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MicroRNA-708-5p regulates mycobacterial vitality and the secretion of inflammatory factors in *Mycobacterium tuberculosis*-infected macrophages by targeting TLR4

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Abstract. – OBJECTIVE: Tuberculosis (TB), a major public health problem worldwide, is induced by *Mycobacterium tuberculosis* (M.tb) infection. Macrophages serve as the cellular home in immunoreaction against M.tb infection, which is tightly adjusted by host microRNAs (miRNAs) expression. The purpose of this research was to investigate the function mechanism of miR-708-5p in mycobacterial vitality and immunoreaction in human macrophages (HTP-1 and U937 cells) after M.tb infection.

MATERIALS AND METHODS: Colony-forming unit (CFU) assay was used to measure mycobacterial survival. The interferon-γ (IFN-γ), interleukin-6 (IL-6), IL-1β, and tumor necrosis factor-α (TNF-α) expression in cell supernatants were detected by enzyme-linked immunosorbent assay (ELISA). The relationship between miR-708-5p and toll-like receptor 4 (TLR4) was predicted and revealed by TargetScan and Dual-Luciferase Reporter Assay.

RESULTS: Our results suggested that the miR-708-5p level was increased in a concentration-dependent and time-dependent manner in M.tb-infected human macrophages. Compared with the control group, miR-708-5p mimic enhanced the intracellular mycobacterial survival during M.tb infection, while miR-708-5p down-regulation suppressed the mycobacteria survival. Moreover, the secretion of the pro-inflammatory factors, including IFN-γ, IL-6, IL-1β, and TNF-α significantly enhanced in M.tb-induced macrophages, while miR-708-5p mimic reduced these inflammatory cytokines. Conversely, miR-708-5p inhibitor dramatically promoted the accumulation of the inflammatory factors in macrophages after M.tb treatment. In addition, evidence indicated that TLR4 was a direct and functional target of miR-708-5p. MiR-708-5p negatively regulated the TLR4 level in macrophages.

CONCLUSIONS: The findings indicated that miR-708-5p level was upregulated in macrophages after M.tb infection. And miR-708-5p could regulate mycobacterial vitality and inflammatory response to M.tb infection in human macrophages by targeting TLR4.

Key Words:
MicroRNA-708-5p, Tuberculosis, *Mycobacterium tuberculosis*, Inflammatory responses, TLR4.

Introduction

Tuberculosis (TB), a chronic infectious disease, is caused by *Mycobacterium tuberculosis* (M.tb) infection[1]. M.tb remains a major bacterial cause of mortality and morbidity worldwide[2]. Macrophages can serve as the cellular hosts for M.tb, and it plays an essential role in immunoreaction against M.tb infection[3,4]. Also, it is reported that macrophages played vital roles in reducing M.tb vitality and secreting inflammatory factors, including IFN-γ, IL-6, IL-1β, and TNF-α[5,6]. Unfortunately, to date, valid diagnoses and treatment for TB are rarely known, and the immunologic mechanism of M.tb-infected macrophages is still unknown. Previously Fu et al[7] have shown that microRNA (miRNA) could modulate the activity of the protein-encoding genes, and regulate the molecular mechanisms of pathogenesis in M.tb-infected macrophages.

MiRNAs are small, non-coding RNAs with 20-22 nucleotides in length, which can regulate various targeted gene expression by binding with the 3′-untranslated region (3′-UTR) of target genes[8,9]. Recently, many reports have indicated that miRNAs participate in immunoreaction in M.tb-induced macrophages, and the secretion of inflammatory elements could be altered by mycobacteria within macrophages, such as miR-132,

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miR-26a, miR-30A, and miR-206[10-13]. Lately, Gu et al[14] indicated that miR-23a-5p could regulate mycobacterial vitality by targeting toll-like receptor 2 (TLR2) after M.tb infection. However, the detailed mediation mechanism of miR-708-5p in the immunoreaction of M.tb-induced macrophages remains to be elucidated.

TLRs play an important role in mediating inborn and compliant host immunoreaction by targeting ligands in causative agent[15]. Lately, reports have demonstrated that miRNAs could modulate the immune response in macrophages *via* altering the TLR signaling pathway[15]. TLR4, which belongs to the TLR family, is situated in the cell membrane and cytoplasm. Muller et al[16] have suggested that M.tb infection can interfere Nfkb nuclear factor kappa B (NF-kB) pathway by mediating TLR4 signaling, which may lead to the increased pro-inflammatory factors secretion in macrophages.

MiR-708-5p has been found to be involved in many diseases, especially in cancers, but also in neurodegeneration and the immune response[17]. Considering the above reports, in this work, we performed the in-depth investigation of the functional mechanism of miR-708-5p in mycobacterial vitality and inflammatory response in M.tb-induced human macrophages, which may provide us with better therapies for tuberculosis treatment.

Materials and Methods

Mycobacterial Preparation for Macrophage Infections

M. tuberculosis H37Rv were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) and were propagated in Middlebrook 7H9 broth medium containing with 10% oleic acid albumin dextrose catalase enrichment (OADC; Difco, Guang Zhou, Guangdong Province, China) at 37°C. The infection was carried out as described previously[18]. The macrophages (THP-1 and U937 cells) were incubated in 24 well plates and the cells were infected by *M. tuberculosis* H37Rv for 48 hours (h) as previous study described[19].

