Altered microRNA Signatures in Sputum of Patients with Active Pulmonary Tuberculosis

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Abstract
Role of microRNA (miRNA) has been highlighted in pathogen-host interactions recently. At present, their role in active pulmonary tuberculosis is unknown. The aim of the study was to delineate miRNA expression in sputum supernatant of patients with active pulmonary tuberculosis. Expression of miRNAs was evaluated by microarray analysis and differentially expressed miRNAs were validated by RT-qPCR. Secreted cytokines TNF-α and IL-6 were measured by ELISA. We found that 95 miRNAs were differentially expressed between tuberculosis group and controls. More miRNAs (52 out of 95 miRNAs) were underexpressed than overexpressed during tuberculosis infection. Overexpression of miR-3179, miR-147 and underexpression of miR-19b-2* in TB group compared with controls were confirmed in the validation cohort. TNF-α and IL-6 levels were not significantly altered between TB group and controls. For the first time, differential expression of miRNAs in sputum was found in active pulmonary tuberculosis. The study provides rationale for identifying the role of miRNAs in the pathogenesis of pulmonary tuberculosis and indicates potential for miRNA-based therapeutic strategies.

Introduction
MicroRNAs (MiRNAs) are small, noncoding RNAs (~22 nt) that have key roles in regulation of many biological processes, such as development and tumorigenesis, via regulating expression of their target mRNAs [1,2,3]. Emerging evidence also throws light into the role of miRNAs in the intricate host-pathogen interaction networks [4,5]. For example, miRNA-155 is essential for the T cell-mediated control of helicobacter pylori infection [6]. Let-7 family members repress the expression of IL-6 and IL-10 during Salmonella infection [7]. MiR-147 attenuates TLR-induced inflammatory responses [8].

Tuberculosis (TB), caused by Mycobacterium tuberculosis (M. tuberculosis), is one of the most deadly infectious diseases [9]. To date, the role of miRNAs in the pathogenesis of active pulmonary TB has not yet been elucidated. MiRNA spectrum in body fluids can reflect altered physiological and/or pathological conditions [10]. Recent studies have shown that miRNAs are stably present in sputum [11,12] and unique miRNA signatures in sputum are altered in many lung diseases, such as lung cancer and chronic obstructive pulmonary disease [13,14]. Taking into account the central role of miRNAs in disease and significance of sputum in the assessment of lung disease [15], we hypothesized that miRNA expression is altered in sputum of patients with active pulmonary TB. Therefore, the study was aimed to detect miRNAs expression in sputum and to develop further understanding of the role of miRNAs in active pulmonary TB.

Materials and Methods
Human subjects
Fifty-eight patients with active pulmonary TB were enrolled from Affiliated Hospital of Weifang Medical University and Weifang Chest Hospital, China, between December 2009 and January 2011. Eligibility for patients entry into the study included typical symptoms of pulmonary TB such as cough, fever, fibrocavitary lung infiltrate on chest radiograph, at least one positive sputum smear and/or positive sputum culture for M. tuberculosis. Biochemical tests such as niacin production and nitrate reduction were carried out to identify M. tuberculosis. Patients were excluded who had other coexisting lung disease. Thirty-two healthy age and sex matched volunteers were recruited as controls.

Sample preparation, RNA isolation and RNA quality control
Early morning sputum samples, a minimum of 1.5 ml, were collected in a sterile, disposable plastic containers before starting chemotherapy [16] and were then solubilized with an equal volume of 0.1% dithiothreitol within 1 h of collection. Samples...
were placed at 37°C for 30 min to ensure complete homogenization, and were subsequently centrifuged to yield cell free supernatant and cell pellet. To exclude salivary contamination, pellet cells were stained with Wright’s stain for differential cell counts. A sputum sample was considered adequate for further analysis when percentage of squamous cells was less than 80% [17,18,19]. Cell free supernatant was aliquoted and stored immediately in liquid nitrogen until analysis.

