Increased microRNA-155 and decreased microRNA-146a may promote ocular inflammation and proliferation in Graves’ ophthalmopathy

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Graves’ ophthalmopathy is an inflammatory autoimmune disease of the orbit, characterized by inflammation and proliferation of the orbital tissue caused by CD4+ T cells and orbital fibroblasts. Despite recent substantial findings regarding its cellular and molecular foundations, the pathogenesis of Graves’ ophthalmopathy remains unclear. Accumulating data suggest that microRNAs play important roles in the pathophysiology of autoimmunity and proliferation. Specifically, microRNA-155 (miR-155) can promote autoimmune inflammation by enhancing inflammatory T cell development. In contrast to miR-155, microRNA-146a (miR-146a) can inhibit the immune response by suppressing T cell activation. Furthermore, miR-155 and miR-146a are involved in cell proliferation, differentiation, and many other life processes. Thus, miR-155 and miR-146a, with opposite impacts on inflammatory responses carried out by T lymphocytes, appear to have multiple targets in the pathogenesis of Graves’ ophthalmopathy. Our previous work showed that the expression of miR-146a was significantly decreased in peripheral blood mononuclear cells from Graves’ ophthalmopathy patients compared with normal subjects. Accordingly, we proposed that the expression of miR-155 increased and the expression of miR-146a decreased in the target cells (CD4+ T cells and orbital fibroblasts), thus promoting ocular inflammation and proliferation in Graves’ ophthalmopathy. The proposed hypothesis warrants further investigation of the function of the differentially expressed microRNAs, which may shed new light on the pathogenesis of Graves’ ophthalmopathy and lead to new strategies for its management.

MeSH Keywords: Autoimmune Disease • Graves’ Ophthalmopathy • MicroRNAs

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HYPOTHESIS

Graves’ ophthalmopathy is an inflammatory autoimmune disease of the orbit. Signs and symptoms of Graves’ ophthalmopathy are caused by inflammation of orbital connective tissue, increased orbital volume due to overproduction of glycosaminoglycans, and enhanced adipogenesis [1]. Despite substantial new findings in its cellular and molecular underpinnings, the pathogenesis of Graves’ ophthalmopathy remains unclear. Many patients with Graves’ ophthalmopathy have to endure the condition for long periods and some severely affected patients are resistant to current treatment regimens [2]. Accumulating data suggest that the proliferation and immune response involved by the CD4^+ T cells and orbital fibroblasts play important roles in the occurrence and development of Graves’ ophthalmopathy [3,4]. On the other hand, microRNAs have emerged as important modulators of immunity and cellular physiology [5–7].

The Immune Response and Molecular Regulation in Graves’ Ophthalmopathy

Evidence from several laboratories suggests that orbital fibroblasts are the autoimmune target and effector cells in Graves’ ophthalmopathy [4]. TSHR expressed on the surface of orbital fibroblasts is a preferential candidate autoantigen. Disease manifestations are the product of a close interaction between orbital fibroblasts and CD4^+ T cells. Various classes of molecules (e.g., HLA antigens, CTLA-4, and cytokines) immunomodulate this interaction [8]. Previous studies showed there were imbalances between the CD4^+ T cells (Th1/Th2/Treg/Th17) and their associated cytokines in the pathogenesis of Graves’ ophthalmopathy [3]. In the early stage of Graves’ ophthalmopathy, the imbalance between Th1 and Th2 shifts to Th1 dominance, and the Th1 cell-mediated immune response play an important role. In the late stage, Th2 cell-mediated immune response is predominant in the fibrosis of orbital tissue. Increased serum interleukin-17 and the correlation of IL-17 concentration with the clinical activity scores suggest that Th17 and IL-17 play a pathophysiological role in Graves’ ophthalmopathy [9]. Treg cells from patients with Graves’ ophthalmopathy showed different immunomodulation response compared with normal controls [10]. In addition, orbital fibroblasts express CD40, ICAM, IGF-1R, and other immunomodulatory molecules, amplifying the immune response [4,11,12].

The Proliferative Response and Molecular Mechanisms in Graves’ Ophthalmopathy

Based on the surface expression of Thy-1, orbital fibroblasts were divided into Thy-1^+ and Thy-1^- subtypes [4]. Thy-1^+ orbital fibroblasts differentiate into myofibroblasts upon activation of TGF-β. Thy-1^+ orbital fibroblasts differentiate into adipocytes under the influence of PPAR γ agonists [4]. Activated CD4^+ T cells secrete IFN-γ, IL-1, and TNF-α, inducing the expression of TSHR and CD40 on the surface of orbital fibroblasts, which promote the secretion of fibronectin, type 1 collagen, glycosaminoglycans, and other extracellular matrices. On the other hand, 15d-PGJ2 expressed on activated CD4^+ T cells are PPARγ ligands, and induce orbital fibroblast differentiation into adipocytes [4,8].

