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A putative short-chain dehydrogenase Rv0148 of *Mycobacterium tuberculosis* affects bacterial survival and virulence

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ABSTRACT

During infection, *Mycobacterium tuberculosis* combats the stress generated by the host cells through the action of short-chain dehydrogenases/reductases (SDRs). Rv0148 belongs to the oxidoreductase family with the SDRs domain, which regulates the homeostasis of *M. tuberculosis.* In our earlier studyusing knockout mutant strain (Δ0148), we reported that Rv0148 is involved in intermediary metabolism, drug resistance and cell homeostasis of *M. tuberculosis*. In the current study, we explored the functional role of Rv0148 using gene knockout mutant *in-vitro* and *in-vivo* models of infection. We report the Δ0148 is attenuated for virulence of *M. tuberculosis.* During human monocyte (THP-1) cell line infection, *M. tuberculosis* Δ0148 displayed reduced intracellular survival compared to the wild type at successive time points. Similarly, in a guinea pig animal model of aerosol infection, Δ0148 displayed a growth attenuation at 5- and 10-week post-infection in the lungs and spleen compared to the wild-type *M. tuberculosis* and Rv0148-complemented Δ0148 strains. Our study suggest that Rv0148 has a distinct role in the intracellular virulence of *M. tuberculosis.*

Introduction

Mycobacterium tuberculosis (*M. tuberculosis*), the causative agent of Tuberculosis (TB) that continues to be the leading infectious disease primarily affecting the lungs. The existence of *M. tuberculosis* in humans was reported thousands of years ago and still prevailing as a verysuccessful pathogen (John, 2019; Bussi and Gutierrez, 2019). In 2020, the World Health Organization (WHO) reported around 10 million active TB cases and 1.3 million deaths (WHO, 2021) . Aerosols containing *M. tuberculosis* causes infection primarily in the lungs and result in the development of pulmonary TB. During pulmonary infection, *M. tuberculosis* enters into the alveolar macrophages and alters the host's innate immune system (Uribe-Quero and Rosales, 2017). Within the macrophages, *M. tuberculosis* resides in the phagosomes and facilitates the environment for its replication by altering the host defense mechanisms (Chai et al., 2018; Simmons et al., 2018; Rosales and Uribe--Querol, 2017). As a result of infection, macrophages generate immune responses by secreting cytokines, chemokines and antimicrobial proteins (Pieters, 2008). Bacterial oxidoreductases are involved in neutralizing oxidative stress generated by the host and, thus, plays a key role in the virulence during infection (He et al., 2017). Recently, we have reported that the *M. tuberculosis* Rv0148 is an oxidoreductase possessing short-chain dehydrogenase (SDRs) domain, and is involved in homeostasis and host immunity (Bhargavi et al., 2020).

During *M. tuberculosis* infection, innate and adaptive immune responses generated by the host against pathogen are regulated by microRNAs (miRNAs) (Singh et al., 2013), which control various genes involved in several immune-associated pathways (Meng et al., 2014; Behrouzi et al., 2019).. Thus, we predicted that function of Rv0148 might be regulated by miRNAs by the data from our earlier study (Bhargavi et al., 2020). Currently, identification of miRNAs regulating *M. tuberculosis* infection using *in-silico* approaches, followed by their characterization by *in-vitro* and *in-vivo* models have contributed to our understanding of host-*M. tuberculosis* interactions (Etna et al., 2018). Based on *in-silico* analysis, a study by Etna et.al reported that the host miRNAs might target mycobacterial genes involved in the survival of bacteria and disease development (Etna et al., 2018). However further studies are needed to validate these findings, which would help to identify potential therapeutic targets, as well as biomarkers for TB.

In the current study, we used *in-vitro* and *in-vivo* models to validate

Abbreviations: SDRS-, Short Chain Dehydrogenase/Reductase; M. tuberculosis-, Mycobacterium tuberculosis; miRNA-, Micro RNA; PCR-, Polymerase Chain Reaction; RT PCR-, Real Time PCR.

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the specific role of Rv0148 in the virulence of *M. tuberculosis* during infection. In addition, we also performed a preliminary analysis using *in-silico* tools to identify the functional target miRNAs of Rv0148We observed that during human macrophage cell line infection, the *M. tuberculosis* Δ0148 strain exhibited attenuated *in-vitro* growth, compared to the wild-type and complemented strains. Our studies in athe guinea pig model of pulmonary infection further confirmed the attenuated growth of this mutant in the lungs and spleen, with these tissues exhibiting reduced gross pathology. In addition, using *in-silico* tools, we predicted potential target miRNAs of Rv0148. Accordingly, identified eight target miRNAs that were not functionally associated with *M. tuberculosis*. Furthermore, we used MiRTargetLink to study the interaction between the eight target miRNAs associated with *M. tuberculosis* Rv0148 and other miRNAs reported earlier (Zheng et al., 2015). Interestingly, we found that miRNA-582–5p, associated with apoptosis, interacted with eight other miRNAs. We observed that during human macrophage cell line infection, the expression of miRNA −582–5p was low in Δ0148, compared to the wild-type and complemented strains. We also confirmed the expression of miRNA-582–5p expression in the lungs, spleen and serum of infected guinea pigs; expression of miRNA-582–5p was considerably low in tissues infected by mutant strain as compared to H_{37}Rv. Based on these results, we propose that the interplay between miRNA-582–5p and Rv0148 is important in regulating the survival and virulence of *M. tuberculosis*. Howeveradditional experiments are required to support this hypothesis. In summary, the current study report that Rv0148 is an important virulence factor that is involved in intracellular survival and pathogenesis of *M. tuberculosis.*