Total RNA was extracted using Trizol reagent (Invitrogen) and further purified with a RNeasy mini kit (Qiagen, Denmark) according to the manufacturer’s instructions. RNA quantity and quality were assessed using NanoDrop Spectrophotometer (ND-1000, Nanodrop Technologies) and electrophoresis, respectively [20]. RNA concentrations ranged from 51 to 67 ng/μl. Equal amounts (300 ng) of RNA from each patient and control were pooled and presented as two groups (TB and control), respectively. MiRNA signature profiles were generated from the above two groups.

Detection of miRNAs expression and data analysis

Exiqon miRCURY™ LNA arrays v.16.0, containing 1,223 capture probes covering all human, were used to quantify genome-wide miRNA expression in the two groups described above. One microgram of each group was 3’-end-labeled with a miRCURY™ Hy3™ power labeling kit (Exiqon, Vedbaek) and hybridized on the LNA arrays according to the manufacturer’s instructions. Images on the chip were scanned using an Axon GenePix 4000B microarray scanner (Axon Instruments, Foster City, CA) and imported into GenePix Pro 6.0 software (Axon) for grid alignment and data extraction. MiRNAs with intensities >50 were used to calculate the normalization factor. Expression data were normalized using the median normalization. After normalization, average values of replicate spots of each miRNA were used for statistical analysis. Hierarchical clustering was performed using MEV software (v4.6, TIGR).

RT-qPCR analysis

To validate the microarray results, RT-qPCR was further performed using individual samples from a randomly selected subgroup (30 patients with active pulmonary TB and 30 healthy controls). miR-19b-2*, miR-3179 and miR-147 were selected for RT-qPCR analysis. First-strand cDNA was generated from 200 ng of total RNA using miRNA-specific primers (Table 2). Approximately 2.5 ng of cDNA was then used for PCR analysis using a GeneAmp PCR System 9700 (Applied Biosystems, USA). The threshold cycle (Ct) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. Each miRNA level was normalized with U6 small nuclear RNA level. All samples were run in triplicate and repeated a minimum of two times.

**Table 1.** Characteristics of participants.

| Characteristics                  | TB (N = 58) | Control (N = 32) |
|----------------------------------|-------------|-----------------|
| Male/female                      | 38/20       | 21/11           |
| Age, mean (range) years          | 40.83±18.04 (12–70) | 37.53±15.16 (15–62) |
| TST test                         | Not applicable | Negative       |
| Both sputum smear and culture positive | 32           | 0               |
| Smear negative while culture positive | 26           | 0               |
| Cough                            | 46           | 0               |
| Fever                            | 42           | 0               |
| Weight loss                      | 39           | 0               |
| Night sweats                     | 32           | 0               |
| Hemoptysis                       | 27           | 0               |

All patients had clinical signs and symptoms of active pulmonary TB; comprising, 79.3% cough, 72.4% fever, 67.2% weight loss, 55.2% night sweats, and 46.6% hemoptysis. Healthy controls involved in the study, were free of active TB infection, latent TB infection and any clinical symptoms of any infectious disease. Both TB patients and healthy controls were non-smokers.

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**Table 2.** Oligonucleotides used in this study.

| Primer set name | Reverse transcriptase reaction primer (5’ to 3’) | Real-time quantitative PCR primer (5’ to 3’) |
|-----------------|-----------------------------------------------|-----------------------------------------------|
| U6              | CGCTTCCAGAATTTGCGTGTACAT                      | Forward: CCTTCCAGACACATAATAACTAAAT Reverse: CGCTTCCAGAATTTGCGTGTACAT |
| hsa-miR-19b-2*  | GTCGTATCCAGGTGGAGTCGGAGACAGACTGGAATG         | Forward: GGGAGTTTTGCCAGGTTTGGTGG Reverse: CAGTGCCGTGCTGAG |
| hsa-miR-3179    | GTCGTATCCAGGTGGAGTCGGAGACAGACTGGAATG         | Forward: GGGAGAAAGGGGTGAAAT Reverse: CAGTGCCGTGCTGAG |
| hsa-miR-147     | GTCGTATCCAGGTGGAGTCGGAGACAGACTGGAATG         | Forward: GGGAGGAGGGGTGAAAT Reverse: CAGTGCCGTGCTGAG |

