Mir-142-3p Regulates Inflammatory Response by Contributing to Increased TNF-α in Chronic Rhinosinusitis With Nasal Polyposis

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Abstract

Objective: Previous studies suggested that microRNAs played an important role in the progression of inflammation and remodeling of chronic rhinosinusitis with nasal polyposis. However, the abnormal expression of microRNAs and regulation cytokine expression in nasal polyposis are not clear. Method: The miR-142-3p and tumor necrosis factor α (TNF-α) expression levels in chronic rhinosinusitis with nasal polyposis were detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR). The miR-142-3p and TNF-α levels in human nasal epithelial cells (HNEpC) after stimulation by lipopolysaccharide (LPS) were detected by qRT-PCR. Moreover, HNEpCs were transfected by miR-142-3p mimics or inhibitor or cotransfected with si-TNF-α to evaluate the regulation of miR-142-3p on TNF-α which affects the production of inflammatory factors. Results: The miR-142-3p and TNF-α were significantly higher in nasal mucosa of chronic rhinosinusitis with polyps patients compared to normal human. MiR-142-3p and TNF-α expression levels were increased after LPS stimulation in a dose- and time-dependent manner. Knockdown of miR-142-3p in HNEpCs downregulated TNF-α expression at both messenger RNA and protein levels. Conclusions: It is indicated that miR-142-3p may participate in the regulation of the body’s inflammatory response through the LPS-TLR-TNF-α signaling pathway in chronic rhinosinusitis with nasal polyposis.

Keywords
chronic rhinosinusitis, nasal polyposis, miRNA-142-3p, TNF-α, inflammation

Introduction

There are 2 subtypes, with polyps (CRSwNP) and without polyps (CRSsNP), in the chronic rhinosinusitis (CRS). The prevalence of CRSwNP ranged from 0.5% to 4.3%, which is less common than CRSsNP in national population surveys.¹ However, there are about 38% to 69% of CRSwNP cases that commonly lead to revision endoscopic sinus surgery therapy.²⁻⁴ Olfactory impairment is the common symptom of CRSwNP patients⁵ and is also likely to endorse nasal obstruction.⁶ MicroRNAs (miRNAs) are a class of short noncoding RNAs negatively regulating gene expression by inducing messenger RNA (mRNA) degradation or translation repression by targeting the 3′ untranslated region (3′UTR) of miRNAs.⁷ Currently, more than 1000 miRNAs have been identified and reported in the RNA database, and many of them are used as a molecular biomarker for diagnosis, prognosis, and treatment.⁸⁻⁹ MicroRNAs are involved in different cellular functions including differentiation, growth, proliferation, and apoptosis.⁹ Recent studies showed that miRNAs are widely involved in immune responses such as maturation and differentiation of immune cells, natural immune response, and so on.

Tumor necrosis factor α (TNF-α) is mainly produced by mononuclear macrophages and eosinophils and could induce interleukin (IL) 6, IL-10, and interferon γ (IFN-γ) release and play a role in cytotoxicity and growth inhibition.¹⁰ In addition, TNF-α could induce nuclear factor κB (NF-κB), C-fos, and C-Jun expression and regulate the immunoreaction, which is a broad spectrum of pro-inflammatory cytokines.¹¹ Previous studies suggested that TNF-α was overexpressed in the lung...
and nasal mucosa, which played an important role in airway inflammatory responses. However, at present, TNF-α roles in Asian patients with CRSwNP have not been illuminated. Chronic rhinosinusitis with polyps is a multifactorial disease because of the result of the persistent inflammations of nasal cavity and sinuses. The pathogenesis of CRSwNP remains unclear. As a newly discovered fast and effective regulatory factor, it is worth studying whether miRNA involved in the immune response and what role it plays on CRSwNP. In the present study, the mechanisms between the miR-142-3p and TNF-α activation in vitro and in vivo were investigated. We had emphasized that miR-142-3p might be a novel target for the development of treatment for CRSwNP diseases.

**Materials and Methods**

**Clinical Samples**

Twenty patients with nasalpolyps (NPs) and 20 healthy controls were enrolled from the Department of Otolaryngology in the Central South University (China). None of the patients were treated with glucocorticoids or other antibodies for 3 months before the study. The mucosal biopsies of the inferior turbinate were collected. Total RNA was extracted for corresponding gene expression. The approval for this study was obtained from the institutional review boards of the participating hospitals in China.

