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Article

In silico analysis of miRNA target genes possibly induced by tuberculosis infection

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Abstract:

The objective was to identify, through *in silico* analysis, the genes to which miR-146a, miR-146b, and miR-155 bind and to analyze the metabolic pathways in which they participate during tuberculosis infection. For the analysis, it was used: miRBase, UniProtKB, TargetScan Human, miRDB, and miRTarBase. miR-146a interacts with or binds to genes important in cell adhesion and the process of phagocytosis (*CLDN16* and *ATP6V1C2*, respectively) ($P< 0.05$); this interaction could have important implications in the pathogenesis of tuberculosis or related diseases. The results of this work suggest that the activation of specific molecular mechanisms in response to tuberculosis is regulated by miR-146a, miR-146b, and miR-155. The genes with which miR-146a and miR-155 interact or bind are involved in the immune response and cellular processes essential during tuberculosis infection.

Keywords: *Mycobacterium*, miR-146a, miR-146b, miR-155, Prediction, Diagnosis.

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Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*) represents a risk to public health and a threat to health security; it is one of the leading causes of morbidity and mortality worldwide[1]. It is an infectious-contagious disease that mainly affects the lungs[2]. The World Health Organization (WHO) reported that 1.4 million people died in 2021 and considers it the deadliest disease after COVID-19; it is estimated that 10.6 million people fell ill with TB in 2021[3]. It is evident that during the COVID-19 pandemic, the TB care, diagnosis, and treatment system was deficient; this increases the possibility of a rebound of the disease, with a possible impact on global health[4]. In most cases, the SARS-CoV-2 virus causes a respiratory infection in the infected individual, which can be complicated in varying degrees, ranging from mild to severe. The use of immunosuppressive drugs in COVID-19 may lead to an increase in *Mtb* gene expression. Some reports describe cases of co-infection between *Mtb* and SARS-CoV-2, with a prevalence of TB between 0.47 and 4.47 % among patients with COVID-19, and it is reported that the mortality rate in patients with TB/COVID-19 was higher than that of patients affected by only one of these pathogens[4-6].

In daily clinical practice, the accurate diagnosis of TB is through the culture of the microorganism, and growing bacteria are required to perform drug susceptibility tests, which is a major medical challenge and slows down the procedure[7]. In addition to this, there is the problem of the progressive development of drug-resistant TB, which reinforces the urgent need to research new molecules for the diagnosis and control of TB[8]. Early diagnosis, as well as effective treatment of infected individuals, could help reduce TB. For this reason, it is essential to conduct research on novel biomarkers to design control methods. In recent years, microRNAs have been studied as promising molecules for such effects due to their high stability, sensitivity, and specificity[9]. miRNAs are small, non-coding regulatory RNAs that act by repressing protein expression at the post-transcriptional level and have important functions in many physiological and pathophysiological processes[10]. The regulatory mechanisms of miRNAs are based on the complementarity of sequences between the miRNA and the target mRNA; if the binding is perfect, it results in the degradation of the mRNA; if the binding is partial, the translation is repressed[11]. mRNA deadenylation leads to mRNA instability and, thus, degradation[10]. After any of these mechanisms, the host's innate immune response is activated, with the production of cytokines and chemokines[11].
Development in omics sciences has allowed the rapid identification and characterization of small non-coding RNAs, which are part of a complex gene regulatory system, and differential expression of these has been found in individuals infected with TB. During infection with

Mtb, the host's immune response is activated; in this host-*Mtb* interaction, the profile of miRNAs is manipulated; this implies the regulation of several biological processes mediated by these molecules[11]. Some miRNAs that are modified during an *Mtb* infection are also produced in immune cells contained in the granuloma and lead to the adaptive immune response; they can also be secreted into the extracellular medium through processes such as apoptosis or necrosis, encapsulation within microvesicles or exosomes, and through binding to high-density lipoproteins (HDL), among others. This allows stable expression patterns of miRNAs associated with TB infection[12].