2. Materials and methods

2.1. Bacterial strains and growth conditions

In the current study, wild type H37Rv, Δ0148 and CΔ0148 strains were grown in Middlebrook 7H9-OADC broth (Difco, USA) supplemented with 0.2% glycerol,0.05% Tween-80 and antibiotics cyclohexiamide (10 μg/ mL), carbenicillin (50 μg/ mL), hygromycin (50 μg/ mL) and kanamycin (25 μg/ mL), whenever necessary, and incubated at 37°C with 180 rpm shaking (Table 1). The gene knockout mutant of *M. tuberculosis* Rv0148 (Δ0148) was constructed using specialized transduction, as we reported previously (Bhargavi et al., 2020). A PCR-confirmed clone of this strain was used for *in-vitro* and *in-vivo* studies reported here. Complementary strain CΔ0148 was constructed using pMV261 (Stover et al., 1991) and electroporated into knockout strain Δ0148.

All other chemicals were purchased from Sigma-Millipore, unless mentioned otherwise.

Table 1
Plasmids, mutant constructs and cell lines used in the study.

Plasmids	Description	Reference/ origin
pMV261	*E. coli* mycobacterial shuttle vector, *kan*^{R}, *hsp*60 promoter carrying 6x- His-tag (GTG-GTG-GTG-GTG-GTG-GTG)	(Stover et al., 1991)
Constructs		
Δ0148	p0004- SacB carrying the left and right arm fragments of Rv0148 gene from *M. tuberculosis*, *hyg*^{R} (four fragment ligation)	Bhargavi et al., 2020
CΔ0148	Complement of Rv0148 gene knockout mutant of *M. tuberculosis*	Bhargavi et al., 2020
Cell lines		
THP-1 cell line	Human leukemia monocytic cell line	Lab Stock

2.2. THP-1 macrophage infections

Intracellular viability of wild type H37Rv, Δ0148 and CΔ0148 strains was assessed using human monocytic cell line THP-1, which has become a valuable *in-vitro* model to determine various host-pathogen studies like intracellular survival, cytokine profiling and immune-associated pathways during *M. tuberculosis* infection. In brief, THP-1 cells were grown in Roswell Park Memorial Institute (RPMI) media supplemented with 10% fetal bovine serum (FBS) (Thermo scientific, USA) and 1% anti mycotic solution (GIBCO, USA). Cells were grown to reach 1×10^6 cells/ml, and cell count was confirmed through trypan blue staining. Viable cells were seeded onto 24-well tissue culture plates to differentiate into macrophages using 50 mM phorbol 12-myristate 13-acetate (PMA). Tissue culture plates were incubated in the presence of 5% CO_2 for 2 days at 37 °C, then washed with RPMI medium containing 10% FBS and incubated for a further 24 h. For infection, the bacterial strains were cultured in 7H9 media to mid-log phase (OD_{600} 0.5 – 0.8) and the number of bacteria were enumerated by plating on 7H10 OADC agar media. Bacterial inoculum (50–70 μl) was added to 10 ml of RPMI, to obtain a final multiplicity of infection of 10:1 (Bacteria: Macrophage). The cells were then infected with wild type H37Rv, Δ0148 and CΔ0148 in 1 ml cultures in triplicate wells. Infection was allowed for 4 h ($t = 0$), and the infected cells were treated with RPMI containing 1 μg/ml streptomycin to eliminate extracellular bacteria. Then the infected cells were washed with 1 ml of RPMI media 3 times and lysed with sterile water. The intracellular survival at day 0, 1, 3, 5 and 7 post infection was analysed by plating the serially diluted lysates of wild type H37Rv, Δ0148 and CΔ0148 strains-infected THP-1 cells onto 7H10 OADC agar without Tween-80. The plates were incubated for 3–4 weeks at 37 °C and the number ofbacterial colony forming units (CFU) were counted and data were represented as CFU per ml of the lysate, present in each well. The experiment was repeated 3 times.

2.3. Guinea pig infection studies

The pathogen-free 6–8 weeks old female Dunkin-Hartley guinea pigs of 300 – 350 gm body weight were maintained in cages provided with water and ad libidum food in National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra, India. The study was conducted as per the institutional animal ethics committee approval (File no: NJIL&OMD/3-IAEC/2019–03). Groups of guinea pigs ($n = 5$) were infected with 50 to 80 bacilli of wild type H_{37}Rv, Δ0148 or CΔ0148 strains through aerosol route using an aerosol chamber (Inhalation Exposure System, Glasscol Inc., IN, USA). The infected animals were euthanized at 5 and 10 weeks using Thiopentone sodium injection (100 mg/kg body weight) (Neon Laboratories Ltd., India). The animals were dissected aseptically and pathological changes in spleen, liver and lung tissues were recordedre. The organs were weighed and homogenized using a tissue homogenizer. The homogenized tissues were plated onto Middlebrook 7H11 agar plates supplemented with amphotericin (20 mg/ml), polymyxin B (15 mg/ml), carbenicillin (50 μg/ml) and cycloheximide (100 μg/ml), and the plates were incubated at 37 °C in 5% CO_2 incubator for 3–4 weeks. The colonies were enumerated and the bacterial load was expressed as $\log_{10}$ CFU/g of tissue.