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Enzyme-linked immunosorbent assay (ELISA) analysis
Sputum samples were collected as described above. Protease inhibitors were used to prevent proteolytic degradation during sputum solubilization. Cytokine levels were measured from 50 μl of cell-free supernatants using commercially available ELISA kits (BioSource, Nivelles, Belgium) according to the manufacturer’s instructions. All samples were assayed in duplicate.

Statistical analysis
Data were presented as mean ± standard deviation (SD). ANOVA test or student’s t test was used for statistical analysis. P<0.05 was regarded as significantly different.

Results
Differential expression of miRNAs between TB patients and controls
A total of 95 miRNAs were differentially expressed between TB group and controls. Forty-three miRNAs were overexpressed and fifty-two miRNAs were underexpressed in TB group compared with controls. MiRNAs levels in TB group were 3.08- to 340- fold overexpression, but 2.56- to 25.0- fold underexpression in comparison with controls (Tables 3, 4), respectively. Cluster analysis based on these differentially expressed miRNAs showed a clear distinction between TB group and controls (Figure 1).

Validation of microarray results by RT-qPCR
Three miRNAs, miR-19b-2*, miR-3179 and miR-147, were selected for further validation of microarray results using RT-qPCR. The reasons for choosing them were that miR-19b-2* and miR-3179 were the most underexpressed and overexpressed miRNAs in TB group compared with controls, respectively. MiR-147 was of an extra interest because it is a negative regulator of inflammatory responses [8]. To better understand the association between TB infection and miR-147, it was selected for validation.

Data analysis showed that miR-19b-2* was underexpressed while miR-3179 and miR-147 were overexpressed in TB group compared with controls. The RT-qPCR results were consistent with those of microarray (Figure 2).

Measurement of cytokines levels
Both microarray and RT-qPCR results showed that miR-147 was overexpressed in TB group compared with controls. Considering the role of miR-147 in reduction of TNF-α and IL-6 [8], we hypothesized that TNF-α and IL-6 levels in TB sputum might not be altered. Consistent with the hypothesis, their levels were not obviously altered between TB group and controls (data not shown).

Discussion
Recent studies have shown that infection of human macrophages with Mycobacterium avium hominisuis causes a specific miRNA response [21] and infection of mice with Mycobacterium bovis bacillus Calmette-Guérin (BCG) downregulates miR-29 expres-
Table 3. Overexpressed miRNAs in TB sputum.