**Cells Lines**

Human nasal epithelial cells (HNEpCs) were purchased from American Type Culture Collection and were grown in standard culture medium (RPMI-1640 containing 10% fetal bovine serum, 2 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin). The cells were cultured in an incubator at 37°C in 5% CO₂.

**MiR-142-3p Mimics and Interference Transfection**

Human nasal epithelial cells transfected by miR-142-3p mimics and inhibitor (Life Technologies Corporation, Shanghai, China) were used for the overexpression and inhibition of miR-142-3p in cells. The used pSicoR/miR-142-3p oligonucleotide primers: forward 5′-TTGTAGTGTTCCTACTTTATGGATTCAAGAGATCCA-TAAAGTAGGAAACACTACAA-3′ and reverse 5′-TCGAGAAAAATGTAGTGTTCCTACTTTATGGATTCAAGAGATCCA-TAAAGTAGGAAACACTACAA-3′, were synthesized, which was based on the sequence of human miR-142-3p from miRBase database. The inhibitor vector (pSicoR/Inh) was generated using oligonucleotides of forward, 5′-TCGAGAAAAATGTAGTGTTCCTACTTTATGGATTCAAGAGATCCA-TAAAGTAGGAAACACTACAA-3′ and reverse, 5′-TTGTAGTGTTCCTACTTTATGGATTCAAGAGATCCA-TAAAGTAGGAAACACTACAA-3′. After 24-hour transfection, the cells were harvested.

**Lipopolysaccharide Stimulation**

Human nasal epithelial cells (1 × 10^5 cells/well) were seeded in 12-well plates and transfected as described above. When HNEpCs reached 80% to 90% confluence, the cells were washed by phosphate-buffered saline (37°C, pH 7.4) and added fresh culture medium. The lipopolysaccharide (LPS; Sigma, St Louis, Missouri) was added to the cultures at the concentrations of 0, 0.1, 0.5, and 1 μg/mL and incubated at 37°C for 0 to 72 hours.

**RNA Extraction and Quantitative Reverse Transcription Polymerase Chain Reaction**

Total RNA of HNEpC cells or frozen nasal tissues were extracted using TRIzol reagent (Invitrogen, Carlsbad, California), according to the manufacturer’s instructions. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis was carried out with Prime Script RT Reagent Kit (TaKaRa, Dalian, China). The miR-142-3p forward primer, 5′-
CGAGGATCCGGAGGTAGAGGAGGCAAG-3', reverse primer, 5'-CCGAATTCAGCTTCGGGAACCAGGAAGG-3'; IL-6 forward primer, 5'-ACTCAACCTCCAGAACAGG-3', reverse primer, 5'-CCATTTTGGGAGTT- CAGGTG-3'; IL-10 forward primer, 5'-TCAAGGCCGC TGTAACCTCC-3', reverse primer, 5'-GATGTCAGACA TCTGTAGGCT-3', reverse primer, 5'-GATGTCAAACT CACTCATGGCT-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3', reverse primer, 5'-CTGGGATGC TTTCGACCTC-3'. All PCR reactions were performed in triplicate. Relative gene expression was calculated using the comparative 2^(-AΔCt) method with the housekeeping gene glyceraldehyde-3-phosphatedehydrogenase (GAPDH) as a reference.

Luciferase Reporter Assay

293T cells were cotransfected with miR-142-3p mimics or inhibitor, pMIR-REPORT-TNF-α-3'UTR luciferase reporter plasmid, or empty pMIR-REPORT and pTK-RL plasmid. Luciferase activity assay was performed using Dual-Luciferase Reporter System (Promega, Berthold Technologies, Germany), according to the manufacturer’s instructions after 48-hour transfection. Relative luciferase activity was measured with a microplate luminometer (Berthold Centro LB 960, Madison, WI) and normalized with Renilla luciferase activity.

Enzyme-Linked Immunosorbent Assay

The IL-6, IL-10, IFN-γ, and TNF-α expression levels in the supernatant were detected by enzyme-linked immunosorbent assay (ELISA) method according to the instruction of ELISA
kits which were purchased from BioLegend (San Diego, California).

