Immune regulation of miR-30 on the *Mycobacterium tuberculosis*-induced TLR/MyD88 signaling pathway in THP-1 cells

YUQING WU, QI SUN and LIANG DAI

Department of Tuberculosis, Jiangxi Province Chest Hospital, Nanchang, Jiangxi 330006, P.R. China

Abstract. The present study aimed to examine the expression of microRNA (miR)-30 family members in THP-1 human monocytes cells during *Mycobacterium tuberculosis* (MTB) H37Rv infection, and to investigate the role of miR-30 in the regulation of MTB-induced Toll-like receptor (TLR)/myeloid differentiation factor 88 (MyD88) activation and cytokine expression. The THP-1 cells were infected with MTB H37Rv and the expression of miR-30 family members was determined by reverse transcription-quantitative polymerase chain reaction analysis. In addition, miR-30a and miR-30e mimics were transfected into THP-1 cells to overexpress miR-30a and miR-30e. The expression of TLR2, TLR4 and MyD88 was determined by western blot analysis, and the expression of the cytokines tumor necrosis factor-α, interleukin (IL)-6, and IL-8 was determined using ELISA assays. A luciferase reporter assay was used to identify the target gene of miR-30a. MTB infection was demonstrated to significantly induce miR-30a and miR-30e expression in THP-1 cells in a time-dependent manner. Forced overexpression of miR-30a, but not miR-30e, exhibited an inhibitory effect on TLR/MyD88 activation and cytokine expression in the uninfected and MTB-infected THP-1 cells. The luciferase reporter assay demonstrated that miR-30a directly regulates the transcriptional activity of the MyD88 3'-untranslated region. In conclusion, the present study, to the best of our knowledge, is the first to demonstrate that miR-30a suppresses TLR/MyD88 activation and cytokine expression in THP-1 cells during MTB H37Rv infection, and that MyD88 is a direct target of miR-30a. The current study may aid in the development of novel therapeutic approaches for treating MTB.

Correspondence to: Dr Liang Dai, Department of Tuberculosis, Jiangxi Province Chest Hospital, 346 Dieshan Road, Nanchang, Jiangxi 330006, P.R. China
E-mail: dai_brighten@126.com

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by the *Mycobacterium tuberculosis* (MTB) bacterium. It may infect any part of the body, but most commonly occurs in the lungs (1). General signs and symptoms include fever, chills, night sweats, loss of appetite, weight loss, and fatigue (1). MTB is a leading cause of infection-associated mortality globally, with ~1/3 individuals globally having been infected with MTB (2-4). Approximately 8.8 million new cases of TB were diagnosed, and 1.20 or 1.45 million deaths occurred in 1990 and 2010, respectively, most of these occurring in developing countries (5). The pathogenic mechanism of MTB is complex and the emergence of drug resistance has led to difficulty in the control of TB (4). Although the bacillus Calmette-Guérin vaccine protects against TB in certain populations, it has limitations in disease control (6-10), as it is insufficient at protecting from adult pulmonary tuberculosis. Therefore, investigating effective novel methods to prevent and cure TB is of high priority worldwide.

MicroRNAs (miRNAs/miRs) are small (21-24 nucleotides) non-coding RNAs that participate in various physiological and pathological processes (11,12). miRNAs bind to partially complementary sequences in the 3'-untranslated region (3'-UTR) of target mRNAs, leading to degradation of the transcript or translational inhibition (13,14). Previous research has demonstrated that miRNAs are important regulators of the immune response that function at the post-transcriptional level (15). A number of miRNAs have been implicated in the pathogenesis of MTB infection, including miR-29, -147, -21 and -125b (16). Das *et al* (17) reported that the differential expression of miRNAs, including miR-30a and miR-30e, was observed between THP-1 human myeloid leukemia cells infected with MTB H37Rv and MTB H37Ra strains. Chen *et al* (18) suggested that miR-30a serves an important role in the elimination of intracellular MTB. However, the regulatory mechanism of miR-30 family induction in response to MTB infection remains unclear.