MicroRNA-155 (miR-155) and MicroRNA-146a (miR-146a) Display a Reciprocal Immunomodulation Function Affecting the Occurrence and Development of Autoimmune Disease

MicroRNAs are single-stranded, small, noncoding RNAs, about 21–25 nucleotides in length [5]. They control gene expression by targeting the 3’-UTR or the coding sequences of specific mRNAs and triggering either translation repression or RNA degradation. MicroRNAs have recently emerged as important modulators of immunity and cellular physiology [5,13–15].

The expression miR-155 and miR-146a can be induced by the TLR4/NF-κB pathway, an important immune and inflammatory response pathway [16]. In turn, both miR-155 and miR-146a have multiple target genes in the TLR4/NF-κB pathway, reciprocally regulating the immune response. Specifically, miR-146a was demonstrated to target the TNF receptor-associated factor 6 (TRAF6), as well as IL-1 receptor-associated kinases (IRAK) 1 and IRAK2 in the NF-κB signaling cascade, as a negative feedback inhibitor. In contrast, miR-155 can promote inflammation by targeted suppression of cytokine signaling 1 (SOCS1) and SH2 domain-containing inositol-5-phosphatase 1 (SHIP1), which are 2 important negative regulators in the TLR4/NF-κB pathway [17]. On the other hand, IL-1β and TNF-α can induce inflammation, hyaluronan production, and adipogenesis through the activation of the TLR/NF-κB pathway in orbital fibroblasts from Graves’ ophthalmopathy [4,18]. Recently, the TLR gene polymorphisms were reported to be associated with susceptibility to Graves’ ophthalmopathy [19].

MiR-155, which is one of the most prominent microRNAs linked to inflammation, is upregulated in both myeloid and lymphoid activated cells. In T cells, miR-155 regulates Treg cell development and competitive fitness through the repression of SOCS1, while also being essential for the development of Th17 cells during autoimmunity [20,21]. Meanwhile, both Treg cells and Th17 cells play pathophysiological roles in Graves’ ophthalmopathy. On the contrary, miR-146a limits T cell activation and promotes decreased inflammatory response. MiR-146a^−/− mice
Graves' ophthalmopathy [1,3,4]. MiR-155 and miR-146a, with important roles in the occurrence and development of response involved in the CD4

Accumulating data suggest that the proliferation and immune functions of miR-155 and miR-146a in Graves' ophthalmopathy. Target validation remains a critical step in defining the shared targets (IGF1R, COL4A3 and MMP16). To date, these tar

IGF1R, TRAF6, FAS, SMAD4, IRAK1, CCND1, MMP16, and COL4A3

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miR-155 and miR-146a have multiple mRNA targets, as revealed by bio
informatics analysis utilizing TargetScan 6.2 software (http://
www.targetscan.org, release date June, 2012) [31,32]. With re
ards to the pathogenesis of Graves' ophthalmopathy, the folowi
ning miR-155 targets may be of particular interest: IGF1R, IGF1, C/EBP-β, TGFBR2, SMAD1, SMAD2, SMAD5, SOCS1, CD28, CD84, IL1RAP, fibrillin 2, LAT2, COL1A2, COL4A3, CTLA4, HLA-
DDB, CCR4, CCR5, CXCL14, and MMP16 [1,4,13,32]. For miR-

miR-155 and miR-146a are typical multifunctional miRNA, with characteristic regulation in the same cellular pathway but according to different conditions, involved in immune regulation, cell proliferation, differentiation, apoptosis, extracellular matrix metabolism, and other life processes [16,29,30]. Both miR-155 and miR-146a have multiple mRNA targets, as revealed by bio-informatics analysis utilizing TargetScan 6.2 software (http://www.targetscan.org, release date June, 2012) [31,32]. With regards to the pathogenesis of Graves' ophthalmopathy, the following miR-155 targets may be of particular interest: IGF1R, IGF1, C/EBP-β, TGFBR2, SMAD1, SMAD2, SMAD5, SOCS1, CD28, CD84, IL1RAP, fibrillin 2, LAT2, COL1A2, COL4A3, CTLA4, HLA-
DDB, CCR4, CCR5, CXCL14, and MMP16 [1,4,13,32]. For miR-

The Hypothesis

MiR-155 and miR-146a were involved in the differentiation of orbital fibroblasts into adipocytes or myofibroblasts [18,28].

