic mechanisms in addition to established mechanisms in order to survive within the host. Understanding of these unconventional survival mechanisms can provide novel targets that can be used as host-directed therapy against tuberculosis. For instance, mycobacterial infection inhibits the IFN-γ-induced expression of HLA-DRα and HLA-DRβ mRNA and partially inhibits CIITA expression in infected macrophages without affecting the expression of IFN regulatory factor-1 mRNA. Inhibition of HDACs with butyric acid or MS-275 results in the inability of *M.tb* to block expression of HLA-DRα and HLA-DRβ mRNA in response to IFN-γ and leads to effective antigen presentation [128].

It is apparent that our increasing knowledge of how epigenetic modifications influence immune function and specialization has unveiled the therapeutic potential of this interestingly novel area. The possible modulations of histone modification and miRNA regulation can be exploited as therapeutic targets in the management of both active and dormant TB using site specific and more potent HDAC inhibitors, bromodomain inhibitors, and CRISPR technology. As the field of phytochemicals seems very promising against TB, the strategy should be to search for phytochemicals and/or small molecules which may be used to modify the host epigenetic signatures to the state which is host protective. Dietary phytochemicals such as tea genistein, sulforaphane, polyphenols, resveratrol, curcumin, and others have been reported to be effective agents against diseases such as cancer and are known to act through epigenetic mechanisms [129]. This would make this area very interesting for our readers who are interested in this field of research. In the case of TB, curcumin, bergenin, allicin, gingerol, and other plant-based bioactive compounds were found to be effective [130–133] although their effect on host epigenetics is still to be explored. Evidence available in other diseases such as cancer opens up new and highly promising dimensions of the use of phytochemicals and other immunogenic agents as host-directed therapy to target host epigenetic mechanisms to manage TB. Thus, in our opinion, modulation of host epigenome may offer a range of potential therapeutic options in future.

Acknowledgements

We would like to acknowledge the funding support provided by Department of Science and Technology, Government of India (DST/INSPIRE/04/2014/002012 to VPD and DST/INSPIRE/04/2014/002069 to AB), and core funding from ICGEB, New Delhi, India. The authors declare no competing financial interests.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

SF, AK, MA, IP, and VY wrote the manuscript. VPD and AB conceptualized the work, provided the resources, and edited the manuscript.

References

1 World Health Organization (2018) Global Tuberculosis Report 2018. World Health Organization. https://apps.who.int/iris/handle/10665/274453.
2 Fatima S, Kumari A, Das G & Dwivedi VP (2020) Tuberculosis vaccine: a journey from BCG to present. *Life Sci* **252**, 117594.
3 Awofeso N (2008) Anti-tuberculosis medication side-effects constitute major factor for poor adherence to tuberculosis treatment. *Bull World Health Organ* **86**, 240.

