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MiR-150-5p regulates EGR2 to promote the development of chronic rhinosinusitis via the DC-Th axis

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ABSTRACT

Background and aims: Accumulating studies indicate that miR-150-5p might play a significant role in dendritic cells (DCs) of peripheral blood in chronic rhinosinusitis (CRS) patients. We sought to investigate the effects and mechanism of miR-150-5p, which regulates early growth response 2 (EGR2) to promote the development of CRS via the DC-Th axis.

Methods: The upregulated expression of miR-150-5p in DCs of CRS was assayed by real-time quantitative polymerase chain reaction (qRT-PCR), and IL-17 cytokines in the supernatants of DC-naïve T cells co-cultures were analysed by enzyme-linked immunosorbent assay (ELISA). Flow cytometry was used to evaluate T cell proliferations. EGR2 was also identified as a direct target of miR-150-5p by establishing a miRNA–mRNA network, and this target was validated with a Dual-Luciferase® Reporter Assay System and Western blot.

Results: MiR-150-5p was up-regulated in DCs in peripheral blood from CRS patients, and this expression was down-regulated by EGR2 expression via the DC-Th axis. Up-regulated miR-150-5p Regulates DCs, and DCs Promote Naïve T Cells Proliferation. MiR-150-5p Further Regulates EGR2 and Inhibits DCs, Which Makes the DC-Th Axis Abnormal in the Peripheral Blood of Patients with CRS.

Conclusion: MiR-150-5p and its identified target, EGR2, are involved in the development of CRS. DCs can promote T cell proliferations of peripheral blood in CRS.

1. Introduction

Chronic rhinosinusitis (CRS) is defined as an inflammation of the nose and the sinus mucosa, and has a morbidity of approximately 14% [1]. CRS is one of the most common presentations of upper airway illness and severely affects patient quality of life. Its frequency is not surprising given levels of environmental exposure to microbes, pollutants, and allergens. Inflammatory cells, inflammatory cytokine and chemokine production, and airway remodeling have been detected in the sinonasal mucosae of CRS patients, although the precise pathophysiological mechanisms causing such persistent inflammation remain unclear. Most guidelines classify CRS into two groups: CRS without nasal polyposis (CRSsNP) and CRS with nasal polyposis (CRSwNP). CRS treatment is challenging for doctors because of its high incidence and unsatisfactory treatment outcomes. Thus, studies of the pathogenesis of CRS are of great importance. We previously demonstrated that the imbalance of Th17/regulatory T cell (Treg) may play an important role in the development of NP and that more intensive in vitro studies are required to further clarify the cellular mechanisms of Th17/Treg in inflammation and atopy [2]. However, further study of the mechanism of CRS is needed. In the present study, the role of miR-150-5p and its target in CRS were investigated.

Human dendritic cells (DCs) are among the most potent antigen-presenting cells in the airway, and they play a crucial role in the link between “innate” (i.e., pathogen recognition) and “adaptive” (i.e., regulation of the type of T cell-mediated response) immune responses [3,4]. There are two major types of DCs: myeloid dendritic cells (mDCs) and plasmacytoid dendtritic cells (pDCs). mDCs stop sampling the microenvironment and become dedicated antigen-presenting cells when they encountered pathogens. pDCs are mainly involved in the differentiation of Tregs. In our research, mDCs were analysed and studied. Mature DCs migrate to secondary lymphoid organs and stimulate T cells proliferation. DC activation by pathogens induces the emergence of pro-inflammatory cytokines, which activates and induces naive T cells, that differentiates into Th1, Th2, and Th17 type T helper cells, or Treg cells. Recent studies have shown that the number of DCs and the percentage

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Table. 1
Clinical features of the controls and the patients with CRS in this study.

Characteristics	Control	CRSsNP	AtopicCRS wNP	Non-topic CRSwNP
Accumulated cases	11	12	12	13
Gender (male:female)	8:3	5:7	11:1	8:5
Age (years)	18–47	24–72	16–84	20–80
Allergen skin prick test	−	+	+	−
Sinus CT	Nasal septum deviation without rhinosinusitis and nasal polyp	Rhinosinusitis without nasal polyp	Rhinosinusitis with nasal polyp	Rhinosinusitis with nasal polyp

Table. 2
qRT-PCR Primers.

Gene name	Primer sequence (5′-3′)	Molecular weight (MW)
has-miR-150-5p	(F)5′-TCTCCCAACCCTTGTACCAGTG-3′	6606.29
	(R) 5′-CTCAACTGGTGTCGTGGTA-3′	5835.75
U6	(F) 5′-CTCGCTTCGGCAGCACA-3′	5131.36
	(R) 5′-AACGCTTCACGAATTTGCGT-3′	6091.99

