



Contents lists available at ScienceDirect

Best Practice & Research Clinical Endocrinology & Metabolism

journal homepage: www.elsevier.com/locate/beem

3

MicroRNAs in autoimmune thyroid diseases and their role as biomarkers

Rebeca Martínez-Hernández (Senior Research Associate)^{a,b},
Mónica Marazuela (Full Professor)^{a,*}

^aDepartment of Endocrinology, Hospital Universitario de la Princesa, Instituto de Investigación Princesa, Universidad Autónoma de Madrid, C/ Diego de León 62, 28006 Madrid, Spain

^bFaculty of Medicine, Universidad San Pablo CEU, CEU Universities, Urbanización Monteprincipe, Alcorcon, Madrid, Spain



ARTICLE INFO

Article history:

Available online 8 February 2023

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at the posttranscriptional level. They are emerging as potential biomarkers and as therapeutic targets for several diseases

Abbreviations: AITD, autoimmune thyroid diseases; AKAP12, A-kinase anchoring protein 12; Akt, AKT serine/threonine kinase 1; CAS, Clinical Activity Score; C/EBP α , CCAAT/enhancer binding protein; CILP, Cartilage Intermediate Layer Protein; CLDN1, Claudin 1; DC, dendritic cells; DEPDC4, DEP domain containing 4; DLX5, Distal-less homeobox 5; EAE, experimental autoimmune encephalomyelitis; EDA, Ectodysplasin A; ENO4, Enolase 4; Foxp3, Forkhead box P3; FFPE, Formalin-Fixed Paraffin-Embedded; FT4, Free Thyroxine; GATM, Glycine Amidinotransferase; GC, Glucocorticoids; GD, Graves' disease; GO, Graves' Ophthalmopathy; GTF2H1, general transcription factor IIH subunit 1; GTF2H2, general transcription factor IIH subunit 2; HAS2, hyaluronan synthase 2; HIF1- α , Hypoxia Inducible Factor 1 subunit alpha; HSPB1, Heat Shock Protein family B (small) member 1; HT, Hashimoto's thyroiditis; IFN- γ , Interferon-gamma; IL, Interleukin; IL-23R, Interleukin 23 receptor; IMID, Immune Mediated Inflammatory Disorders; INTU, inturnd planar cell polarity protein; ITK, IL-2-inducible T-cell kinase; JNK-1/2, c-Jun N-terminal kinases-1/2; KIF27, kinesin family member 27; MiRNAs, MicroRNAs; MV, Microvesicles; NF- κ B, Nuclear factor kappa-light-chain-enhancer; NGS, Next Generation Sequencing; NK, natural killer cells; Notch1, Notch receptor 1; NOX4, NADPH oxidase 4; NUMB, NUMB endocytic adaptor protein; OF, Orbital Fibroblasts; PACRG, parkin co-regulated; PBMC, Peripheral blood mononuclear cells; PBX3, PBX homeobox 3; PDCD4, Tumor Suppressor Programmed Cell Death 4; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PPAR, peroxisome proliferator activated receptor gamma; PTEN, Phosphatase and Tensin homolog; qRT-PCR, quantitative Real-Time Reverse Transcription; RA, Rheumatoid Arthritis; RARA, retinoic acid receptor alpha; RARB, retinoic acid receptor beta; RHBDL2, Rhomboid like 2; RXRA, retinoid X receptor alpha; SDC1, Syndecan 1; SIRT1, Sirtuin 1; SMAD4, SMAD family member 4; STAT, Signal Transducer and Activator of Transcription; STK36, serine/threonine kinase 36; T3, Triiodothyronine; T-bet, T-box transcription factor 21; Tfh, Follicular helper T cell; Tg, Thyroglobulin; TGF β , Transforming Growth Factor beta 1; Th, T helper cell; TLR, Toll-like receptors; TNF- α , tumor necrosis factor alpha; TPO, Thyroperoxidase; Tr1, Type 1 regulatory cells; TRAF4, TNF receptor associated factor 4; Treg, T regulatory lymphocytes; TSH-R, Thyrotropin receptor; TSH-R-Ab, Thyrotropin receptor antibodies; VEGF, Vascular Endothelial Growth Factor; VIP, Vasoactive Intestinal Peptide; VPAC, Vasoactive intestinal peptide receptor; ZFPM2, Zinc Finger Protein, FOG family member 2; ZNRF3, Zinc and Ring Finger 3

* Corresponding author.

E-mail addresses: rebeca.martinez@salud.madrid.org (R. Martínez-Hernández), monica.marazuela@salud.madrid.org, monica.marazuela@uam.es (M. Marazuela).

<https://doi.org/10.1016/j.beem.2023.101741>

1521-690X/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords:

Autoimmune thyroid diseases
Biomarkers
Hashimoto thyroiditis
Graves' disease
Graves' ophthalmopathy
MicroRNAs

including autoimmune thyroid diseases (AITD). They control a wide range of biological phenomena, including immune activation, apoptosis, differentiation and development, proliferation and metabolism. This function makes miRNAs attractive as disease biomarker candidates or even as therapeutic agents. Because of their stability and reproducibility circulating miRNAs have been an interesting area of research in many diseases, and studies describing their role in the immune response and in autoimmune diseases have progressively developed. The mechanisms underlying AITD remain elusive. AITD pathogenesis is characterized by a multifactorial interplay based on the synergy between susceptibility genes and environmental stimulation, together with epigenetic modulation. Understanding the regulatory role of miRNAs could lead to identify potential susceptibility pathways, diagnostic biomarkers and therapeutic targets for this disease. Herein we update our present knowledge on the role of microRNAs in AITD and discuss on their importance as possible diagnostic and prognostic biomarkers in the most prevalent AITDs: Hashimoto's thyroiditis (HT), Graves' disease (GD) and Graves' Ophthalmopathy (GO). This review provides an overview of the state of the art in the pathological roles of microRNAs as well as in possible novel miRNA-based therapeutic approaches in AITD.

© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Autoimmune diseases

Tolerance is the mechanism by which the immune system avoids activation by self-antigens. "Central" tolerance takes place in the thymus and bone marrow and plays a key role in immune system homeostasis by inactivating or deleting autoreactive T and B lymphocytes. However, as small numbers of potentially self-reacting lymphocytes can still "leak out" into the periphery, additional "peripheral" mechanisms are required to maintain tolerance [1]. Any defect or failure in tolerance mechanisms can lead to breakdown of tolerance and to the development of autoimmunity [1,2].

Autoimmune diseases include several human disorders characterized by the loss of immunological tolerance to self-antigens and the presence of autoreactive immune cells and (or) autoantibodies against healthy cells and normal tissues. The activation and proliferation of auto-reactive cells that recognize self-antigens and are present in the peripheral blood is prevented in the periphery by different immune regulatory mechanisms, including the suppressive effect of T regulatory (Treg) lymphocytes. However, in certain individuals, auto-reactive T cells escape from the control of immune regulatory mechanisms and are subsequently activated, proliferate, and differentiate (autoimmune response) [3].

Autoimmune thyroid disorders

Autoimmune thyroid disorders (AITD) are a group of heterogeneous disorders characterized by the dysregulation of the immune system leading to a loss of tolerance to organ-specific self-antigens including thyroglobulin (Tg), thyroperoxidase (TPO) and the thyrotropin receptor (TSH-R) [4]. This leads to an abnormal lymphocytic activation in the thyroid gland, and occasionally the orbit, involved in a broad spectrum of diseases. The two main pathological conditions in AITD are Hashimoto's thyroiditis (HT) and Graves' disease (GD). Both conditions are characterized by the presence of circulating thyroid antibodies and infiltration of the thyroid gland and sometimes the orbit by autoreactive lymphocytes. It has been traditionally described that HT is mainly mediated by a cellular autoimmune response, whereas GD has been considered to be mainly mediated by a humoral response, i.e. by autoantibodies directed against the thyrotropin receptor (TSH-R-Ab) that lead to development of goiter and hyperthyroidism [4–6].

However, an evident humoral autoimmune response is also observed in HT, where antibodies against Tg and TPO are present, while a variable degree of T cell activation is also observed in GD, with variable levels of thyroid cell infiltration [5,6]. Regarding Graves' ophthalmopathy (GO), both immune responses, including TSH-R-Ab, have been involved in its pathogenesis. GO is the most common extra-thyroidal manifestation of AITD, clinically appearing in up to 25% of patients with GD [7] (Fig. 1).

The interaction of thyroidal follicular cells, antigen presenting cells, and autoreactive T and B lymphocytes is involved in the pathogenesis of AITD, which are mediated by different T helper cell (Th) subsets, mainly Th1, Th2, and Th17 [8,9]. Recent significant advances in the knowledge of T regulatory (Treg) lymphocytes have unveiled a relevant role of these cells in the pathogenesis of autoimmune and chronic inflammatory diseases, through their ability to suppress immune responses.