The present study evaluated and analyzed the expression of miR-30 family members in THP-1 cells during MTB H37Rv infection, and assessed the role of miR-30 in regulating MTB-induced Toll-like receptor (TLR)/myeloid differentiation factor 88 (MyD88) signaling pathway activation and cytokine expression.

Materials and methods

Cell culture and transfection. THP-1 human monocyte and HEK293 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (both Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37˚C with 5% CO_2. For infection, the THP-1 cells were exposed to MTB H37Rv (multiplicity-of-infection, 1:10; ATCC, Manassas, VA, USA) at 37˚C for 0, 6, 12 or 24 h. The miR-30a mimic (sense, UGUAAA CAUCCUCGACUGGAAG; antisense, UCCAGUCGAGGA UGUUUACAUU), miR-30e mimic (sense, UGUAAACAU CCUUGACUGGAAG; antisense, UCCAGUCAAGGAUGU UUACAUU) and miR negative control (miR-NC) (UUGUAC UACACAAAAGUACUG) were synthesized by GenePharma Co., Ltd. (Shanghai, China). Cells were transfected with 100 nM miR-30a mimic, miR-30e mimic and miR-NC using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. After 4 h, the media was replaced and the cells were maintained in culture prior to analysis.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. The expression of miR-30a, miR-30b, miR-30c, miR-30d and miR-30e was analyzed by RT-qPCR. U6 gene was used as the control. Primers specific to miR-30a (forward, 5'-TGTAAACATCCTCGA CTGGAAG-3'; reverse, 5'-TGGTGTCGTGGAGTCG-3'), miR-30b (forward, 5'-TGTAAACATCCTACACTCAGCT-3'; reverse, 5'-TGGTGTCGTGGAGTCG-3), miR-30c (forward, 5'-TGTAAACATCCTACACTCTCAGC-3'; reverse, 5'-TGG TGTCGTGGAGTCG-3'), miR-30d (forward, 5'-TGTAAA CATCCCCGACTGGAAG-3'; reverse, 5'-TGGTGTCGTGGA GTCG-3'), miR-30e (forward, 5'-AAGTGTAAACATCCT CGACTGGAAG; reverse, 5'-TGGTGTCGTGGAGTCG-3'), U6 (forward, 5'-TGGTGTCGTGGAGTCG-3'; reverse, 5'-TGG TGTCGTGGAGTCG-3'). Total RNA was isolated from cells using an miRNeasy Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. Total RNA (1 mg) was reverse transcribed into cDNA using a RevertAid™ First Strand cDNA Synthesis kit (Fermentas; Thermo Fisher Scientific, Inc., Pittsburgh, PA, USA). The PCR reaction was performed on a 7300 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the SYBR-Green PCR Master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.). qPCR was performed in a 25 μl reaction containing 12.5 μl 2x SYBR-Green Mix, 100 nM of forward and reverse primers and 1 μl cDNA. Thermocycling conditions for PCR were 5 min at 95˚C followed by 40 cycles of 5 min at 95˚C, 10 sec at 58˚C and 10 sec at 72˚C. Fold-changes in target mRNA expression were determined using the $2^{-\Delta\Delta Cq}$ method (19).

Western blot analysis. Cells were harvested and lysed in RIPA lysis buffer (Sangon Biotech Co., Ltd., Shanghai, China). The total protein concentration was determined using a BCA Protein assay kit (Sangon Biotech Co., Ltd.) according to the manufacturer's protocol. Total protein (20 μg) was separated by SDS-PAGE using a 10% (wt/vol) gel and then transferred to polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat milk at 4˚C overnight, followed by incubation with the following rabbit polyclonal primary antibodies at 37˚C for 1 h: Anti-TLR2 (1:800; cat. no. 2229; Cell Signaling Technology, Inc., Danvers, MA, USA), anti-TLR4 (1:500; cat. no. sc-30002; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), anti-MyD88 (1:800; cat. no. 3699; Cell Signaling Technology, Inc.). Following washing three times with Tris-buffered saline with 0.1% Tween-20 (TBST) (10 min/wash), the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G secondary antibodies (1:10,000; cat. no. sc-2004; Santa Cruz Biotechnology, Inc.) at 37˚C for 1 h. Protein bands were detected using a Pierce ECL western blotting substrate (Pierce; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The band intensity was quantified using Image J 1.48u software (National Institutes of Health, Bethesda, MD, USA) and the experiments were performed three times independently.