2.4. Prediction of miRNA targets

The Rv0148 gene sequence was obtained from Mycobrowser (https://mycobrowser.epfl.ch/), and the translated sequence was used to query miRDB database with reference set as humans. The functional miRNA targets for Rv0148 were identified through miRDB (http://mirdb.org/miRDB/) (Wang, 2008; Chen and Wang, 2020) and miRBase (http://www.mirbase.org/) (Kozomara et al., 2019). Further, the interaction analysis between functionally targeted miRNAs of Rv0148 and reported miRNAs of *M. tuberculosis* was performed using MiRTargetLink Human 2.0 (Liu and Wang, 2019).

2.5. miRNA primer design

The miRNA sequence was obtained from the Sanger database. Since the miRNA sequence was too short, the primer synthesis was done by adding adaptors at specific ends by the manufacturer (Imperial life Sciences). The adaptor sequence of miRNA, which was additionally provided along with primers, was used during cDNA synthesis. To analyze the expression of miRNA, qRT-PCR was performed using 2 μg of RNA, 1 μl of 25 mM concentration dNTP with 1 μl of adapter sequence, and miRNA-specific primers (Table 2).

2.6. Extraction and quantification of miRNA from THP-1 macrophages

miRNA extraction from infected THP-1 cells was performed using miRNeasy mini kit as per the manufacturer's protocol (Qiagen, CA, USA). Briefly, 1 ml of uninfected, wild-type H37Rv, Δ0148, or CΔ0148 infected cells were combined with the Trizol and lysed using 0.1 mm beads in a bead beater,followed by chloroform extraction. The supernatant was precipitated with 100% ethanol and the pellet waswashed with 70% ethanol. The purified nucleic acid components were transferred to miRNA extraction column and extraction was done as per manufacturer's protocol and eluted with RNase free water (Qiagen, CA, USA). Eluted product was quantified using a Nanodrop spectrophotometer and used for cDNA synthesis with the addition of 10 pmoles of adaptor primer to each of the samples (uninfected, wild-type H37Rv, Δ0148, and CΔ0148 strains). Quantitative qPCR was performed using SYBR Green Master Mix (Thermo Scientific) as per suggestions provided by manufacturer using Applied Biosystems 7300 real-time PCR system. The reaction was performed using miRNA-582–5p forward and reverse primers and cDNA template. Conditions for amplification were: 1 cycle at 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. A distinct curve was noted, which represents the amplification and detection of miRNA-582–5p based on dissociation and calculated threshold. The expression of miRNA-582–5p was calculated from the Ct values of uninfected and infected samples.

2.7. Extraction of miRNA from guinea pig infected lung, spleen and serum

Serum was separated from the whole blood of euthanised guinea pigs infected with wild type H37Rv, Δ0148 or CΔ0148 strains at 5 weeks post infection. A 500 μl aliquot of serum was taken for miRNA extraction. The lung and spleen tissues were homogenized using lysing matrix D beads in a FastPrep-24 5 G instrument with a speed of 6.0 m/*sec* for 40 s. One ml of homogenized tissue wascombined with Trizol and used for miRNA extraction. Eluted miRNA from serum, lung and spleen were used to synthesize cDNA, and qRT-PCR was performed as mentioned above and analyzed to determine the expression of miRNA-582–5p.

2.8. Statistical analysis

The graphs represented in the manuscript were produced from three independent experiments, using. GraphPad Prism 5.0 (GraphStat Technologies). Data obtained from macrophage infection and intracellular survival in infected guinea pig lungs and spleen were analysed to compute the mean, standard deviation.Two-way ANOVA was performed to determine the statistical difference between the groups and the significance was reported as $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. The miRNA expression data was analysed using SDS software (Applied Biosystems) and the fold change in expression was determined using the relative quantity method between multiple groups. One way ANOVA was performed to calculate the significance between the groups and the significance was reported as $**p < 0.01$ and $***p < 0.001$.

Table 2
miRNA primers used in the study.

Name of primer	Sequence of miRNA primer	Purpose
hsa-miR-582–5p F	ACACTCCAGCTGGGTTACAGTTGTTCAACCA	Real Time PCR
hsa-miR-582–5pR	TGGTGTCGTGGAGTCG	Real Time PCR
hsa-miR-582–5p-RT (adaptor)	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTT GAGAGTAACTG	For cDNA synthesis

3. Results

3.1. Intracellular survival of Δ0148 in THP-1 cells

The intracellular survival of Δ0148 was determined in the human macrophage infection model (Theus et al., 2004). As shown in Fig. 1, although the CFU count of the mutant was reduced at 4 h after post infection, it was not statistically significant. However, the intracellular survival of Δ0148 on day 4, 24, 48, 72 and 96 h post infection was reduced significantly compared to wild type H37Rv and the complemented strains ($p < 0.001$) (Fig. 1).