Treg cells play an important role in immune tolerance to self-antigens and exert immunosuppressive effects through different mechanisms. Different Treg cell subsets are involved in the pathogenesis of AITD including natural Treg cells (CD4+CD25+Foxp3+ and CD4+CD69+Foxp3-) and peripheral Tregs (CD4+Foxp3-IL10+ type 1 Treg cells or Tr1 cells). In this regard, several reports have described a decreased number and/or defective function of Treg cells in AITD [9–15].

Upon antigen recognition, the presence of Interleukin -12 (IL-12), Interferon-gamma (IFN- γ), Interleukin 2 (IL-2) and the expression of the T-box transcription factor 21 (T-bet) induce the differentiation of naïve CD4+ T cells into Th1 cells (which mainly synthesize IFN- γ and IL-2). Conversely, the presence of interleukin-4 (IL-4) inhibits T helper 1 (Th1) lymphocyte differentiation and favors the generation of Th2 lymphocytes, which mainly synthesize interleukins IL-4, IL-5, IL-6, and IL-13. T helper

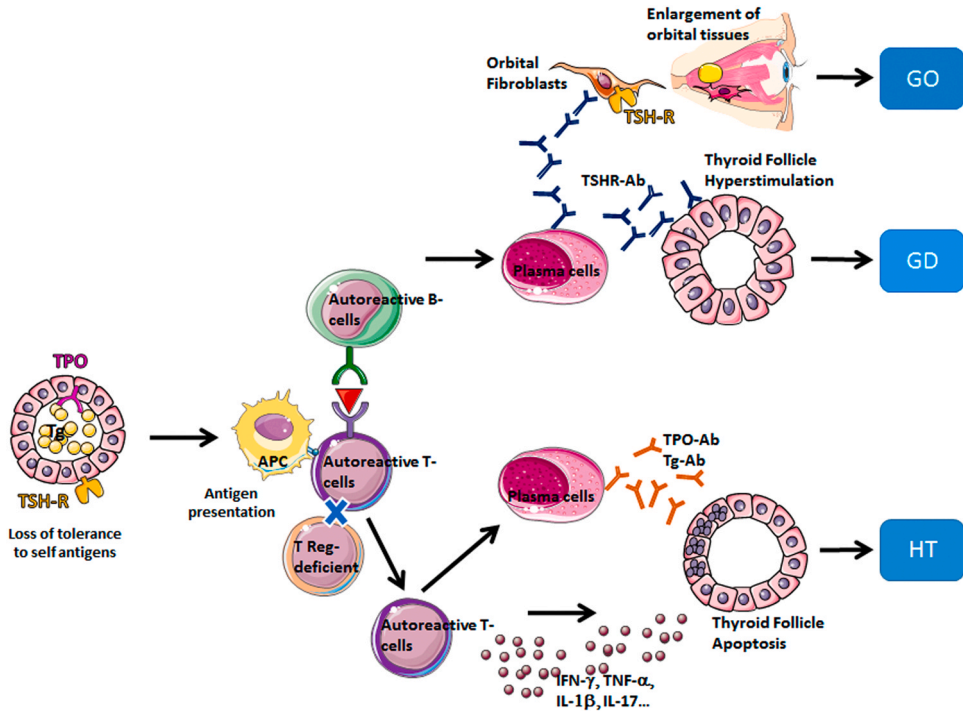


Fig. 1. Loss of immune tolerance to organ-specific self-antigens, including thyroglobulin (Tg), thyroperoxidase (TPO), and the thyrotropin receptor (TSH-R) allows subsequent activation, proliferation, and differentiation of auto-reactive cells. HT is mediated by a predominant cellular autoimmune response, with a heavy inflammatory cell infiltrate, secretion of proinflammatory cytokines, and anti-Tg and anti-TPO antibodies (Ab), leading to subsequent tissue destruction and thyroid gland failure. GD is mediated by a predominant humoral autoimmune response, with the synthesis of agonistic auto-antibodies specific for the TSH-R leading to hyperplasia and increased production of thyroid hormones. Anti-TSH-R-Ab also recognize the TSH-R on orbital fibroblasts initiating tissue changes characteristic of Graves' ophthalmopathy (GO).

17 (Th17) lymphocytes are mainly characterized by the synthesis of interleukins IL-17A, IL-17F, IL-21, and IL-22, which contribute to the release of other pro-inflammatory mediators (such as chemokines, tumor necrosis factor alpha (TNF- α)-like and IL- β by stimulating epithelial cells, fibroblasts, and macrophages. In this regard, HT has long been described as a Th1-mediated disease with heavy T cell infiltration and progressive destruction of thyrocytes. In contrast, GD has been reported to be mainly mediated by a Th2 response with autoantibody production and gland hyperplasia [8,16]. In both cases, and also in GO, there is an increase of Th17 cells, strongly supporting a relevant role for these cells in the pathogenesis of the chronic inflammatory process and tissue damage observed in AITD [17–19]. Thus, nowadays both Treg and Th17 cells are considered as plausible potential targets for novel therapeutic approaches to auto-immune and chronic inflammatory conditions [20,21].

Introduction to microRNAs (miRNAs)

One of the main discoveries when the genome was deciphered was the fact that more than 80% of the genome specific biological functions were primarily associated with regulation of protein-coding gene expression. miRNAs are the most commonly identified post-transcriptional regulators of gene expression [22,23]. Research on miRNA was initially slow; although the first miRNA (lin-4) was identified in 1993, the second miRNA (let-7) had to wait until 2000 [24]. However, this research later increased and to date, miRNAs have been actively studied in animals, plants, protists, and viruses [25].

MiRNAs are a class of short (19–25 nucleotides) non-coding RNA molecules that are synthesized in the nucleus through the transcription of double-stranded pri-miRNA (1–3 kb) that ends as a single-stranded 'mature miRNA' in the cytoplasm. After initial processing in the nucleus by the RNase Drosha, the pre-miRNA is transported to the cytoplasm, where the miRNA hairpin is cleaved by the endoribonuclease Dicer, forming a miRNA duplex. One of the miRNA strands is loaded into the RNA inducing silencing complex (RISC), which regulates mRNA transcription and protein translation through various mechanisms. Within the cell cytoplasm, miRNAs regulate gene expression post-transcriptionally by binding to complementary sequences in the target mRNAs. miRNA binding to its target usually results in mRNA degradation or translational inhibition, thereby regulating expression of target genes [26] (Fig. 2).

miRNAs belong to one of the most abundant classes of human genome regulators, as more than 30% of human genes are regulated by miRNAs through degradation of target mRNAs [27]. Currently, nearly 3000 human miRNAs have been annotated in the miRBase [28]. Because of high similarity and binding to different mRNA sequences, each miRNA may suppress multiple mRNA targets (average 200) and one mRNA can be targeted by many miRNAs [26,29]. Accordingly, by targeting a plethora of mRNAs, miRNAs can simultaneously regulate multiple pathways and biological processes [26].

miRNA functions

miRNAs participate in the regulation of almost every aspect of cell physiology [29,30]. Hence, it is difficult to discern the specific function/effect of a specific miRNA. miRNAs are involved in several biological processes, including immune functions, cellular apoptosis, cell differentiation and development, proliferation, and metabolism. Based on the crucial role of miRNAs in human physiology, their abnormal expression (upregulation or downregulation) may lead to the development of diverse diseases such as cancer, immune-mediated disorders, cardiovascular disorders, neurological disorders, musculoskeletal disorders, lung diseases and developmental abnormalities [31] (Fig. 2).

miRNA function in the immune system

Recent studies showed that miRNAs play a crucial role in the development and function of the immune system, where they are involved in the differentiation and survival of immune cells, antibody production and release of inflammatory mediators. miRNAs are a crucial part of both innate and adaptive immunity [32–34].

Innate immunity acts as the first line of the body's immune defences against infectious agents. It has been demonstrated that several miRNAs play an essential role in innate immunity in responses involving