Cytokine quantification. The concentration of tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-8 in cell supernatants were determined using the Human TNF-α (cat. no. DTA00C), IL-6 (cat. no. D6050) and IL-8 (cat. no. D8000C) Quantikine ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's protocol. Briefly, the cells were harvested and centrifuged at 1,000 x g at room temperature for 5 min, then the cell supernatants were collected. Standard curves were generated from serial dilutions of recombinant cytokine standards in PBS. The detection range for TNF-α, IL-6 and IL-8 using these kits are 15.6-1,000, 3.1-300 and 31.2-2,000 pg/ml, respectively.

Luciferase reporter assay. The miRanda program (www.microrna.org/microrna/home.do) was used to identify the targets of miR-30a. Subsequently, yhe DNA fragment of MyD88 wild-type 3'-UTR or mutant 3'-UTR (NM_001172566.1) was cloned into the pMIR-REPORT™ miRNA Expression Reporter Vector (Applied Biosystems; Thermo Fisher Scientific, Inc.) to construct reporter plasmids. The HEK293 cells were co-transfected with 400 ng reporter plasmids and 100 nM miR-30a mimic or miR-NC using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The pRL-SV40 *Renilla* luciferase plasmid (Promega Corporation, Madison, WI, USA) was transfected as an internal control. The cells were harvested and lysed with Passive Lysis Buffer (Promega Corporation) 24 h after transfection. Subsequently, luciferase activity was measured using the Dual-Luciferase® Reporter Assay system (Promega Corporation) according to the manufacturer's protocol.

Statistical analysis. Statistical analysis was performed using SPSS software (version 19.0; IBM Corp., Armonk, NY, USA). All data are presented as the mean ± standard deviation of ≥3 independent experiments. The Student's t-test was used to compare the statistical significance of results between two groups, and one-way analysis of the variance followed by a Fisher's least significant difference test was used for the comparison of multiple groups. P<0.05 was considered to indicate a statistically significant difference.
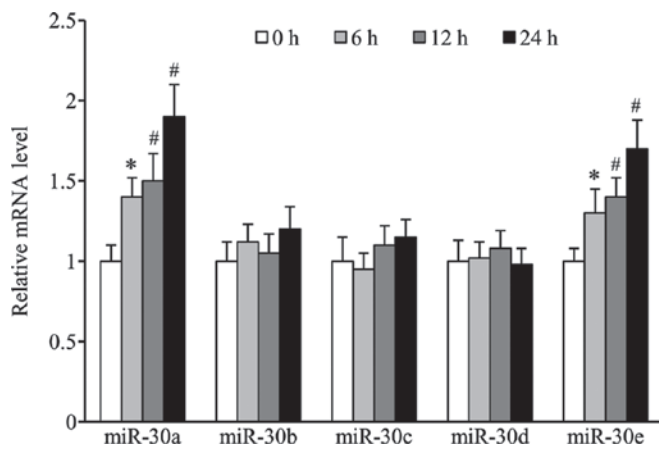
Figure 1. Expression of miR-30 family members in THP-1 cells infected with *Mycobacterium tuberculosis*. *P<0.05 and #P<0.01 vs. 0 h. mRNA/miR, microRNA.

Results

Expression of miR-30 family members in THP-1 cells infected with MTB. The expression of miR-30a, -30b, -30c, -30d and -30e in THP-1 cells was examined by RT-qPCR at 0, 6, 12 and 24 h following H37Rv infection (Fig. 1). The expression of miR-30a and miR-30e was significantly increased in H37Rv-infected THP-1 cells at 6, 12 and 24 h after infection compared with the uninfected THP-1 cells (all P<0.05) in a time-dependent manner. However, MTB infection had no significance effect on the expression of miR-30b, miR-30c or miR-30d in THP-1 cells.

