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LncRNA MZF1 Antisense RNA 1 Promotes *Mycoplasma Pneumoniae* Pneumonia in Children via miR-16-5p

Qin Wang[1], Bing Han[1], Yanan Ji[1], Cuicui Zhang[1], Lianying Ruan[1], Hengrui Hao[2*]

[1] Department of Pediatrics, Xingtai People's Hospital, Xingtai, Hebei 054000, China

[2] Pediatric Intensive Care Unit, Xingtai People's Hospital, Xingtai, Hebei 054000, China

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ABSTRACT

Objective: To determine the expression, as well as the underlying mechanism, of lncRNA MZF1 antisense RNA 1 (MZF1-AS1) in *Mycoplasma pneumoniae* pneumonia (MPP).

Methods: GSE179051 was analyzed for differentially expressed lncRNAs in MP infection. The levels of MZF1-AS1 were determined by quantitative Real-time PCR in children with MPP and healthy subjects. The diagnostic value of MZF1-AS1 was assessed using ROC curves. The effects of MZF1-AS1 knockdown on A549, human primary alveolar macrophages (hpAM) and human airway smooth muscle (ASM) cell function were assessed. The downstream miRNA for MZF1-AS1 was predicted and validated.

Results: MZF1-AS1 was a differentially expressed lncRNA in MP infection and significantly increased in MPP children, especially in those with new-onset asthma. ROC results revealed that MZF1-AS1 can distinguish MPP children from the controls with an area under the ROC curves (AUC) of 0.87 (sensitivity: 77.6%, specificity: 90.5%) at a threshold of 1.3451 and an AUC of 0.83 (sensitivity: 77.1%, specificity: 91.0%) at a threshold of 1.3539. MZF1-AS1 can distinguish the MPP combined with new asthma from the MPP without asthma with an AUC of 0.83 (sensitivity: 75.3%, specificity: 77.8%) at the threshold of 1.7888. Downregulation of MZF1-AS1 can alleviate inflammatory factor release in A549 and hpAM cells. miR-16-5p was a target of MZF1-AS1.

Conclusion: MZF1-AS1 is upregulated in MPP children and might result in increased levels of inflammatory cytokines through targeting miR-16-5p, thus contributing to MPP.

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*** Corresponding Author:** Hengrui Hao, *hanghengrui054000@163.com*

Introduction

Prior to the COVID-19 pandemic, *Mycoplasma pneumoniae* (MP) was a common cause of respiratory infections.[1,2] MP infection accounts for about 40% of pneumonia cases in children.[3] The results of physical examination are non-specific, making it difficult to distinguish MP pneumonia (MPP) from other respiratory infections.[3,4] MPP is usually a benign self-limiting condition. However, the complex situations that may be encountered in clinical practice are antibiotic resistance of MPP and refractory MPP with an exaggerated immune response to MP.[5] Alveolar macrophages are the most abundant innate immune cells in the lung.[6] They are the first to initiate an immune response when pathogens invade the lungs. MP is believed to be implicated in the mechanisms of new-onset asthma and exacerbation of asthma.[4,7] Existing mouse models have shown that MP infection can lead to bronchial constriction and increased airflow resistance.[7] In asthma patients, MP may exacerbate asthma symptoms and cause asthma exacerbations.[8] MP can be detected in 20% of children hospitalized due to worsening asthma.[9] Among children with newly developed wheezing symptoms, a high rate of the cases was found to be infected with MP.[10] MP infection can stimulate various cells (epithelial cells, endothelial cells, macrophages, and smooth muscle cells) and cellular components to participate in the pathological and physiological processes of asthma.[11] Therefore, the molecular mechanism of MPP and the rapid and accurate diagnosis of MPP are important.

Epigenetics is a branch of biology that describes genetic expressivity in response to environmental cues and the expression of age- and sex-related phenotypic traits.[12] Epigenetic modifications can regulate gene expression and signaling pathways, ultimately, altering cellular physiology. The epigenetic mechanisms of long non-coding RNAs (lncRNA), represented by miRNA and lncRNA, have been proven to be widely involved in the regulation of gene expression. More and more evidence suggests that these ncRNAs are differentially expressed in MP infection and affect various biological processes, playing an indispensable role in the initiation and promotion of MP infection.[13] The mechanism of miRNA- and lncRNA-mediated MP infection may include inflammatory response, cell apoptosis, etc.[14] Focusing on miRNAs and lncRNAs associated with MP infection may provide new insights for the early diagnosis and treatment of MPP.

This study focused on the differentially expressed lncRNAs caused by MP in epithelial cells based on the GSE179051 dataset. Among them, MZF1-AS1 has been reported to be associated with obesity-related asthma.[15] Therefore, we investigated the expression level and diagnostic value of MZF1-AS1 in children with MPP. We then investigated the potential mechanism of MZF1-AS1 in MPP combined with asthma using three cell lines.