| Name              | TB patients versus control | Chromosome |
|-------------------|----------------------------|------------|
| hsa-miR-2116*     | 3.08                       | 15         |
| hsa-miR-409-3p    | 3.09                       | 14         |
| hsa-miR-1204      | 3.10                       | 8          |
| hsa-miR-3131      | 3.15                       | 2          |
| hsa-miR-491-5p    | 3.20                       | 9          |
| hsa-miR-499-5p    | 3.22                       | 20         |
| hsa-miR-147       | 3.34                       | 9          |
| hsa-miR-518f*     | 3.40                       | 19         |
| hsa-miR-3156      | 3.45                       | 10         |
| hsa-miR-3170      | 3.63                       | 13         |
| hsa-miR-20b       | 4.18                       | X          |
| hsa-miR-151-5p    | 4.29                       | 8          |
| hsa-miR-1         | 4.44                       | 20         |
| hsa-miR-920       | 4.47                       | 12         |
| hsa-miR-3913      | 4.48                       | 12         |
| hsa-miR-190b      | 4.50                       | 1          |
| hsa-miR-616*      | 4.54                       | 12         |
| hsa-miR-3662      | 4.61                       | 6          |
| hsa-miR-376c      | 4.75                       | 14         |
| hsa-miR-4325      | 4.85                       | 20         |
| hsa-miR-29a       | 5.21                       | 7          |
| hsa-miR-96*       | 5.24                       | 7          |
| hsa-miR-1246      | 5.26                       | 2          |
| hsa-miR-325       | 5.96                       | X          |
| hsa-miR-3610      | 6.27                       | 8          |
| hsa-miR-633       | 6.27                       | 17         |
| hsa-miR-3673      | 6.34                       | 8          |
| hsa-miR-631       | 6.71                       | 15         |
| hsa-miR-1911*     | 7.03                       | X          |
| hsa-miR-548d-5p   | 7.32                       | 17         |
| hsa-miR-3692*     | 7.54                       | 6          |
| hsa-miR-151-3p    | 7.60                       | 8          |
| hsa-miR-3163      | 7.65                       | 11         |
| hsa-miR-367*      | 10.1                       | 4          |
| hsa-miR-302d      | 11.7                       | 4          |
| hsa-miR-526b      | 11.9                       | 19         |
| hsa-miR-3609      | 13.1                       | 7          |
| hsa-miR-23a       | 14.2                       | 19         |
| hsa-miR-539       | 18.2                       | 14         |
| hsa-miR-1289      | 20.8                       | 20         |
| hsa-miRPlus-11247*| 24.4                      | Unknown    |
| hsa-let-7g*       | 25.8                       | 3          |
| hsa-miR-3179      | 347                        | 16         |

Column “Name” contained the name of miRNA; Column “TB patients versus control” contained level ratio of TB/control; Column “Chromosome” meant distribution of each miRNA on chromosome. Each miRNA spot was replicated for four times on the same slide and two microarray chips were used for each group. After normalization, obtained average values for each miRNA spot were used for statistics. The P values for these miRNAs were less than 0.05 in TB group compared with controls.

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Table 4. Underexpressed miRNAs in TB sputum.

| Name              | TB patients versus control | Chromosome |
|-------------------|----------------------------|------------|
| hsa-miR-19b-2*    | 0.04                       | X          |
| hsa-miR-3152      | 0.06                       | 9          |
| hsa-miR-3656      | 0.09                       | 11         |
| hsa-miR-3180-3p   | 0.10                       | 16         |
| hsa-miR-1203      | 0.15                       | 17         |
| hsa-miR-548l      | 0.16                       | 11         |
| hsa-miR-1248      | 0.17                       | 3          |
| hsa-miR-3652      | 0.18                       | 12         |
| hsa-miR-512-5p    | 0.19                       | 19         |
| hsa-miR-3667-3p   | 0.20                       | 22         |
| hsa-miR-149       | 0.20                       | 2          |
| hsa-miR-3927      | 0.20                       | 9          |
| hsa-let-7b        | 0.21                       | 22         |
| hsa-miR-301a      | 0.21                       | 17         |
| hsa-miR-1909*     | 0.22                       | 19         |
| hsa-miR-125b-1*   | 0.22                       | 11         |
| hsa-miR-582-3p    | 0.23                       | 5          |
| hsa-miR-663       | 0.24                       | 20         |
| hsa-miR-515-3p    | 0.26                       | 19         |
| hsa-miR-1228*     | 0.27                       | 12         |
| hsa-miR-4296      | 0.28                       | 10         |
| hsa-miR-3195      | 0.29                       | 20         |
| hsa-miR-1260b     | 0.29                       | 11         |
| hsa-miR-380*      | 0.29                       | 14         |
| hsa-miR-3175      | 0.29                       | 15         |
| hsa-miR-371-3p    | 0.30                       | 19         |
| hsa-miR-585       | 0.30                       | 5          |
| hsa-miR-4320      | 0.31                       | 18         |
| hsa-miR-504       | 0.32                       | X          |
| hsa-miR-200b      | 0.33                       | 1          |
| hsa-miR-181c      | 0.33                       | 19         |
| hsa-let-7c        | 0.34                       | 21         |
| hsa-miR-4276      | 0.34                       | 4          |
| hsa-miR-625       | 0.35                       | 14         |
| hsa-miR-3664      | 0.35                       | 11         |
| hsa-miR-3648      | 0.35                       | 21         |
| hsa-miR-373       | 0.35                       | 19         |
| hsa-miR-3678-5p   | 0.35                       | 17         |
| hsa-miR-744       | 0.36                       | 17         |
| hsa-miR-769-3p    | 0.36                       | 19         |
| hsa-miR-649       | 0.36                       | 22         |
| hsa-miR-586       | 0.37                       | 6          |
| hsa-miR-498       | 0.38                       | 19         |
| hsa-miR-708*      | 0.38                       | 11         |
| hsa-miR-99b       | 0.38                       | 19         |
| hsa-miR-935       | 0.38                       | 19         |
| hsa-miR-802       | 0.38                       | 21         |
| hsa-miR-618       | 0.38                       | 12         |
| hsa-miR-181a      | 0.38                       | 9          |
MiR-301a overexpression contributes to NF-kB activation in innate immune response [26] and miR-let-7c is a potent anti-inflammatory miRNA [8]. Recent studies have shown that miR-let-7c in innate immune response [26] and miR-let-7c is underexpressed in sputum of patients with chronic obstructive pulmonary disease [14]. Our results showed that miR-let-7b and miR-let-7c were underexpressed while miR-let-7g* was overexpressed in TB sputum compared with controls, which suggests that let-7 family members are also involved in regulation of anti-TB immune response.