4 Tobin DM (2015) Host-directed therapies for tuberculosis. *Cold Spring Harb Perspect Med* **5**, a021196.
5 Zhai W, Wu F, Zhang Y, Fu Y & Liu Z (2019) The immune escape mechanisms of *Mycobacterium tuberculosis*. *Int J Mol Sci* **20**, 340.
6 Kathirvel M & Mahadevan S (2016) The role of epigenetics in tuberculosis infection. *Epigenomics* **8**, 537–549.
7 Zheng L, Leung ET, Wong HK, Lui G, Lee N, To KF, Choy KW, Chan RC & Ip M (2016) Unraveling methylation changes of host macrophages in Mycobacterium tuberculosis infection. *Tuberculosis* **98**, 139–148.
8 Kolloli A & Subbian S (2017) Host-directed therapeutic strategies for tuberculosis. *Front Med (Lausanne)* **4**, 171.
9 Smith I (2003) *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. *Clin Microbiol Rev* **16**, 463–496.
10 Philips JA & Ernst JD (2012) Tuberculosis pathogenesis and immunity. *Annu Rev Pathol* **7**, 353–384.
11 Woo M, Wood C, Kwon D, Park KP, Fejer G & Delorme V (2018) *Mycobacterium tuberculosis* infection and innate responses in a new model of lung alveolar macrophages. *Front Immunol* **9**, 438.
12 Sia JK & Rengarajan J (2019) Immunology of *Mycobacterium tuberculosis* infections. *Microbiol Spectr* **7**. https://doi.org/10.1128/microbiolspec.gpp3-0022-2018
13 Laufer JM, Kindinger I, Artinger M, Pauli A & Legler DF (2018) CCR7 is recruited to the immunological synapse, acts as co-stimulatory molecule and drives LFA-1 clustering for efficient T cell adhesion through ZAP70. *Front Immunol* **9**, 3115.
14 Sakai S, Kauffman KD, Schenkel JM, McBerry CC, Mayer-Barber KD, Masopust D & Barber DL (2014) Cutting edge: control of *Mycobacterium tuberculosis* infection by a subset of lung parenchyma-homing CD4 T cells. *J Immunol* **192**, 2965–2969.
15 Nikitina IY, Panteleev AV, Kosmiadi GA, Serdyuk YV, Nenasheva TA, Nikolaev AA, Gorelova LA, Radaeva TV, Kiseleva YY, Bozhenko VK *et al.* (2018) Th1, Th17, and Th1Th17 lymphocytes during tuberculosis: Th1 lymphocytes predominate and appear as low-differentiated CXCR3(+)CCR6(+) cells in the blood and highly differentiated CXCR3(+/-)CCR6(-) cells in the lungs. *J Immunol* **200**, 2090–2103.
16 Sakamoto K (2012) The pathology of *Mycobacterium tuberculosis* infection. *Vet Pathol* **49**, 423–439.
17 Noble D (2015) Conrad Waddington and the origin of epigenetics. *J Exp Biol* **218**, 816–818.
18 Bierne H, Hamon M & Cossart P (2012) Epigenetics and bacterial infections. *Cold Spring Harb Perspect Med* **2**, a010272.
19 Singh M, Yadav V & Das G (2018) Chapter 4 – host epigenetic modifications in *Mycobacterium tuberculosis* infection: a boon or bane. In The Value of BCG and TNF in Autoimmunity, 2nd edn (Faustman DL, ed.), pp. 39–55. Academic Press, London, United Kingdom.
20 Meaney MJ & Szyf M (2005) Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. *Dialogues Clin Neurosci* **7**, 103–123.
21 Adhikari S & Curtis PD (2016) DNA methyltransferases and epigenetic regulation in bacteria. *FEMS Microbiol Rev* **40**, 575–591.
22 Bird A (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* **16**, 6–21.
23 Illingworth RS & Bird AP (2009) CpG islands–'a rough guide'. *FEBS Lett* **583**, 1713–1720.
24 Okano M, Bell DW, Haber DA & Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* **99**, 247–257.
25 Fuso A, Ferraguti G, Scarpa S, Ferrer I & Lucarelli M (2015) Disclosing bias in bisulfite assay: MethPrimers underestimate high DNA methylation. *PLoS One* **10**, e0118318.
26 Huh I, Zeng J, Park T & Yi SV (2014) Correction: DNA methylation and transcriptional noise. *Epigenetics Chromatin* **7**, 13.
27 Zhu L, Zhong J, Jia X, Liu G, Kang Y, Dong M, Zhang X, Li Q, Yue L, Li C *et al.* (2016) Precision methylome characterization of *Mycobacterium tuberculosis* complex (MTBC) using PacBio single-molecule real-time (SMRT) technology. *Nucleic Acids Res* **44**, 730–743.
28 Bosshart H & Heinzelmann M (2016) THP-1 cells as a model for human monocytes. *Ann Transl Med* **4**, 438.
29 Mujtaba S, Winer BY, Jaganathan A, Patel J, Sgobba M, Schuch R, Gupta YK, Haider S, Wang R & Fischetti VA (2013) Anthrax SET protein: a potential virulence determinant that epigenetically represses NF-κB activation in infected macrophages. *J Biol Chem* **288**, 23458–23472.
30 Shell SS, Prestwich EG, Baek SH, Shah RR, Sassetti CM, Dedon PC & Fortune SM (2013) DNA methylation impacts gene expression and ensures hypoxic survival of Mycobacterium tuberculosis. *PLoS Pathog* **9**, e1003419.
31 Randall SE, Martini MC, Zhou Y, Joubran SR & Shell SS (2020) MamA essentiality in *Mycobacterium smegmatis* is explained by the presence of an apparent cognate restriction endonuclease. *BMC Res Notes* **13**, 462.
32 Cole ST (1999) Learning from the genome sequence of Mycobacterium tuberculosis H37Rv. *FEBS Lett* **452**, 7–10.