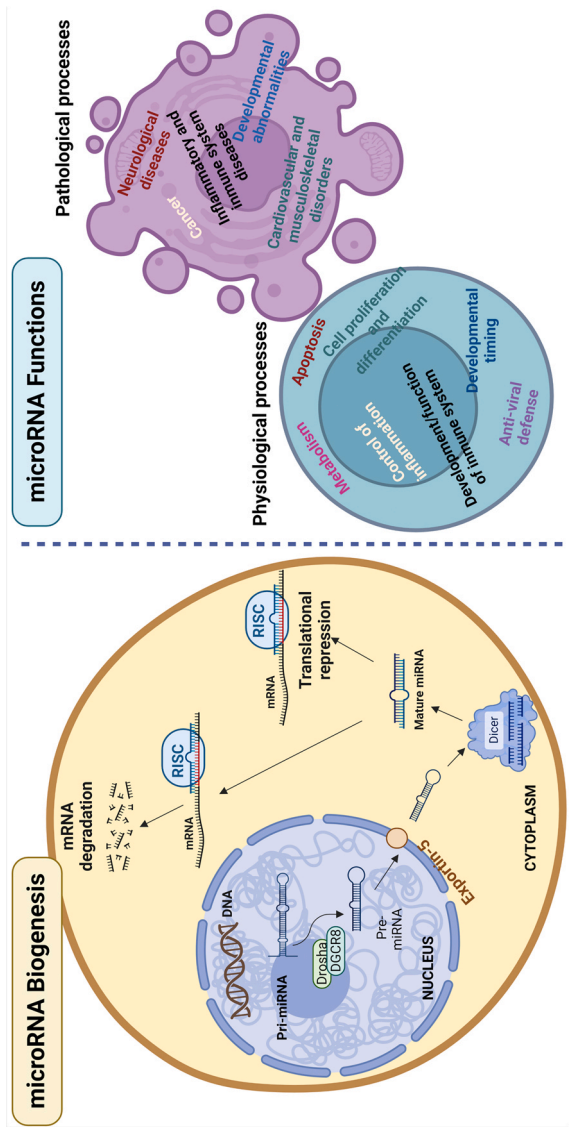


Fig. 2. Biogenesis and functions of miRNAs. Primary miRNAs (Pri-miRNAs) are transcribed by RNA polymerase II and cleaved by Drosha/DGCR8 microprocessor complex subunit to a precursor miRNA (pre-miRNA). Pre-miRNAs are then transported to the cytoplasm by exportin. The pre-miRNAs are cleaved by RNase III Dicer into miRNA duplexes. One of the strand of these double-stranded RNA forms a mature miRNA (miRNA), which is loaded on RNA induced silencing complex (RISC) containing target messenger RNA (mRNA), thereby resulting in mRNA degradation and/or translation repression.

monocytes, macrophages, dendritic cells (DC), granulocytes, and natural killer (NK) cells. Monocytes and DC trigger anti-pathogen inflammatory reactions by recognizing microbial components via Toll-like receptors (TLR). Multiple miRNAs, e.g. miR-146a, miR-155 and miR-21, are induced by TLR activation in monocytes and DC [22] and, together with other mechanisms, can control the inflammatory response by targeting the mRNAs of certain TLR signaling components [32,35]. miRNAs can both promote the initiation of an inflammatory reaction and prevent excessive inflammation thus allowing the return to immune homeostasis and tissue repair.

Several studies have reported an essential role of miRNAs in the development and function of lymphocytes in mouse models [32,36]. A specific deletion of the gene encoding Dicer, the protein regulator of miRNA synthesis, in a T-cell specific Dicer knockout mouse strain leads to the development of an uncontrolled autoimmunity secondary to the disruption of Treg cells [37]. Dicer-deficient Treg cells have a decrease in the transcription factor FoxP3, along with an altered expression of multiple key regulators of the T-cell function [37]. Deletion of Dicer in mutant mice can also lead to defects in B cell proliferation and survival [38].

“Changed” miRNAs can also initiate the secretion of inflammatory cytokines. In this regard, some miRNAs such as let-7a, miR-26, miR-146a, miR-146b, miR-150 and miR-155 can lead to Th17 differentiation and promote several autoimmune disorders mediated by IL-17 [39,40].

miRNAs in immune mediated disorders

Given the role of miRNAs in processes involved in the immune system physiology including T cell reactivity, antibody response [41] and sustained inflammation, it is conceivable that deregulation miRNAs may lead to impaired self-tolerance and to the development of autoimmune diseases [30]. In this regard, miRNAs have emerged as regulators of a wide range of immune disorders, including autoimmunity and chronic inflammatory diseases [29]. As many immune-mediated diseases involve cytokines, extracellular matrix and / or impaired apoptosis, several identical miRNAs have been found to be involved in deregulation these pathways across various immune-related diseases (e.g. miR-146, miR-155, miR-150 and miR-21). Accumulating evidence suggests that miRNAs can be used as disease biomarkers, and several publications have reported their value as diagnostic and prognostic tools in different diseases, including various autoimmune disorders [42] such as rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome (SS) and psoriasis, amongst others [29,43–46].

Extracellular miRNAs as potential signaling mediators and biomarkers

Several studies have revealed that miRNAs can be secreted into the bloodstream and affect other cells and may be key mediators of various cell-to-cell signaling pathways. In fact, extracellular miRNAs have been found in a variety of mammalian body fluids including blood plasma, urine, breast milk, amniotic fluid, and saliva [47].

Since miRNAs are stable in body fluids such as serum or plasma, where they can be reproducibly detected, they have emerged as potential prognostic biomarkers for risk assessment and as a valuable diagnostic tool for diagnosis and prognosis [42]. Circulating tissue-derived miRNAs are not only specific and stable, but they are also protected from endogenous RNase activity, and could be useful as minimally invasive diagnostic tools for various diseases as well as for research on novel therapeutic targets [48]. The advantage of using circulating miRNA is that the diagnostic approach could be minimized to a single blood test [48]. Some of these circulating miRNAs could be originally synthesized and act in the diseased tissue and then be released to human body fluids, including serum, plasma, urine, and saliva, where they provide a potential utility as biomarkers of altered tissues. It is worth highlighting that in some pathologies one miRNA biomarker may be sufficient to predict the clinical outcome, whereas in most cases only well-defined miRNA panels would be useful as diagnostic or prognostic tools.

In addition, miRNAs are now known to master cell-to-cell signaling via association with extracellular vesicles that protect them from degradation and allow efficient entry into neighboring cells [2,47,49]. Many extracellular miRNAs are present in exosomes, which are lipid bilayer-bound microvesicles (30–120 nm in size) released by different cell types, to different body fluids [50,51]. Exosomes in turn can be transported to distant locations where they are captured by recipient cells where they liberate their

cargo miRNA [52] thereby playing a messenger role in cell-to-cell communication [53,54]; in addition, exosomal miRNAs may be useful as potential biomarkers for human diseases. miRNAs can also be transported by microvesicles (MV), which are gaining importance as a source of miRNAs in systemic circulation. MV retain certain membrane and cytoplasmic components, including cytosolic proteins, lipids, DNA, mRNA, and miRNAs from their parental cells and can transfer bioactive molecules to neighboring cells modulating biological processes such as inflammatory and autoimmune disorders.

miRNAs and autoimmune thyroid disorders

miRNAs can be differentially expressed in AITD patients in different tissues including thyroid tissue, the orbit and/or serum or plasma. Thyrocytes, immune cells, and other different cells types including cells from the thyroid stroma play a role in the pathophysiology of AITD as both target and effector cells (Fig. 3). Thus, the role of miRNAs can be linked to different pathways depending on the reference sample used in the study. Therefore, studying the expression of miRNAs, their distribution and the relationship between circulating miRNAs and those in various tissues is important to better understand the physiological and pathological mechanisms of disease. Differences between tissue-specific miRNA patterns should be considered when exploring different miRNA signatures as possible disease biomarkers (Tables 1–4).

Circulating miRNAs IN AITD

Several studies have reported differentially expressed miRNAs in peripheral blood mononuclear cells (PBMCs), serum, plasma and T cells in AITD using different methodological approaches such as quantitative Real-Time Reverse Transcription (qRT-PCR), microarrays, sequencing, reporter assays, and miRNA transfection (Table 1).

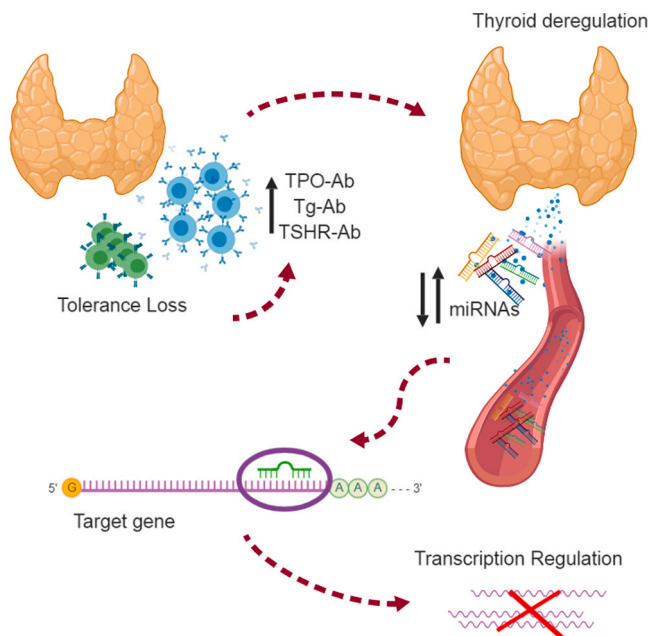


Fig. 3. In AITD, immune tolerance to thyroid antigens is lost causing thyroid cell damage (mediated by humoral or cellular immune responses). Thyroid deregulation may cause a deregulation of miRNA expression profiles (in the thyroid tissue or in the circulation). MicroRNAs regulate gene expression by mRNA degradation or translational inhibition.

Table 1
Circulating mirna profiles from HT and GD patients.