Little is known about the molecular pathogenesis of the disease, but there are recent reports that demonstrate the importance of miRNAs in pulmonary TB and that they can be detected in the blood of infected patients, so they are currently indicated as candidates for diagnosis. The miRNAs that are modulated in response to *Mtb* infection are miR-125b, miR-155, miR-144, miR-3179, miR-147, miR-146a/b, miR-886-5p, let-7e, and let-7i[13,14]. The presence and regulation of these miRNAs in TB-infected humans indicates their importance in the pathogenesis and survival of the bacillus, so their study during infection is indispensable.

miRNA 146b has been associated with the regulation of various signaling pathways, and some of its described target genes are AKT3, IL6, IRAK1, NFKB1, and TLR4[15]; and it has been reported to be overexpressed in the serum of patients with active TB[16]. miR-155 modulates the production of inflammatory mediators in response to microbial stimuli by downregulating the expression of TAK1 and the TRAF6-binding protein. It has also been observed that, in TB infection, miR-155 is overexpressed and inhibits IFN-γ-induced autophagy; some of the target genes with which it has been associated are AKT1, APAF1, ATP6V1H, and CASP3[15]. Interestingly, miR-155 has a dual function during TB infection; on the one hand, it maintains the survival of *Mtb*-infected macrophages and, on the other hand, promotes the survival and function of *Mtb*-specific T cells[17]. Considering the importance of miRNAs in the pathogenesis of human TB, where they have been detected in the blood of sick patients, in this work, the genes to which miR-146a, miR-146b, and miR-155 bind were predicted, and the metabolic pathways in which they participate were analyzed.

Material and methods

Selection of study miRNAs

The three miRNAs used for the analysis were selected through a bibliographic search in the NCBI database, through the collection of biomedical journals of PubMed Central (https://www.ncbi.nlm.nih.gov/pmc/)[18], the selected miRNAs were those studied in humans, those consistently reported in at least five scientific journals and those that were

found to be overexpressed in infected individuals in experimental trials; of these, three were selected at random. The miRBase database[19] was used to obtain data on the miRNAs of interest, such as the access number and mature sequence of each miRNA, which were as follows: hsa-mir-146a-3p: MIMAT0004608 (CCUCUGAAAUUCAGUUCUUCAG), hsa-miR-146b-3p: MIMAT0004766 (GCCCUGUGGACUCAGUUCUGGU), hsa-miR-155-3p: MI0000681 MIMAT0004658 (CUCCUACAUAUUAGCAUUAACA).

Prediction of miRNA pathways and target genes

Three ontological models were used to determine the target genes of the miRNAs analyzed, which allowed to obtain the most accurate meta-information and describe the semantics of the most objective data. The following programs were used: TargetScan Human[20], miRDB[21,22], particularly analyzed with the target ontology[23] and miRTarBase tools[23,24]. The inclusion criteria of the target genes to be studied were as follows. For the TargetScan Human software[20], only genes with context scores above -0.20 were included for analysis. In the case of Target ontology[23], only target genes that met a target score above 77 were selected. For the miRTarBase software[23,24], the target genes of each miRNA were chosen according to the experimental evidence validated by at least two methods and reported in papers related to the study topic. In addition, the genes that were found consistently in at least two of the programs used were the ones that were considered for their review. Finally, of these, at least two target genes were randomly selected to review their relevance in TB infection. Each gene was assigned a metabolic or regulatory pathway using information from the Kyoto Gene and Genome (KEGG) library[25].

Results

Prediction of target genes and analysis of the metabolic pathways of miRNAs

Bioinformatic analyses allowed the prediction of target genes for miR-146a, miR-146b, and miR-155 (Table 1); some of these genes have major implications during TB infection. miR-146a can regulate genes involved in cell adhesion and phagosome formation processes (*CLDN16*, *ATP6V1C2*) (Figure 1). miR-146b is involved in the metabolic pathways of degradation of valine, leucine, and isoleucine and the thyroid hormone signaling pathway, among others (Figure 2). miR-155 is involved in the pathways of the sulfur relay system and

tryptophan metabolism, according to the predictions of the KEGG program (January 2022) (Figure 3).

All three miRNAs have target genes validated in the database. In the specific case of miR-146a, its main KEGG metabolic pathways are cell adhesion molecules and phagosome, but it also participates in the regulation of cancer metabolism; one of the genes that stand out in this process is *ZEB2*, which is a transcription factor that plays a role in transforming growth factor-β signaling pathways, which are essential during early fetal development, and their dysregulation has been characterized in different types of cancer[26]. The miRTarBase software does not show an intersection of miRNAs in metabolic pathways, as they do not share predicted target genes.