Materials and Methods

Exploration of lncRNAs

The GSE179051 chip data was obtained from GEO, and the whole transcriptome changes caused by MP in epithelial cells were screened using online GEO2R. After downloading the obtained results, the encoding genes were removed and the abnormally expressed ncRNAs were screened. The obtained ncRNAs were displayed using a volcano plot.

Participants and sampling

The sample size was calculated using PASS (version 2021) software and the Tests for One ROC Curve mode. The total number of MPP cases was calculated based on the proportion of 46% of MPP cases that may develop asthma. The total numerical result for the AUC test with continuous data was 172. Then, with a 20% expansion, this study finally identified a retrospective inclusion of 210 cases.

The Medical Research Ethics Committee of Xingtai People's Hospital has reviewed and approved our research protocol (Serial No. 2021009106) and informed patient consent form. All guardians of the participants provided written informed consent forms. Children diagnosed with MPP under the age of 10 years were included in this study based on the following inclusion criteria: clinical manifestations are consistent with pneumonia, including fever, cough, dyspnoea, dry or productive sputum, and radiological lung abnormalities; positive results of serological tests (MP IgM detection ≥ 1:40, YHLO Biotech, China)) and 7500 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, United States) were diagnosed as MP infection; MP is the only pathogen. If a child was diagnosed with pre-existing asthma, chronic lung disease, bronchopulmonary malformations, immunodeficiency, immunosuppression, cardiovascular disease, or co-infection with other

pathogens, they were excluded. If a child had already been treated with antibiotics or had symptoms related to pneumonia or asthma, they were excluded. These patients were distributed from October 2022 to March 2023. After obtaining the consent of their guardians, blood samples of these children were collected upon admission. In addition, 210 healthy children who underwent routine health examinations in our hospital during the same period were used as controls, upon written informed consent forms from the guardians.

Cell culture, transfection and infection

This study used three types of cells: Lung epithelial cell A549 (No. CCL-185, ATCC, USA), human primary alveolar macrophages (No. JY-J1130, Shanghai Jinyuan Biotechnology, China), and human airway smooth muscle (ASM) cells (No. 3410, ScienCell, USA). All cells were cultured according to the manufacturer's recommendations.

At passages five to eight, cells were seeded with a medium containing Lipofectamine RNAiMAX (Invitrogen, USA) and transfection vectors, such as MZF1-AS1 siRNA (si-MZF1-AS1) or negative control (si-NC).

The MP standard strain M129 (South China University, China) was cultured in PPLO broth medium (BD Biosciences, USA) until orange or yellow. After amplification and cultivation in a new broth medium, lipid-associated membrane proteins (LAMPs) were prepared as described by Ding et al [16]. The prepared LAMPs were evaluated for concentration using an enhanced BCA protein assay kit (Beyotime Biotech, China). For cell treatment, 8 μg/mL LAMPs were used to culture the three kinds of cells, respectively, for 16 hours.

Co-culture of LAMPs-infected human primary alveolar macrophages and ASM cells

A co-culture model in vitro was constructed using a 0.4 μm of polyethylene terephthalate (PET) Transwell insertion system. In short, human primary alveolar macrophages with different transfections were infected with LAMPs and then inoculated into the upper chamber, while ASM cells were inoculated into the lower chamber, to establish a co-culture system. After co-culture, ASM cells were washed and subjected to subsequent measurements.

Measurement of A549 cell growth inhibition

Based on the absorbance at 450nm, we conducted a cell growth inhibition experiment using a 96-well plate using Cell Count Kit-8 (CCK-8; Rockville, USA). Firstly, the cells are digested and resuspended. After dilution, 100 μL of cell suspension was added to the plate well. Cell inhibition rate=[(Ac-As)/(Ac-Ab)] × 100%. Ac, As, and Ab mean absorbance value in control, experiment, and blank.

Apoptosis measurement

The analysis of apoptosis was done by Annexin V-FITC/PI Apoptosis Kit (Elabscience Biotechnology, China), followed by flow cytometry analysis and quantification by FlowJo software.

Inflammatory mediator assays

Inflammatory mediators, IL-1β, and TNF-α in cell culture supernatant were assessed by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, USA). Data were expressed as pg/mL.

RNA expression

Total RNA was isolated by Trizol (Ambion, USA). Then complementary DNA RNA was obtained by reverse translation using PCR Master Mix (Vazyme Biotech, China). Expression of RNAs was quantified by SYBR green-based, quantitative real-time (qRT)-PCR using an AceR qPCR SYBR Green Master Mix (Vazyme Biotech). Housekeeping genes included GAPDH, U6, and β-actin, for lncRNA, miRNA, and mRNA, respectively. Calculation was done by $2^{-\Delta\Delta Ct}$ method.