Source of samples	miRNA assays	miRNAs studied	miRNA results	Associated genes if studied	Reference
PBMC	Microarrays and qRT-PCR	miR-154, miR-376b, miR-431, miR-329, miR142-3p, miR-31, miR-222, miR-590-5p, miR-186, miR-192, miR-449a, miR-485-3p, miR-187, miR-26b, miR-30c-2, let-7b	GD: ↓miR-154, miR-376b and miR-431	DEPDC4 for miR-154* DLX5, HAS2 and RHBDL2 for miR-376b	[55]
PBMC and CD4+ T cells	qRT-PCR	miRNA 34a_1, miRNA 143_1, miRNA 146a_1, miRNA 155*_1 and 155_2, miRNA 181a*_1, miRNA 181b_1, miRNA 200a_1 and 200a_2	GD PBMC: ↑ miRNA 146a_1 GD and HT CD4+ and CD8+: ↓miR-200a_1 and miR-200a2		[56]
T regulatory cells	Microarrays and qRT-PCR	miR-155, miR-519e, miR30a, miR-19b, miR-146a	GD & HT CD8+: ↓miR-155_2 and miR-155_1 Initial GD: ↑miR-636, miR-30a, miR-181a, miR-155, miR-519e ↓ miR-19b, miR-146a	RARA, RARB, RXRA for miR-636 and miR-30a GTF2H1 and GTF2H2 for miR-181a	[63]
PBMC	qRT-PCR and reporter assay	miR-125a	HT: ↓ miR-125a	IL-23R	[64]
PBMC	qRT-PCR	Let-7e	TH: ↑ Let-7e	IL-10	[65]
CD4+ T cells and plasma	qRT-PCR, reporter assay	miR-346	GD: ↓ miR-346	Bcl-6	[67]
CD4+ T cells	Microarrays, qRT-PCR	2006 miRNAs	GD: ↑ miR-10a and miR-125b ↓ miR-4443	TRAF4	[68]
Peripheral blood T cells	qRT-PCR	miR-9-5p, miR-29a-3p, and miR-210-3p	HT: ↓ miR-29a-3p	T-bet	[66]
T regulatory cells	qRT-PCR	miR-23a-3p	GD: ↓ miR-23a-3p	SIRT1	[69]
T regulatory cells	qRT-PCR	miR-363-5p	GD: ↑ miR-363-5p	Notch1 signaling pathway through STAT4 and HSPB1	[70]
Serum	Microarrays and qRT-PCR	miR-16, miR-22, miR-375 and miR-451	HT: ↑ miR-22, miR-375 and miR-451 GD: ↑miR-16, miR-22, miR-375 and miR-451		[72]
Serum and Exosomes	Microarrays, qRT-PCR	Let-7g -3p, miR-23b -5p, miR-92a -3p, miR-92b -3p, miR-183-3p, miR -339-5p	GD in remission ↑ miR-23b -5p, miR-92a-3p ↓ let-7g-3p and miR-339-5p		[73]
Serum	qRT-PCR	miR-146a, miR-155, miR-210	GD ↑ miR-210 ↓ miR146a, miR-155		[74]
Thyroid tissue and Serum	Sequencing and qRT-PCR	3431 miRNAs	AJTD (HT and GD): ↑ miR-21 -5p, miR-96-5p, miR-142-3p, and miR-301a-3p ↓ miR-Let7d-5p		[44]
Thyroid tissue and Serum	qRT-PCR	miR-142-3p, miR-154-3p, miR-431-3p, miR-590-5p, and let-7b	GD: ↑ miR-142-3p and let-7b	PLZF	[76]

(continued on next page)

Table 1 (continued)

Source of samples	miRNA assays	miRNAs studied	miRNA results	Associated genes if studied	Reference
Serum	qRT-PCR	miR-10a, miR-19a-3p, miR-19b-3p, miR-21-5p, miR-23a-3p, miR-26b-5p, miR-27a-3p, miR-29a-3p, miR-93-3p, miR-101-3p, miR-125b-5p, miR-126-3p, miR-127-3p, miR-142-3p, miR-143-3p, miR-146a-5p, miR-150-5p, miR-155-5p, miR-191-5p, miR-210-3p, miR-326, miR-451	GD: ↓ miR-19b and miR-26b GD severe: ↑ miR19a and miR-143		[75]
Serum	qRT-PCR	miR21-5p, miR-22-3p, miR-22-5p, miR-142-3p, miR-146a-5p, miR-301-3p and miR-451	HT: ↑ miR21-5p, miR-22-3p, miR-22-5p, miR-142-3p, miR-146a-5p, miR-301-3p and miR-451		[77]
Plasma Microvesicles	qRT-PCR	miR-326, miR150, miR-155 and miR-146a	AITD (GD and HT); ↑ miR146a	IL8 and SMAD4	[78]
Plasma	Sequencing	3025 miRNAs	HT: ↑ miR-155 GD: ↑ miR-497, miR-320b-1 and miR-320b-2 GO: ↑ miR-27a-3p GD and GO: ↓ miR-22-3p novel: 19-15038, miR182-5p, miR-22-3p, miR-27a-3p, miR-6748-3p and 20 proteins as prection model		[79]
Plasma	qRT-PCR	168 miRNAs	HT: ↑ miR-205, miR-20a-3p, miR-375, miR-296, miR-451, miR-500a		[80]
Plasma	Microarrays and qRT-PCR	miR-16-1-3p, miR-122-5p, miR221-3p, miR-144-3p and miR-762	GD: ↑ miR-762 ↓ miR144-3p		[81]

Abbreviations: PBMcs = Peripheral blood mononuclear cells; qRT-PCR = quantitative Real-Time Reverse Transcription; AITD = Autoimmune Thyroid Disease; HT = Hashimoto's Thyroiditis; GD = Graves 'Disease; DEPDc4 = DEP domain containing 4; DLX5 = Distal-less homeobox 5; HAS2 = hyaluronan synthase 2; RHBDL2 = Rhomboid like 2; RARA = retinoic acid receptor alpha; RARB= retinoic acid receptor beta; RXRA = retinoid X receptor alpha; GTF2H1 = general transcription factor IIH subunit 1; GTF2H2 = general transcription factor IIH subunit 2; IL-23R = Interleukin 23 receptor; TRAF4 = TNF receptor associated factor 4; T-bet = T-box transcription factor 21; SIRT1 = Sirtuin 1; Notch1 = Notch receptor 1; STAT4 = Signal Transducer and Activator of Transcription 4; HSPB1 = Heat Shock Protein family B (small) member 1; PLZF = pro-myelocytic leukemia zinc finger; IL8 = interleukin 8; SMAD4 = SMAD family member 4.

Table 2
Thyroid tissue miRNA profiles from HT and GD patients.

Source of samples	miRNA assays	miRNAs studied	miRNA Results	Associated genes if studied	Reference
FFPE thyroid tissue: Thyrocytes vs Lymphocytic infiltrate (LCM) Aspiration	qRT-PCR	Mir-141	HT: ↓ mir-141	TGFBP1	[82]
Thyroid Gland Fine-Needle	qRT-PCR	miR-146a, miR-155_2 and miR-200a1	GD: ↓ miR-146a HT: ↓miR-155_2 and ↑miR-200a1		[62]
Thyroid tissue	Microarrays and qRT-PCR	miR-22, -183, -625*, -3613-3miR-29a*, -92b, -361-5p, -101, -708, -1179, -210, -192, -505, -29c*, -660, -26a, -324-3p, -4324, -455-5p, -197, -191 and let- 7b*	miR-22, miR-183 ↓ miR-101, miR-197 and miR-660	EDA, GATM, MLLT4 for miR-22 AKAP12 and ZFPM2 for miR-183 PBX3 for miR-101, CILP for miR-197 and SDC1 for miR-660	[85]
FFPE Thyroid tissue	Microarrays, qRT-PCR	miR-142-5p	TH: ↑ miR-142-5p, miR-142-3p and miR-146a	CLDN1	[83]
FFPE Thyroid tissue	qRT-PCR	miR-146b, miR-221, miR-222	GD: ↓miR-146b, miR-221, miR-222		[84]
Thyroid tissue and Serum	Sequencing and qRT-PCR	3431 miRNAs	AITD (HT and GD): ↑ miR-21-5p, miR-96-5p, miR-142-3p, and miR-301a-3p ↓ miR-let7d-5p		[44]
Thyroid tissue and Serum	qRT-PCR	miR-142-3p, miR-154-3p, miR-431-3p, miR-590-5p, and let-7b	GD: ↑ miR-142-3p and let-7b	PLZF	[76]
Thyroid tissue	Sequencing and qRT-PCR	3431 miRNAs	AITD: ↑ miR-21-5p, miR-146b-3p, miR-5571-3p and miR-6503-3p	ENO4, INTU, KIF27, PACRG, STK36	[86]

Abbreviations: FFPE = Formalin-Fixed Paraffin-Embedded; AITD = Autoimmune Thyroid Disease; HT = Hashimoto's Thyroiditis; GD = Graves' Disease; qRT-PCR = quantitative Reverse Transcription; TGFBP = Transforming Growth Factor beta 1 Receptor; EDA = Ectodysplasin A; GATM = Glycine Amidinotransferase; AKAP12 = A-kinase anchoring protein 12; ZFPM2 = Zinc Finger Protein, FOG family member 2; PBX3 = PBX homeobox 3; CILP = Cartilage Intermediate Layer Protein; SDC1 = Syndecan; CLDN1 = Claudin 1; PLZF = pro-myelocytic leukemia zinc finger; ENO4 = Enolase 4; INTU = intuned planar cell polarity protein; KIF27 = kinesin family member 27; PACRG = parkin co-regulated; STK36 = serine/threonine kinase 36.