Cell Culture

Human macrophages (THP-1 and U937 cells) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and maintained in the Roswell Park Memorial Institute medium-1640 (RPMI-1640; Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA), 1% penicillin-streptomycin, L-glutamine (2 mM), HEPES (10 mM) and sodium pyruvate (1 mM; Sigma-Aldrich, Saint Louis, MO, USA). The human macrophages were maintained at 37°C in a humidified atmosphere of 5% CO_2 incubator.

The HEK293 cells (ATCC, Manassas, VA, USA) were maintained in DMEM medium (Gibco, Grand Island, NY, USA), containing 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), 1% penicillin/streptomycin (Beyotime, Shanghai, China) under a humidified atmosphere containing 5% CO_2 at 37°C.

Cell Transfection

MiR-708-5p mimic (sense: 5'-AAGGAGCUUACAAUCUAGCUGGG-3' and anti-sense: 5'-CAGCUAGAUUGUAAGCUCCUUUU-3'), miR-708-5p inhibitor (5'-CCCAGCUAGAUUGUAAGCUCCUU), mimic control (sense: 5'-UUCUCCGAACGUGUCACGUTT-3' and anti-sense: 5'-ACGUGACACGUUCGGAGAATT-3') and inhibitor control (5'-CAGUACUUUUGUGUAGUACAA-3') were obtained from GenePharma Company (Shanghai, China). After 48 h of M.tb infection, different oligonucleotides were transfected into cells for 48 h by using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) following the instruction of the manufacturer. The qRT-PCR assay was carried out to examine the efficiency of cell transfection.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total RNA Extraction Kit (Generay Biotech, Shanghai, China) was carried out to extract the total RNA from the cells following the instruction of the manufacturer. Then, the total RNA was reverse transcribed into cDNAs using PrimeScript™ RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) and qRT-PCR assay was performed using the SYBR Green Master Mix (TaKaRa, Otsu, Shiga, Japan) on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 small nuclear RNA was performed as an internal control. The reaction conditions were as follows: 95°C for 5 min, 35 cycles of denaturation at 94°C for 15 sec, annealing at 50°C for 30 sec, chain extension at 72°C for 60 sec and the last extension step at 72°C for 10 min. The

primers were obtained from Sangon Biotechnology (Shanghai, China) and listed as follows: U6, forward 5'-GCTTCGGCAGCACATATACTAAAAT-3'; reverse 5'-CGCTTCACGAATTTGCGTGTCAT-3'; GAPDH, forward 5'-TGTTGCCATCAATGACCCCTT-3'; reverse 5'-CTCCACGACGTACTCAGCG-3'; miR-708-5p, forward 5′-GGCGCGCAAGGAGCTTACAATC-3′; reverse 5′-GTGCAGGGTCCGAGGTAT-3′; TLR4, forward 5′-TGAGCAGTCGTGCTGGTATC-3′; reverse, 5′-CAGGGCTTTTCTGAGTCGTC-3′. The relative miRNA and mRNA expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method.

Western Blot Assay

The cell proteins were collected using Radio Immunoprecipitation Assay (RIPA) buffer (Beyotime Biotechnology, Shanghai, China). The bicinchoninic acid (BCA) protein analysis kit (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used to measure the concentration of the extracted proteins according to the manufacturer's instructions. The proteins were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Then, the membranes were incubated with 5% skim milk in Phosphate-Buffered Solution-0.1% Tween-20 (PBST), and the membrane was further incubated with primary antibodies overnight at 4°C. β-actin was used as an internal control. After that, the membranes were washed with PBST three times and blocked with secondary antibody for 2.5 h at room temperature. Finally, these proteins were assessed using enhanced chemiluminescence detection system (Beyotime Institute of Biotechnology, Shanghai, China).

Colony-Forming Unit (CFU) Assay

CFU analysis was used to evaluate the bacterial survival in human macrophages. The cells were infected with M.tb at 10 MOI for 48 h, and the cellular bacteria was discarded by washing thrice with PBS. Then, sterile distilled water was used to lyse the infected cells and 10-fold serial dilutions were plated on Middlebrook 7H11 agar plates supplemented 10% oleic acid albumin dextrose catalase enrichment and incubated at 37°C for 3 weeks.

ELISA

10 MOI M.tb was used to infect U937 cells and THP-1 cells for 48 h. Then, the cells were transfected with miR-708-5p inhibitor, miR-708-5p mimic, inhibitor control, or mimic control for 48 h. The cell culture supernatants were harvest and the levels of IFN-γ, IL-6, IL-1β, and TNF-α in supernatants were detected by ELISA kit (all from Beyotime Institute of Biotechnology, Shanghai, China) according to the manufacturer's protocol.

Dual-Luciferase Reporter Assay

TargetScan bioinformatics software (www.targetscan.org/vert_71) was used to search the potential targets of miR-708-5p, and we observed the binding sites between TLR4 and miR-708-5p. The TLR4-3'UTR containing the miR-708-5p binding sites or mutated target sites were amplified by genomic PCR and sub-cloned into a pGL3-Luciferase promoter vector (Promega, Madison, WI, USA) to construct the reporter vector TLR4-wild-type (TLR4-WT) or TLR4-mutated-type (TLR4-MUT). For the Luciferase reporter assay, the human embryonic kidney 293 (HEK293) cells were co-transfected with mimic control or miR-708-5p mimic and pGL3-Luciferase promoter vector containing wild-type (WT) or mutated (MUT) TLR4 3'UTR using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. After 48 h of transfection, the relative Luciferase activity was measured by Dual-Luciferase® Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Statistical Analysis

All the results were confirmed in three independent experiments. The data were expressed as the means ± standard deviation (SD) and analyzed by using GraphPad Prism 6.0 statistical software. The Student's *t*-test was performed to compare the differences between the two groups and the One-way analysis of variance (ANOVA) followed by Tukey's test was used to compare multiple groups. $*p<0.05$ indicated statistically significant difference.

