

Received: 2014.03.14  
Accepted: 2014.04.03  
Published: 2014.04.18

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

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

ADEF 1 **Kaijun Li**  
DF 1 **Yi Du**  
BD 2 **Ben-Li Jiang**  
ADG 1 **Jian-Feng He**

1 Department of Ophthalmology, First Affiliated Hospital, Guangxi Medical University, Nanning, Guangxi, China  
2 Department of Ophthalmology, First People's Hospital of Nanning, Nanning, China





**Corresponding Author:** Jian-Feng He, e-mail: hejianf@163.com

**Source of support:** This work was supported by grants from the National Natural Science Foundation of China (No. 81360152, No. 81260149) and the Guangxi Natural Science Foundation (No. 2013GXNSFBA019168)

Graves' ophthalmopathy is an inflammatory autoimmune disease of the orbit, characterized by inflammation and proliferation of the orbital tissue caused by CD4<sup>+</sup>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<sup>+</sup>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**

**Full-text PDF:** <http://www.medscimonit.com/download/index/idArt/890686>

 1649  —  1  36

## Background

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<sup>+</sup>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<sup>+</sup>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<sup>+</sup>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<sup>+</sup> and Thy-1<sup>-</sup> subtypes [4]. Thy-1<sup>+</sup> orbital

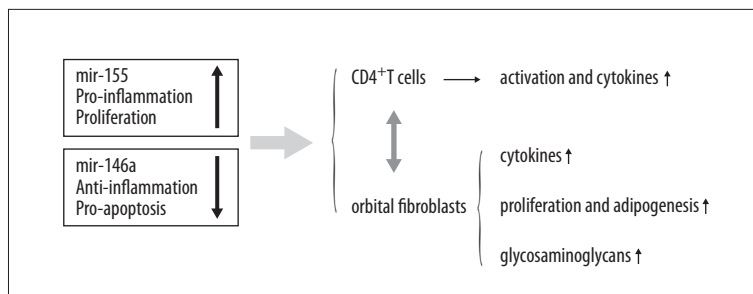
fibroblasts differentiate into myofibroblasts upon activation of TGF- $\beta$ . Thy-1<sup>-</sup> orbital fibroblasts differentiate into adipocytes under the influence of PPAR  $\gamma$  agonists [4]. Activated CD4<sup>+</sup> T cells secrete IFN- $\gamma$ , IL-1, and TNF- $\alpha$ , inducing the expression 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<sup>+</sup> T cells are PPAR $\gamma$  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', 5' 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- $\kappa$ B 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- $\kappa$ B 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- $\kappa$ B 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- $\kappa$ B pathway [17]. On the other hand, IL-1 $\beta$  and TNF- $\alpha$  can induce inflammation, hyaluronan production, and adipogenesis through the activation of the TLR/NF- $\kappa$ B 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<sup>-/-</sup> mice



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

develop spontaneous autoimmunity due to hyperactivation of T cells [22,23]. In addition, miR-155 promotes the proliferation of tumor cells by inhibiting the target gene SHIP1; while miR-146a inhibits the tumor cell proliferation by target CXCR4 and Smad4 [22,24]. Furthermore, miR-155 inhibits the differentiation of adipocyte by target C/EBP- $\beta$  [25]. CXCR4 is a chemokine receptor with potent chemotactic activity in lymphocytes. The expression of CXCR4 by T cells and fibroblasts from patients with Graves' ophthalmopathy was significantly greater than that from controls [26,27]. Interaction between CXCR4 and its ligand may maintain an active immune response in the late stage of Graves' ophthalmopathy. TGF- $\beta$ /Smad pathway and C/EBP- $\beta$  were involved in the differentiation of orbital fibroblasts into adipocytes or myofibroblasts [18,28].

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 bioinformatics 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- $\beta$ , TGFBR2, SMAD1, SMAD2, SMAD5, SOCS1, CD28, CD84, IL1RAP, fibrillin 2, LAT2, COL1A2, COL4A3, CTLA4, HLA-DMB, CCR4, CCR5, CXCL14, and MMP16 [1,4,13,32]. For miR-146a, the following targets may be particularly interesting: IGF1R, TRAF6, FAS, SMAD4, IRAK1, CCND1, MMP16, and COL4A3 [1,4,13]. It is interesting that miR-155 and miR-146a have some shared targets (IGF1R, COL4A3 and MMP16). To date, these targets have not been verified in patients with Graves' ophthalmopathy. Target validation remains a critical step in defining the functions of miR-155 and miR-146a in Graves' ophthalmopathy.