Table 3
Circulating miRNA profiles from GO patients.

Source of samples	miRNA assays	miRNAs studied	miRNA results	Associated genes if studied	Reference
CD4+ T cells	qRTPCR	miR-146a	GO: ↓ miR-146a	Th1 cytokines	[87]
CD4+ T cells	qRTPCR	miR-146a	GO: ↓ miR-146a	NUMB	[88]
CD4+ T cells	qRT-PCR	miR-96 and miR-183	GO: ↑ miR-96 and miR-183	PTEN, Akt phosphorylation and proliferation of T cells	[89]
Serum	Microarrays, qRTPCR and reporter assay	miR-155, miR-224	Resistant GC GD: ↓ miR-224	GR, GSK-3b	[90]
Serum	Sequencing and qRT-PCR	3431 miRNAs	GO: ↓ miR-Let7d		[44]
Serum	qRT-PCR	miR21-5p, miR27a-3p, miR100-5p, miR145-5p, miR146a-5p, miR153-3p, miRNA92a-3p, miR199a-5p, miR223-3p, miR7-5p, and miR99a-5p	GO: ↑ miR-96-5p and miR-301a-3p miR21-5p, miR27a-3p, miR100-5p, miR145-5p, miR146a-5p, miR153-3p, miRNA92a-3p, miR199a-5p, miR223-3p, miR7-5p, and miR99a-5p		[91]
Plasma	qRTPCR	miR-146a	Inactive and Active GO: ↓ miR-146a	IL17	[92]
Plasma	Sequencing and qRT-PCR	miR-6721-5p, miR-5096, miR-4446-3p, miR-885-3p, miR-4433b-3p, miR-671-3p, miR-615-3p, miR-4474-3p, miR-143-5p, miR-5581-3p	GO responding to ivGC: ↑ miR-885-3p and ↓ miR-4474-3p and miR-615-3p	AKT/NFκB	[93]

Abbreviations: qRT-PCR = quantitative Real-Time Reverse Transcription; GO = Graves' Ophthalmopathy; CC = Glucocorticoids; ivGC = intravenous Glucocorticoids; NUMB = NUMB endocytic adaptor protein; PTEN = Phosphatase and Tensin homolog; Akt = AKT serine/threonine kinase 1; GR = Glucocorticoid Receptor; GSK-3b = Glycogen synthase kinase 3 beta; IL17 = Interleukin 17; NF-κB = Nuclear factor kappa-light-chain-enhancer.

Table 4
Retroorbital tissue mirna profiles from GO patients.

Source of samples	miRNA assays	miRNAs studied	miRNA Results	Associated genes if studied	Reference
Orbital Fibroblasts	qRT-PCR and miRNA transfection	miR-21	GO: ↑ miR-21	TGFβ1 and Smad3	[95]
Orbital Fibroblasts	RNA transfection, WB, qRT-PCR	miR-21	GO: upregulation of miR21 by PDGF-BB	PDCD4	[96]
Orbital Fibroblasts	Microarrays and qRT-PCR	2578 miRNAs	GO: ↑ miR-146a	IL1B, IL6, ICAM-1	[97]
Orbital Fibroblasts	qRT-PCR, WB	miR-146a	GO: ↑ miR-146a	TGF-β	[99]
TSH- stimulated-Orbital Fibroblasts	qRT-PCR	miR-146a and miR-155	GO: ↑ miR-146a and miR-155	ZNRF3 AND PTEN	[100]
Orbital Fibroblasts	qRT-PCR, WB	miR-27a and miR-27b	GO: ↓ miR-27a and miR-27b	PPARγ, CCAAT/enhancer binding protein (C/EBP)α and C/EBPβ	[101]
Thyroid and Orbital tissue	qRT-PCR, In Situ Hybridization	miR-199a	GD and GO: ↓ miR-199-3p and miR-199-5p	NOX4/HIF1-α/VEGF	[104]
Orbital Fibroblasts	qRT-PCR	miR-130a	Thy1-: ↑ miR-130a than Thy1+	AMPK	[102]
Orbital tissue	qRT-PCR	miR-155	GO: ↑ miR-155	ITK	[98]

Abbreviations: qRT-PCR = quantitative Real-Time Reverse Transcription; WB = western blot; GO=Graves' Ophthalmopathy; TGFβ1 = Transforming Growth Factor beta 1; PDGF-BB = platelet-derived growth factor; PDCD4 = Tumor Suppressor Programmed Cell Death 4; IL1B = Interleukin 1B; IL6 = Interleukin 6; ICAM-1 = Interleukin adhesion molecule 1; ZNRF3 = Zinc and Ring Finger 3; PTEN = Phosphatase and Tensin homolog; PPARγ=peroxisome proliferator activated receptor gamma; C/EBPα and C/EBPβ = CCAAT/enhancer binding protein α and β; NOX4 = NADPH oxidase 4; HIF1-α = Hypoxia Inducible Factor 1 subunit alpha; VEGF = Vascular Endothelial Growth Factor; AMPK = adenosine monophosphate-activated protein kinase; ITK = IL-2-inducible T-cell kinase.

Circulating miRNAs in peripheral blood mononuclear cells (PBMC)

Liu et al. studied a total of sixteen miRNAs differentially expressed in PBMC from GD patients at diagnosis compared with normal subjects [55]. The authors showed that the expression of miR-154, miR-376b, and miR-431 was decreased in PBMC; moreover, these three miRNAs were suppressed by incubation of primary PBMC cultures with triiodothyronine (T3), providing evidence of T3 as a possible modulator of the immune system via miRNAs. Another study of Bernecker et al., analyzed variations of key immunoregulatory miRNAs in PBMC and in CD4+ and CD8+ T-cells from AITD patients [56]. An increase of miR-146a was reported in PBMC from GD patients. This miRNA has been associated with the regulation of the immune response in several autoimmune disorders [57–59]. Furthermore, miR-155 was significantly decreased in CD8 T cells of HT and GD patients. miR-155 has been involved in Treg cell development and in the differentiation of Th17 cells [60,61]. miR-200a-1 and miR-200a-2 were also downregulated in CD4+ and CD8+ T-cells of GD and HT patients. The function of these two miRNAs has been associated with the increase of proinflammatory Th1 cytokines in these patients [56,62].

Many studies have explored the expression of certain miRNAs and the regulatory mechanisms of their target genes. The purpose of these studies was to establish relations between miRNAs and their targets in order to find candidate biomarkers for the treatment of AITD. Wang et al. using microarrays studied mRNA and miRNA expression profiles of Treg cells in newly diagnosed GD patients [63]. The study showed 23 upregulated and 14 downregulated miRNAs and validated a significant upregulation of miR-636, miR-30a, miR-181, miR155 and miR-519 and downregulation of miR-19b and miR-146a in GD compared to controls. Integrating these data with the transcriptome, the authors concluded that the retinoic acid pathway was enriched significantly in Tregs of GD patients, pointing to a role of this pathway in the dysfunction of Treg cells described in GD.

Several studies have investigated the role of microRNAs in the regulation of their target genes by analyzing their anti-correlating expression levels. Using PBMC from HT patients, inverse correlations were found between miR-125a-3p and interleukin 23 receptor (IL-23R) [64], Let-7e and IL10 [65] and miR-29a-3p and T-bet [66]. IL23R and T- bet play important roles in T cell proliferation, cytokine production and Th17 cell survival. On the contrary, the reduction of IL-10 expression by Let-7e may play an important role in the negative regulation of the immune response in HT. Therefore, miR-125a-3p, Let-7e and miR29a may serve as potential candidate targets for treatment and clinical diagnosis in patients with HT.

In GD samples, miR-346 downregulation has been associated with the increase of Bcl-6 transcription repressor and increased percentages of follicular helper T cells participating in the regulation of both effector and memory B cell responses as well as antibody production [67]. A similar study found an increase of miR-4443 in GD patients at diagnosis [68]. This miRNA targets the TNF receptor associated factor 4 (TRAF4), and can induce the overexpression of cytokines, chemokines and the proliferation of CD4+ T cells, activating the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway.

Several studies have demonstrated the functional deficiency of Tregs in AITD. In this regard, significantly down-regulated miR-23a-3p and up-regulated sirtuin 1(SIRT1) have been described in GD CD4+ T cells pointing to an abnormal acetylation of FoxP3, which could lead to Tregs malfunction [69]. Recently, Yin et al. have reported that Treg cell proliferation, differentiation and function could be also regulated by miR-363–5p through the signal transducer and activator of transcription 4 (STAT4)- heat shock protein family B (small) member 1 (HSPB1)- notch receptor 1 (Notch1) axis [70]. These results point to important roles played by miRNAs in abnormalities in immune mechanisms found in AITD patients.