**Statistical Analysis**

All statistical analysis was carried out using the SPSS version 18.0 software (SPSS, Inc, Chicago, Illinois). All values were expressed as the mean (standard deviation) from at least 3 repeated individual experiments for each group. The differences among groups were analyzed by one-way analysis of variance followed by Bonferroni multiple comparison tests or t test, as appropriate. All data were expressed as mean (SD). Differences were considered significant when $P < .05$.

**Results**

**MiR-142-3p and TNF-α Overexpression in CRSwNP Patients**

The miR-142-3p and TNF-α expression levels were detected by qRT-PCR assay on CRSwNP patient’s tissues. The results showed that miR-142-3p was significantly higher in nasal mucosa of CRSwNP patients compared to normal human (Figure 1A). At the same time, we also observed the expression of inflammatory factor TNF-α in CRSwNP patients was increased compared to normal control group (Figure 1B).

**Lipopolysaccharide Stimulation Increased miR-142-3p and TNF-α Expressions in the HNEpCs**

The miR-142-3p and TNF-α expression levels were determined after LPS stimulation at different concentrations (0-1 μg/mL) and different time points (0-72 hours). The results showed that miR-142-3p and TNF-α expression levels were increased after LPS stimulation in a dose- and time-dependent manner (Figure 2).

**Tumor Necrosis Factor-α Is a Target of miR-142-3p in HNEpCs**

To test whether miR-142-3p targets TNF-α in HNEpCs, we constructed a reported plasmid by cloning the human TNF-α 3’UTR into the pMIR-REPORT luciferase vector. The TNF-α reporter or a control plasmid was then cotransfected together with miR-142-3p mimics into the HNEpCs. The luciferase activity indicated that TNF-α was a potential target of miR-142-3p (Figure 3A). In addition, knockdown of miR-142-3p in

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**Figure 3.** Cytokine changes in LPS-treated HNEpCs transfected with miR-142-3p mimics or miR-142-3p inhibitors. A, Relative luciferase activities in HNEpCs transfected with plasmids of TNF-α 3’UTR. The levels of TNF-α mRNA (B) or protein (C) were measured in HNEpCs transfected with miR-142-3p mimics or miR-142-3p inhibitors. *$P < .05$. HNEpCs indicates human nasal epithelial cells; LPS, lipopolysaccharide; mRNA, messenger RNA; TNF-α, tumor necrosis factor-α; 3’UTR, 3’ untranslated region.
HNEpCs downregulated TNF-α expression at both the mRNA and protein levels. MiR-142-3p downexpression reversed TNF-α expression during LPS treatment (Figure 3B). Similarly, TNF-α protein levels in the cell supernatants were significantly reduced after miR-142-3p knockdown (Figure 3C). Conversely, overexpression of miR-142-3p significantly upregulated TNF-α expression at both the mRNA and protein levels (Figure 3B and C).

**MiR-142-3p Regulated Inflammatory Cytokines by Inducing TNF-α Expression**

In order to observe whether the regulation of miR-142-3p on TNF-α affects the production of inflammatory factors, we examined the changes of various inflammatory cytokines in HNEpC cells by ELISA. The results showed that LPS induced upregulation of IL-6, IL-10, and IFN-γ compared to the untreated cells served as controls; cells treated with LPS alone for 24 hours; cells were transfected with siRNA of TNF-α for 24 hours; cells were transfected with si-TNF-α for 24 hours, LPS stimulated for another 24 hours; cells were transfected with miR-142-3p mimics for 24 hours; and cells were cotransfected with miR-142-3p mimics and si-TNF-α for 24 hours. A-C, The relative mRNA levels of IL-6, IL-10, and IFN-γ in HNEpC cells were detected by real-time quantitative PCR. D-F, The ELISA results of IL-6, IL-10, and IFN-γ proteins of cell culture supernatant. *P < .05. ELISA indicates enzyme-linked immunosorbent assay; HNEpCs, human nasal epithelial cells; IFN-γ, interferon gamma; IL, interleukin; LPS, lipopolysaccharide; mRNA, messenger RNA; PCR, polymerase chain reaction; TNF-α, tumor necrosis factor-α.
untreated control cells. However, knockdown of TNF-α attenuated changes in inflammatory factors caused by LPS. MiR-142-3p-mimic transfected cells produced similar inflammatory factor changes as LPS treatment. Interestingly, when we cotransfect miR-142-3p mimics with si-TNF-α, the upregulation of inflammatory factors by miR-142-3p mimics is significantly reduced (Figure 4A-C). To better validate this result, we examined the mRNA changes of IL-6, IL-10, and IFN-γ. The results were consistent with the results of ELISA (Figure 4D-F). Therefore, miR-142-3p may regulate inflammatory cytokines by inducing TNF-α expression.