3.2. In-vivo survival of Δ0148

Since we observed a decrease in the intracellular survival of Δ0148 in THP-1 cells, we were interested in checking the virulence of Δ0148 during *in-vivo* infection. We choose a guinea pig model, which is considered to be an effective animal model to study the pathogenesis and bacterial burden during *M. tuberculosis* infection. We observed that at five week post-infection, the lungs of animals infected by mutant strains exhibited fewer granulomatous lesions and lower gross disease pathology, compared tothose guinea pigs infected by wild type H37Rv or CΔ0148 (Figure S1). The bacterial load in the guinea pig lungs infected with Δ0148, wild type H37Rv and CΔ0148 was 3 log10 CFUs, 4.9 log10 CFUs and 4.8 log10 CFUs (Fig. 2A), respectively, at five week post-infection. The bacillary load in the lungs of Δ0148 infected animals reduced by about 1.9 folds, which was statistically significant compared to the lungs of wild type H37Rv or CΔ0148 infected animals. Similarly, the bacterial load in the spleen of Δ0148, wild type H37Rv and CΔ0148 infected guinea pigs at five week post-infection was 2.5 log10 CFUs, 4.2 log10CFUs and 4 log10CFUs, respectively with a statistically significant reduction of about 1.7 logs CFU in the Δ0148-infected animals compared to the wild type H37Rv infected animals (Fig. 2B).

At ten weeks post-infection, the lungs and spleen of Δ0148-infeced animals exhibited minimal gross pathology and granulomatous lesions compared to H37Rv-infected animals (Figure S2). The lung bacterial load in Δ0148, H37Rv and CΔ0148 infected animals were 3.5 log10 CFUs, 5.5 log10 CFUs and 5.4 log10 CFUs, respectively, which showed a statistically significant 2 folds reduction in the mutant group compared to wild type H37Rv infected animals (Fig. 2C). Similarly, the bacterial load in the spleen at ten weeks post- infection was 3 log10 CFUs, 4.5 log10 CFUs and 4.2 log10 CFUs, respectively, in animals infected with Δ0148, H37Rv and CΔ0148. This shows a statistically significant 1.6 folds reduction in bacterial burden in the Δ0148 compared to wild type H37Rv infected animals (Fig. 2D). Thus, a statistically significant decrease in the lung and spleen bacterial load was observed in animals infected with mutant strain at 5- and 10-weeks post infection,compared to the wild type H37Rv infected animals. Further, the animals infected with complement strain displayed bacterial burden equivalent to wild type H37Rv with similar pathological damages and lesions (Fig. 2. A–D). Since the deletion of Rv0148 appears to weaken bacterial survival and virulence, this gene is involved in the pathogenesis of *M. tuberculosis*.

3.3. In silico identification of miRNAs

The miRDB database predicted eight functional miRNA targets associated with Rv0148; miRNA-6775, miRNA-770–5p, miRNA-

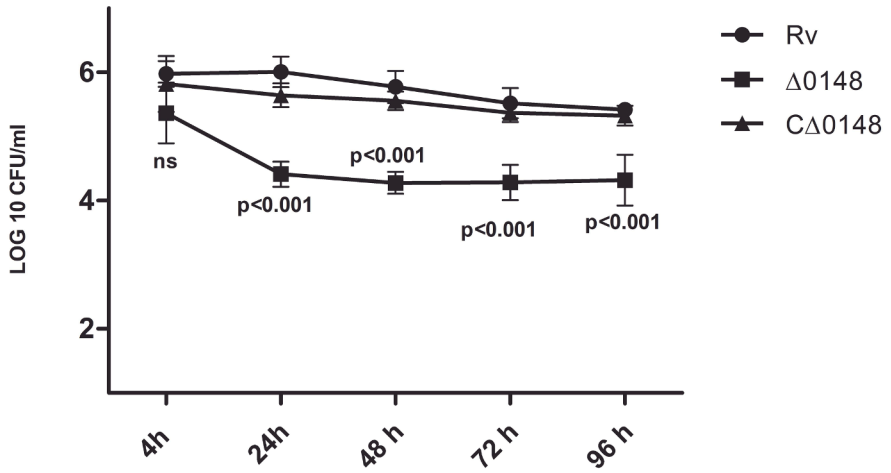

Fig. 1. Macrophage infection of Δ0148. The graphs representing log10 CFUs of H37Rv, Δ0148 and CΔ0148 strains were plotted by taking the mean and standard deviation using Two-way ANOVA. Significance at ** $p < 0.01$ and ***$p < 0.001$. The values represented in the graph were the mean of three independent experiments carried out using replicates.