Effect of miR-30a and miR-30e on TLR2, TLR4 and MyD88 expression in MTB-infected THP-1 cells. THP-1 cells were transfected with miR-30a mimic or miR-30e mimic, and were then infected with H37Rv for 24 h. As presented in Fig. 2, the expression of miR-30a and miR-30e was significantly increased in THP-1 cells following transfection with the mimics compared with the miR-NC-transfected control group (P<0.01; Fig. 2A and B, respectively).

The expression of TLR2, TLR4 and MyD88 protein in MTB-infected THP-1 cells following transfection with the miR-30a or miR-30e mimics was examined by western blot analysis. Compared with the control group, MTB infection led to significantly increased expression of TLR2, TLR4 and MyD88 in THP-1 cells (all P<0.05; Fig. 3). Transfection of the miR-30a mimic significantly suppressed the expression of TLR2, TLR4 and MyD88 in the uninfected and H37Rv-infected THP-1 cells compared with the control group and the MTB infected group, respectively (both P<0.05; Fig. 3). Transfection of miR-30e mimic did not exert any significant effects on TLR2, TLR4 and MyD88 expression in the uninfected or H37Rv-infected THP-1 cells (Fig. 3).

Effect of miR-30a and miR-30e on TNF-α, IL-6 and IL-8 expression in MTB-infected THP-1 cells. The expression of TNF-α, IL-6 and IL-8 in MTB-infected THP-1 cells following transfection with the miR-30a or miR-30e mimic was examined using an ELISA assay. As presented in Fig. 4, the expression of TNF-α, IL-6 and IL-8 was significantly
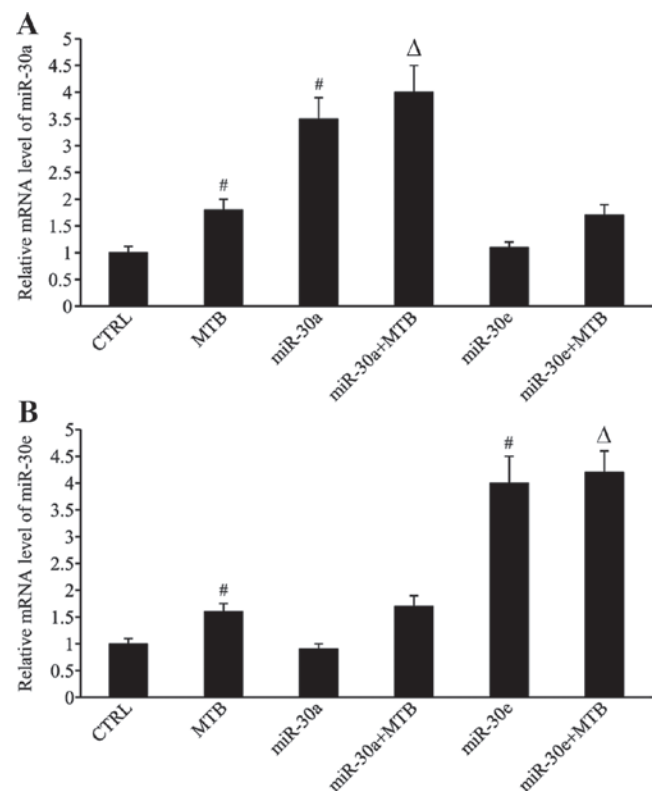

Figure 2. Effect of miR-30a and miR-30e mimics on the expression of miR-30a and miR-30e in THP-1 cells. Relative expression of (A) miR-30a and (B) miR-30e in THP-1 cells following transfection with miR-30a and miR-30e mimics. #P<0.01 vs. CTRL; ΔP<0.01 vs. MTB. mRNA/miR, microRNA; MTB, *Mycobacterium tuberculosis*; CTRL, control.

increased in H37Rv-infected THP-1 cells compared with the control group (all P<0.01). In H37Rv infected cells, expression of TNF-α, IL-6 and IL-8 was significantly suppressed by transfection with the miR-30a mimic (P<0.01). Transfection of the miR-30a mimic also significantly inhibited TNF-α, IL-6 and IL-8 expression in uninfected THP-1 cells compared with the control group (P<0.05). The expression of TNF-α, IL-6 and IL-8 was not significantly altered in uninfected THP-1 cells following transfection with miR-30e.