Luciferase assay

MZF1-AS1 cDNA fragment, which encompassed microRNA binding sites, was inserted into the pmirGLO plasmids (Promega, USA), defined as wt-MZF1-AS1. Mutant MZF1-AS1 (mut-MZF1-AS1) was generated by site-directed mutagenesis PCR with platinum pfx DNA polymerase. miR-16-5p mimic or negative control (NC) and luciferase reporter plasmids were co-transfected into A549 cells using Engreen Entranster™ H4000. At 48 hours after transfection, luminescent signals were quantified by Dual-Luciferase Reporter Assay System (Promega, USA) on a microplate reader. Each value from the firefly luciferase constructs was normalized by corresponding Renilla luciferase value, as relative luciferase activity.

Biotin-coupled probe pull-down assay

Two biotinylated probes were specifically

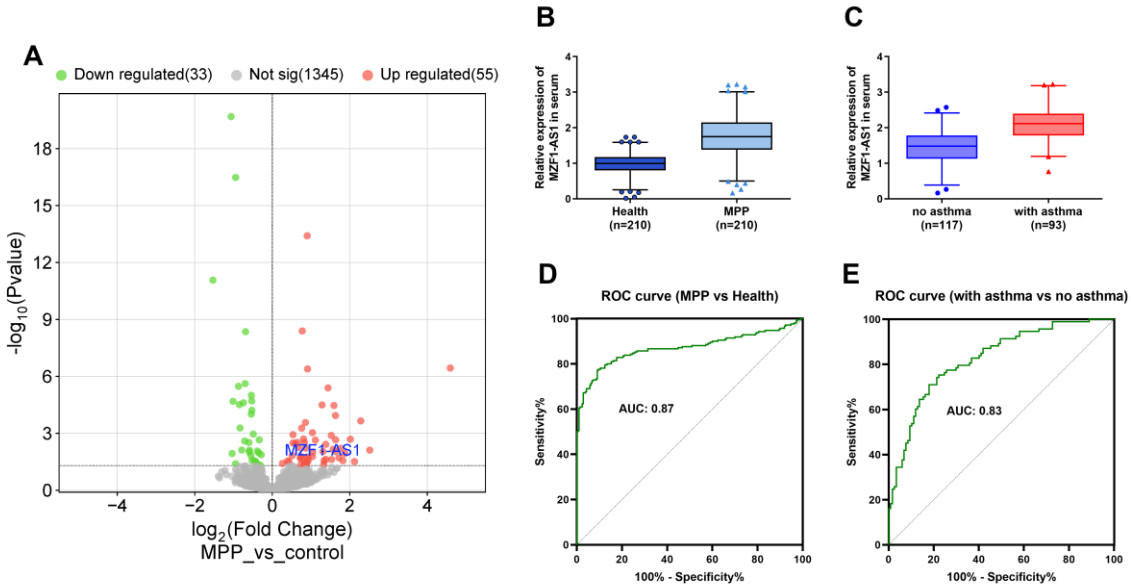

Figure 1. The expression and diagnostic significance of LncRNA MZF1-AS1 in MPP. (A) Volcano plot of differentially expressed lncRNAs in MP-infected epithelial cells based on GSE179051 dataset. (B) MZF1-AS1 expression in serum from MPP children and healthy children was quantified by qRT-PCR. The error band indicates the 2.5-97.5 percentile. ***$P <$ 0.001 (C) MZF1-AS1 expression in serum from MPP children with or without new-onset asthma was quantified by qRT-PCR. The error band indicates the 2.5-97.5 percentile. ***$P < 0.001$. (D) ROC curve of MZF1-AS1 in serum yielded an area under the curve (AUC) of 0.87 for MPP children from the health. (E) ROC curve of MZF1-AS1 in serum yielded an AUC of 0.83 for MPP with newly asthma versus MPP without asthma. MPP, *Mycoplasma pneumoniae* pneumonia.

designed, a positive probe to bind to the junctional region of miR-16-5p and a negative probe used as a control. The cells were washed in cold PBS, lysed in lysis buffer, and incubated with 3 μg of biotinylated probe for 2 hours. By incubating the cell lysate with magnetic beads (Life Technologies, USA) for 4 hours, the pulled-down RNA was extracted using Trizol reagent, and analyzed by quantitative RT-PCR.

RNA immunoprecipitation (RIP) assay

Cells were lysed and incubated with EZ-Magna RIP RNA binding protein immunoprecipitation buffer (Millipore, USA) containing magnetic beads conjugated with anti-Ago2 antibodies and negative control immunoglobulin G (IgG; Abcam). RT-PCR analysis of extracted RNA was performed to identify the enrichment of MZF1-AS1.

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway analyses *Target predictions and gene annotations*

miRNAs for MZF1-AS1 and targets for miR-16-5p were predicted on ENCORI (https://rnasysu.com/encori/). GO and KEGG pathway analyses were performed using OE cloud tools (https://cloud.oebiotech.com).