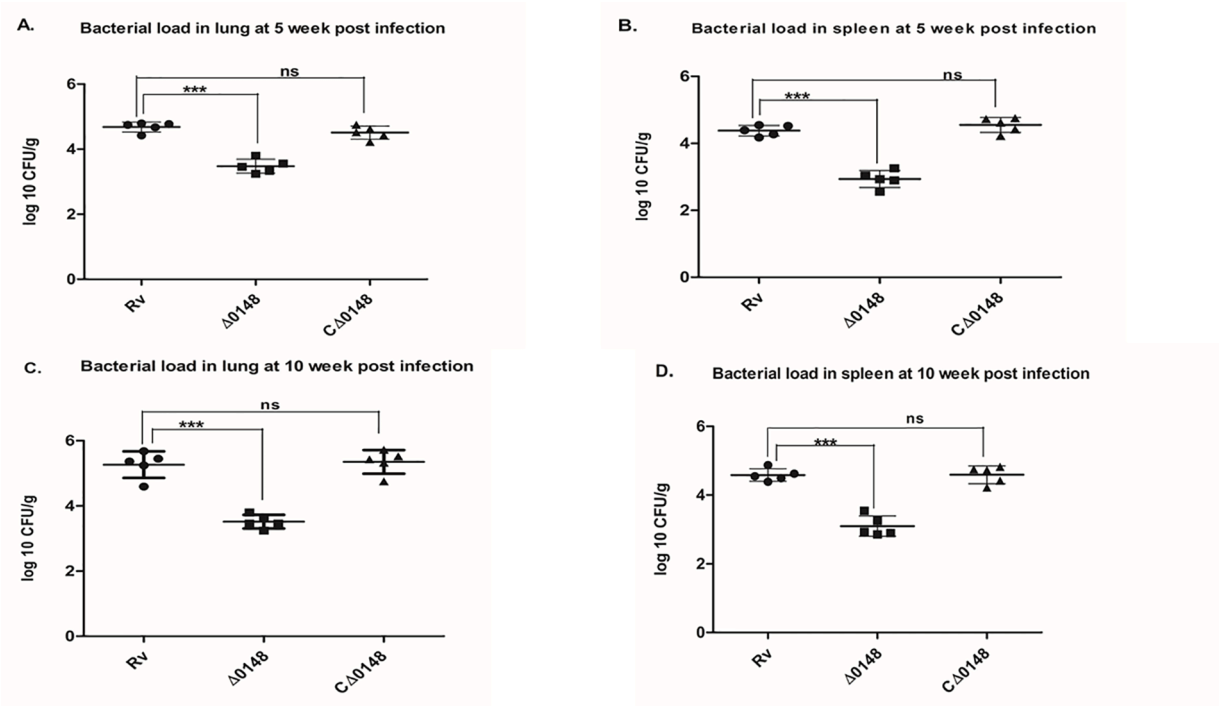

Fig. 2. A–D. Survival of Δ0148 in guinea pig lung and spleen at 5 and 10 weeks post infection. A. The graph represents bacterial load as log10 CFUs in guinea pig lungs infected with wild type H37Rv, Δ0148 or CΔ0148 strains at 5 weeks post infection. B. The graph represents bacterial load as log10 CFUs in guinea pig spleen after infection with wild type H37Rv, Δ0148 or CΔ0148 strains at 5 weeks post infection.. The graphs were plotted with the mean SEM (error bars) of 5 independent animals in each group. C. The graph represents bacterial load as log10 CFUs in guinea pig lungs infected with wild type H37Rv, Δ0148 or CΔ0148 strains at 10 weeks post infection. D. The graph represents bacterial load as log10 CFUs in guinea pig spleen after infection with wild type H37Rv, Δ0148 or CΔ0148 strains at 10 weeks post infection. The graphs were plotted by considering the mean of 5 independent animals in each group.

4712–5p, miRNA-5084, miRNA-4750–5p, miRNA-3613–5p, miRNA-5090 and miRNA-4715–3p (Fig. S3). We determined the probable interactions (weak or strong) between the target miRNAs from our studies and those miRNAs reported previously to be associated with *M. tuberculosis* pathogenesis, by (Zheng et al., 2015). To perform the interaction analysis between the miRNAs, we used MIRTarget link. The miRNAs were grouped under the common gene cluster Eph-family receptor-interacting protein B2 (EFNB2) based on their sequence and structure, and we predicted that miRNA-582–5p interacts with functional miRNA targets of Rv0148 (Fig. S4).

3.4. miRNA levels in infected THP-1 cells

Variable levels of miRNA-582–5p expression were detected in the lysate of THP-1 cells infected with different strains. miRNA-582–5p was upregulated in CΔ0148 and wild type compared to uninfected cells (Fig. 3. A).The level of miRNA-582–5p expression was downregulated by 2 folds in Δ0148 mutant, compared to the wild type H37Rv infected cells.