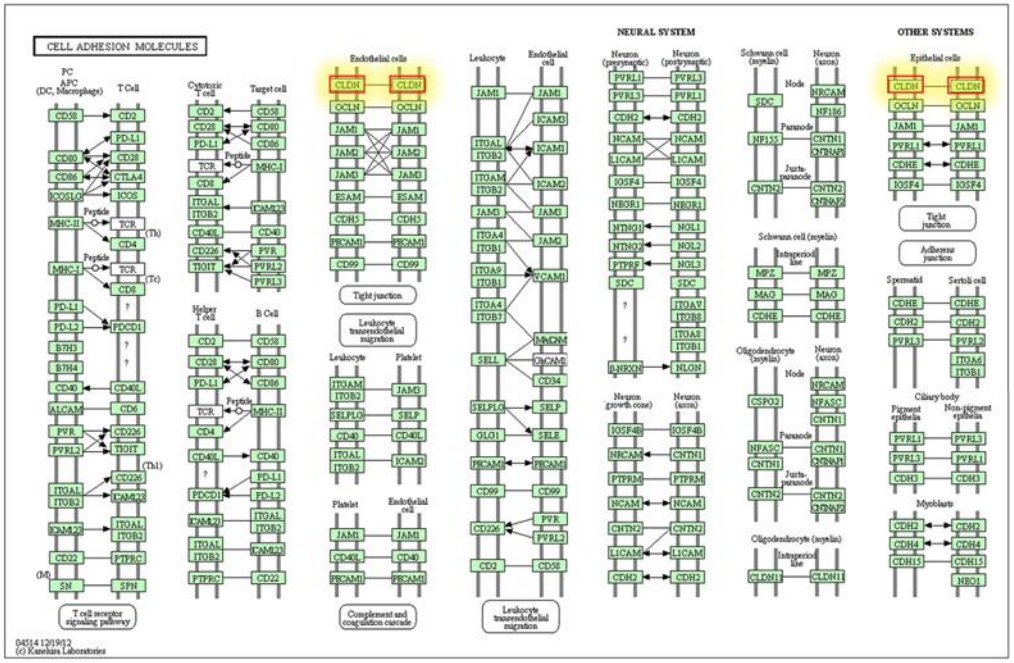
Table 1: Prediction of the main metabolic pathways and genes to which miR-146a, miR-146b, and miR-155 miRNAs bind and intercept

miRNA	KEGG metabolic pathway	Outstanding target genes	*P*-Value
miR-146a	Cell adhesion molecules	CLDN16 (claudin 16)	0.02
	Phagosome	ATP6V1C2 (ATPase H+ transporting V1 subunit C2)	0.03
miR-146b	Degradation of valine, leucine, and isoleucine	BCKDHB (branched chain keto acid dehydrogenase) ABAT (4-aminobutyrate aminotransferase)	0.006
	Thyroid hormone signaling pathway	THRA (thyroid hormone receptor alpha) RXRB (retinoid X receptor beta)	0.01
miR-155	Sulfur relay system	MOCS2 (molybdenum cofactor synthesis 2)	0.006
	Tryptophan metabolism	IDO-1 (indoleamine 2,3-dioxygenase 1)	0.01

The analysis was performed from the mature sequence -3p of each miRNA.

Figure 1: KEGG metabolic pathways predicted for miRNA 146a according to the genes it binds to. Panel A) Cell adhesion molecules, Panel B) Phagosome. The genes that stand out in this research are highlighted (yellow)

A)



B)