Statistical analysis

GraphPad Prism software (version 7.04) was used for statistical analysis. Shapiro-Wilk test was used to test the normal distribution of each dataset. Comparisons between two groups were performed using a non-parametric Mann-Whitney U-test or t-test, according to data distribution. Comparisons among more than two groups were achieved using one- or two-way analysis of variance (multiple-comparison tests). To test the clinical significance of MZF1-AS1, receiver operating characteristic (ROC) curves were generated in different patient sets. A *P* value of less than 0.05 was used to determine significance.

Results

Upregulation of MZF1-AS1 in pneumonia potentially distinguished pneumonia from health

In the GSE179051 dataset, based on the screening conditions of logFC absolute value greater than 1 and adjP less than 0.05, 31 lncRNAs were found to exhibit abnormal expression in MP-treated epithelial cells (including 4 downregulated lncRNAs and 27 upregulated lncRNAs), with MZF1-AS1 appearing as an upregulated lncRNA (**Figure 1A**). In our pediatric patient cohort, MZF1-AS1 showed elevated expression levels in the blood of MPP

children (**Figure 1B**). Among our 210 pediatric MPP patients, 93 of them had newly developed asthma. The expression level of MZF1-AS1 in children with MPP combined with newly diagnosed asthma was higher than that in MPP children without asthma (**Figure 1C**). Then we assessed the values of MZF1-AS1 in distinguishing MPP from the health. The areas under the ROC curves were 0.87, with a sensitivity of 77.6% and specificity of 90.5% at the threshold of 1.3451 and a sensitivity of 77.1% and specificity of 91.0% at the threshold of 1.3539, for MPP children from the healthy ones (**Figure 1D**). The areas under the ROC curves were 0.83 with a sensitivity of 75.3% and specificity of 77.8% at the threshold of 1.7888, for the MPP combined with new-onset asthma from the MPP without asthma (**Figure 1E**).

MZF1-AS1 inhibited lung cell growth but promoted inflammation

Next, we used lung epithelial A549 cells to explore the role of MAF1-AS1 in MPP. The expression level of MAF1-AS1 in A549 cells increased after MP infection; then, MAF1-AS1 siRNA can knock down the expression level of MAF1-AS1 (**Figure 2A**). The results of the cell growth inhibition test showed that MP infection can inhibit the growth of A549 cells, but knockdown of MAF1-AS1 can alleviate its growth inhibition on A549 cells (**Figure 2B**). In addition, MP can