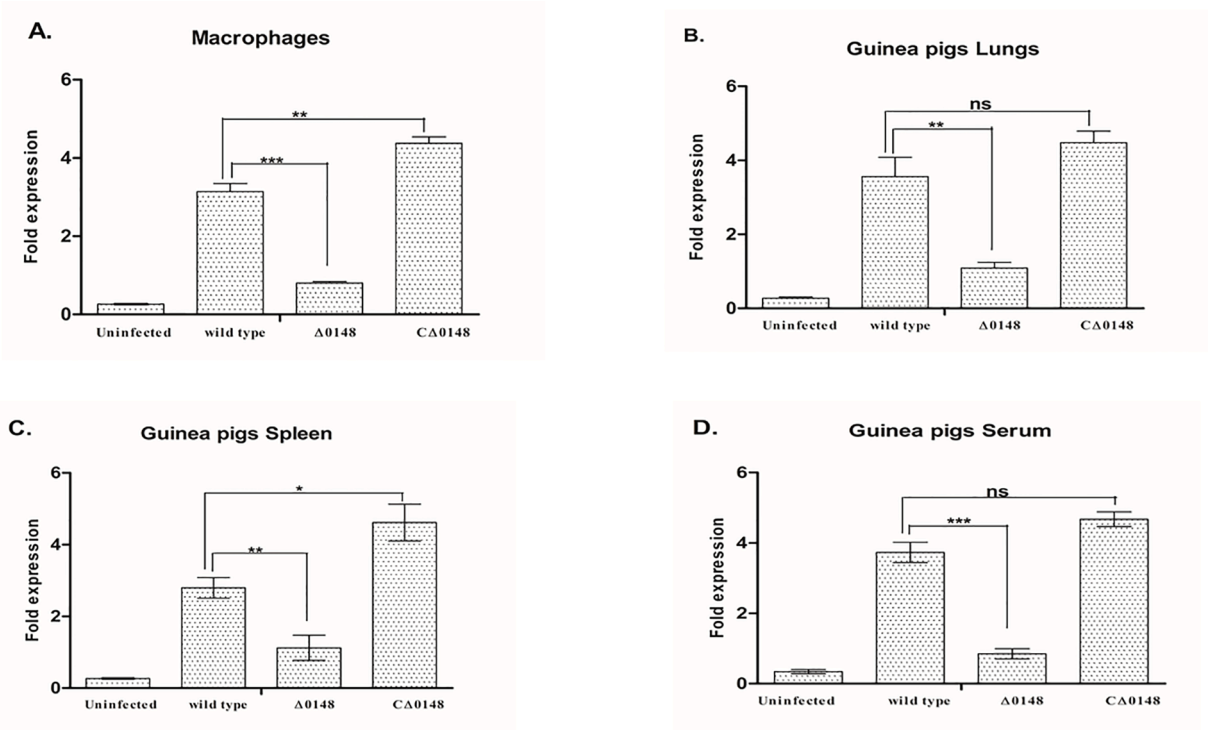

Fig. 3. A–D. Expression of miRNA-582–5p in THP-1 macrophages and in infected guinea pig tissues and serum. A. Expression of miRNA-582–5p in infected THP-1 macrophages. B. miRNA-582–5p expression in the lungs of guinea pigs infected with wild type H37Rv, Δ0148 and CΔ0148 strainsat 5 weeks post infection. C. Expression of miRNA-582–5p in the spleen of guinea pigs infected with wild type H37Rv, Δ0148 and CΔ0148 strains at 5 weeks post infection. D. Expression of miRNA-582–5p in the serum of guinea pigs infected with wild type H37Rv, Δ0148 and CΔ0148 strains at 5 weeks post infection. The obtained CT values were analysed using SDS software and relative quantification method was used to calculate the fold expression of miRNA between the groups. One way ANOVA was performed between the groups and the significance was reported as ** $p < 0.01$ and ***$p < 0.001$ and values plotted are mean and standard deviation from 3 independent experiments performed in triplicate (for THP-1 cells) or $n = 5$ animals per group (guinea pig samples).

3.5. miRNA levels in infected guinea pig lungs, spleen and serum

The expression of miRNA-582–5p was observed in the lungs, spleen and serum of guinea pigs infected with wild type H37Rv, Δ0148 and CΔ0148 strains at variable levels. Compared to the naïve, uninfected animals, the infected guinea pig tissues and sera had a higher level of miRNA-582–5p expression (Fig. 3. B–D). The mutant (Δ0148) infected animals displayed nearly 2.2-fold in lungs, 2-fold in spleen, and 2.5 -folds in serum lowerexpression of miRNA-582–5p compared to H37Rv. Although the expression of miRNA-582–5p was slightly higher in all the tested tissues and serum of the complemented strain- compared to H37Rv-infected samples, the difference was not statistically significant (Fig. 3. B–D).

4. Discussion

Mycobacterium tuberculosis Rv0148 is a putative short-chain dehydrogenases/reductases (SDRs), belonging to the oxidoreductase family. The functional role of oxidoreductases and SDRs in *M. tuberculosis* pathogenesis is not well understood. In our earlier study, we reported the examined the functional role of Rv0148 by constructing gene knockout mutant in *M. tuberculosis* using specialized transduction, reporting that this gene plays an important role in bacterial drug resistance, intermediary metabolism and homeostasis (Bhargavi et al., 2020). In addition to the above findings, the current study demonstrated that Rv0148 is involved in the survival of *M. tuberculosis*, since mutation in this gene attenuated bacterial growth in the THP-1 macrophage infection model and in the guinea pig infection model. These results suggest that Rv0148 is involved in the pathogenesis of *M. tuberculosis* by enabling the bacteria to survive intracellularly

Infection of host cells by *M. tuberculosis* induces changes in host cell metabolism that differentially regulates the expression of cytokines that impacts the clearance or progression of infection. To assess the infection and survival outcomes, we used THP-1 cell lineto infect with wild type H37Rv, Rv0148 mutant and Rv0148-complimenting *M. tuberculosis* strains and determined that the intracellular viability of the mutant was compromised, compared to wild type and complemented strains. Previously, we have reported that infection of THP-1 cesll by Δ0148 is was associated with enhanced secretion of pro inflammatory cytokines IL-6, TNF-α and IL-1β (Bhargavi et al., 2020). These cytokines are crucial in establishing antibacterial host response during *M. tuberculosis* infection. In this study, we observed impaired intracellular survival of the Δ0148 mutant in THP-1 cells. Together, these observations suggest that the proinflammatory cytokine levels induced by Δ0148 may be associated with the attenuated intracellular survival. Indeed, a study on gene knockout mutant of *M. tuberculosis zmp1* of, also reported increased IL-1β secretion, which was associated with reduced bacterial survival during macrophage infection (Sharon et al., 2008).