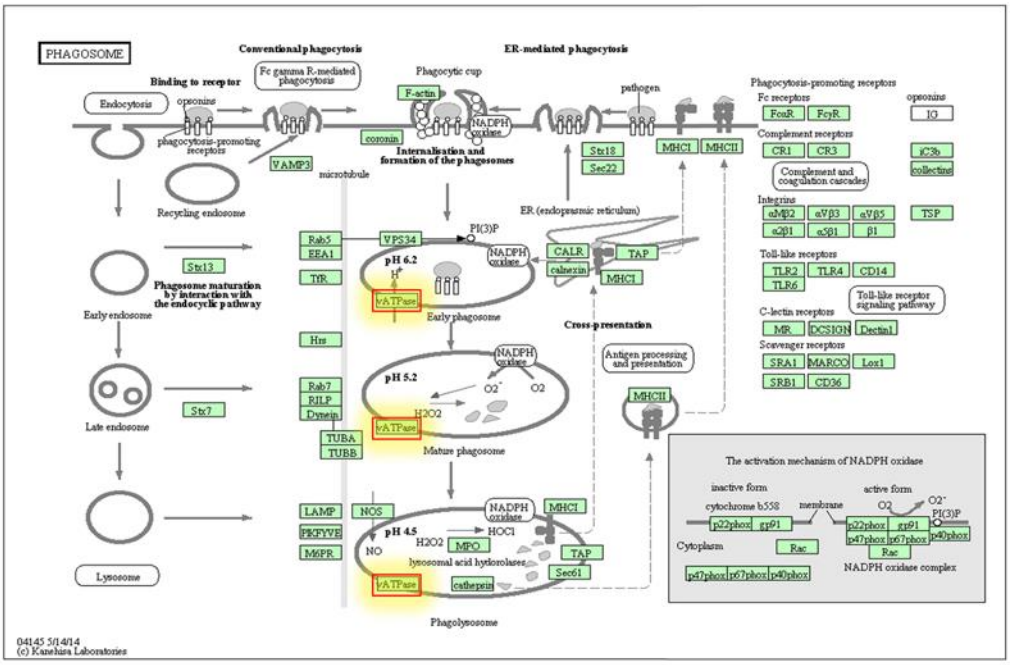
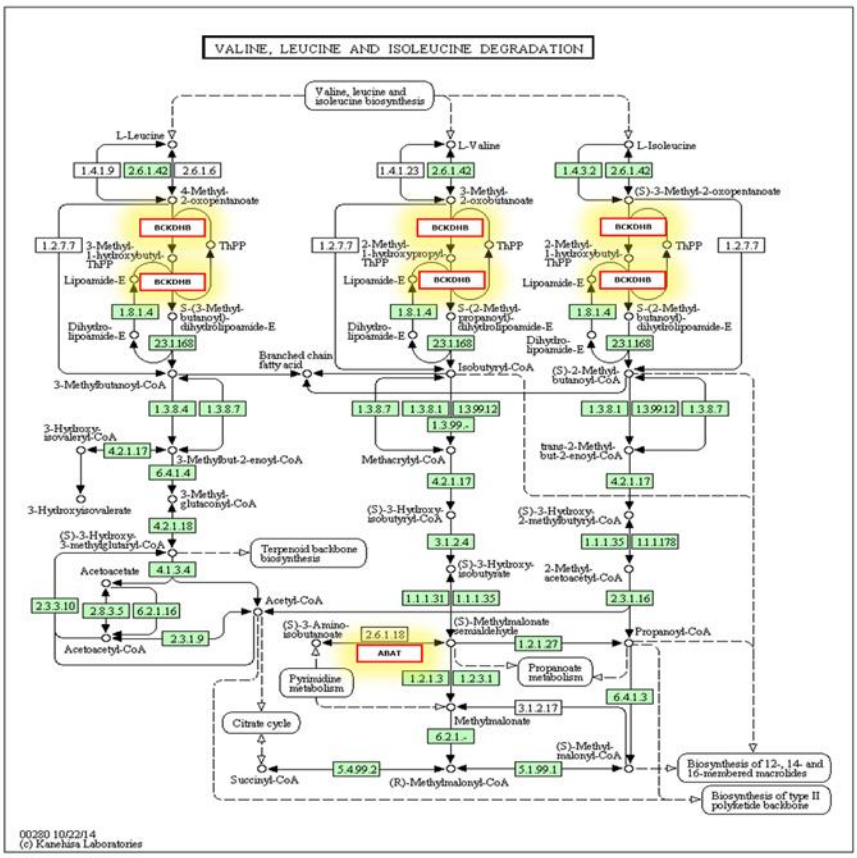


Figure 2: KEGG metabolic pathways predicted for miRNA 146b according to the genes it binds to. Panel A) Degradation of valine, leucine, and isoleucine, Panel B) Thyroid hormone signaling pathway. The genes that stand out in this research are highlighted (yellow)

A)



B)