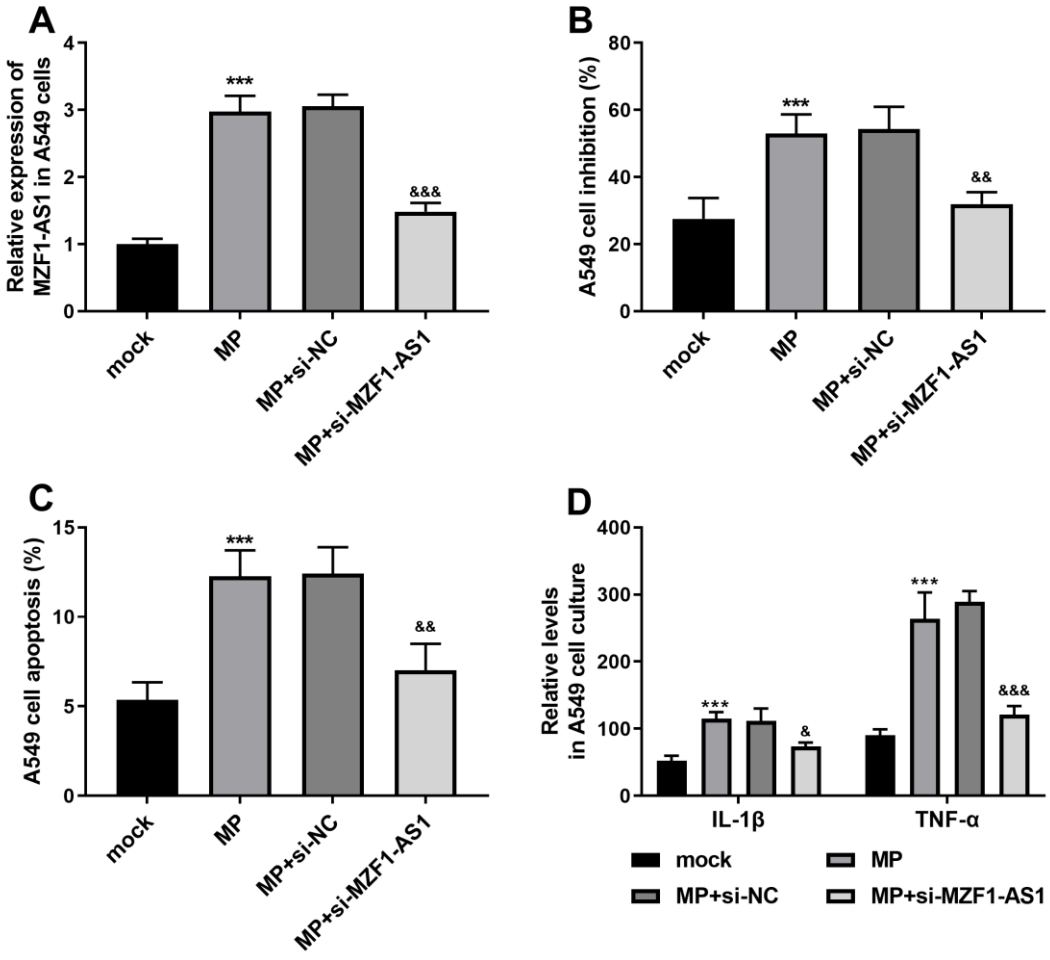

Figure 2. The effect of MZF1-AS1 inhibition on A549 cell growth and inflammation. (A) MZF1-AS1 expression in MP-infected A549 cells was quantified by qRT-PCR. (B) Effect of MZF1-AS1 knockdown on the growth inhibition rate of MP-infected A549 cells. (C) Effect of MZF1-AS1 knockdown on the apoptotic rate of MP-infected A549 cells. (D) Effect of MZF1-AS1 knockdown on the release of TNF-α and IL-1β. ***$P < 0.001$, MP vs mock. &$P < 0.05$, &&$P < 0.01$, &&&$P < 0.001$, MP+si-MZF1-AS1 vs MP+si-NC. MP, *Mycoplasma pneumoniae*.

promote apoptosis of A549 cells, but knocking down MAF1-AS1 can inhibit its promotion of A549 apoptosis (**Figure 2C**). The results of inflammatory factors showed that the elevation of TNF-α and IL-1β after MP infection can be suppressed by knocking down MAF1-AS1 (**Figure 2D**).

MZF1-AS1 influenced alveolar macrophages and ASM cell function

Next, we measured the impact of MZF1-AS1 on the function of human primary alveolar macrophages (hpAM). The expression level of MZF1-AS1 was increased in hpAM infected with MP; this increase can be reversed by its specific siRNA (**Figure 3A**). The levels of TNF-α and IL-1β in hpAM cell culture medium were increased due to MP infection but decreased due to MZF1-AS1 knockdown (**Figure 3B**). The M1 phenotype markers iNOS and CXCL1 mRNAs in hpAM increased after MP infection, whereas MZF1-AS1 knockdown can reduce the expression levels of these markers (**Figure 3C**). After co-culturing with various processed hpAM, it was found that MP-infected hpAM can increase the expression level of MZF1-AS1 in ASM cells, while MZF1-AS1 knockdown of hpAM can reduce the expression level of MZF1-AS1 (**Figure 3D**). The level of MUC5AC mRNA, a major component of airway gel-forming mucin, in ASM cells was increased by MP infection, but MZF1-AS1 knockdown can inhibit this increase (**Figure 3E**). MP-infected hpAM can promote the growth of ASM cells, but knockdown of MZF1-AS1 can counteract the growth of ASM cells promoted by MP-infected hpAM (**Figure 3F**).