33 Looney M, Lorenc R, Halushka MK & Karakousis PC (2021) Key macrophage responses to infection with *Mycobacterium tuberculosis* are co-regulated by microRNAs and DNA methylation. *Front Immunol* **12**, 685237.

34 Jiang C, Zhu J, Liu Y, Luan X, Jiang Y, Jiang G & Fan J (2017) The methylation state of VDR gene in pulmonary tuberculosis patients. *J Thorac Dis* **9**, 4353–4357.

35 Bai X, Feldman NE, Chmura K, Ovrutsky AR, Su WL, Griffin L, Pyeon D, McGibney MT, Strand MJ, Numata M *et al.* (2013) Inhibition of nuclear factor-kappa B activation decreases survival of Mycobacterium tuberculosis in human macrophages. *PLoS One* **8**, e61925.

36 Fallahi-Sichani M, Kirschner DE & Linderman JJ (2012) NF-κB signaling dynamics play a key role in infection control in tuberculosis. *Front Physiol* **3**, 170.

37 Aletta JM, Cimato TR & Ettinger MJ (1998) Protein methylation: a signal event in post-translational modification. *Trends Biochem Sci* **23**, 89–91.

38 Ng SS, Yue WW, Oppermann U & Klose RJ (2009) Dynamic protein methylation in chromatin biology. *Cell Mol Life Sci* **66**, 407–422.

39 Wagner KW, Alam H, Dhar SS, Giri U, Li N, Wei Y, Giri D, Cascone T, Kim JH, Ye Y *et al.* (2013) KDM2A promotes lung tumorigenesis by epigenetically enhancing ERK1/2 signaling. *J Clin Invest* **123**, 5231–5246.

40 Yamagata K, Daitoku H, Takahashi Y, Namiki K, Hisatake K, Kako K, Mukai H, Kasuya Y & Fukamizu A (2008) Arginine methylation of FOXO transcription factors inhibits their phosphorylation by Akt. *Mol Cell* **32**, 221–231.

41 Tsai YC & Weissman AM (2011) Dissecting the diverse functions of the metastasis suppressor CD82/KAI1. *FEBS Lett* **585**, 3166–3173.

42 Artavanis-Tsakonas K, Kasperkovitz PV, Papa E, Cardenas ML, Khan NS, Van der Veen AG, Ploegh HL & Vyas JM (2011) The tetraspanin CD82 is specifically recruited to fungal and bacterial phagosomes prior to acidification. *Infect Immun* **79**, 1098–1106.

43 Koh HJ, Kim YR, Kim JS, Yun JS, Kim S, Kim SY, Jang K & Yang CS (2018) CD82 hypomethylation is essential for tuberculosis pathogenesis via regulation of RUNX1-Rab5/22. *Exp Mol Med* **50**, 1–15.

44 Fox W, Ellard GA & Mitchison DA (1999) Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946–1986, with relevant subsequent publications. *Int J Tuberc Lung Dis* **3**, S231–S279.

45 Ennassiri W, Jaouhari S, Sabouni R, Cherki W, Charof R, Filali-Maltouf A & Lahlou O (2018) Analysis of isoniazid and rifampicin resistance in *Mycobacterium tuberculosis* isolates in Morocco using GenoType(®) MTBDRplus assay. *J Glob Antimicrob Resist* **12**, 197–201.

46 Chen L, Li H, Chen T, Yu L, Guo H, Chen Y, Chen M, Li Z, Wu Z, Wang X *et al.* (2018) Genome-wide DNA methylation and transcriptome changes in *Mycobacterium tuberculosis* with rifampicin and isoniazid resistance. *Int J Clin Exp Pathol* **11**, 3036–3045.

47 Warrier T, Kapilashrami K, Argyrou A, Ioerger TR, Little D, Murphy KC, Nandakumar M, Park S, Gold B, Mi J *et al.* (2016) N-methylation of a bactericidal compound as a resistance mechanism in Mycobacterium tuberculosis. *Proc Natl Acad Sci USA* **113**, E4523–E4530.

48 Verma D, Parasa VR, Raffetseder J, Martis M, Mehta RB, Netea M & Lerm M (2017) Anti-mycobacterial activity correlates with altered DNA methylation pattern in immune cells from BCG-vaccinated subjects. *Sci Rep* **7**, 12305.

49 Hasso-Agopsowicz M, Scriba TJ, Hanekom WA, Dockrell HM & Smith SG (2018) Differential DNA methylation of potassium channel KCa3.1 and immune signalling pathways is associated with infant immune responses following BCG vaccination. *Sci Rep* **8**, 13086.