MyD88 is a direct target of miR-30a. Using the miRanda program, it was identified that MyD88 is a direct target of miR-30a. The wild-type or mutant MyD88 3'-UTR was constructed and co-transfected with the miR-30a mimic or miR-NC into the HEK293 cells, and a dual-luciferase assay was performed. When compared with the control group, the miR-30a mimic significantly suppressed the luciferase activity of wild-type MyD88 (P<0.01); however, the miR-30a mimic had to effect on the luciferase activity of the mutant MyD88 (Fig. 5).

Discussion

The miR-30 family consists of five members, miR-30a-e. Previous studies have highlighted the importance of miR-30 in the development and progression of cancer (20-23). It has previously been demonstrated that miR-30 is associated with inflammatory responses (24-26). MTB infection may modulate immune responses by affecting the expression of miRNAs. A
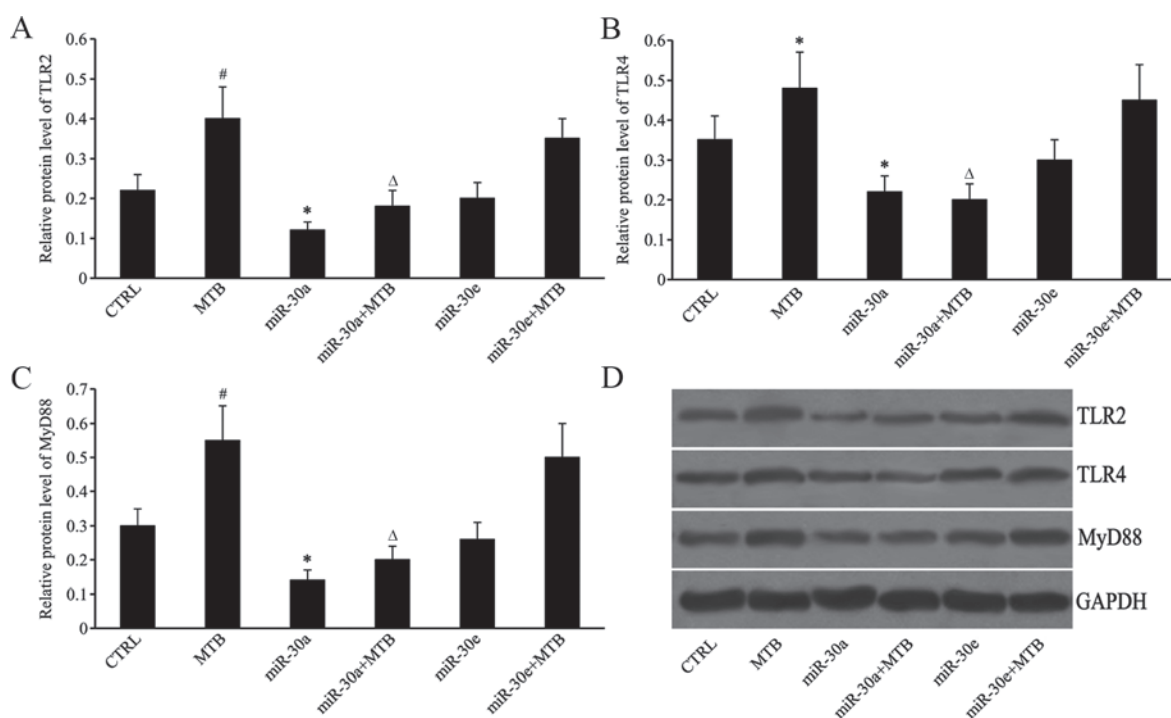
Figure 3. Expression of TLR2, TLR4 and MyD88 in MTB-infected THP-1 cells following transfection with miR-30a or miR-30e mimics assessed using western blot analysis. Quantification of expression of (A) TLR2, (B) TLR4 and (C) MyD88. (D) Western blot of TLR2, TLR4 and MyD88 expression. *P<0.05, #P<0.01 vs. CTRL; ΔP<0.01 vs. MTB. miR, microRNA; MTB, *Mycobacterium tuberculosis*; CTRL, control; TLR, Toll-like receptor; MyD88, myeloid differentiation factor 88; lane 1, CTRL; lane 2, MTB; lane 3, miR-30a; lane 4, miR-30a+MTB; lane 5, miR-30e; lane 6, miR-30e+MTB.
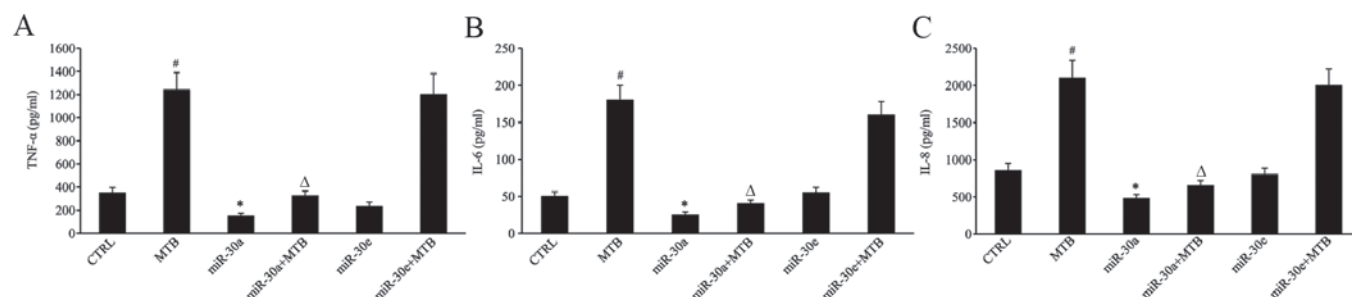