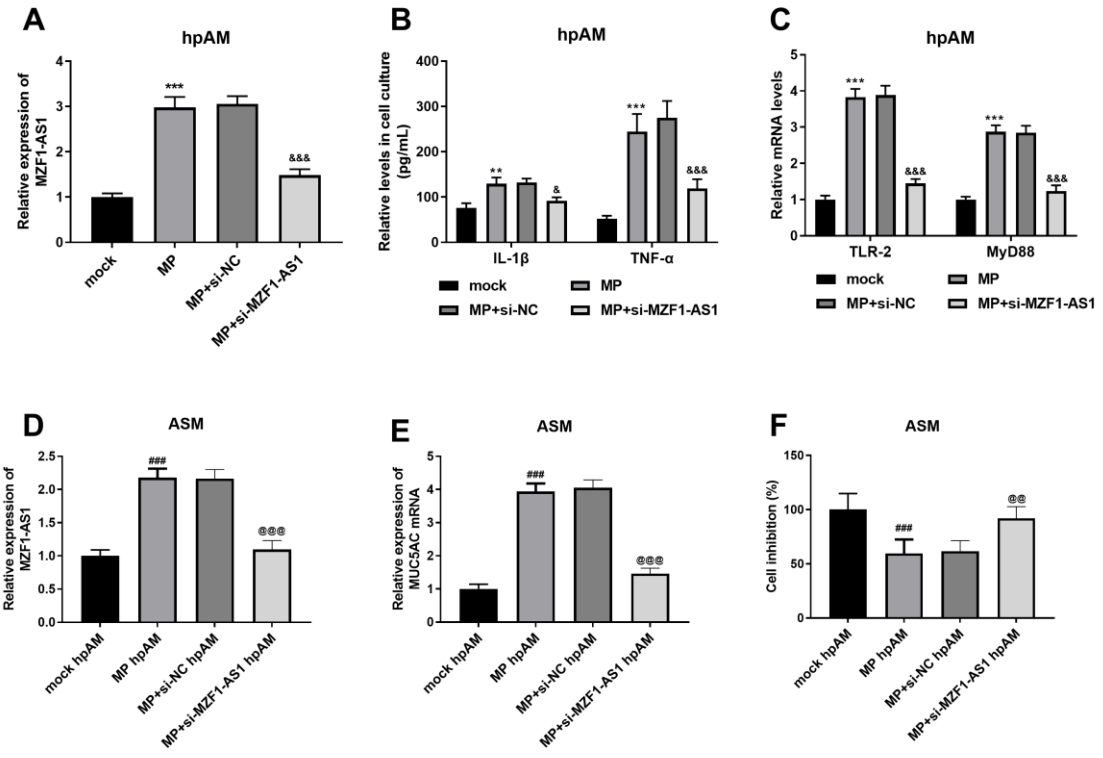

Figure 3. The effect of MZF1-AS1 inhibition on hpAM and ASM cell function. (A) MP-infected hpAM cells were analyzed for MZF1-AS1 expression by qRT-PCR. (B) MP-infected hpAM cells were analyzed for TNF-α and IL-1β. (C) TLR-2 and MyD88 mRNA expression was assayed by qRT-PCR in MP-infected hpAM cells after transfection. (D) MZF1-AS1 expression was assayed by qRT-PCR in ASM cells co-cultured with MP-infected hpAM. (E) The levels of MUC5AC mRNA in ASM cells co-cultured with MP-infected hpAM. (F) The ASM cell growth inhibition was determined after co-cultured with MP-infected hpAM. $^{**}P < 0.01$, $^{***}P < 0.001$, MP vs mock. $\&P < 0.05$, $\&\&\&P < 0.001$, MP+si-MZF1-AS1 vs MP+si-NC. $\#\#\#P < 0.001$, MP hpAM vs mock hpAM. $@P < 0.05$, $@@@P < 0.001$, MP+si-MZF1-AS1 hpAM vs MP+si-NC hpAM. hpAM, human primary alveolar macrophages. ASM, airway smooth muscle. MP, *Mycoplasma pneumoniae*.