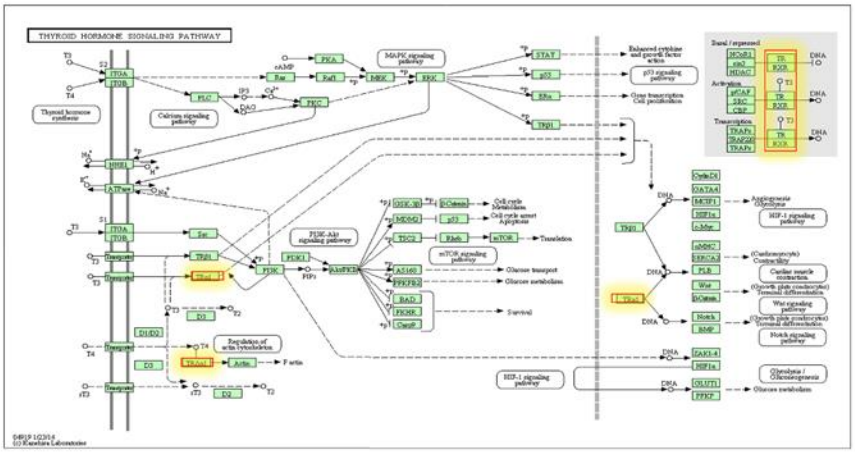
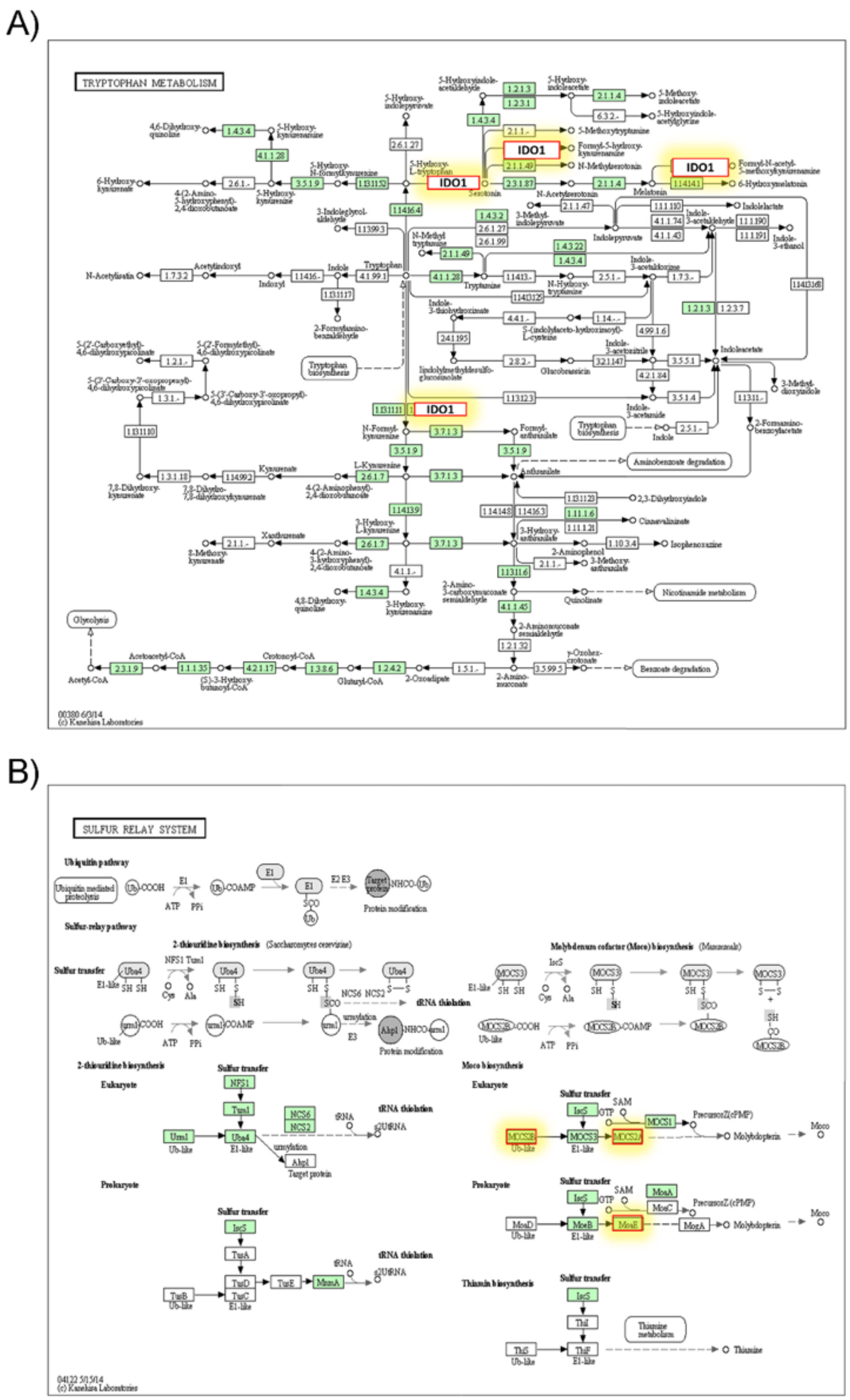