It is worth mentioning that several miRNAs, such as miR-150 and miR-155 [27,28], have been shown to regulate adaptive immune response, while our results showed that their levels in both sputum and serum were not changed significantly between TB group and controls, which suggests that other miRNAs may be involved in regulation of anti-TB immune response.

A recent study shows that miR-29a is specifically overexpressed after mycobacterial infection of human macrophages [21], which is in good consistency with our results that miR-29a was 5.21- and 11.9-fold overexpression in TB sputum and serum compared with controls, respectively. Interferon-γ (IFN-γ) has a critical role in immune responses to intracellular bacterial infection and miR-29 is found to suppress immune responses to intracellular pathogens by downregulating IFN-γ [22]. Overexpressed miR-29a in our study could partly explain one mechanism by which M. tuberculosis avoids macrophage killing through inhibition of IFN-γ-mediated signaling. These data suggest that miR-29a might act as a negative regulator of immune response against TB infection.

The preferred niche of M. tuberculosis is the macrophages [29]. Macrophages recognize invading M. tuberculosis primarily through Toll-like receptors (TLRs) [30]. TLR signals can activate NF-kB, which results in induction of multiple pro-inflammatory cytokines, such as TNF-α and IL-6 [31]. A recent study shows that, upon activation of TLRs/NF-kB signaling pathway, miR-147 is induced in murine macrophages and attenuates expression of proinflammatory cytokines, such as TNF-α and IL-6, which indicates that miR-147 appears to have potent anti-inflammatory properties [8]. Our results showed that miR-147 was 3.94-fold overexpression in TB sputum compared with controls. Considering the role of miR-147 in down-regulating excessive inflammatory responses, we hypothesized that both TNF-α and IL-6 levels in TB sputum are not overexpressed in comparison with controls. Consistent with the hypothesis, levels of TNF-α and IL-6 did not differ significantly between TB group and controls. MiR-147 attenuates TLRs stimulation-induced-inflammatory response, however, the detailed mechanism is unknown. Targets for miR-147 identified by computational prediction programs, such as TargetScan, have not previously been shown to be directly involved in TLR signaling events. Thus, the mechanism by which miR-147 regulates TLR-induced inflammatory responses requires further investigation.