50 Das J, Verma D, Gustafsson M & Lerm M (2019) Identification of DNA methylation patterns predisposing for an efficient response to BCG vaccination in healthy BCG-naïve subjects. *Epigenetics* **14**, 589–601.

51 Hamon MA & Cossart P (2008) Histone modifications and chromatin remodeling during bacterial infections. *Cell Host Microbe* **4**, 100–109.

52 Tremethick DJ (2007) Higher-order structures of chromatin: the elusive 30 nm fiber. *Cell* **128**, 651–654.

53 Mohrmann L & Verrijzer CP (2005) Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. *Biochim Biophys Acta* **1681**, 59–73.

54 Rae W (2017) Indications to epigenetic dysfunction in the pathogenesis of common variable immunodeficiency. *Arch Immunol Ther Exp (Warsz)* **65**, 101–110.

55 Pennini ME, Pai RK, Schultz DC, Boom WH & Harding CV (2006) Mycobacterium tuberculosis 19-kDa lipoprotein inhibits IFN-gamma-induced chromatin remodeling of MHC2TA by TLR2 and MAPK signaling. *J Immunol* **176**, 4323–4330.

56 Kumar P, Agarwal R, Siddiqui I, Vora H, Das G & Sharma P (2012) ESAT6 differentially inhibits IFN-γ-inducible class II transactivator isoforms in both a TLR2-dependent and -independent manner. *Immunol Cell Biol* **90**, 411–420.