Circulating miRNA in serum

miRNAs are detectable in plasma or serum in a remarkably stable form, encapsulated into extracellular vesicles or bound to special lipid proteins, which protect them from RNase digestions [71]. Yamada et al. studied by microarrays miRNA expression patterns in serum obtained from HT, GD patients and healthy controls. The authors found increased serum levels of miR-22, miR-375 and miR-451 in HT patients and upregulation of miR-16, miR-22, miR-375 and miR-451 in GD patients compared to controls [72]. However, the study had some limitations regarding lack of correlations of miRNA results with patients' clinical data.

Further studies have analyzed miRNA profiles and correlated them with patients' clinical variables. In this regard, Hiratsuka et al. conducted a thorough study analyzing the relation between changes in circulating miRNAs and clinical activity in GD [73]. GD patients in remission had increased serum levels of miR-23b-5p and miR-92a-39 and decreased let-7g-3p and miR-339-5p levels when compared with patients with persistent positive TSH-R-Ab. In addition, Zheng et al. found in GD patients significant changes in miR-210, miR-155 and miR-146a serum levels associated with clinical outcomes such as thyroid size and TSH-R-Ab and free thyroxine (FT4) levels [74]. Recently, Martínez-Hernández et al. studied the expression of immunologically relevant miRNAs in serum samples from patients with different immune mediated inflammatory disorders (IMID), including GD, unraveling miRNAs that were most consistently associated with dysregulation of the immune system leading to autoimmune disorders [75]. In this report, the authors showed two miRNA signatures, one of them (miR-19b and miR-26b) predicted the development of autoimmune disease, while the other (miR19-a and miR143) was associated with a more severe disease.

Since miRNAs can be transferred between different cells, the differential miRNA expression in specific cells of damaged tissue in AITD could lead to differential levels of circulating miRNAs. In this regard, two studies have investigated miRNA expression profiles in thyroid tissue and then validated them in serum samples. A recent report described a five-miRNA signature (miR-Let7d-5p, miR-21-5p, miR-96-5p, miR-142-3p, and miR-301a-3p) significantly expressed in AITD that correlated, particularly in patients with GD, with more severe disease outcomes, including active GO, goiter, higher antibody titers and higher recurrence rates [44]. In another study, let-7b was found to be upregulated in serum, thyroid tissue and PBMC in patients with GD and was hypothesized to participate in TSH-R-Ab production via pro-myelocytic leukemia zinc finger [76]. Interestingly, a recent study has validated some of the previously described miRNAs reporting that miR-21-5p, miR-22,3p, miR-22-5p, miR-142-3p, miR-146a-5p, miR-301-3p and miR-451 are upregulated in HT patients [77]. These findings suggest that these miRNA signatures could have a great value for diagnosis, and as potential biomarkers for disease activity and treatment response in AITD patients.

Circulating miRNA in plasma and microvesicles

Rodríguez et al. assessed the function of microRNAs from circulating microvesicles in AITD plasma samples [78]. They found that miR-146 and miR-155 were upregulated in AITD and may have a relevant role in the inhibition of Treg cell differentiation and in the induction of Th17 cell differentiation. A recent study combined different 'omics data (miRNA and proteomics) using different prediction models, finding that Novel miR:19-15038, miR-182-5p, miR-22-3p, miR-27a-3p, miR-6748-3p and 20 proteins associated with fibrosis and gut permeability were robust blood biomarkers potentially able to discriminate between healthy controls, GD and GO patients [79]. Zhao et al. using microarrays and two different cohorts as training and testing stages identified upregulated miRNAs, such as miR-205, miR-20a-3p, miR-375, miR-296, miR-451, and miR-500a, that could discriminate HT patients from normal individuals [80]. Another similar study conducted with GD samples, showed that miR-144-3p and miR-762 were good discriminators of GD patients from healthy controls [81].

miRNAs IN AITD thyroid tissue

miRNA in thyroid formalin-fixed paraffin-embedded samples

One of the first studies exploring microRNAs in thyroid tissues was conducted by Dorris et al. in formalin-fixed paraffin-embedded (FFPE) samples from HT and papillary thyroid carcinoma patients [82]. They validated a decrease in miR-141 expression and associated this decrease with the transforming growth factor beta 1 (TGFβ) signaling pathway in HT. Zhu reported 39 miRNAs dysregulated in HT, and validated a downregulation of claudin 1 expression related with miR-142-5p [83]. This downregulation may participate in an increased permeability of thyrocytes that could allow the exposure of auto-antibodies, thereby contributing to the autoimmune condition of the disease. Studies performed by FFPE in GD samples showed that decreased expression of miR146b, miR-221 and miR-222 could play a role in the development of papillary thyroid carcinoma [84] (Table 2).

miRNA in thyroid fine-needle aspiration biopsies

Bernecker et al. used thyroid gland fine-needle aspiration biopsies of patients with HT and GD and described a decreased expression of miR146a in GD and miR-155 in HT as well as an upregulated miR-200a expression in HT [62]. However, this study had some limitations such as the small sample size and the lack of clinical data correlating with the miRNA results.

miRNA in fresh thyroid tissue

miRNAs may control a plethora of biological phenomena by targeting multiple genes. Hence, modeling context-specific miRNA-mRNA networks has the potential to identify mechanisms that are involved in the pathogenesis of AITD. In this regard, two studies have analyzed the integration of miRNA and mRNA profiles to explore molecular signatures involved in important mechanisms underlying AITD. Using microarrays, Qin et al. found in GD samples a significant upregulation of miR-22 and miR183 and downregulation of miR-101, miR-197 and miR-660, which anti-correlated with the expression of ectodysplasin A (EDA), glycine amidinotransferase (GATM), A-kinase anchoring protein 12 (AKAP12), zinc finger protein, FOG family member 2 (ZFPM2), PBX homeobox 3 (PBX3), cartilage intermediate layer protein (CILP) and syndecan 1 (SDC1) [85]. However, no further studies were conducted to verify the relationship between these miRNAs and their potential targets, and their possible roles in the pathogenesis of GD. Recently, Martínez-Hernández et al. performed an anti-correlation analysis of miRNAs and mRNAs in thyroid samples from patients with HT, GD without GO and GD with GO by next generation sequencing (NGS) [86]. The interest in this latter technique, in comparison to microarrays, relies on its greater ability to capture the scale and complexity of whole transcriptomes. Furthermore, NGS allows the discovery of novel miRNAs and can identify miRNAs that are expressed at levels that fall below microarrays' detection threshold. The authors found a molecular signature composed of miR-21-5p, miR-146b-3p, miR-5571-3p and miR-6503-3p and their anti-correlated genes: Enolase 4 (ENO4), intuned planar cell polarity protein (INTU), kinesin family member 27 (KIF27), parkin co-regulated, and serine/threonine kinase 36 (STK36). These genes are involved in ciliogenesis, which is a novel susceptibility pathway capable of controlling the pathogenesis of AITD. The study of miRNAs and their anti-correlated-genes is a very novel tool to discover new pathogenic pathways in AITD.

miRNAs and Graves' ophthalmopathy

GO or thyroid-associated ophthalmopathy, is the most common extrathyroidal manifestation of GD. Its pathogenesis includes a plethora of molecular and cellular mechanisms including autoantigens, cellular immunity, heterogeneity of orbital fibroblasts, and also genetic and epigenetic factors such as miRNAs. Orbital fibroblasts are the primary cell targets of the immune attack and their heterogeneity may determine whether fibrosis or adipogenesis predominates (Tables 3 and 4).

Circulating miRNAs in GO

Peripheral blood mononuclear cells miRNAs in GO

Several studies have reported the association of miRNA profiles with the development of GO. MiR-146a expression was found to be downregulated in CD4+ T lymphocytes from GO patients in two studies [87,88]. The authors found that this downregulation promoted ocular inflammation by targeting NUMB endocytic adaptor protein (NUMB) and also by the inducing Th1 differentiation leading to an excess of Th1 cytokines. Thiel et al. demonstrated an enhanced expression of miR-96 and miR-183 associated with an increased proliferative activity of T cells in vitro [89].

Serum miRNAs in GO

Another study analyzed miRNA levels to predict GO outcome after glucocorticoid therapy (GC) [90]. In the study, the authors revealed that miR-224-5p and TSH-R-Ab levels were independently associated with glucocorticoid (GC) response and could effectively predict GC sensitivity in GO patients.