Results

MiR-708-5p Expression Was Significantly Increased in Macrophages After M.tb Infection

In order to explore the level of miR-708-5p after M.tb infection, the macrophages were treated with M.tb with different MOI (0, 1, 5, or 10) for 48 h, or infected with M.tb at 10 MOI for differ-

ent periods of time as indicated (0, 12, 24, and 48 h), and the level of miR-708-5p was detected using qRT-PCR analysis. The results showed that the expression of miR-708-5p significantly enhanced in M.tb infection macrophages in a dose-dependent manner compared with non-infected macrophages (Figures 1A and 1B). Similarly, we found that miR-708-5p level was also remarkably elevated in a time-dependent manner in M.tb-infected human macrophages (Figures 1C and 1D). In summary, these data indicated that M.tb infection significantly increased the miR-708-5p level in human macrophages, suggesting an underlying relationship between miR-708-5p and M.tb infection.

The Effects of MiR-708-5p on the M.tb Vitality in Human Macrophages

To gain insight into the biological behavior of miR-708-5p on THP-1 and U937 cells after M.tb infection, we determined the effects of miR-708-5p on the M.tb vitality in macrophages. Firstly, we transfected miR-708-5p mimic, mimic control, inhibitor control, or miR-708-5p inhibitor into macrophages for 48 h. The qRT-PCR assay was used to detect the transfection efficiency. Our results suggested that the miR-708-5p level was significantly enhanced in the miR-708-5p mimic transfected group (Figures 2A and 2B), whereas miR-708-5p inhibitor suppressed the level of miR-708-5p in macrophages compared to the control group (Figures 3A and 3B). Besides, the mycobacterial survival was also determined by CFU assay in human macrophages after transfecting with miR-708-5p mimic, miR-708-5p inhibitor, mimic control, or inhibitor control after M.tb infection (10 MOI) for 48 h. The CFU assay showed that miR-708-5p mimic transfection greatly facilitated the viability of mycobacteria in M.tb-infected macrophages compared to the control group (Figures 2C and 2D). Conversely, the viability of mycobacteria in miR-708-5p inhibitor group was lower than that in the control group (Figures 3C and 3D). These data revealed that miR-708-5p enhanced the intracellular mycobacteria vitality in M.tb-infected macrophages.