## The Hypothesis

Accumulating data suggest that the proliferation and immune response involved in the CD4<sup>+</sup>T cells and orbital fibroblasts play important roles in the occurrence and development of Graves' ophthalmopathy [1,3,4]. 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 [13,16]. 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 [33]. Taken together, miR-155 and miR-146a may influence the development of Graves' ophthalmopathy by modulating: (1) the TLR4/NF- $\kappa$ B pathway; (2) the imbalances between CD4<sup>+</sup>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<sup>+</sup>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

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  $\geq 4$ ) 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<sup>+</sup>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<sup>+</sup>T cells and orbital fibroblasts from Graves'

ophthalmopathy patients by real-time RT-PCR as we described previously [33]; (2) The effects of miR-155 and miR-146a on CD4<sup>+</sup>T cells can be assessed by transfection with their inhibitors or mimics, respectively. Then the expression of CD40L, CXCR4 and secretion of IL-1 $\beta$ , IL-2, IL-6, IL-10, IL-17, IL-23, IFN- $\gamma$ , TGF- $\beta$ , TNF- $\alpha$  will be assessed and the NF- $\kappa$ B, 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- $\alpha$ , IL-8, IL-6; collagen 1, hyaluronic acid, MMP-9; lipid droplets; PPAR- $\gamma$ , C/EBP- $\alpha$ , C/EBP- $\beta$  will be assessed by flow cytometry, ELISA, oil staining, PCR, and Western blot and the TGF- $\beta$ /Smad and NF- $\kappa$ B pathways will be analyzed by PCR and Western blot; (4) The effects of miR-155 and miR-146a on the mixed culture of CD4<sup>+</sup>T cells and orbital fibroblasts can be evaluated; (5) Screening and verification of the target genes of miR-155 and miR-146a in CD4<sup>+</sup>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.