Figure 4. Expression of TNF-α, IL-6 and IL-8 in MTB-infected THP-1 cells following transfection with miR-30a or miR-30e mimics. ELISA analysis of (A) TNF-α, (B) IL-6 and (C) IL-8 levels. *P<0.05, #P<0.01 vs. CTRL; ΔP<0.01 vs. MTB. miR, microRNA; MTB, *Mycobacterium tuberculosis*; CTRL, control; TNF-α, tumor necrosis factor α; IL, interleukin.
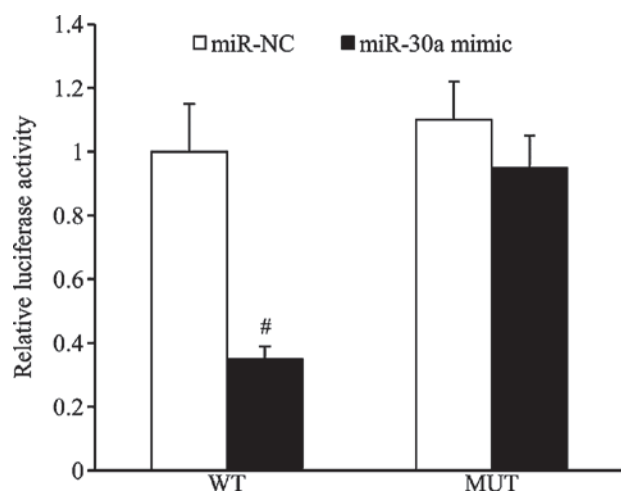

Figure 5. MyD88 is a direct target of miR-30a. #P<0.01 vs. miR-NC. miR, microRNA; NC, negative control; MyD88, myeloid differentiation factor 88; WT, wild-type; MUT, mutant.

previous study by Chen *et al* (18) revealed that the expression of miR-30a was induced in MTB-infected macrophages, and that this overexpression of miR-30a suppressed the ability of host cells to eradicate intracellular MTB. Anti-TB treatment of these cells led to the decreased expression of miR-30a (18). In the present study, H37Rv-infected THP-1 cells were used to investigate the effect of MTB infection on the expression of miR-30 family members. In agreement with previous findings, the current study demonstrated that MTB infection significantly induced miR-30a and miR-30e expression in THP-1 cells in a time-dependent manner. However, the expression of miR-30b, -30c and -30d was not altered following MTB infection. These findings indicate that miR-30a and miR-30e may interact with MTB in THP-1 cells. Subsequently, the regulatory mechanism of miR-30 induction in response to MTB infection was investigated.