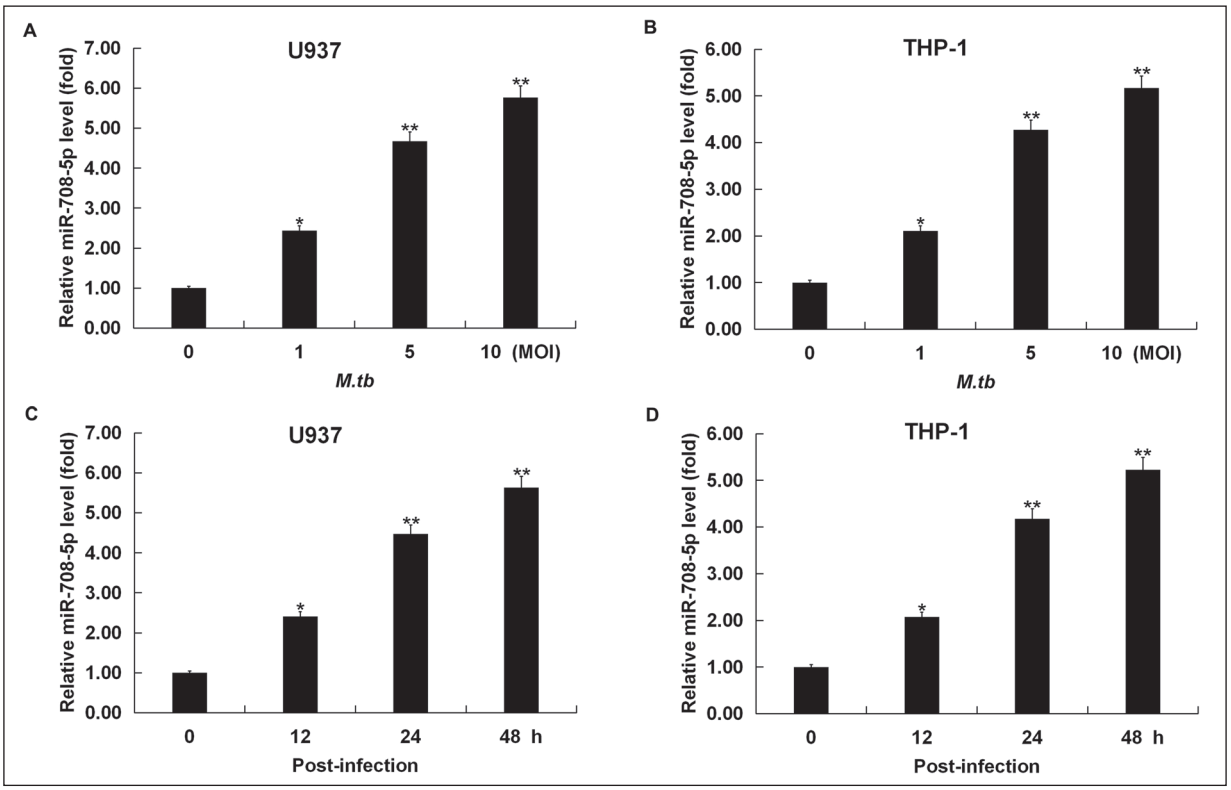

Figure 1. Effect of M.tb infection on the expression level of miR-708-5p in macrophages. The U937 cells **(A)** and THP-1 **(B)** were infected with M.tb at 0, 1, 5, or 10 MOI for 48 h, respectively. The qRT-PCR analysis was performed to examine the expression levels of miR-708-5p. The U937 **(C)** and THP-1 cells **(D)** were infected with M.tb at an MOI of 10 for 0, 12, 24, and 48 h, respectively; then, the miR-708-5p expression was evaluated using the qRT-PCR analysis. All results expressed the mean ± SD from three determinations. *p<0.05, **p<0.01 *vs.* Control.

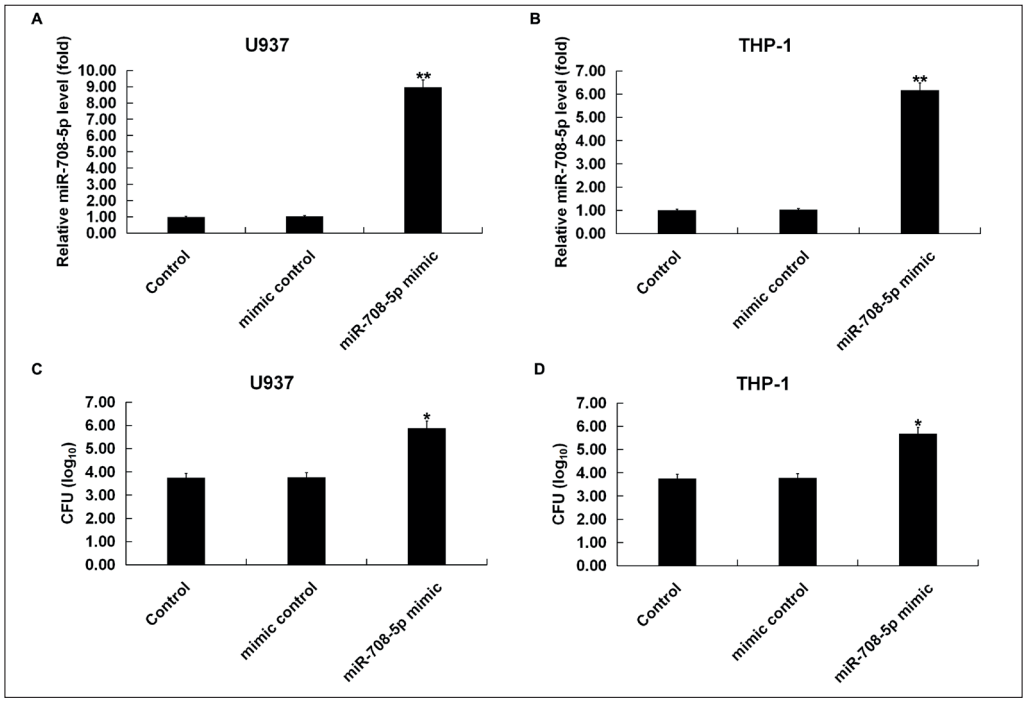

Figure 2. Effect of miR-708-5p mimic on the mycobacterial survival in M.tb-infected macrophages. The U937 **(A)** and THP-1 cells **(B)** were transfected with miR-708-5p mimic or mimic control, and the miR-708-5p levels were determined by qRT-PCR analysis. The U937 cells **(C)** and THP-1 **(D)** were transfected with miR-708-5p mimic or mimic control for 48 h, followed by 10 MOI M.tb infection, and the CFU assay was carried out to evaluate the mycobacterial survival. All results were shown as the mean ± SD from three independent experiments. *p<0.05, **p<0.01 *vs.* Control.