Figure 3: KEGG metabolic pathways predicted for miRNA 155 according to the genes it binds to. Panel A) Tryptophan metabolism, Panel B) Sulphur relay system. The genes that stand out in this research are highlighted (yellow)



Discussion

In the analysis of miR-146a, two important predicted metabolic pathways were found, where there are two genes with which this miRNA interacts in independent pathways; one of them is the *CLDN16* gene, which encodes claudin 16. Claudins are proteins with transmembrane domains found in the area of tight junction between epithelial and endothelial cells; together with other proteins, they form pores and are key components of the paracellular channel. Paracellular channels at the tight junction have properties of ionic selectivity, pH dependence, and other effects[27]. The overexpression of claudin-16 has been associated with ovarian cancer and other diseases, and its importance has been determined in cellular magnesium reabsorption[27].

One study determined the expression pattern of some claudins (2 and 4) and analyzed structural changes in colon biopsies in patients with TB. The results show that claudin-2 expresses itself in the area of tight junction between cells, and no structural changes were observed in the tissue analyzed[28]. Recently, the effect of TB infection on the expression of cell-binding proteins in the central nervous system (CNS) was studied. These results suggest that Claudin-5 decreases its expression and changes its location within the cell in response to infection with *Mtb* of the N15 strain, suggesting that *Mtb* affects the expression of brain proteins at cell junctions. This damage consisted of cellular changes suggestive of toxicity due to the observation of signs of necrosis[29].

The *ATP6V1C2* gene encodes an enzyme that is an ATPase; some studies have suggested the importance of P-type ATPases in the physiology and intracellular survival of mycobacteria[30]. A human transcriptional profile of ATPases under conditions of hypoxia, oxidative stress, starvation, and intoxication by chemical agents and infection processes *in vitro* and *in vivo* evidenced the differential expression of these transporters in these conditions. ATPase is a highly conserved proton pump that expresses itself in cells[31]. Recently, a study was conducted where two compounds that inhibit the growth of drug-sensitive and drug-resistant TB strains were studied; in this study, through transcriptomic assays, changes in the expression of certain genes in response to TB infection were shown; one of these genes was *ATP6V1C2*, which was found to be overexpressed in response to TB infection[32].

miR-146b interacts with prominent genes involved in energy production in cells, such as dehydrogenase (*BCKDHB*), and with aminotransferase (ABAT), which is involved in the degradation of valine, leucine, and isoleucine; some reports indicate that increased serum lactate dehydrogenase activity is an indicator of presumptive diagnosis of pneumonia and other infections such as TB[33].

miR-155 showed interaction with the *MOCS*2 and *IDO*-1 genes, which come from the sulfur relay system and tryptophan metabolism, respectively. The *MOCS2* gene encodes two different proteins, MOCS2A and MOCS2B; these two together form the molybdopterin synthase enzyme, which is involved in the biosynthesis of the molybdenum cofactor (MoCo), which is a prosthetic group. MoCo-dependent enzymes are involved in many biological processes; interestingly, MoCo works directly on ethylbenzene dehydrogenases and other enzymes[34]. Molybdenum (Mo) is necessary for several enzymes, such as sulfite oxidase and aldehyde oxidase, among others, to have their function. The function of those enzymes is the breakdown of substances in the body, some of which are toxic if not metabolized. Some mycobacteria have genes that code for MoCo. *Mtb* possesses multiple homologs that encode synthase in the biosynthesis of MoCo; this suggests that its expansion may fulfill different cellular functions[35].