TLRs are most potent inducers of inflammatory responses (27). Among the cell surface TLRs, TLR2 and TLR4

have been the focus of research due to their unique capacity to identify extracellular and intracellular pathogens (28). TLR2 and TLR4 are able to trigger signal transduction by recruiting MyD88, which drives activation of the transcription factor nuclear factor-κB, leasing to the expression of proinflammatory cytokines. It has been demonstrated that TLR2 and TLR4 may interact with MTB, and that they are associated with the immune response to MTB infection (29-31).

In the present study, an miR-30a or miR-30e mimic were transfected into THP-1 cells to investigate the effect of miR-30 on the TLR/MyD88 signaling pathway. This revealed that miR-30a exhibits a significant inhibitory effect on TLR, MyD88 and cytokine expression in uninfected THP-1 cells. Furthermore, this effect was also observed in MTB-infected THP-1 cells. miR-30e did not exhibit a significant effect on TLR, MyD88 or cytokine expression in the untreated or MTB-infected THP-1 cells. These results indicate that miR-30a participates in MTB-induced immune responses via suppressing TLR/MyD88 activation and cytokine expression.

To identify the molecules targeted by miR-30a in the TLR/MyD88 signaling pathway, the present study used the miRanda program and identified that MyD88 was a predicted target gene of miR-30a. The luciferase reporter assay demonstrated that miR-30a directly regulates the transcriptional activity of MyD88 3'-UTR. These results suggest that miR-30a suppresses the TLR/MyD88 signaling pathway by directly targeting MyD88.

In conclusion, the current study presents, to the best of our knowledge, the first evidence that miR-30a suppresses TLR/MyD88 activation and cytokine expression in THP-1 cells during MTB H37Rv infection, and that MyD88 is a direct target of miR-30a. The present study highlights the role of miR-30a in the immune response against MTB in monocytes, and may aid the development of novel therapeutic approaches for treating MTB.