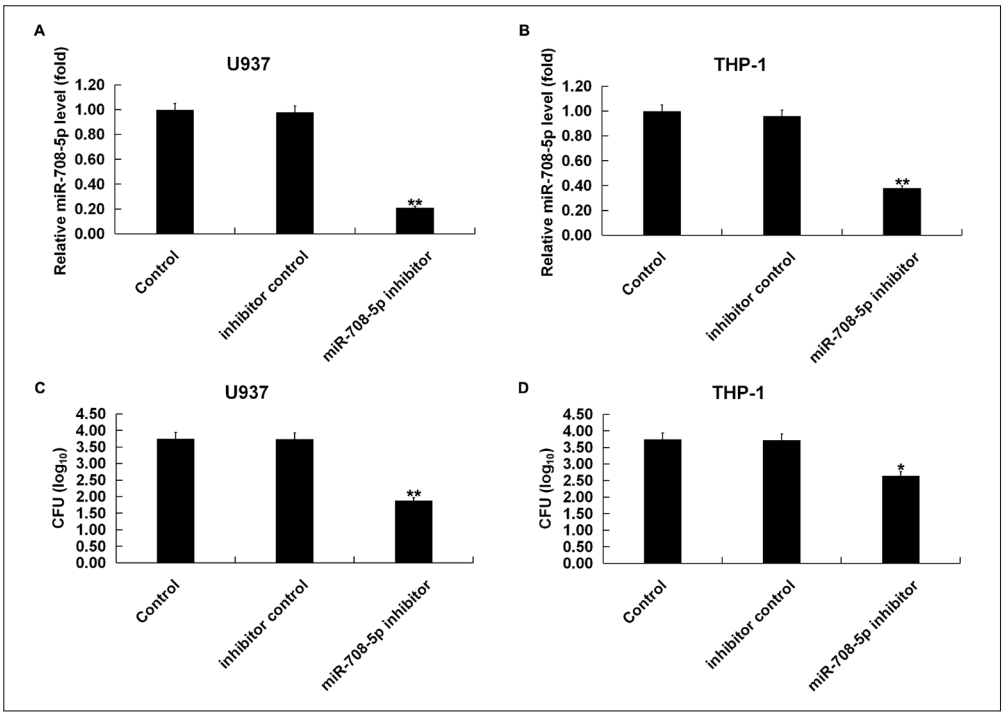

Figure 3. Effect of miR-708-5p inhibitor on the mycobacterial survival in M.tb-infected macrophages. The U937 **(A)** and THP-1 cells **(B)** were transfected with miR-708-5p inhibitor or inhibitor control for 48 h, and the expression levels of miR-708-5p was determined by qRT-PCR. The U937 cells **(C)** and THP-1 **(D)** were transfected with miR-708-5p inhibitor or inhibitor control for 48 h, followed by 10 MOI M.tb infection, and a CFU assay was carried out to evaluate the mycobacterial survival. Data were expressed as the mean ± SD from three individual experiments. *p<0.05, **p<0.01 *vs.* Control.

The Effect of MiR-708-5p on the Cytokines Levels in M.tb-Infected Macrophages

To further demonstrate the molecular mechanism of miR-708-5p in M.tb-infected macrophages, we further determined the roles of miR-708-5p in the inflammatory factors section in the M.tb-infected macrophages. The results demonstrated that M.tb-infected macrophages (10 MOI) at 48 h post-infection could significantly increase the protein expression of cytokines, including IFN-γ, IL-6, IL-1β, and TNF-α. However, miR-708-5p mimic transfection attenuated these effects in both THP-1 (Figures 4A-4D) and U937 (Figures 4E-4H) cells. Meanwhile, miR-708-5p inhibitor strikingly enhanced the expression of IFN-γ, IL-6, IL-1β, and TNF-α in both THP-1 (Figures 5A-5D) and U937 (Figure 5E-5H) cells during M.tb-infection. Our data were consistent with those of Shi et al[20]. These data suggested that miR-708-5p could negatively mediate the production of cytokines in human macrophages.

TLR4 Was a Target of MiR-708-5p

Previous reports have shown that miRNA exerts their roles by targeting their targets. In order to better comprehend the underlying immune response of miR-708-5p after M.tb treatment, we used a bioinformatic tool to predict the target gene of miR-708-5p. Our data demonstrated that TLR4 was a potential target of miR-708-5p and the binding sites between TLR4 and miR-708-5p were exhibited in Figure 6A. Then, we conducted The Luciferase reporter assay to verify the computational prediction. As presented in Figure 6B, miR-708-5p mimic decreased the Luciferase activity in HEK293 cells co-transfected with TLR4-WT and mR-708-5p mimic. However, the Luciferase activity of the cells co-transfected with TLR4-MUT and mR-708-5p mimic has no significant changes, indicating that TLR4 was a direct target of miR-708-5p.

MiR-708-5p Regulated the Expression of TLR4 in M.tb-Infected Macrophages

We then speculated that there was a relationship between miR-708-5p and the level of TLR4 in U937 and THP-1 cells infected with M.tb. To further confirm this hypothesis, we transfected miR-708-5p mimic, miR-708-5p inhibitor, mimic control or inhibitor control into the M.tb-infected macrophages, then, qRT-PCR and Western blot were conducted to evaluate the levels of TLR4. Western blot assay demonstrated that the TLR4 protein expression level in U937 (Figure 7A) and THP-1 (Figure 7A) cells was decreased in the miR-708-5p mimic group, while miR-708-5p inhibitor enhanced the TLR4 protein expression level (Figure 7D) in U937 and THP-1 cells. qRT-PCR analysis indicated that miR-708-5p mimic transfection significantly decreased the mRNA level of TLR4 in the macrophages compared to control (Figures 7B and 7C). Meanwhile, the mRNA level of TLR4 in the macrophages was significantly increased after miR-708-5p inhibitor transfection (Figures 7D and 7E).

Discussion

Tuberculosis (TB) is a frequent infectious disease, which is caused by *Mycobacterium tuberculosis* infection[21,22]. Also, macrophages participate in host immunoreaction against M.tb infection and inflammatory cytokines secretion[6,23]. Many studies[24] suggested that miRNAs regulate the host defense to intracellular mycobacteria, but till now, the role of miR-708-5p during M.tb infection remains largely unknown. In this study, we infected the macrophage cell lines with M.tb with indicated MOI for 48 h, or 10 MOI for different periods of time, and then, the qRT-PCR analysis was performed to evaluate the miR-708-5p level. Our results indicated that the miR-708-5p level in M.tb-infected macrophages was significantly enhanced in a time-dependent and dose-dependent manner. These results indicated a potential relationship between miR-708-5p and mycobacterial infection.