57 Tarashi S, Badi SA, Moshiri A, Ebrahimzadeh N, Fateh A, Vaziri F, Aazami H, Siadat SD & Fuso A

(2020) The inter-talk between *Mycobacterium tuberculosis* and the epigenetic mechanisms. *Epigenomics* **12**, 455–469.
58 Sharma G, Upadhyay S, Srilalitha M, Nandicoori VK & Khosla S (2015) The interaction of mycobacterial protein Rv2966c with host chromatin is mediated through non-CpG methylation and histone H3/H4 binding. *Nucleic Acids Res* **43**, 3922–3937.
59 Yaseen I, Kaur P, Nandicoori VK & Khosla S (2015) Mycobacteria modulate host epigenetic machinery by Rv1988 methylation of a non-tail arginine of histone H3. *Nat Commun* **6**, 8922.
60 Khosla S, Sharma G & Yaseen I (2016) Learning epigenetic regulation from mycobacteria. *Microb Cell* **3**, 92–94.
61 Shen Y, Liu WW, Zhang X, Shi JG, Jiang S, Zheng L, Qin Y, Liu B & Shi JH (2020) TRAF3 promotes ROS production and pyroptosis by targeting ULK1 ubiquitination in macrophages. *FASEB J* **34**, 7144–7159.
62 Naime ACA, Bonfitto PHL, Solon C, Lopes-Pires ME, Anhê GF, Antunes E & Marcondes S (2019) Tumor necrosis factor alpha has a crucial role in increased reactive oxygen species production in platelets of mice injected with lipopolysaccharide. *Platelets* **30**, 1047–1052.
63 Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC & Kouzarides T (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410**, 120–124.
64 Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, Schreiber SL, Mellor J & Kouzarides T (2002) Active genes are tri-methylated at K4 of histone H3. *Nature* **419**, 407–411.
65 Asaad M, Abo-Kadoum MA, Nzungize L, Uae M, Nzaou SAE & Xie J (2020) Methylation in Mycobacterium-host interaction and implications for novel control measures. *Infect Genet Evol* **83**, 104350.
66 Chen YC, Chao TY, Leung SY, Chen CJ, Wu CC, Fang WF, Wang YH, Chang HC, Wang TY, Lin YY *et al.* (2017) Histone H3K14 hypoacetylation and H3K27 hypermethylation along with HDAC1 up-regulation and KDM6B down-regulation are associated with active pulmonary tuberculosis disease. *Am J Transl Res* **9**, 1943–1955.
67 Gringhuis SI, den Dunnen J, Litjens M, van Het Hof B, van Kooyk Y & Geijtenbeek TB (2007) C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-kappaB. *Immunity* **26**, 605–616.
68 Sánchez A, Espinosa P, García T & Mancilla R (2012) The 19 kDa *Mycobacterium tuberculosis* lipoprotein (LpqH) induces macrophage apoptosis through extrinsic and intrinsic pathways: a role for the mitochondrial apoptosis-inducing factor. *Clin Dev Immunol* **2012**, 950503.
69 Kim KH, An DR, Song J, Yoon JY, Kim HS, Yoon HJ, Im HN, Kim J, Kim DJ, Lee SJ *et al.* (2012) *Mycobacterium tuberculosis* Eis protein initiates suppression of host immune responses by acetylation of DUSP16/MKP-7. *Proc Natl Acad Sci USA* **109**, 7729–7734.
70 Ghosh S, Padmanabhan B, Anand C & Nagaraja V (2016) Lysine acetylation of the *Mycobacterium tuberculosis* HU protein modulates its DNA binding and genome organization. *Mol Microbiol* **100**, 577–588.
71 Anand C, Garg R, Ghosh S & Nagaraja V (2017) A Sir2 family protein Rv1151c deacetylates HU to alter its DNA binding mode in *Mycobacterium tuberculosis*. *Biochem Biophys Res Commun* **493**, 1204–1209.
72 Yaseen I, Choudhury M, Sritharan M & Khosla S (2018) Histone methyltransferase SUV39H1 participates in host defense by methylating mycobacterial histone-like protein HupB. *EMBO J* **37**, 183–200.
73 Green KD, Biswas T, Pang AH, Willby MJ, Reed MS, Stuchlik O, Pohl J, Posey JE, Tsodikov OV & Garneau-Tsodikova S (2018) Acetylation by Eis and deacetylation by Rv1151c of *Mycobacterium tuberculosis* HupB: biochemical and structural insight. *Biochemistry* **57**, 781–790.
74 Jose L, Ramachandran R, Bhagavat R, Gomez RL, Chandran A, Raghunandanan S, Omkumar RV, Chandra N, Mundayoor S & Kumar RA (2016) Hypothetical protein Rv3423.1 of *Mycobacterium tuberculosis* is a histone acetyltransferase. *FEBS J* **283**, 265–281.
75 Campo M, Seshadri C, Stein CM, Peterson G, Wells RD, Mayanja-Kizza H, Boom WH & Hawn T (2016) The role of histone deacetylases in the innate immune response to *Mycobacterium tuberculosis* infection. *J Immunol* **196**, 126.15.
76 Wang X, Tang X, Zhou Z & Huang Q (2018) Histone deacetylase 6 inhibitor enhances resistance to *Mycobacterium tuberculosis* infection through innate and adaptive immunity in mice. *Pathog Dis* **76**, fty064.
77 Campo M, Heater S, Peterson GJ, Simmons JD, Skerrett SJ, Mayanja-Kizza H, Stein CM, Boom WH & Hawn TR (2021) HDAC3 inhibitor RGFP966 controls bacterial growth and modulates macrophage signaling during Mycobacterium tuberculosis infection. *Tuberculosis* **127**, 102062.
78 Moreira JD, Koch BEV, van Veen S, Walburg KV, Vrieling F, Mara Pinto Dabés Guimarães T, Meijer AH, Spaink HP, Ottenhoff THM, Haks MC *et al.* (2020) Functional inhibition of host histone deacetylases (HDACs) enhances in vitro and in vivo anti-mycobacterial activity in human macrophages and in zebrafish. *Front Immunol* **11**, 36.
79 Kleinnijenhuis J, Quintin J, Preijers F, Joosten LA, Ifrim DC, Saeed S, Jacobs C, van Loenhout J, de Jong