The Hypothesis: We propose that the expression of miR-155 increased and expression of miR-146a decreased in CD4+ T cells and orbital fibroblasts in patients with Graves' ophthalmopathy, thus promoting the autoimmune inflammation and proliferative response in the orbital tissue (Figure 1).

The study protocol was approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University (No. 2013-ky-048). Diagnoses of Graves' ophthalmopathy were based on Bartley's criteria [34]. To test the hypothesis, retrobulbar orbital adipose tissues will be obtained from 20 patients with severe and active Graves' ophthalmopathy (with cornea or optic nerve involvement and clinical activity score ≥4) during orbital decompression surgery [35,36]. Patients with cancer, other active inflammation, or autoimmune disease will be excluded. All patients will be euthyroid at the time of enrollment. For the control group, orbital adipose tissues will be obtained from 20 age- and sex-matched patients with atrophic globe but normal retrobulbar structure, during enucleation and orbital implantation surgery. Patients with thyroid diseases, autoimmune diseases, active inflammation, cancer, or a history of immunosuppressive therapy will be excluded from the control group. The retrobulbar orbital adipose tissues will be used for the culture of orbital fibroblasts. CD4+ T cells will be obtained and isolated from the peripheral blood.

The following experiments can be done to test our hypothesis: (1) Quantify the expression of miR-155 and miR-146a in peripheral CD4+ T cells and orbital fibroblasts from Graves' ophthalmopathy patients compared with normal subjects [33]. Taken together, miR-155 and miR-146a may influence the development of Graves' ophthalmopathy by modulating: (1) the TLR4/NF-κB pathway; (2) the imbalances between CD4+ T cells (Th1/Th2/Treg/Th17) and their associated cytokines; and (3) the differentiation and proliferation of orbital fibroblasts. Accordingly, we propose that the expression of miR-155 increased and the expression of miR-146a decreased in CD4+ T cells and orbital fibroblasts in patients with Graves' ophthalmopathy, thus promoting autoimmune inflammation and proliferative response in the orbital tissue (Figure 1).

Testing the Hypothesis

miR-146a inhibits the tumor cell proliferation by target CXCR4 and tumor cell prolifera-
tion and adipogenesis at the same cellular pathway but ac-

Opposite impacts on inflammatory responses carried out by T lymphocytes, appear to have multiple targets in the pathogen-

Testing the Hypothesis: We propose that the expression of miR-155 increased and expression of miR-146a decreased in CD4+ T cells and orbital fibroblasts in patients with Graves' ophthalmopathy, thus promoting the autoimmune inflammation and proliferative response in the orbital tissue.
ophthalmopathy patients by real-time RT-PCR as we described previously [33]; (2) The effects of miR-155 and miR-146a on CD4+ T cells can be assessed by transfection with their inhibitors or mimics, respectively. Then the expression of CD40L, CXCR4 and secretion of IL-1β, IL-2, IL-6, IL-10, IL-17, IL-23, IFN-γ, TGF-β, TNF-α will be assessed and the NF-kB, Jak/Stat pathway will be analyzed; (3) The effects of miR-155 and miR-146a on orbital fibroblasts can be assessed by transfection with their inhibitors or mimics, respectively. Then the expression of CD40, CXCR4, TSHR, IGF-1R; TNF-α, IL-8, IL-6; collagen 1, hyaluronic acid, MMP-9; lipid droplets; PPAR-γ, C/EBP-α, C/EBP-β will be assessed by flow cytometry, ELISA, oil staining, PCR, and Western blot and the TGF-β/Smad and NF-kB pathways will be analyzed by PCR and Western blot; (4) The effects of miR-155 and miR-146a on the mixed culture of CD4+ T cells and orbital fibroblasts can be evaluated; (5) Screening and verification of the target genes of miR-155 and miR-146a in CD4+ T cells and orbital fibroblasts in patients with Graves’ ophthalmopathy can be performed to elucidate the molecular mechanism. The differences between the Graves’ ophthalmopathy patients and the control group will be compared by independent samples t-test.

Consequences of the Hypothesis

Due to the poor understanding of the pathogenesis of this disease, substantial obstacles have prevented the development of specific medical therapies for Graves’ ophthalmopathy. Studies to test our hypothesis will generate new knowledge and concepts about the role of miR-155 and miR-146a in the pathogenesis of Graves’ ophthalmopathy. Further elucidation of the function of the differentially expressed microRNAs may shed new light on the pathogenesis of Graves’ ophthalmopathy, resulting in new management targets and improving patient outcomes.

Conflict of interest statement

All authors declared no conflict of interest.

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