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Micro124-mediated AHR expression regulates the inflammatory response of chronic rhinosinusitis (CRS) with nasal polyps

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ABSTRACT

MicroRNAs represent a component of the innate immune responses that can restrain inflammatory signaling, miR124 is an important member of inflammation-associated miRNAs, and abnormal miR124 expression is observed in many inflammatory diseases and immune disorders. However, the role and signaling pathways of miR124 in chronic rhinosinusitis with nasal polyps (CRSwNPs) have not been studied in detail. The aryl hydrocarbon receptor (AHR) is a ligand-inducible transcription factor that is highly conserved in evolution and plays important roles in the inflammatory response process. In our study, we describe the role of miR124 in the inflammatory response of CRS with nasal polyps. We found that the expression of miR124 was decreased in nasal polyps, and negatively correlated with the expression of AHR. MiR124 can inhibit AHR expression by directly target 3′ untranslated region (3′-UTR) of AHR. To further investigate the relationship between miR124, AHR and CRS inflammatory response, we transfect HNEpC cells with miR124 mimic, miR124 inhibitors or siRNA of AHR, then all the results showed that miR124 could regulates cellular inflammatory response through negatively regulating AHR expression. This study demonstrated that the regulation of AHR expression by miR124 is critical to the development of inflammatory response in CRSwNPs.

1. Introduction

Chronic rhinosinusitis with nasal polyposis (CRSwNP) is a chronic heterogeneous disease of epithelial inflammation and tissue eosinophilic infiltration [1,2]. In patients with nasal polyposis (NPs), the nasal mucosal epithelium is histologically characterized by persistent inflammation and irreversible structural changes, which leads to remodeling in the nasal mucosal [3]. Although there are many studies on the role of chronic inflammation in the development of nasal polyps, but the underlying mechanism is still not clear.

The aryl hydrocarbon receptor (AHR) is a member of the basic helix-loop-helix Per-Arnt-Sim (bHLH-PAS) transcription factor family. In previous studies, some chemicals or xenobiotics can respond to the immune response by mediating AHR [4–6]. In the immune system, it is well known that the expression of AHR needs to be tightly controlled, aberrant expression of AHR can cause system disorders [7–10]. AHR activation can facilitate the generation of Treg or Th17 cells based on the disease model, tissue context and type of AHR ligand, then directly influencing the Th17/Treg balance [11–13]. AHR plays a very important role in inflammation and immune response, and it is involved in the regulation of a variety of signaling pathways.

MicroRNAs (miRNAs) are noncoding transcripts of 18–25 nucleotides (nts) derived from initially long primary transcripts. mature miRNAs can specifically bind to 3′-untranslated regions (3′-UTRs) of target mRNAs, leading to either mRNA degradation or inhibition of translation [11]. We used quantitative PCR to determine the expression of a series of inflammation-associated miRNAs in the nasal mucosa of CRSwNPs. It was found that the expression of miR124 was most significantly reduced in CRSwNPs in nasal mucosa, which attracted us enough attention [Fig. S1]. According to TargetScan analysis, AHR 3′-UTR contains the binding site of the

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miR-124/124ab family and is highly conserved among humans, mice and most species. Research has shown that miR124 is one of the most abundantly expressed miRNAs in the nervous system and it is also highly expressed in the immune cells and organs [12–14]. MiR124 can inactivate macrophages, thereby promoting microglial quiescence and inhibiting experimental autoimmune encephalomyelitis (EAE) [15]. And miR124 inhibits the differentiation of TH1 and TH17 cells by inhibiting STAT1 and STAT3 activation in the presence of protein inhibitor cytokine signaling 5 (SOCS5) [16]. In our study, we found that the expression of miR124 is reduced in NPs, which prompts us to investigate the relationship between miR124 and inflammation in NPs. In the present study, our results demonstrated that miR124 can negatively regulate the expression of AHR and inflammatory cytokine, and inhibition of AHR expression results in loss of miR124 regulating inflammatory factors. This study has identified that Micro124-mediated AHR expression regulates the inflammatory response of chronic rhinosinusitis (CRS) with nasal polyps, and which acts as a novel essential regulator of inflammatory response. In future miR124/AHR pathway may be acted as a new therapeutic target and clinical strategies to modulate inflammation-related diseases.

2. Materials and methods

2.1. Study population

Biopsies of patients with NPs (n = 20) and healthy controls (n = 20) were obtained from the Department of Otolaryngology in the Second Hospital of Shandong University (China). The patient's specific information is described in detail in Table 1. None of the patients were treated with glucocorticoids or other antibodies for 3 months prior to the study. The mucosal biopsy of the inferior turbinate (IT) was obtained from 20 non-NP patients with septal deviation and those as healthy controls. All tissues were fixed in formalin for histological evaluation. RNA and protein were extracted from healthy controls (n = 20) and NPs (n = 20) for the detection of corresponding gene and protein expression. The approval for this study was obtained from the institutional review boards of the participating hospitals in China.