D, Stunnenberg HG *et al.* (2012) Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proc Natl Acad Sci USA* **109**, 17537–17542.
80 Lella RK & Sharma C (2007) Eis (enhanced intracellular survival) protein of *Mycobacterium tuberculosis* disturbs the cross regulation of T-cells. *J Biol Chem* **282**, 18671–18675.
81 Moores RC, Brilha S, Schutgens F, Elkington PT & Friedland JS (2017) Epigenetic regulation of matrix metalloproteinase-1 and -3 expression in *mycobacterium tuberculosis* infection. *Front Immunol* **8**, 602.
82 Levenson VV & Melnikov AA (2012) DNA methylation as clinically useful biomarkers-light at the end of the tunnel. *Pharmaceuticals (Basel)* **5**, 94–113.
83 Wieczorek G, Asemissen A, Model F, Turbachova I, Floess S, Liebenberg V, Baron U, Stauch D, Kotsch K, Pratschke J *et al.* (2009) Quantitative DNA methylation analysis of FOXP3 as a new method for counting regulatory T cells in peripheral blood and solid tissue. *Cancer Res* **69**, 599–608.
84 Esterhuyse MM, Linhart HG & Kaufmann SH (2012) Can the battle against tuberculosis gain from epigenetic research? *Trends Microbiol* **20**, 220–226.
85 Ahmad S (2011) Pathogenesis, immunology, and diagnosis of latent Mycobacterium tuberculosis infection. *Clin Dev Immunol* **2011**, 814943.
86 O'Connell RM, Rao DS, Chaudhuri AA & Baltimore D (2010) Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* **10**, 111–122.
87 Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**, 281–297.
88 Watanabe Y & Kanai A (2011) Systems biology reveals microRNA-mediated gene regulation. *Front Genet* **2**, 29.
89 Maute RL, Dalla-Favera R & Basso K (2014) RNAs with multiple personalities. *Wiley Interdiscip Rev RNA* **5**, 1–13.
90 Krol J, Loedige I & Filipowicz W (2010) The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* **11**, 597–610.
91 Subramanian S & Steer CJ (2010) MicroRNAs as gatekeepers of apoptosis. *J Cell Physiol* **223**, 289–298.
92 Nana-Sinkam SP & Croce CM (2013) Clinical applications for microRNAs in cancer. *Clin Pharmacol Ther* **93**, 98–104.
93 Hammond SM (2015) An overview of microRNAs. *Adv Drug Deliv Rev* **87**, 3–14.
94 Fu Y, Yi Z, Li J & Li R (2014) Deregulated microRNAs in CD4+ T cells from individuals with latent tuberculosis versus active tuberculosis. *J Cell Mol Med* **18**, 503–513.
95 Etna MP, Sinigaglia A, Grassi A, Giacomini E, Romagnoli A, Pardini M, Severa M, Cruciani M, Rizzo F, Anastasiadou E *et al.* (2018) *Mycobacterium tuberculosis*-induced *miR-155 subverts autophagy by targeting ATG3 in human dendritic cells. PLoS Pathog* **14**, e1006790.
96 Ghorpade DS, Leyland R, Kurowska-Stolarska M, Patil SA & Balaji KN (2012) MicroRNA-155 is required for *Mycobacterium bovis* BCG-mediated apoptosis of macrophages. *Mol Cell Biol* **32**, 2239–2253.
97 Rothchild AC, Sissons JR, Shafiani S, Plaisier C, Min D, Mai D, Gilchrist M, Peschon J, Larson RP, Bergthaler A *et al.* (2016) MiR-155-regulated molecular network orchestrates cell fate in the innate and adaptive immune response to *Mycobacterium tuberculosis. Proc Natl Acad Sci USA* **113**, E6172–E6181.
98 Wang J, Wu M, Wen J, Yang K, Li M, Zhan X, Feng L, Li M & Huang X (2014) MicroRNA-155 induction by *Mycobacterium bovis* BCG enhances ROS production through targeting SHIP1. *Mol Immunol* **62**, 29–36.
99 Thai TH, Calado DP, Casola S, Ansel KM, Xiao C, Xue Y, Murphy A, Frendewey D, Valenzuela D, Kutok JL *et al.* (2007) Regulation of the germinal center response by microRNA-155. *Science* **316**, 604–608.
100 Liu PT, Wheelwright M, Teles R, Komisopoulou E, Edfeldt K, Ferguson B, Mehta MD, Vazirnia A, Rea TH, Sarno EN *et al.* (2012) MicroRNA-21 targets the vitamin D-dependent antimicrobial pathway in leprosy. *Nat Med* **18**, 267–273.
101 Wu Z, Lu H, Sheng J & Li L (2012) Inductive microRNA-21 impairs anti-mycobacterial responses by targeting IL-12 and Bcl-2. *FEBS Lett* **586**, 2459–2467.
102 Harapan H, Fitra F, Ichsan I, Mulyadi M, Miotto P, Hasan NA, Calado M & Cirillo DM (2013) The roles of microRNAs on tuberculosis infection: meaning or myth? *Tuberculosis* **93**, 596–605.
103 Hackett EE, Charles-Messance H, O'Leary SM, Gleeson LE, Muñoz-Wolf N, Case S, Wedderburn A, Johnston DGW, Williams MA, Smyth A *et al.* (2020) *Mycobacterium tuberculosis* limits host glycolysis and IL-1β by restriction of PFK-M via microRNA-21. *Cell Rep* **30**, 124–136.e4.
104 Rajaram MV, Ni B, Morris JD, Brooks MN, Carlson TK, Bakthavachalu B, Schoenberg DR, Torrelles JB & Schlesinger LS (2011) *Mycobacterium tuberculosis* lipomannan blocks TNF biosynthesis by regulating macrophage MAPK-activated protein kinase 2 (MK2) and microRNA miR-125b. *Proc Natl Acad Sci USA* **108**, 17408–17413.
105 Kim JK, Yuk JM, Kim SY, Kim TS, Jin HS, Yang CS & Jo EK (2015) MicroRNA-125a inhibits