Using next generation sequencing (NGS) with AITD samples, Martínez-Hernández et al. described a significant downregulation of miR-Let7d-5p and an increase of miR-96-5p, and miR-301a-3p in serum samples from patients with GO compared to controls [44]. Interestingly, miR-Let7d-5p levels were

inversely correlated with severity and a higher clinical activity. The study concluded that miR-Let7 may exert inhibitory effects on inflammation and accordingly its reduction could contribute to the development of GO. GO shares common pathogenic mechanisms with other IMID such as psoriasis, rheumatoid arthritis, and spondyloarthritis. In this regard, a recent report described the association of miRNA profiles with the expression of vasoactive intestinal peptide (VIP) (a potent anti-inflammatory factor, both in innate and adaptive immunity) and its receptors vasoactive intestinal peptide receptors 1 and 2 (VPAC1 and VPAC2) in IMID patients [91]. The authors showed that miR21-5p, miR27a-3p, and miR100-5p correlated with VIP serum levels and that miR145-5p, miR146a-5p, miR153-3p, miRNA92a-3p, miR199a-5p, miR223-3p, miR7-5p, and miR99a-5p were associated with VPAC2 mRNA expression levels. Their results showed the potential of using the VIP/VPAC axis in the diagnosis of IMID and its association with miRNA signatures to provide novel approaches in the management of immune diseases.

Plasma miRNAs in GO

Wei and colleagues reported a decreased expression of miR-146a plasma levels that correlated with increased levels of IL17 in active GO compared with inactive GO [92]. Their expression levels also correlated with the clinical activity score (CAS) in GO patients. The authors hypothesized a role of miR-146a in the upregulation of IL17 levels in GO progression, pointing to both molecules as potential biomarkers of active GO. Sun et al. studied possible plasma biomarkers to predict the response of GO patients to intravenous GC [93]. They found increased miR-885-3p levels in patients responsive to intravenous GC, in which this miRNA targeted the AKT serine/threonine kinase 1 (Akt)/NFkB signaling pathway. These results provide a potential novel biomarker for the selection of treatment methods for GO patients.

Retro-orbital tissue miRNAs in GO

The extensive orbital tissue remodeling observed in GO is caused by fibroblast activation and differentiation in the orbit. Upon activation, fibroblasts expressing the cell surface marker Thy1+(CD90) can differentiate into myofibroblasts, while fibroblasts without Thy1 (Thy1-) differentiate into adipocytes [94]. Regarding orbital fibroblasts (OF), Tong et al. found an increased expression of miR-21-5p in OF from GO, suggesting that miR-21-5p could promote TGF- β -induced collagen production in OF [95]. A later study demonstrated that miR-21 could enhance the proliferation of OF by suppressing the tumor suppressor programmed cell death 4 (PDCD4) [96].

Young Jang et al. and Jeong et al., performed microarray analyses of orbital tissue from GO patients compared to control subjects and found an increased expression of miR-146a [97] and miR-155 [98]. Further experiments demonstrated that interleukin 1 β induced a concentration dependent increase of both miRNAs. They proposed that miR-146a and miR-155 have a positive effect on the anti-inflammatory process by regulation of c-Jun N-terminal kinases-1/2 (JNK-1/2) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathways and by inhibiting IL-2-inducible T-cell kinase (ITK), which are important mediators of inflammation. Subsequent reports have demonstrated the inhibitory effects of miR-146a on orbital fibrosis by downregulation of the TGF- β signaling pathway [99]. However, TSH-stimulated OF promote an increase in cell proliferation by inducing miR-146 and miR-155, which target zinc and ring finger 3 (ZNR-F3) and phosphatase and tensin homolog (PTEN) respectively (inhibitors of the PI3K/Akt signaling pathway) [100]. The authors suggested the importance of the critical balance between both miRNAs. Thus, it is important to assess different miRNA signatures as the expression of distinct profiles may interact to regulate different functions.

Regarding adipocytes, miR-27a and miR-27b can impair adipocyte differentiation by targeting peroxisome proliferator activated receptor gamma (PPAR γ), CCAAT/enhancer binding protein α and β (C/EBP α and C/EBP β) in OF, representing potential therapeutic targets for GO [101]. Another study investigated miR-130a expression differences between Thy1+ and Thy1- OF from GO patients. The authors found a significant increase of miR-130a in Thy1- OFs that correlated with an enhanced lipid accumulation in these cells, which may play a role in the expansion of orbital adipose tissue [102].

Many studies have supported the involvement of oxidative stress in the pathogenesis of GO. Reactive oxygen species stimulate orbital fibroblast proliferation and differentiation, hyaluronan synthesis, and upregulation of inflammatory mediators [103]. Based on this, downregulated miR-199a in GO orbital adipose tissue may play a role in the regulation of oxidative stress and angiogenesis by targeting NADPH

oxidase 4 (NOX4) / hypoxia inducible factor 1 subunit alpha (HIF1- α) / vascular endothelial growth factor (VEGF) [104].

Possible roles of the miRNAs reported in AITDs and GO

Among all the miRNAs studied in AITD, miR-146a is one of the most frequently de-regulated in all the compartments analyzed (PBMC, serum, plasma and thyroid tissue, Figs. 4 and 5). These results suggest that circulating miRNAs could be synthesized and act in thyroid tissue and then be released to the blood or vice-versa, as a form of intercellular communication. In addition, miR-146a could be a master biomarker and a potentially targetable factor in AITD. Indeed, miR-146a is implicated in several innate (through the NF- κ B signaling pathway) and adaptive immune responses (targeting the signal transducer and activator of transcription 1 [STAT1]) and participates in T regulatory cell development and regulation [105,106]. In miR-146a knock-out models, suppression of miR-146a increases Treg frequency but disrupts their functions leading to immune tolerance failure [58,107]. Lack of miR-146a in T cells has also been correlated with high Th17 differentiation, an increase of Th cells, survival of B cells and an increase in germinal centers leading to autoimmune reactions [108]. Treg cell homeostasis is essential for limiting the activation of mature B cells and effector T cells such as Th17 cells. In this regard, it has been widely described that the immunosuppressive function of Tregs is compromised and Th17 function is increased in thyroid autoimmunity, including the inflammatory process in GO [9]. Accordingly, it is plausible to speculate that miR-146a could be one of the molecular mechanism causing Treg dysfunction and increased levels of Th17 cells in AITD. Regarding the special role of miR-146a as a negative regulator of inflammatory reactions, abnormal expression patterns of this miRNA have also been observed in other autoimmune conditions such as psoriasis, rheumatoid arthritis, Crohn disease, Sjögren's syndrome, and systemic lupus erythematosus, among others [106]. The increase or reduction of these patterns results in the activation and inactivation of a wide spectrum of signaling pathways. Therefore, a comprehensive and precise approach is needed to explore and interpret the role of miR-146a in various autoimmune and/or inflammatory diseases. Hence, miR-146a can be considered as a relevant biomarker for diagnosis of different immune-mediated disorders.

Another miRNA studied by several authors that has shown overlap between different samples analyzed is miR-155. miR-155 is a well-established regulator of immunity that is expressed in spleen, thymus, liver, lung and kidney, as well as in monocytes, macrophages, dendritic cells (DC), B cells and T cells. miR-155 inhibits apoptosis of CD4+ T cells and promotes their migration. In addition, it promotes Th1, Th17, Treg, and follicular helper T cell (Tfh) differentiation [109,110]. The induction of miR-155

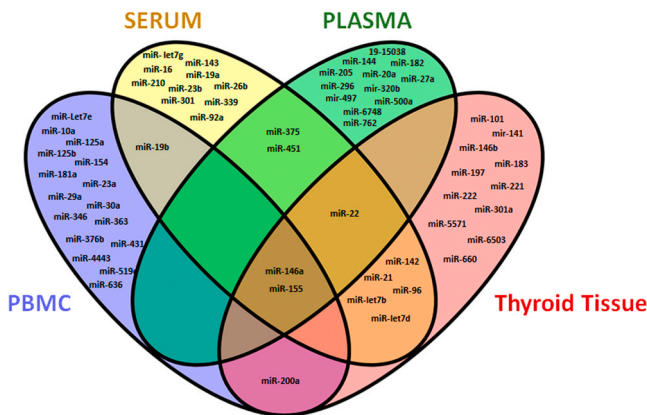


Fig. 4. Venn Diagram showing the exclusive and common dysregulated miRNAs in peripheral blood mononuclear cells (PBMC), serum, plasma and thyroid tissue in AITD patients. From all miRNA profiles, miR-146a and miR-155 have been validated in the four types of samples.

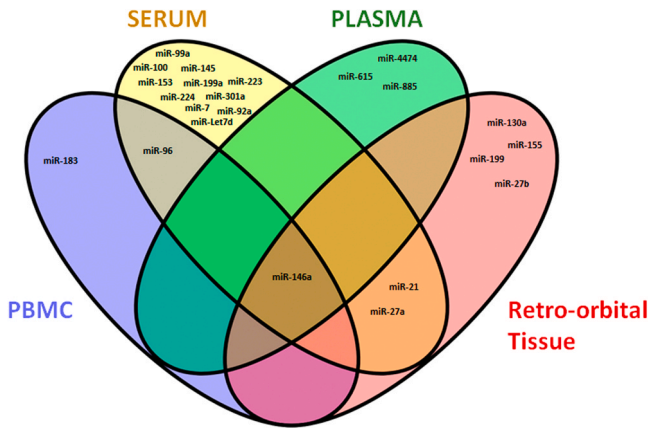


Fig. 5. Venn Diagram showing the exclusive and common dysregulated miRNAs in peripheral blood mononuclear cells (PBMC), serum, plasma and retro-orbital tissue in GO patients. miR-146a is shared by all miRNA profiles from all samples studied.

together with miR-146a in stimulated orbital fibroblasts increases their proliferation, thereby explaining, at least in part, the expansion of the orbital tissue observed in GO [98,100].