**Discussion**

Tumor necrosis factor α was produced by epithelial and immunocompetent cells, such as mast cells, macrophages, and eosinophils, levels of which are obviously increased in nasal secretions of patients with chronic sinusitis. In our study, TNF-α is significantly higher in nasal polyps compared to normal tissues, and there was a positive correlation with the expression changes of miR-142-3p. We change the expression of miR-142-3p that can affect the expression of TNF-α and the IL-6, IL-10, and IFN-γ release. The abovementioned evidence suggests that miR-142-3p may regulate the body’s inflammatory response through regulating the expression of TNF-α.

During the process of respiratory movement, nasal mucosa is the first to be exposed to many microorganisms as well as their degradants, such as LPS. Respiratory diseases such as rhinosinusitis and asthma are characterized by aberrant immune function of airway epithelial cells. Lipopolysaccharide has already been regarded as a main regulator of inflammation. High exposure level of LPS is associated with the occurrence and development of many airway diseases.

Previous studies reported that miRNAs regulate inflammatory responses to pathogens at both the transcriptional and the translational levels. Several miRNAs, such as miR-146, miR-21, miR-9, and miR-155, are thought to play a role in regulating LPS-induced inflammatory responses. MiR-142-3p is one of the most highly expressed miRNAs in LPS-stimulated HNEpCs and can modulate TNF-α expression in response to LPS. In the present study, expression of miR-142-3p is high in CRSwNP patients, which is increased by LPS stimulation and is associated with high levels of TNF-α expression. These suggested that aberrant miR-142-3p expression may be a systemic feature of CRSwNP patients, leading to inflammatory responses of HNEpCs.

Many studies have shown that miR-142-3p plays a pivotal role in the expression of pro-inflammatory cytokines in many kinds of cell lines. Zhang et al reported that transfection of miR-142-3p mimic significantly increased the release of TNF-α, IL-6, and MCP-1 in THP-1 cells. Guo and Lin reported that miR-142-3p mimic transfection was remarkably induced TNF-α and IL-1 expression in MLE-12 cells. However, Xu et al reported that miR-142-3p was a potential to negatively regulate the production of pro-inflammatory mediator NF-κB, TNF-α, and IL-6 in the macrophages. What’s more, previous studies showed that TNF-α overexpression played a significant role in inflammatory response in the nasal cavities not only in CRSwNP patients but also in murine models. However, the miR-142-3p expression regulation and the relationship with cytokine release, such as TNF-α, IL-6, and IL-10 in CRSwNP, are unclear. According to previous reports, we hypothesized that miR-142-3p expression and cytokine release could lead to regulation of airway inflammation and remodeling.

It is a better therapeutic strategy for understanding the precise mechanisms in the progression of allergic airway inflammation and remodeling. Some cytokines, such as IL-6, were considered as remodeling-associated mediators in the allergic airway. The present study showed that TNF-α was a key mediator in these remodeling. Consequently, simultaneously affecting multiple cytokines (IL-6, IL-10, and IFN-γ) by targeting TNF-α may be more effective than only targeting one particular cytokine at blocking remodeling.

In conclusion, our finding indicated that miR-142-3p may participate in the regulation of the body’s inflammatory response through the LPS-TLR-TNF-α signaling pathway, which requires further study. The downregulation of miR-142-3p results in decreased TNF-α expression and reduced inflammatory responses. Our results suggest that miR-142-3p may be used as a new biomarker for predicting chronic nasosinusitis-targeted drugs.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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