**Figure 2. Confirmation miRNA level by RT-qPCR.** RT-qPCR analysis confirmed microarray data. After normalization to U6 RNA, data were presented as mean ± SD (n = 30) and obtained average value for each miRNA was used for statistics. MiR-19b-2* was underexpressed while miR-3179 and miR-147 were overexpressed in TB sputum compared with controls. The experiment was conducted in triplicate. *P<0.05 versus control. doi:10.1371/journal.pone.0043184.g002
Taken together, we showed for the first time that miRNA expression profiles in sputum were significantly altered during TB infection, which provides rationale for studying the role of miRNAs in the pathogenesis of active pulmonary TB and indicates potential for improving diagnosis, prognosis and their impact on future therapeutic strategies. However, it is difficult to synthesize the study results to reach a definitive conclusion based on this single study. The association between TB infection and miRNAs is just beginning to be explored and further investigation is required to determine the role of miRNAs in active pulmonary tuberculosis.

References

1. Lagos-Quintana M, Rauhut R, Lendelewel W, Tuschel T (2001) Identification of novel genes coding for small expressed RNAs. Science 294: 853–856.
2. Pattyn A, Lai EC (2008) Biological principles of microRNA-mediated regulation: shared themes amid diversity. Nat Rev Genet 9: 831–842.
3. Ma R, Jiang T, Kang X (2012) Circulating microRNAs in cancer: origin, function and application. J Exp Clin Cancer Res 31: 36.
4. Chea XM, Splinter PL, O’Hara SP, LaRusso NF (2007) A cellular micro-RNA, let-7i, regulates Toll-like receptor 4 expression and contributes to cholangiocyte immune responses against Cryptosporidium parvum infection. J Biol Chem 282: 20929–20938.
5. Zhou R, Hu G, Liu J, Gong YG, Drescher KM, et al. (2009) NF-kappaB p65-dependent transactivation of microRNA genes following Cryptosporidium parvum infection stimulates epithelial cell immune responses. PLoS Pathog 5: e1000611.
6. Oertli M, Engler DB, Kohler E, Koch M, Meyer TF, et al. (2011) MicroRNA-155 is essential for the T cell-mediated control of helicobacter pylori infection and for the induction of chronic gastritis and colitis. J Immunol 187: 3578–3586.
7. Schulte LN, Furlno A, Moellenkopf HJ, Reinhardt R, Vogel J (2011) Analysis of the host microRNA response to Salmonella uncovers the control of major cytokines by the let-7 family. EMBO J 30: 1977–1989.
8. Liu G, Friggeri A, Yang Y, Park YJ, Tsuruta Y, et al. (2009) miR-147, a microRNA that is induced upon Toll-like receptor stimulation, regulates murine macrophage inflammatory responses. Proc Natl Acad Sci U S A 106: 15019–15024.
9. Dye C, Williams BG (2010) The population dynamics and control of tuberculosis. Science 326: 856–861.
10. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, et al. (2008) Serum microRNAs are promising novel biomarkers. PLoS One 3: e1348.
11. Xie Y, Todd NW, Liu Z, Zhan M, Fang H, et al. (2010) Altered microRNA expression in sputum for diagnosis of non-small cell lung cancer. Lung Cancer 67: 170–176.
12. Yu L, Todd NW, Xing L, Xie Y, Zhang H, et al. (2010) Early detection of lung adenocarcinoma in sputum by a panel of microRNA markers. Int J Cancer 127: 2870–2878.
13. Moghazy KE, McElvany NG, Greene CM (2010) MicroRNAs in inflammatory lung disease–master regulators or target practice? Respir Res 11: 148.