It has been suggested[25] that the macrophage activity is vital in the immunity defense against mycobacterial infection. Recently, Wang et al[26] demonstrated that much more intracellular mycobacteria resulted in the necrocytosis of macrophage that may accelerate mycobacterial vitality. Based on the above researches, we further explored the effect of miR-708-5p level on mycobacterial viability in M.tb-infected macrophages. We transfected the mimic control, miR-708-5p mimic, inhibitor control, or miR-708-5p inhibitor into human macrophage THP-1 and U937. qRT-PCR was carried out to analyze the miR-708-5p level and the CFU assay was used to detect the mycobacterial activity. Our results indicated that the upregulation of miR-708-5p enhanced the miR-708-5p level in macrophages, and the mycobacterial survival was remarkably enhanced. Meanwhile, miR-708-5p inhibitor demonstrated

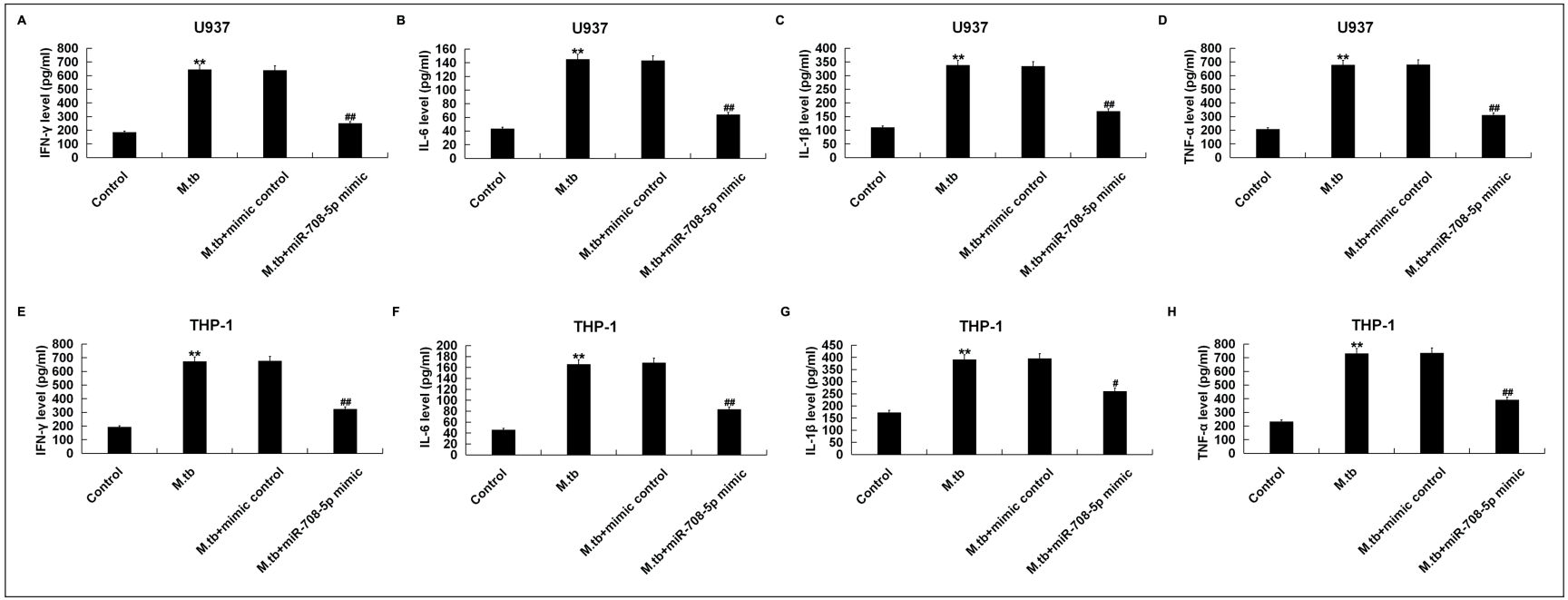

Figure 4. Effect of miR-708-5p mimic on the cytokines secretion in M.tb infected macrophages. ELISA assay was performed to determine the protein expression of IFN-γ **(A)**, IL-6 **(B)**, IL-1β **(C)**, and TNF-α **(D)** in U937 cells. The protein expression of IFN-γ **(E)**, IL-6 **(F)**, IL-1β (**G**), and TNF-α **(H)** in THP-1 cells were determined by ELISA assay. All results above was expressed as mean ± SD. $^{**}p<0.01$ vs. Control; $^{\#}$, $^{\#\#}p<0.05$, 0.01 *vs.* M.tb.