References

1. Duncan AH: The Sedimentation test in pulmonary tuberculosis: A review of 635 cases. Can Med Assoc J 34: 655-659, 1936.
2. Shenoi S and Friedland G: Extensively drug-resistant tuberculosis: A new face to an old pathogen. Annu Rev Med 60: 307-320, 2009.
3. Chaisson RE and Martinson NA: Tuberculosis in Africa-combating an HIV-driven crisis. N Engl J Med 358: 1089-1092, 2008.
4. Zumla A, Raviglione M, Hafner R and von Reyn CF: Tuberculosis. N Engl J Med 368: 745-755, 2013.
5. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, et al: Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 380: 2095-2128, 2012.
6. Colditz GA, Berkey CS, Mosteller F, Brewer TF, Wilson ME, Burdick E and Fineberg HV: The efficacy of bacillus Calmette-Guérin vaccination of newborns and infants in the prevention of tuberculosis: Meta-analyses of the published literature. Pediatrics 96: 29-35, 1995.
7. Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV and Mosteller F: Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. JAMA 271: 698-702, 1994.
8. Lambert PH, Hawkridge T and Hanekom WA: New vaccines against tuberculosis. Clin Chest Med 30: 811-826, x, 2009.
9. Hussey G, Hawkridge T and Hanekom W: Childhood tuberculosis: Old and new vaccines. Paediatr Respir Rev 8: 148-154, 2007.
10. Pitt JM, Blankley S, McShane H and O'Garra A: Vaccination against tuberculosis: How can we better BCG? Microb Pathog 58: 2-16, 2013.
11. Friedman JM and Jones PA: MicroRNAs: Critical mediators of differentiation, development and disease. Swiss Med Wkly 139: 466-472, 2009.
12. Bartel DP: MicroRNAs: Target recognition and regulatory functions. Cell 136: 215-233, 2009.
13. Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R and Pasquinelli AE: Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 122: 553-563, 2005.
14. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS and Johnson JM: Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433: 769-773, 2005.
15. Baltimore D, Boldin MP, O'Connell RM, Rao DS and Taganov KD: MicroRNAs: New regulators of immune cell development and function. Nat Immunol 9: 839-845, 2008.
16. Harapan H, Fitra F, Ichsan I, Mulyadi M, Miotto P, Hasan NA, Calado M and Cirillo DM: The roles of microRNAs on tuberculosis infection: Meaning or myth? Tuberculosis (Edinb) 93: 596-605, 2013.
17. Das K, Saikolappan S and Dhandayuthapani S: Differential expression of miRNAs by macrophages infected with virulent and avirulent Mycobacterium tuberculosis. Tuberculosis (Edinb) 93 (Suppl): S47-S50, 2013.
18. Chen Z, Wang T, Liu Z, Zhang G, Wang J, Feng S and Liang J: Inhibition of autophagy by MiR-30A induced by mycobacteria tuberculosis as a possible mechanism of immune escape in human macrophages. Jpn J Infect Dis 68: 420-424, 2015.
19. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
20. Li W, Liu C, Zhao C, Zhai L and Lv S: Downregulation of β3 integrin by miR-30a-5p modulates cell adhesion and invasion by interrupting Erk/Ets-1 network in triple-negative breast cancer. Int J Oncol 48: 1155-1164, 2016.
21. Li L, Yang C, Liu X, Yang S, Ye S, Jia J, Liu W and Zhang Y: Elevated expression of microRNA-30b in osteoarthritis and its role in ERG regulation of chondrocyte. Biomed Pharmacother 76: 94-99, 2015.
22. Ye Z, Zhao L, Li J, Chen W and Li X: miR-30d blocked transforming growth factor β1-Induced Epithelial-Mesenchymal transition by targeting snail in ovarian cancer cells. Int J Gynecol Cancer 25: 1574-1581, 2015.
23. Che S, Sun T, Wang J, Jiao Y, Wang C, Meng Q, Qi W and Yan Z: miR-30 overexpression promotes glioma stem cells by regulating Jak/STAT3 signaling pathway. Tumour Biol 36: 6805-6811, 2015.
24. Demolli S, Doebele C, Doddaballapur A, Lang V, Fisslthaler B, Chavakis E, Vinciguerra M, Sciacca S, Henschler R, Hecker M, et al: MicroRNA-30 mediates anti-inflammatory effects of shear stress and KLF2 via repression of angiopoietin 2. J Mol Cell Cardiol 88: 111-119, 2015.
25. Bauer M, Kinkl N, Meixner A, Kremmer E, Riemenschneider M, Förstl H, Gasser T and Ueffing M: Prevention of interferon-stimulated gene expression using microRNA-designed hairpins. Gene Ther 16: 142-147, 2009.
26. Li G, Zhao Y, Wen L, Liu Z, Yan F and Gao C: Identification and characterization of microRNAs in the spleen of common carp immune organ. J Cell Biochem 115: 1768-1778, 2014.
27. Kawai T and Akira S: Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34: 637-650, 2011.
28. Blasius AL and Beutler B: Intracellular toll-like receptors. Immunity 32: 305-315, 2010.
29. Kumar P, Tyagi R, Das G and Bhaskar S: Mycobacterium indicus pranii and Mycobacterium bovis BCG lead to differential macrophage activation in Toll-like receptor-dependent manner. Immunology 143: 258-268, 2014.
30. Saraav I, Singh S and Sharma S: Outcome of Mycobacterium tuberculosis and Toll-like receptor interaction: Immune response or immune evasion? Immunol Cell Biol 92: 741-746, 2014.
31. Kleinnijenhuis J, Oosting M, Joosten LA, Netea MG and Van Crevel R: Innate immune recognition of Mycobacterium tuberculosis. Clin Dev Immunol 2011: 405310, 2011.