14. Pottecher GE, Mesulaghi P, Bracke KR, Thos O, Durme YM, et al. (2011) MicroRNA expression in induced sputum of smokers and patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 183: 898–906.
15. Gray RD, MacGregor G, Noble D, Innis M, Dewar M, et al. (2008) Sputum proteomics in inflammatory and suppurative respiratory diseases. Am J Respir Crit Care Med 178: 444–452.
16. Casado R, Ladarola P, Pannell I, Lainetti M, Corinco A, et al. (2007) Protein expression in sputum of smokers and chronic obstructive pulmonary disease patients: a pilot study by CapLC-ESI-Q-TOF. J Proteome Res 6: 4615–4623.
17. Gershman NH, Liu H, Wong HH, Liu JT, Fahy JV (1999) Fractional analysis of sequential induced sputum samples during sputum induction: evidence that different lung compartments are sampled at different time points. J Allergy Clin Immunol 104: 322–328.
18. Sagel SD, Kapner R, Osberg I, Sonntag MK, Accurso FJ (2001) Airway inflammation in children with cystic fibrosis and healthy children assessed by sputum induction. Am J Respir Crit Care Med 164: 1425–1431.
19. Soman AJ, Lindner RA, Prasad SS, Sebastian LT, Pedersen SK, et al. (2005) Proteomic analysis of sputum from adults and children with cystic fibrosis and from control subjects. Am J Respir Crit Care Med 172: 1416–1426.
20. Thunnissen FB (2003) Sputum examination for early detection of lung cancer. J Clin Pathol 56: 853–856.
21. Sharbati J, Lewin A, Kutz-Lohoff B, Kamal E, Einspanier R, et al. (2011) Integrated microRNA-mRNA-analysis of human monocye derived macrophages upon mycobacterium avium subsp. hominisuus infection. PLoS One 6: e20252.
22. Ma F, Xu S, Liu X, Zhang Q, Xu X, et al. (2011) The microRNA miR-29 controls innate and adaptive immune responses to intracellular bacterial infection by targeting interferon-γ. Nat Immunol 12: 861–869.
23. Fu Y, Yi Z, Wu X, Li J, Xu F (2011) Circulating microRNAs in patients with pulmonary tuberculosis. J Clin Microbiol 49: 4246–4251.
24. Lu Z, Li Y, Takwi A, Li B, Zhang J, et al. (2011) miR-301a as an NF-kB activator in pancreatic cancer cells. EMBO J 30: 57–67.
25. Huang Q, Gumireddy K, Schrier M, Le Sage C, Nagel R, et al. (2008) The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol 10: 202–210.
26. Witwer KW, Sink JM, Gama L, Clemments JE (2010) MicroRNA regulation of TNF-beta protein expression: rapid and sensitive modulation of the innate immune response. J Immunol 184: 529–537.
27. Xiao C, Calado DP, Galler G, Thai TH, Patterson HC, et al. (2007) MIR-150 controls B cell differentiation by targeting the transcription factor c-Myc. Cell 131: 146–159.
28. Thai TH, Calado DP, Casola S, Ansel KM, Xiao C, et al. (2007) Regulation of the germinal center response by microRNA-155. Science 316: 604–608.
29. Loquillet C, Martinon F, Perez C, Munoz M, Thome M, et al. (2006) Mycobacterium tuberculosis salivary immune innnunity to evade specific effectors. J Immunol 177: 6245–6255.
30. Kleemunajhuis J, Oosting M, Joosten LA, Netea MG, Van Crevel R (2011) Innate immune recognition of Mycobacterium tuberculosis. Clin Dev Immunol 2011: 465310.
31. Moynagh PN (2005) The NF-kappaB pathway. J Cell Sci 118: 4589–4592.

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Author Contributions

Conceived and designed the experiments: YF ZY. Performed the experiments: YF ZY RJ RL ZG. Analyzed the data: YF ZY RJ RL ZG. Contributed reagents/materials/analysis tools: YF ZY RJ RL ZG. Wrote the paper: YF ZY RJ RL ZG.