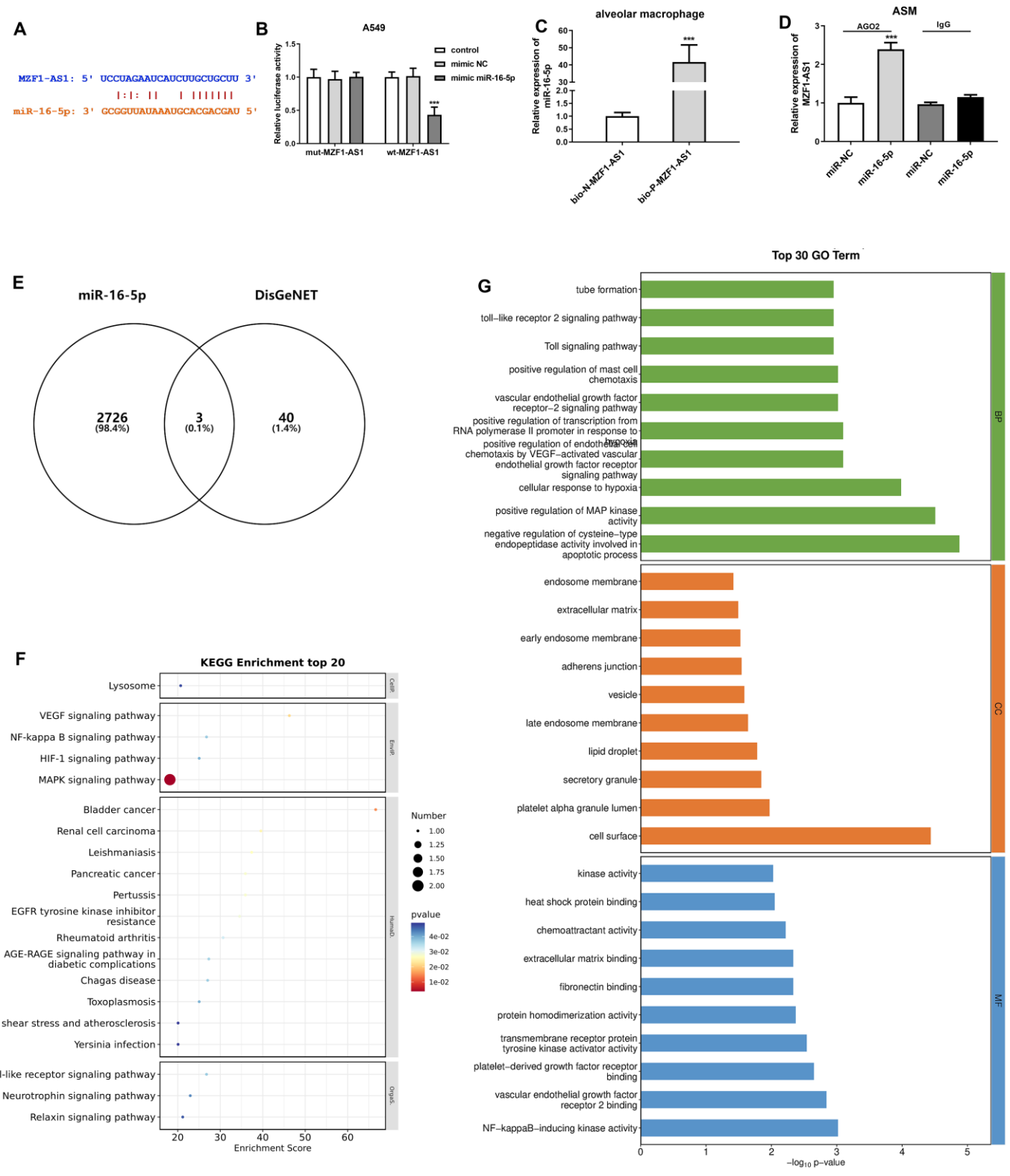

Figure 4. The investigation of MZF1-AS1-downstream factors. (A) The complementary binding sites between MZF1-AS1 and miR-16-5p. (B) The results of Luciferase assay in A549 cells. (C) The results of biotin-coupled probe pull-down assay in hpAM cells. (D) The results of RNA immunoprecipitation (RIP) assay in ASM cells. (E) The VENN diagram of common genes between miR-16-5p targets and MPP-related genes. (F) The enriched KEGG pathways for LAMP3, VEGFA, and IRAK1. (G) The enriched GO terms for LAMP3, VEGFA, and IRAK1. ASM, airway smooth muscle.

MZF1-AS1 may contribute to MPP via miR-16-5p

To understand the mechanism of MZF1-AS1 in the MPP, its interacting miRNA was predicted. miR-16-5p, an obesity-related asthma factor, was found to have binding sites with MZF1-AS1 (**Figure 4A**). To confirm the MZF1-AS1-miR-16-5p interaction, luciferase report assay, pull-down assay, and RIP assay were conducted in A549, hpAM, and ASM, respectively. The luciferase assay showed decreased luciferase activity of MZF1-AS1 in miR-16-5p-overexpressed cells (**Figure 4B**). Pull-down assays confirmed the interaction between MZF1-AS1 and miR-16-5p in hpAM (**Figure 4C**). Based on the RIP-PCR assay results, endogenous MZF1-AS1 was significantly enriched in AGO2-containing bead samples when compared with control IgG immunoprecipitants, suggesting that miR-16-5p directly targeted MZF1-AS1 in an AGO2-dependent manner (**Figure 4D**). The MPP-related genes were obtained from DisGeNET database. Three target genes, including LAMP3, VEGFA, and IRAK1, for miR-16-5p, were shared with the MPP-related genes (**Figure 4E**). These three genes were enriched in MAPK signaling pathway, VEGF signaling pathway, NF-kappa B signaling pathway, Toll-like receptor signaling pathway, HIF-1 signaling pathway, etc. (**Figure 4F**). The enriched GO terms included regulation of cysteine-type endopeptidase activity involved in apoptotic process, positive regulation of mast cell chemotaxis, NF-kappaB-inducing kinase activity, transmembrane receptor protein tyrosine kinase activator activity, fibronectin binding, extracellular matrix binding, etc. (**Figure 4G**).

Discussion

After analyzing the GSE179051 dataset, this study found that the expression level of MZF1-AS1 was elevated in MP-infected epithelial cells. We then retrospectively collected MPP pediatric patients and found through testing that the expression level of MZF1-AS1 in the blood of MPP pediatric patients increased, which has diagnostic value for MPP. MZF1-AS1 has been reported to be associated with obesity-related asthma.[15] Therefore, we investigated the expression level of MZF1-AS1 in MPP children with or without new-onset asthma and found that its expression level was elevated in MPP children with new-onset asthma. It can distinguish MPP children with or without new-onset asthma. Then, we explored the possible mechanisms of MZF1-AS1 in MPP and asthma using three cell lines.