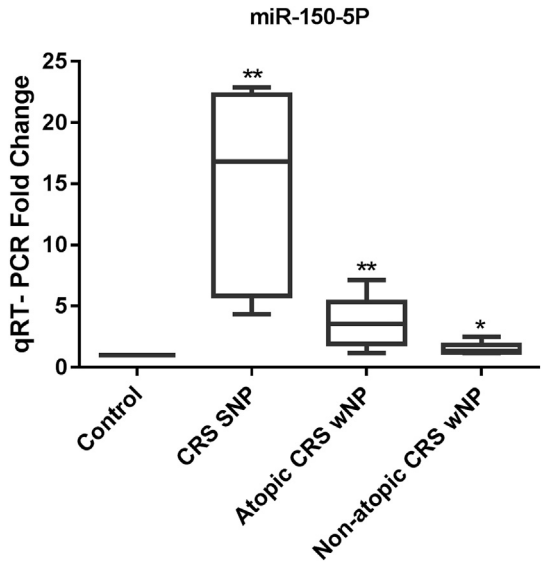

Fig. 1. Validation of the up-regulation of miR-150-5p expression in DCs from the peripheral blood of patients with CRS by qRT-PCR.
DCs were derived from the peripheral blood of patients with CRS and matured with LPS, and miRNAs were extracted from the DCs. qRT-PCR was used to analyse miR-150-5p expression. The miR-150-5p expression level was up-regulated compared with that of the controls. QPCR fold change of each group: CRS sNP 4.32–22.08, atopic CRS wNP 1.16–7.13, non-atopic CRS 1.16–2.52 wNP. ***P < 0.001, **P < 0.01, *P < 0.05. The data are expressed as the means ± SD of three independent experiments.

of activated DCs are increased in the sinonasal mucosa of CRS patients, and lesional DCs demonstrate different phenotypes and distinct abilities to regulate Th cell polarization in different types of CRSwNP, indicating that DCs are crucial for the development and persistence of inflammation in CRS patients [5–7]. Thus DCs play a key role in the reconstruction of T cells and are regarded as upstream regulatory cells. Analysing the mechanism of the abnormal DC-Th axis is crucial for understanding the development of CRS.

MicroRNAs (miRNAs) can regulate DCs differentiation, maturation, antigen presentation, and cytokine profiles [8]. MicroRNAs are highly conserved small non-coding RNAs of 18–22 nucleotides that suppress target genes at the post-transcriptional level by degrading mRNA. Abnormal miRNA expression has recently been implicated in the development of cancer and inflammation [9]. MiR-150 levels in leukocytes and plasma correlate with the aggressiveness of sepsis and can be used as an early marker of sepsis [10]. We previously characterized the miRNA expression profile of mature mDCs in CRS, including miR-150-5p. MiR-150 expression is up-regulated in patients with allergic rhinitis, as demonstrated by quantitative real time polymerase chain reaction (qRT-PCR) [11]. However, it is not clear how miR-150-5p regulates DCs in inflammatory disorders, especially in CRS. Therefore, we predicted the target genes of miR-150-5p. Some studies have identified miR-150 targets in different cellular systems and microenvironments, and these targets may play a role in phenotypic diversity [12]. Interestingly, some studies have indicated that early growth response 2 (EGR2), which is expressed in the restricted stages of lymphocyte development, plays dynamic but similar roles in the development of T, natural killer T cell (NKT) and B cells [13]. Moreover, EGR2 acts as an essential negative STAT3 regulator including in IL-17 expression and Th17 expansion [14]. However, the role of EGR2 in CRS is unclear. We hypothesised that miR-150-5p regulates EGR2 via the DC-TH axis.

Given its high prevalence and considerable associated morbidity, continued research into CRS is necessary to increase our understanding of factors likely to contribute to its pathogenesis, and facilitate the development of novel therapeutic strategies to improve treatment. In this study, we investigated the effects and possible mechanism of miR-150-5p to promote the development of CRS. The DC-Th axis was designated as the main line of research, and the regulation of miR-150-5p in DC was designated as the entry point. This study aimed to evaluate the upstream events of the Th17/Treg imbalance in CRS and its immune regulatory factors. In addition, by regulating the DC-Th axis, we aimed to explore the regulatory role of miR-150-5p with regard to the DC-Th axis and its dysfunction in the pathogenesis of CRS to clarify the role of miR-150-5p in the pathogenesis of CRS. By establishing CRS control strategies, this study has important clinical value and provides new therapeutic targets for CRS.

2. Methods and materials

2.1. Patients and specimens

CRS patients were recruited from the Department of Otorhinolaryngology at the First Affiliated Hospital of Chongqing Medical University. The participants include 37 CRS patients (24 male and 13 female, 16–80 years of age) and 11 patients with nasal septum deviation but without CRS (8 male and 3 female, 18–47 years of age); the participants underwent sinus CT, nasal endoscopy and skin prick test (SPT) (Table 1). The CRS diagnosis followed the diagnostic criteria of the European Nasal Sinusitis Nasal Polyps Guidelines (EPOS 2012). According to the SPT results, (+) indicated atopy because it is associated with an allergic constitution, and (−) indicated a non-atopic patient because it is not associated with an allergic constitution. Peripheral blood samples were collected from patients who had stopped using systemic or topical corticosteroids for at least one month and patients with no choanal polyps, fungal nasal sinusitis, cystic fibrosis, acute upper airway infections, or other systemic diseases. Patients with only nasal septum deviations were used as the controls, and patients with a history of respiratory and allergic diseases were excluded. According to the diagnostic test results, the research subjects were divided into four groups: the control group and the CRSsNP, atopic CRSwNP, and non-atopic CRSwNP groups [15].