Mo enzymes are catalysts in energy generation and detoxification reactions, among other functions. It is known that the substrates converted by bacterial Mo enzymes, which are important for virulence, are of the group that is generated in the host during inflammation or signaling network. This suggests that they could be important drug targets[36]. Mo enzymes catalyze important redox reactions. Mycobacteria have several enzymes that contain Mo; they help regulate *Mtb* latency. The MoCo cofactor is the common cofactor of Mo enzymes in mycobacteria[37]. In some experiments, a novel pathway that uses *Mtb* for resistance to host-imposed stress (hypoxia) has been identified; this ability of *Mtb* to persist under hypoxia conditions contributes to TB latent in the host. Through horizontal transfer, *Mtb* acquired the moaA1-D1 gene, which is involved in the biosynthesis of MoCo; namely, these genes have homologs present in the entire genus *Mycobacterium*; interestingly, the moaA1-D1 genes are induced under hypoxia conditions[38]. Evolutionarily, the *Mtb* complex has developed mechanisms for success, in part by acquiring genes involved in pathogenesis. Deciphering and knowing the mechanisms through which *Mtb* causes a disease is relevant to identifying unknown targets of interest for developing new methods of control, diagnosis, and therapy of the disease. The broad study of the biosynthesis of the MoCo enzymes of *Mtb* will help identify promising drug targets to control TB, especially latent TB.

The *IDO*-1 gene encodes an enzyme found mainly in macrophages; the enzyme participates in the degradation of tryptophan that generates kynurenine; this metabolic pathway constitutes a mechanism of modulation of the immune response. Indolamine 2,3-dioxygenase (IDO-1) is an enzyme found in numerous cells[39]. IDO-1 helps break down tryptophan into kynurenine inside cells and thus regulates the availability of tryptophan. This has broad implications for the body's immune response. The IDO-1 protein has been reported to be overexpressed in response to *Mtb* infection in human and murine macrophages *in vitro*. The overexpression of IDO-1 has also been correlated with the expression of other inflammatory markers, such as C-reactive protein, and the poor diagnosis of patients with TB[40,41].

In vitro studies show that the activity of IDO-1 in antigen-presenting cells inhibits the proliferation of mycobacterial antigen-specific T cells. The activity of IDO-1 could play an important role in inhibiting the *Mtb*-specific adaptive immune response and could help the pathogen survive in the infected host. Tryptophan metabolism is a means of regulating T cell functions, such as tumor-induced immune system evasion, peripheral tolerance, and inflammation during infection[40,42]. Activation of tryptophan metabolism is an antimicrobial mechanism that occurs against some pathogenic bacteria. The activation of IDO-1 and tryptophan metabolism in macrophages within the CNS is related to AIDS-associated dementia and other inflammatory brain diseases[43]. Intervening the activity of the IDO-1 enzyme is a promising strategy for developing treatments for HIV-associated neurological disorders. In relation to the high prevalence of individuals infected with HIV and *Mtb*. It is also an effective (host-directed) therapy against TB.

Although this analysis was performed on the miRNAs that have been described in humans, it is important to note that the genome of *M. bovis* has similarity of more than 99.95 % with *M. tuberculosis* at the nucleotide level; nevertheless, *M. bovis* has lost part of its genome due to genetic mutations, through deletion mechanisms; this makes it smaller (*M. bovis* AF2122/97: 4’345,492 bp) compared to *M. tuberculosis* (CDC1551: 4’403,836 bp)[44,45]. Interestingly, it has been suggested that *M. tuberculosis* arose from *M. bovis* during the period when man domesticated cattle, approximately 10-15,000 years ago, when it infected humans[46]. This assertion is based on the observation of infection (caused by several strains of *M. bovis*) in different animal hosts, including humans; on the other hand, natural infection of *M. tuberculosis* is, up to the date of this publication, apparently restricted to humans[47]. This close similarity between these two species makes the study of gene products, protein products, and miRNAs that may be analogous or equivalent between *M. tuberculosis* and *M. bovis* viable to try to understand a little more about the pathogenesis of tuberculosis caused by these two species, and perhaps to lay the foundations for the design of new biomarkers or possible therapeutic targets.

Conclusions and implications

The findings shown here suggest that miR-146a, miR-146b, and miR-155 are associated with activating specific molecular mechanisms in response to TB. The genes with which miR-146a and miR-155 interact or bind are involved in the immune response and cellular processes essential during TB infection.

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Conflict of interest

The authors declare that they have no conflict of interest.

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