Alveolar macrophages, alveolar epithelial cells, and airway smooth muscle cells have been implicated in MPP.[17] Alveolar macrophages are the most abundant innate immune cells in the lungs. In general, when alveolar macrophages are stimulated by MP, they recruit neutrophils and amplify inflammation through pro-inflammatory cytokines [18,19]. Alveolar macrophages can secrete pro-inflammatory cytokines (such as TNF-α and IL-1β) that are associated with neutrophil infiltration.[18] In this study, we found that MP infection can induce the release of TNF-α and IL-1β in hpAM cells. This is consistent with previous research findings. Moreover, we found that knocking down MZF1-AS1 can inhibit the release of pro-inflammatory cytokines induced by MP. In MPP, it has been reported that TLR-2 signaling is involved in the activation of inflammatory cells by MP-derived lipoproteins. After MP infection, the expression of TLR-2 mRNA and protein on alveolar macrophages, as well as the recruitment of the adapter protein MyD88, would increase.[18] This is also what we have found in our research. Furthermore, we found that knocking down MZF1-AS1 can inhibit the upregulation of TLR-2 and MyD88 mRNA expression induced by MP.

The persistent inflammatory response induced by alveolar macrophages can lead to local tissue damage and epithelial cell damage.[18] During inflammation, alveolar macrophages can attach to alveoli to form gap junction channels. Research shows that, after MP infection, the increase in secretion of TNF-α and IL-1β can upregulate the expression and adhesion connexin 43 in macrophages, forming Cx43 gap junction channels between alveolar macrophages and alveolar epithelial cells.[18] Clinical studies have found that peripheral blood TNF-α levels are higher in children with MPP than those in uninfected children.[20] The upregulation of TNF-α was found in A549 after MP infection in this study, whereas MZF1-AS1 knockdown can alleviate this upregulation. A previous in vitro MPP model experiment found that the viability of A549 cells infected with MP decreased and apoptosis significantly increased [21]. We also found that MP infection induces inflammation and apoptosis in A549 cells, and has an inhibitory effect on A549 growth. Furthermore, we found that MZF1-AS1 knockdown can resist the effect of MP infection on A549. IL-1β is a major pro-inflammatory cytokine. It can participate in many

important cellular functions, such as proliferation, differentiation, and activation of different cell types. Research has shown that MP can induce IL-1β in human lung epithelial A549 cell lines. After MP infection, IL-1β mRNA and protein were induced in A549 cells, but not in uninfected cells.[22] In this study, we found that MP induces IL-1β in A549 cell culture medium, and knocking down MZF1-AS1 can reduce the level of IL-1β induced by MP.

Mycoplasma pneumoniae infection can induce the onset of asthma.[23,24] ASM cells are the core participants in airway remodelling in asthma patients. The inflammatory factors caused by MP infection can lead to excessive proliferation and migration of ASM, resulting in ASM proliferation and airway remodelling in asthma patients.[23] We found an increase of ASM growth under MP infection, which was suppressed by MZF1-AS1 knockdown. Airway epithelial cells can secrete mucus to form a protective film to capture foreign microorganisms. Mucin is the main component of mucus, and MUC5AC is a major class of macromolecular mucins in the airways.[25] The lack of a mucus barrier usually makes the lungs vulnerable to damage, but abnormal mucin secretion and accumulation play a negative role in the pathogenesis of respiratory diseases, such as asthma.[26] Mucus blockage is the main cause of airway stenosis and death in asthma. Therefore, excessive secretion of MUC5AC is harmful to asthma. A previous study showed that MP infection can induce MUC5AC secretion in mouse airway epithelial cells.[25] Our study found that inhibition of MZF1-AS1 can reduce the expression level of MUC5AC.

miR-16-5p was predicted as a downstream miRNA. miR-16-5p is not only associated with cancer cell proliferation and viral replication but also with many inflammatory responses.[27] miR-16 can control macrophage interactions and T-cell activity.[28] miR-16-5p can confront lipopolysaccharide-induced inflammation in the lungs.[28] In addition, miR-16-5p can inhibit cell proliferation and cell cycle of Mycoplasma gallisepticum-infected DF-1 cells and promote apoptosis by targeting PIK3R1 to inhibit the PI3K/Akt/NF-κB pathway, resulting in anti-inflammatory effects.[29] It has been reported that a low level of miR-16-5p can predict the survival of community-acquired pneumonia patients after 30 days of follow-up.[30] We predicted LAMP3, VEGFA, and IRAK1 as target genes of miR-16-5p in MPP. These three genes can be enriched in NF-κB signaling pathway in this study. LAMP3 is cell-type-specific in alveolar cells in mice and plays a critical role in the regulation of pulmonary surfactant homeostasis and normal lung function.[31] VEGF has been identified to be upregulated in children with mycoplasma pneumonia and wheezing.[32] Downregulation of IRAK1 can reduce inflammation in refractory *Mycoplasma pneumoniae* pneumonia.[33]

Conclusion

This study identified the high expression levels of MZF1-AS1 in children with MPP. We also found high expression of MZF-AS1 in children with newly diagnosed MPP asthma, as well as its diagnostic and stratification value. Cell experiment results revealed that MZF1-AS1 may promote MP infection in alveolar epithelial cells, pulmonary macrophages, and ASM through targeting miR-16-5p.

Declarations

Acknowledgments

None declared.

Conflict of interest

None declared

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