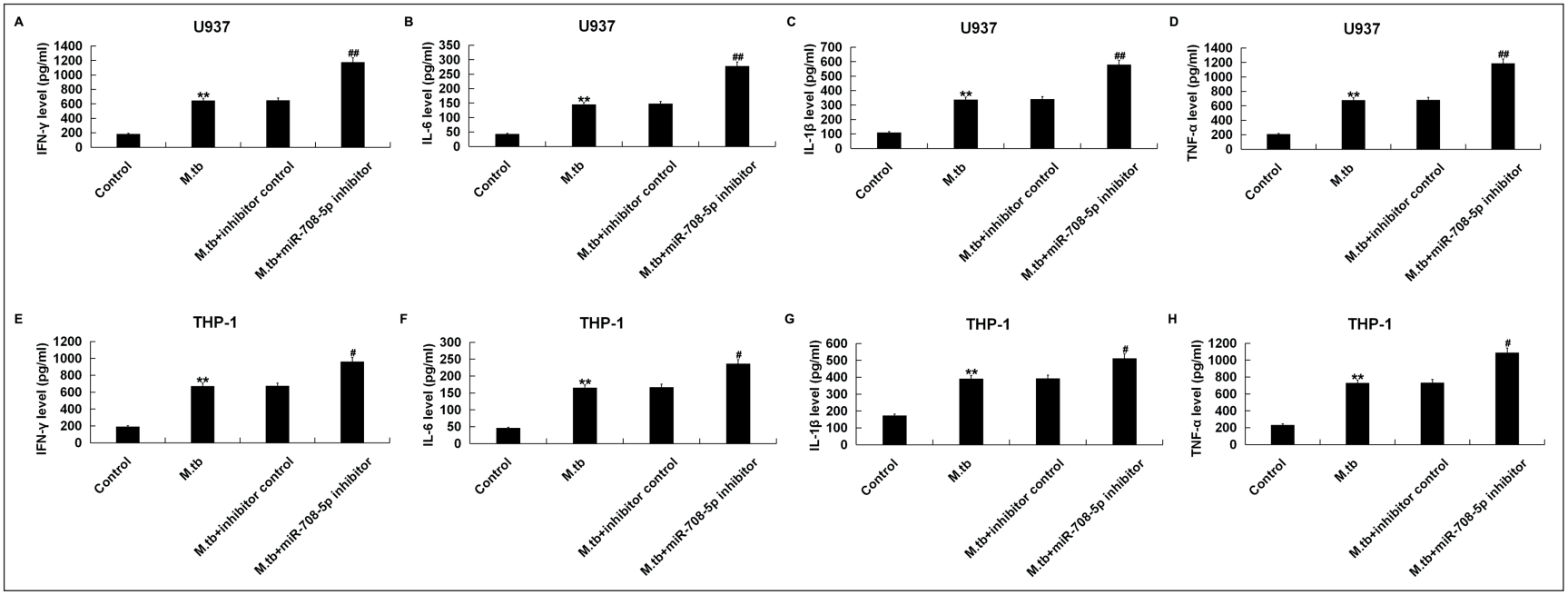

Figure 5. Effect of miR-708-5p inhibitor on the cytokines secretion in M.tb infected macrophages. The protein expression of IFN-γ **(A)**, IL-6 **(B)**, IL-1β **(C)**, and TNF-α **(D)** in U937 cells was examined by ELISA assay. The protein expression of IFN-γ **(E)**, IL-6 **(F)**, IL-1β **(G)**, and TNF-α **(H)** in THP-1 cells were determined by ELISA assay. All the data above was represented as mean ± SD. **p<0.01 vs. Control; $^{\#}$, $^{\#\#}p$<0.05, 0.01 *vs.* M.tb.

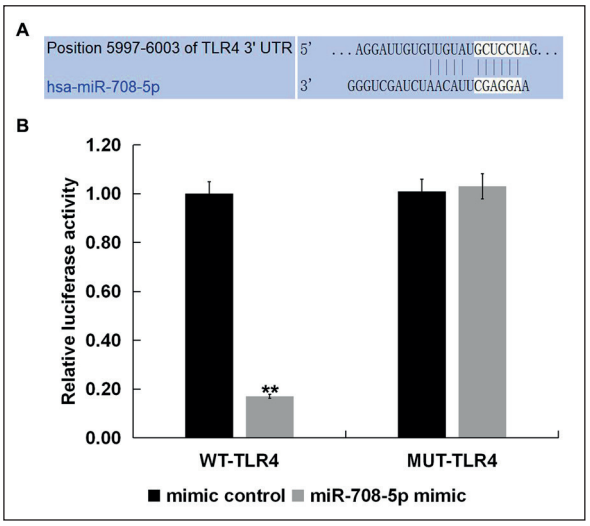

Figure 6. TLR4 was a target of miR-708-5p. **A,** Binding sites of miR-708-5p in the 3'UTR of TLR4 predicted by bioinformatics tools. **B,** Dual-Luciferase assay was used to detect the relative Luciferase activity of a reporter containing wild type (WT) 3'UTR of TLR4 or mutant (MUT) 3'UTR of TLR4. The results were depicted as the mean ± SD of three individual experiments; $^{**}p<0.01$ *vs.* mimic control.

the opposite effects. These findings showed a valid effect of miR-708-5p in the cellular immunologic response and the regulation of miR-708-5p level was responsible for the M.tb intracellular infection.