2.2. Reagents

LPS were purchased from Sigma (Cat. No.L2880, Sigma). AlamarBlueTM Cell Viability Reagent was from Invitrogen (Cat. No. DAL1100). Rabbit polyclonal anti-AHR antibody (Cat. No. bs-1416R, 1:3000 diluted for WB, 1:100 diluted for IF) was from Bioss, and rabbit polyclonal anti-GAPDH antibody (Cat. No. AC027, 1:5000 diluted) was from ABclonal. Rabbit polyclonal anti-TNF antibody (Cat. No. A0277, 1:3000 diluted) was from ABclonal.

2.3. Immunofluorescence (IF)

All nasal biopsy specimens (from NP patients and healthy controls) were washed with PBS and then embedded in paraffin. Tissue wax blocks were sectioned to a thickness of 4 mm with a Leica microtome (Leica, Wetzlar, Germany). Then Expression of AHR on paraffin sections of nasal biopsies was studied by immunofluorescent (IF) staining. The Paraffin sections were imaged with a confocal microscope (LSM 700, Zeiss).

2.4. Cell culture and transfection

Human nasal epithelial cells HNEpC were purchased from Cellbio (Cat. No. CBR-130634), it was grown and maintained in RPIM-1640 medium supplemented with 10% fetal bovine serum (FBS) (Cat.No.10099141, Invitrogen). HNEpC cells were plated at a density of 3 × 105 cells per 60 mm dish and cultured for 24 h. Then cultured cells were transfected with jetPRIME® Transfection Agent (Cat. No. PT-114-15, Polyplus) according to the manufacturer's instructions.

2.5. RNA interference, mimics and inhibitor of microRNA

AHR siRNAs were obtained from Biotech of shanghai. Three pairs of si-*AHR* sequences by used: CAGACAGUAGUCUGUUAUAAC, UAUAACAGACUACUGUCUGGG; GCUGAAGGAAUCAAGUCAAAU, UUGACUUGAUUCCUUCAGCUG; CACAAGAUGUUAUUAAUAAGU, UUAUUAAUAACAUCUUGUGGG. Three pairs of si-*AHR* sequences were mixed 1: 1 prior to transfecting the cells, and for efficient gene silencing, siRNAs were transfected twice. MiR124 mimics were composed of RNA duplexes with the following sequences: sense 5′-UAAGGCACGCGGUGAAUGCC-3′, Anti-sense 5′-CAUUCACCGCGUGCCUUAUU-3'. The sequences of miR-124 inhibitor were as follows: 5′-GGCAUUCACCGCGUGCCUUA-3'. The cells were transfected using jetPRIME® Transfection Agent according to the manufacturer's instruction.

2.6. RNA extraction and quantitative real-time PCR

Total RNA of HNEpC cells or frozen nasal tissues in RNA later were extracted using TRIzol according to the manufacturer's protocol (Cat.No.15596026, Invitrogen). The Mir-X miRNA First-Strand Synthesis Kit is used for converting miRNA into cDNA according to the manufacturer's protocol (Cat.No. 638313,Takara). The other cDNA was performed by RT reactions with PrimeScriptTM RT reagent Kit with gDNA Eraser (Cat.No. RR047A, Takara). And mRNA or miRNA levels were detected by SYBR Green gene expression assays. Quantitative real-time PCR (qPCR) was performed with the following primers: *AHR* forward primer, AATACTTCCACCTCAGTTGGCTTT, reverse primer, AAGCCGGAAAACTATCATGCCACTT (289bp); *IL-6* forward primer, ACTCACCTCTTCAGAACGAATTG,

Table 1
Patients' characteristics.

	Nasal mucosa Healthy controls	Nasal polyps
Sample sizes	20	20
Median age, years (IQR)	46.8 (35–61)	52.3 (36–67)
Gender, male/female	14/6	13/7
Atopy, n/N	5/20	10/20
Asthma, n/N	0	0
Median CT score (IQR)	0	10.7 (5–14)***
Median endoscopy score (IQR)	0	5.5 (3–9)***

The level of significance (p) was obtained from the Student *t*-test. P value of <0.05 was considered statistically significant. *Means the P value is < 0.05, while symbol. **Means the P value is < 0.01.
Gender comparison was performed using the $\chi 2$ test. CT, computed tomography; IQR, interquartile range.

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