To further validate the virulence of Δ0148 in vivo, we used a guinea pig model of pulmonary *M. tuberculosis* infection. We observed that infection by Δ0148 displayed attenuated growth in guinea pig tissues, with reduction of bacterial burden and gross disease pathology in the lungs and spleen, compared to wild type H37Rv infected animals at 5 and 10 weeks post infection. Similar to our findings, an earlier gene deletion study on oxidoreductase type-2 NADH dehydrogenase (NDH-2) in *M. tuberculosis* reported partial attenuation in a mouse model of *M. tuberculosis* infection (Beites et al., 2019). In addition, mutants that are defective in oxidoreductases in other bacteria such as *Salmonella enterica* also reported to display conditional virulence suppressor phenotype in the mice model (Anwar et al., 2013). Thus, we propose that Rv0148 is involved in the regulation of bacterial growth and virulence, since mutation of this gene attenuates *M. tuberculosis* growth in

THP-1 cells and *in-vivo* survival in the lungs and spleen, which is also associated with fewer granuloma, The mycobacterial SDRs constitute a large family of oxidoreductases with NAD or NADP-dependent enzymes. Their role as diagnostic markers of TB and their involvement in drug resistance and virulence has been reported previously (Kakhki et al., 2019; Kaakoush et al., 2007). However, the precise functional role of Δ0148 in *M. tuberculosis* pathogenesis was not reported previously. In this study, we showed that Rv0148, a member of oxidoreductase (SDRs), is involved in the intracellular survival and virulence of *M. tuberculosis*.

To understand the mechanistic role of Rv0148 in modulating the host cell response, we have identified potential miRNA targets of Rv0148 using *in-silico* and wet lab experiments. Our preliminary data suggest that miRNA-582–5p is affected by Rv0148, particularly during intracellular survival, and that miRNA-582–5p regulates the attenuation of intracellular Δ0148 mutant survival. However, this hypothesis needs to be verified through additional experimental datas. We also predict that apoptotic miRNA-582–5p of *M. tuberculosis* regulates the expression of Rv0148. Indeed, we observed differential expression of apoptotic miRNA-582–5p in the human macrophage cell line and in guinea pig infected with wild type H37Rv, Δ0148 and complement strains (CΔ0148) of Rv0148. The significant downregulation of miRNA-582–5p in Δ0148 suggests that this miRNA might negatively regulate the intracellular survival of the mutant during THP-1 cell infection and guinea pig lungs and spleen. Further studies on the role of miRNA-582–5p in relation to the function of Rv0148 would help to understand the mechanistic interplay between Rv0148 and miRNA-582–5p during *M. tuberculosis* infection.

5. Conclusion

Overall, our findings suggest that the oxidoreductase Rv0148 gene is involved in intracellular survival, virulence and pathogenesis of *M. tuberculosis*.

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Ethics approval

The detailed procedure for the animal studies added in this study along with the animal requirements were assessed and approved by the Animal Ethics Committee of National Jalma Institute for Leprosy & Other Mycobacterial Disease, Agra, India. (File no: NJIL&OMD/3-IAEC/2019–03). Animals were maintained according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

CRediT author statement

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Project administration: Kannan Palaniyandi, Gunapati Bhargavi
Resources: Kannan Palaniyandi.
Supervision: Kannan Palaniyandi.
Visualization: Gunapati Bhargavi.
Writing – original draft: Gunapati Bhargavi.
Writing – review & editing: Kannan Palaniyandi.

Availability of data and materials: All data generated during the current study are included in this article and its supplementary files were included.

Declaration of Competing Interest

The authors state that they have no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2022.100113.

References

Anwar, N., Sem, X.H., Rhen, M., 2013. Oxidoreductases that act as conditional virulence suppressors in *salmonella enterica serovar typhimurium*. PLoS ONE 8, 64948. https://doi.org/10.1371/journal.pone.0064948.

Behrouzi, A, Alimohammadi, M, Nafari, A H, Yousefi, M H, et al., 2019. The role of host miRNAs on *Mycobacterium tuberculosis*. BMC EXRNA, 40. https://doi.org/10.1186/s41544-019-0040-y.

Beites, T., O'Brien, K., Tiwari, D., Engelhart, C.A., Walters, S., Andrews, J., Yang, H.J., Sutphen, M.L., Weiner, D.M., Dayao, E.K., Zimmerman, M., Prideaux, B., Desai, P.V., Masquelin, T., Via, L.E., Dartois, V., Boshoff, H.I., Barry, C.E., Ehrt, S., Schnappinger, D., 2019. Plasticity of the *Mycobacterium tuberculosis* respiratory chain and its impact on tuberculosis drug development. Nat. Commun. 10, 1–12. https://doi.org/10.1038/s41467-019-12956-2.

Bhargavi, G., Hassan, S., Balaji, S., Tripathy, S.P., Palaniyandi, K., 2020. Protein-protein interaction of Rv0148 with Htdy and its predicted role towards drug resistance in *Mycobacterium tuberculosis*. BMC Microbiol 20, 93. https://doi.org/10.1186/s12866-020-01763-1.

Bussi, C., Gutierrez, M.G., 2019. *Mycobacterium tuberculosis* infection of host cells in space and time. FEMS Microbiol. Rev. 43, 341–361. https://doi.org/10.1093/femsre/fuz006.

Chai, Q., Zhang, Y., Liu, C.H., 2018. *Mycobacterium tuberculosis*: an adaptable pathogen associated with multiple human diseases. Front. Cell. Infect. Microbiol. 8, 158. https://doi.org/10.3389/fcimb.2018.00158.

Chen, Y., Wang, X., 2020. miRDB: an online database for prediction of functional microRNA targets. Nucleic Acids Res 48, D127–D131. https://doi.org/10.1093/NAR/GKZ757.