The excitation of macrophages resulted in the secretion of many factors to regulate the inflammatory response[27]. Pro-inflammatory cytokines exert a vital role in wiping out the bacterial invaders, including IFN-γ, IL-6, IL-1β, and TNF-α[28]. Chen et al[29] have reported that distinct cytokines secretion could be induced by various M.tb strains. Thus, our study further explored the function of miR-708-5p in immunoreaction. Our findings agree with previous data[30,31], which demonstrated that M.tb infection promoted the production of pro-inflammatory cytokines IFN-γ, IL-6, IL-1β, and TNF-α in macrophages. While upregulation of miR-708-5p significantly suppressed the production of these inflammatory elements after M.tb infection, thus improving inflammatory responses in human macrophages. By contrast, the inflammatory factors secretion was noteworthy enhanced in miR-708-5p inhibitor group after M.tb treatment. These results indicated that miR-708-5p was involved in the regulation of the inflammatory response induced by M.tb when the parasitifer congenital defense against mycobacteria. Our findings indicated that the up-expression of miR-708-5p decreased the M.tb vitality and promoted the secretion of cytokines[32].

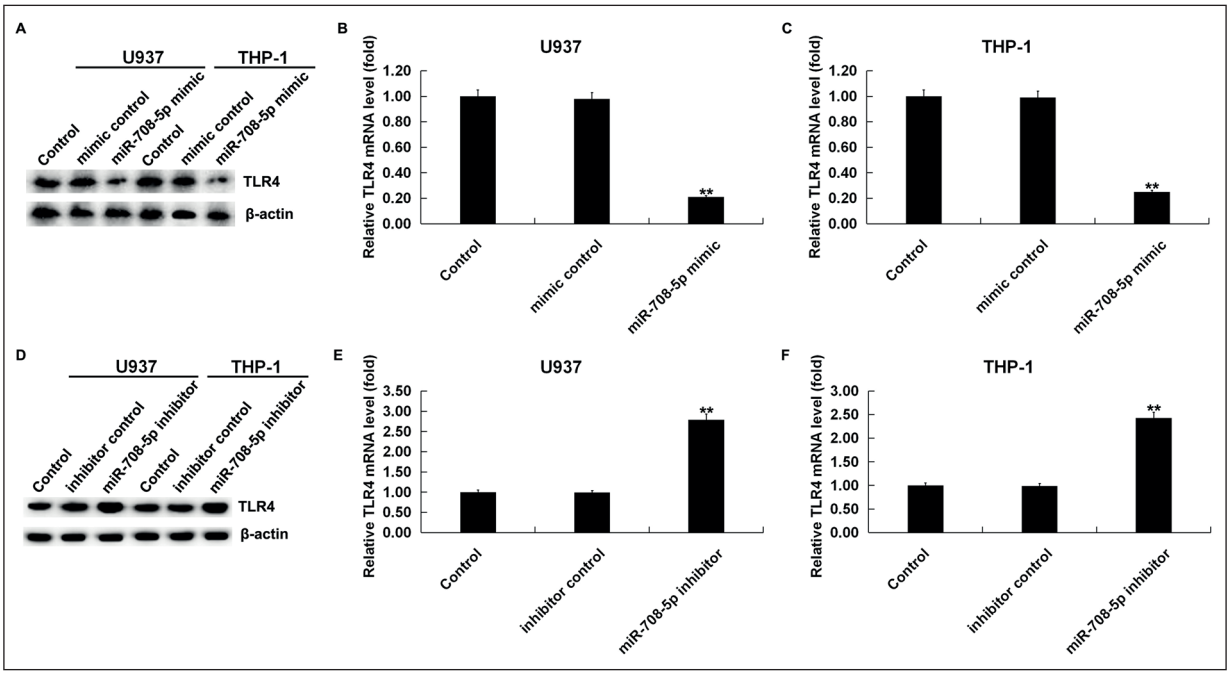

Figure 7. Effects of miR-708-5p on TLR4 expression in human macrophages. **A,** and **E,** Western blot assay was performed to detect the protein level of TLR4 in U937 and THP-1 cells. **B,** and **E,** qRT-PCR assay was performed to detect the mRNA level of TLR4 in human macrophages cell line U937. **C,** and **F,** qRT-PCR assay was performed to detect the mRNA level of TLR4 in human macrophages cell line THP-1. The results were depicted as the mean ± SD of three individual experiments; $^{**}p<0.01$ *vs.* Control.

In order to further understand the potential mechanism of miR-708-5p in the M.tb-induced immune response, we predicted the target genes of miR-708-5p and the results showed that TLR4 was a direct target of miR-708-5p. Many reports[33] have shown that TLRs participated in parasitifer congenital recognition of M.tb. Lately, a report showed that TLR4 was indispensable and exerted protective roles during M.tb infection. Besides, miRNAs could mediate the macrophages immune responses *via* controlling the TLR pathway[34]. In the current study, we found that miR-708-5p overexpression alleviated the TLR4 expression in M.tb-infected macrophages and the downregulation of miR-708-5p showed the opposite effects. These results demonstrated that miR-708-5p might exert an anti-inflammatory effect in M.tb-infected macrophages by targeting TLR4.

Taken together, these results have illuminated the vital function of the miR-708-5p/TLR4 signaling pathway in M.tb-infected macrophages. This study elucidated a vital role of miR-708-5p/TLR4 pathway in mediating immune response and mycobacterial vitality, which might provide a valuable theoretical foundation for tuberculosis diagnosis and treatment.

Conclusions

MiR-708-5p was upregulated in macrophages after M.tb infection and it could regulate mycobacterial vitality and immunoreaction in M.tb-infected macrophages at least partly by targeting TLR4. Our study may provide a better comprehension of the role mechanism of tuberculosis and potential therapeutic target for anti-tuberculosis therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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