autophagy activation and antimicrobial responses during mycobacterial infection. *J Immunol* **194**, 5355–5365.
106 Liu F, Chen J, Wang P, Li H, Zhou Y, Liu H, Liu Z, Zheng R, Wang L, Yang H *et al.* (2018) MicroRNA-27a controls the intracellular survival of *Mycobacterium tuberculosis* by regulating calcium-associated autophagy. *Nat Commun* **9**, 4295.
107 Wang J, Jia Z, Wei B, Zhou Y, Niu C, Bai S, Tong C, Song J & Li Y (2017) MicroRNA-27a restrains the immune response to mycobacterium tuberculosis infection by targeting IRAK4, a promoter of the NF-κB pathway. *Int J Clin Exp Pathol* **10**, 9894–9901.
108 Liang S, Song Z, Wu Y, Gao Y, Gao M, Liu F, Wang F & Zhang Y (2018) MicroRNA-27b modulates inflammatory response and apoptosis during *Mycobacterium tuberculosis* infection. *J Immunol* **200**, 3506–3518.
109 Li S, Yue Y, Xu W & Xiong S (2013) MicroRNA-146a represses mycobacteria-induced inflammatory response and facilitates bacterial replication via targeting IRAK-1 and TRAF-6. *PLoS One* **8**, e81438.
110 Liu Z, Zhou G, Deng X, Yu Q, Hu Y, Sun H, Wang Z, Chen H, Jia C & Wang D (2014) Analysis of miRNA expression profiling in human macrophages responding to Mycobacterium infection: induction of the immune regulator miR-146a. *J Infect* **68**, 553–561.
111 Li M, Wang J, Fang Y, Gong S, Li M, Wu M, Lai X, Zeng G, Wang Y, Yang K *et al.* (2016) microRNA-146a promotes mycobacterial survival in macrophages through suppressing nitric oxide production. *Sci Rep* **6**, 23351.
112 Dorhoi A, Iannaccone M, Farinacci M, Faé KC, Schreiber J, Moura-Alves P, Nouailles G, Mollenkopf HJ, Oberbeck-Müller D, Jörg S *et al.* (2013) MicroRNA-223 controls susceptibility to tuberculosis by regulating lung neutrophil recruitment. *J Clin Invest* **123**, 4836–4848.
113 Liu Y, Wang R, Jiang J, Yang B, Cao Z & Cheng X (2015) miR-223 is upregulated in monocytes from patients with tuberculosis and regulates function of monocyte-derived macrophages. *Mol Immunol* **67**, 475–481.
114 Singh Y, Kaul V, Mehra A, Chatterjee S, Tousif S, Dwivedi VP, Suar M, Van Kaer L, Bishai WR & Das G (2013) *Mycobacterium tuberculosis* controls microRNA-99b (miR-99b) expression in infected murine dendritic cells to modulate host immunity. *J Biol Chem* **288**, 5056–5061.
115 Ouimet M, Koster S, Sakowski E, Ramkhelawon B, van Solingen C, Oldebeken S, Karunakaran D, Portal-Celhay C, Sheedy FJ, Ray TD *et al.* (2016) *Mycobacterium tuberculosis* induces the miR-33 locus to reprogram autophagy and host lipid metabolism. *Nat Immunol* **17**, 677–686.
116 Lv Y, Guo S, Li XG, Chi JY, Qu YQ & Zhong HL (2016) Sputum and serum microRNA-144 levels in patients with tuberculosis before and after treatment. *Int J Infect Dis* **43**, 68–73.
117 Liu Y, Wang X, Jiang J, Cao Z, Yang B & Cheng X (2011) Modulation of T cell cytokine production by miR-144* with elevated expression in patients with pulmonary tuberculosis. *Mol Immunol* **48**, 1084–1090.
118 Kim JK, Lee HM, Park KS, Shin DM, Kim TS, Kim YS, Suh HW, Kim SY, Kim IS, Kim JM *et al.* (2017) MIR144* inhibits antimicrobial responses against Mycobacterium tuberculosis in human monocytes and macrophages by targeting the autophagy protein DRAM2. *Autophagy* **13**, 423–441.
119 Kim JK, Kim TS, Basu J & Jo EK (2017) MicroRNA in innate immunity and autophagy during mycobacterial infection. *Cell Microbiol* **19**, e12687.
120 Chen DY, Chen YM, Lin CF, Lo CM, Liu HJ & Liao TL (2020) MicroRNA-889 inhibits autophagy to maintain mycobacterial survival in patients with latent tuberculosis infection by targeting TWEAK. *MBio* **11**, e03045.
121 Goldberg MF, Saini NK & Porcelli SA (2014) Evasion of innate and adaptive immunity by *Mycobacterium tuberculosis*. *Microbiol Spectr* **2**, https://doi.org/10.1128/microbiolspec.MGM2-0005-2013
122 Fol M, Włodarczyk M & Druszczyńska M (2020) Host epigenetics in intracellular pathogen infections. *Int J Mol Sci* **21**, 4573.
123 Chandran A, Antony C, Jose L, Mundayoor S, Natarajan K & Kumar RA (2015) *Mycobacterium tuberculosis* infection induces HDAC1-mediated suppression of IL-12B gene expression in macrophages. *Front Cell Infect Microbiol* **5**, 90.
124 Sterner DE & Berger SL (2000) Acetylation of histones and transcription-related factors. *Microbiol Mol Biol Rev* **64**, 435–459.
125 Verdin E & Ott M (2015) 50 years of protein acetylation: from gene regulation to epigenetics, metabolism and beyond. *Nat Rev Mol Cell Biol* **16**, 258–264.
126 Cheng CY, Gutierrez NM, Marzuki MB, Lu X, Foreman TW, Paleja B, Lee B, Balachander A, Chen J, Tsenova L *et al.* (2017) Host sirtuin 1 regulates mycobacterial immunopathogenesis and represents a therapeutic target against tuberculosis. *Sci Immunol* **2**, eaaj1789.
127 Bhaskar A, Kumar S, Khan MZ, Singh A, Dwivedi VP & Nandicoori VK (2020) Host sirtuin 2 as an immunotherapeutic target against tuberculosis. *eLife* **9**, e55415.
128 Wang Y, Curry HM, Zwilling BS & Lafuse WP (2005) Mycobacteria inhibition of IFN-gamma induced HLA-DR gene expression by up-regulating histone deacetylation at the promoter region in human THP-1 monocytic cells. *J Immunol* **174**, 5687–5694.