## References:

- Iyer S, Bahn R: Immunopathogenesis of Graves' ophthalmopathy: the role of the TSH receptor. *Best Pract Res Clin Endocrinol Metab*, 2012; 26: 281–89
- Estcourt S, Quinn AG, Vaidya B: Quality of life in thyroid eye disease: impact of quality of care. *Eur J Endocrinol*, 2011; 164: 649–55
- Xia N, Zhou S, Liang Y et al: CD4<sup>+</sup> T cells and the Th1/Th2 imbalance are implicated in the pathogenesis of Graves' ophthalmopathy. *Int J Mol Med*, 2006; 17: 911–16
- van Steensel L, Dik WA: The orbital fibroblast: a key player and target for therapy in graves' ophthalmopathy. *Orbit*, 2010;29: 202–6
- Contreras J, Rao DS: MicroRNAs in inflammation and immune responses. *Leukemia*, 2012; 26: 404–13
- Zhang WW, Wang T, Su YP et al: Recombinant adenoviral microRNA-206 induces myogenesis in C2C12 cells. *Med Sci Monit*, 2011;17(12): BR364–71
- Shang C, Lu YM, Meng LR: MicroRNA-125b down-regulation mediates endometrial cancer invasion by targeting ERBB2. *Med Sci Monit*, 2012; 18(4): BR149–55
- Eckstein AK, Johnson KT, Thanos M et al: Current insights into the pathogenesis of Graves' orbitopathy. *Horm Metab Res*, 2009; 41: 456–64
- Kim SE, Yoon JS, Kim KH, Lee SY: Increased serum interleukin-17 in Graves' ophthalmopathy. *Graefes Arch Clin Exp Ophthalmol*, 2012; 250: 1521–26
- Kahaly GJ, Shimony O, Gellman YN et al: Regulatory T-cells in Graves' orbitopathy: baseline findings and immunomodulation by anti-T lymphocyte globulin. *J Clin Endocrinol Metab*, 2011; 96: 422–29
- Zhao LQ, Wei RL, Cheng JW et al: The expression of intercellular adhesion molecule-1 induced by CD40-CD40L ligand signaling in orbital fibroblasts in patients with Graves' ophthalmopathy. *Invest Ophthalmol Vis Sci*, 2010; 51: 4652–60
- Smith TJ, Hegedus L, Douglas RS: Role of insulin-like growth factor-1 (IGF-1) pathway in the pathogenesis of Graves' orbitopathy. *Best Pract Res Clin Endocrinol Metab*, 2012; 26: 291–302
- Ammari M, Jorgensen C, Apparailly F: Impact of microRNAs on the understanding and treatment of rheumatoid arthritis. *Curr Opin Rheumatol*, 2013; 25: 225–33
- Liu Y, Zhao J, Zhang PY et al: MicroRNA-10b targets E-cadherin and modulates breast cancer metastasis. *Med Sci Monit*, 2012; 18(8): BR299–308
- Wang JF, Zha YF, Li HW et al: Screening plasma miRNAs as biomarkers for renal ischemia-reperfusion injury in rats. *Med Sci Monit*, 2014; 20: 283–89
- Huffaker TB, Hu R, Runtsch MC et al: Epistasis between microRNAs 155 and 146a during T cell-mediated antitumor immunity. *Cell Rep*, 2012; 2: 1697–709
- Ma XD, Buscaglia LEB, Barker JR, Li Y: MicroRNAs in NF-kappa B signaling. *J Mol Cell Biol*, 2011; 3: 159–66
- Yoon JS, Lee HJ, Choi SH et al: Quercetin inhibits IL-1beta-induced inflammation, hyaluronan production and adipogenesis in orbital fibroblasts from Graves' orbitopathy. *PLoS One*, 2011; 6: e26261
- Liao WL, Chen RH, Lin HJ et al: Toll-like receptor gene polymorphisms are associated with susceptibility to Graves' ophthalmopathy in Taiwan males. *BMC Med Genet*, 2010; 11: 154
- Leng RX, Pan HF, Qin WZ et al: Role of microRNA-155 in autoimmunity. *Cytokine Growth Factor Rev*, 2011; 22: 141–47
- Lu LF, Thai TH, Calado DP et al: Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. *Immunity*, 2009; 30: 80–91
- Labbaye C, Testa U: The emerging role of MIR-146A in the control of hematopoiesis, immune function and cancer. *J Hematol Oncol*, 2012; 5: 13
- Yang L, Boldin MP, Yu Y et al: miR-146a controls the resolution of T cell responses in mice. *J Exp Med*, 2012; 209: 1655–70
- Neilsen PM, Noll JE, Mattiske S et al: Mutant p53 drives invasion in breast tumors through up-regulation of miR-155. *Oncogene*, 2013; 32: 2992–3000
- Skarn M, Namlos HM, Noordhuis P et al: Adipocyte differentiation of human bone marrow-derived stromal cells is modulated by microRNA-155, microRNA-221, and microRNA-222. *Stem Cells Dev*, 2012; 21: 873–83
- Douglas RS, Gianoukakis AG, Goldberg RA et al: Circulating mononuclear cells from euthyroid patients with thyroid-associated ophthalmopathy exhibit characteristic phenotypes. *Clin Exp Immunol*, 2007; 148: 64–71
- Smith TJ, Padovani-Claudio DA, Lu Y et al: Fibroblasts expressing the thyrotropin receptor overarch thyroid and orbit in Graves' disease. *J Clin Endocrinol Metab*, 2011; 96: 3827–37
- Koumas L, Smith TJ, Feldon S et al: Thy-1 expression in human fibroblast subsets defines myofibroblastic or lipofibroblastic phenotypes. *Am J Pathol*, 2003; 163: 1291–300
- Schulte LN, Westermann AJ, Vogel J: Differential activation and functional specialization of miR-146 and miR-155 in innate immune sensing. *Nucleic Acids Res*, 2013; 41: 542–53

30. Xu SJ, Jin CY, Shen XH et al: MicroRNAs as potential novel therapeutic targets and tools for regulating paracrine function of endothelial progenitor cells. *Med Sci Monit*, 2012; 18(7): HY27–31
31. Friedman RC, Farh KKH, Burge CB, Bartel DP: Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*, 2009;19: 92–105
32. Elton TS, Selemon H, Elton SM, Parinandi NL: Regulation of the MIR155 host gene in physiological and pathological processes. *Gene*, 2013; 532: 1–12
33. Yang WJ, Ban SG, He JF: Expression of microRNA-146a in peripheral blood mononuclear cells of patients with thyroid-associated ophthalmopathy and its clinical significance. *Yan Ke Xin Jin Zhan*, 2012; 32: 414–17
34. Bartley GB, Gorman CA: Diagnostic criteria for Graves' ophthalmopathy. *Am J Ophthalmol*, 1995; 119: 792–95
35. Mourits MP, Koornneef L, Wiersinga WM et al: Clinical criteria for the assessment of disease activity in Graves' ophthalmopathy: a novel approach. *Br J Ophthalmol*, 1989; 73: 639–44
36. Werner SC: Modification of the classification of the eye changes of Graves' disease: recommendations of the Ad Hoc Committee of the American Thyroid Association. *J Clin Endocrinol Metab*, 1977; 44: 203–4