Etna, M.P., Sinigaglia, A., Grassi, A., Giacomini, E., Romagnoli, A., Pardini, M., Severa, M., Cruciani, M., Rizzo, F., Anastasiadou, E., Di Camillo, B., Barzon, L., Fimia, G.M., Manganelli, R., Coccia, E.M., 2018. *Mycobacterium tuberculosis*-induced miR-155 subverts autophagy by targeting ATG3 in human dendritic cells. PLOS Pathog 14, e1006790. https://doi.org/10.1371/journal.ppat.1006790.

He, L., He, T., Farrar, S., Ji, L., Liu, T., Ma, X., 2017. Cellular physiology and biochemistry cellular physiology and biochemistry antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. Cell Physiol. Biochem. 44, 532–553. https://doi.org/10.1159/000485089.

John, C.A., 2019. Realizing the World Health Organization's end TB strategy (2016–2035): how can social approaches to tuberculosis elimination contribute to progress in Asia and the Pacific? Trop. Med. Infect. Dis. 4 (1), 28. https://doi.org/10.3390/tropicalmed4010028.

Kaakoush, N.O., Kovach, Z., Mendz, G.L., 2007. Potential role of thiol:disulfide oxidoreductases in the pathogenesis of *Helicobacter pylori*. FEMS Immunol. Med. Microbiol. 50, 177–183. https://doi.org/10.1111/j.1574-695X.2007.00259.x.

Kakhki, R.K., Neshani, A., Sankian, M., Ghazvini, K., Hooshyar, A., Sayadi, M., 2019. The short-chain dehydrogenases/reductases (SDR) gene: a new specific target for rapid detection of *Mycobacterium tuberculosis* complex by modified comparative genomic analysis. Infect. Genet. Evol. 70, 158–164. https://doi.org/10.1016/j.meegid.2019.01.012.

Kozomara, A., Birgaoanu, M., Griffiths-Jones, S., 2019. miRBase: from microRNA sequences to function. Nucleic Acids Res 47, D155–D162. https://doi.org/10.1093/NAR/GKY1141.

Liu, W., Wang, X., 2019. Prediction of functional microRNA targets by integrative modeling of microRNA binding and target expression data. Genome Biol 20. https://doi.org/10.1186/S13059-019-1629-Z.

Meng, Q.L., Liu, F., Yang, X.Y., Liu, X.M., Zhang, X., Zhang, C., Zhang, Z.De, 2014. Identification of latent tuberculosis infection-related microRNAs in human U937 macrophages expressing *Mycobacterium tuberculosis* Hsp16.3. BMC Microbiol 14, 37. https://doi.org/10.1186/1471-2180-14-37.

Pieters, J., 2008. *Mycobacterium tuberculosis* and the macrophage: maintaining a balance. Cell Host Microbe 3 (6), 399–407. https://doi.org/10.1016/j.chom.2008.05.006.

Rosales, C., Uribe-Querol, E., 2017. Phagocytosis: a fundamental process in immunity. Biomed Res. Int. https://doi.org/10.1155/2017/9042851.

Simmons, J.D., Stein, C.M., Seshadri, C., Campo, M., Alter, G., Fortune, S., Schurr, E., Wallis, R.S., Churchyard, G., Mayanja-Kizza, H., Boom, W.H., Hawn, T.R., 2018. Immunological mechanisms of human resistance to persistent *Mycobacterium tuberculosis* infection. Nat. Rev. Immunol. 18 (9), 575–589. https://doi.org/10.1038/s41577-018-0025-3.

Singh, Y., Kaul, V., Mehra, A., Chatterjee, S., Tousif, S., Dwivedi, V.P., Suar, M., Van Kaer, L., Bishai, W.R., 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. https://doi.org/10.1074/jbc.C112.439778.

Sharon, S., M., Silvana, K., R., Alexander, S., D., Christine, K., Stefan, E., Burkhard, S., Graham, S., T., Peter, S., Vojo, D., 2008. *Mycobacterium tuberculosis* prevents inflammasome activation. Cell Host Microbe 3, 224–232. https://doi.org/10.1016/J.CHOM.2008.03.003.

Stover, C K, de la Cruz, V F, Fuerst, T R, Burlein, J E, Benson, L A, et al., 1991. New use of BCG for recombinant vaccines. Nature.

Theus, S.A., Cave, M.D., Eisenach, K.D., 2004. Activated THP-1 cells: an attractive model for the assessment of intracellular growth rates of *Mycobacterium tuberculosis* isolates. Infect. Immun. 72, 1169. https://doi.org/10.1128/IAI.72.2.1169-1173.2004.

Uribe-Quero, E., Rosales, C., 2017. Control of phagocytosis by microbial pathogens. Front. Immunol. https://doi.org/10.3389/fimmu.2017.01368.

Wang, X., 2008. miRDB: a microRNA target prediction and functional annotation database with a wiki interface. RNA 14, 1012. https://doi.org/10.1261/RNA.965408.

WHO, 2021. Global Tuberculosis Report. Tuberculosis Report.

Zheng, L., Leung, E., Lee, N., Lui, G., To, K.-F., Chan, R.C.Y., Ip, M., 2015. Differential microRNA expression in human macrophages with *Mycobacterium tuberculosis* infection of Beijing/W and non-Beijing/W strain types. PLoS ONE 10, e0126018. https://doi.org/10.1371/journal.pone.0126018.