129 Hardy TM & Tollefsbol TO (2011) Epigenetic diet: impact on the epigenome and cancer. *Epigenomics* **3**, 503–518.
130 Kumar S, Sharma C, Kaushik SR, Kulshreshtha A, Chaturvedi S, Nanda RK, Bhaskar A, Chattopadhyay D, Das G & Dwivedi VP (2019) The phytochemical bergenin as an adjunct immunotherapy for tuberculosis in mice. *J Biol Chem* **294**, 8555–8563.
131 Bhaskar A, Kumari A, Singh M, Kumar S, Kumar S, Dabla A, Chaturvedi S, Yadav V, Chattopadhyay D & Prakash Dwivedi V (2020) [6]-Gingerol exhibits potent anti-mycobacterial and immunomodulatory activity against tuberculosis. *Int Immunopharmacol* **87**, 106809.
132 Tousif S, Singh DK, Mukherjee S, Ahmad S, Arya R, Nanda R, Ranganathan A, Bhattacharyya M, Van Kaer L, Kar SK *et al.* (2017) Nanoparticle-formulated curcumin prevents posttherapeutic disease reactivation and reinfection with *Mycobacterium tuberculosis* following isoniazid therapy. *Front Immunol* **8**, 739.
133 Dwivedi VP, Bhattacharya D, Singh M, Bhaskar A, Kumar S, Fatima S, Sobia P, Kaer LV & Das G (2019) Allicin enhances antimicrobial activity of macrophages during *Mycobacterium tuberculosis* infection. *J Ethnopharmacol* **243**, 111634.