Regarding miR-21, recent reports have strongly implicated miR-21 in the regulation of immune function and in the development of several autoimmune disorders, such as multiple sclerosis, lupus erythematosus, type 1 diabetes and myasthenia gravis [111–114], suggesting a shared mechanism of action of this miRNA in different immune cells. The effects of miR-21 expression on T cells, including T cell activation, the Th1/Th2 balance, and Th17 differentiation [63,95,105,115,116], may explain these findings. miR-21 plays also an indispensable role in the process of fibrosis in many diseases [95]. In this context, miR-21 has been associated to a higher risk of developing GD, and to the presence of GO indicating that miR-21 may be involved in the pathogenic progression of fibrosis in GO [44,95] (Fig. 6).

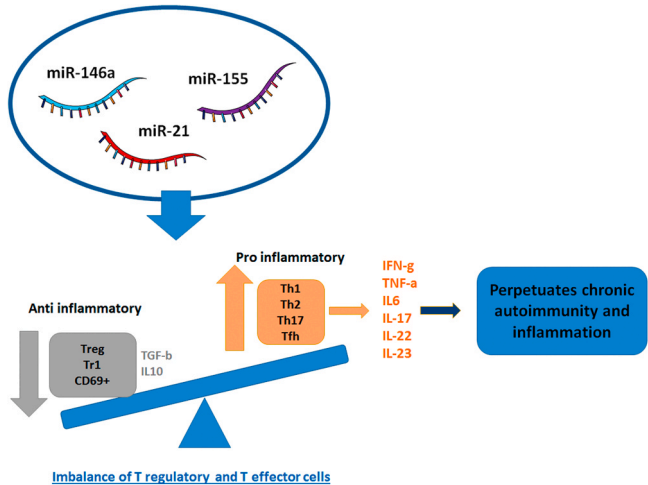


Fig. 6. Possible master regulator roles of miRNAs in AITD. Dysregulation of miR-146a, miR155 and miR21 could impair the inhibitory effect of T regulatory (Treg) cells on the proliferation of pro-inflammatory T helper (Th) cells. The imbalance of T regulatory and T effector cells leads to a loss of immune tolerance perpetuating the autoimmune condition of AITD.

Role as possible therapeutic targets

The discovery of miRNAs as regulatory agents for gene expression and their widespread deregulation in different pathological conditions boosted the idea to exploit them as potential targets for drug development [117,118]. They are currently an emerging area in the study of potential targets for new therapeutic strategies in autoimmune disorders. Reports have shown that correction of miRNA deficiencies by either antagonizing or restoring miRNA function may provide a therapeutic benefit in human cells [119]. The delivery of miRNAs that are highly expressed and tolerated in normal tissues but lost in diseased cells may provide a general strategy for miRNA replacement therapies [29,120].

The first evidence about the usefulness of miRNA-based therapy was obtained in pre-clinical mouse models using an miR-122 antisense oligonucleotide, which was able to decrease hepatic fatty acid and cholesterol synthesis in a diet-induced obesity mouse model [121]. In addition, silencing miR-155 ameliorated the clinical severity of experimental autoimmune encephalomyelitis (EAE) by affecting Th1 and Th17 cells [105,122,123]. It was reported recently that silencing miR-21 conferred striking EAE resistance [116]. Others have demonstrated that silencing miR-126 suppressed the asthmatic phenotype in a mouse model of allergic asthma by decreasing Th2 responses, airway hyperresponsiveness, and mucus secretion [124]. First reports in human cells have focused on cancer therapy. Pancreatic cancer cells with repressed miR-143 / miR-145 levels lost tumorigenicity after restoration of these miRNAs by miRNA therapeutic technologies [125]. Similarly, the replacement of miR-26a, expressed at low levels in hepatocellular carcinoma cells induced cell cycle arrest by targeting cyclins [120].

miRNAs possess unique characteristics that render them very attractive in terms of drug development [118]. They are small, have conserved sequences, and can be easily up or downregulated using artificial agonists or antagonists. In addition, they are easily administered and they can regulate multiple target genes that may be involved in different related processes or signaling pathways. Therefore, multiple different genes can be targeted by miRNAs at the same time [49]. This is not very concerning, as the expression of several miRNAs has been shown to be tissue- and/or cell-specific, probably reflecting underlying pathological conditions.

However, the implementation of miRNA-based therapy to the clinic is not easy, because the biology of miRNA and its interactions with the human genome, transcriptome and proteome remain to be fully understood. Also, the identification and validation of miRNA signatures is not yet accomplished for most diseases. Moreover, a number of additional challenges need to be addressed such as predicting possible off-target effects and toxicity, improving stability and optimizing the delivery systems.

Cytokines are crucial immune mediators that activate the immune response for defence and for recovery of homeostasis. However, excessive or persistent cytokine production can deregulate immune activation and initiate or amplify autoimmune disorders [49]. This key role of cytokines in autoimmune disorders represents the rationale for therapeutic cytokine targeting with biologicals, an approach that has led to major successes in the treatment of diseases such as rheumatoid arthritis (RA) and psoriasis [49,126]. Available literature confirms that cytokines, mostly pro-inflammatory cytokines including TNF- α , IL-1 β , and IL-6, are relevant miRNA targets. Because these cytokines share most of the inducing stimuli and downstream pathways, miRNAs can regulate all of them acting via indirect mechanisms. Thus, miRNAs could represent relevant deregulators of pro-inflammatory cytokines and, as such, interesting therapeutic targets for controlling aberrant cytokine production involved in the onset and amplification of autoimmunity. However, at present, it is not possible to identify signature miRNAs for cytokines, i.e., the miRNAs mainly responsible for cytokine deregulation in specific autoimmune diseases and therefore possible therapeutic candidate/s [49].

Although the emergence of miRNA therapeutics has not provided yet FDA-approved medical interventions based on miRNAs, candidate drugs are in clinical development or in phase 1 and phase 2 clinical trials for diseases with no current effective treatments, such as some cancers [127]. However, due to the development of serious immune-mediated adverse effects, most have been withdrawn or terminated [127]. Therefore, to improve the utility of these therapies in the long-term treatment of these diseases more effective and safer miRNA drugs need to be developed.

Funding

This work was funded by Proyectos de Investigación en Salud (PI) PI19-00584, PI22/01404 and PMP22-00021 (funded by Instituto de Salud Carlos III) and P2022/BMD7379 (funded by la Comunidad de Madrid) and cofinanced by FEDER funds to Mónica Marazuela and Rebeca Martínez-Hernández.

Acknowledgments

We warmly thank Manuel Gómez for English corrections.

References

- [1] Wang L, Wang F-S, Gershwin ME. Human autoimmune diseases: a comprehensive update. *J Intern Med* 2015;278:369–95. <https://doi.org/10.1111/joim.12395>
- *[2] Salvi V, Gianello V, Tiberio L, et al. Cytokine targeting by miRNAs in autoimmune diseases. *Front Immunol* 2019;10. <https://doi.org/10.3389/fimmu.2019.00015>
- [3] Theofilopoulos AN, Kono DH, Baccala R. The multiple pathways to autoimmunity. *Nat Immunol* 2017;18:716–24. <https://doi.org/10.1038/ni.3731>
- [4] Antonelli A, Ferrari SM, Corrado A, et al. Autoimmune thyroid disorders. *Autoimmun Rev* 2015;14:174–80. <https://doi.org/10.1016/j.autrev.2014.10.016>
- [5] Morshed SA, Latif R, Davies TF. Delineating the autoimmune mechanisms in Graves' disease. *Immunol Res* 2012;54:191–203. <https://doi.org/10.1007/s12026-012-8312-8>
- [6] Li H, Wang T. The autoimmunity in Graves's disease. *Front Biosci Landmark Ed* 2013;18:782–7. <https://doi.org/10.2741/4141>
- [7] Bartalena L, Fatourechi V. Extrathyroidal manifestations of Graves' disease: a 2014 update. *J Endocrinol Invest* 2014;37:691–700. <https://doi.org/10.1007/s40618-014-0097-2>
- [8] Weetman AP. Cellular immune responses in autoimmune thyroid disease. *Clin Endocrinol Oxf* 2004;61:405–13. <https://doi.org/10.1111/j.1365-2265.2004.02085.x>
- *[9] González-Amaro R, Marazuela M. T regulatory (Treg) and T helper 17 (Th17) lymphocytes in thyroid autoimmunity. *Endocrine* 2016;52:30–8. <https://doi.org/10.1007/s12020-015-0759-7>
- [10] Marazuela M, García-López MA, Figueroa-Vega N, et al. Regulatory T cells in human autoimmune thyroid disease. *J Clin Endocrinol Metab* 2006;91:3639–46. <https://doi.org/jc.2005-2337> [pii] 10.1210/jc.2005-2337.
- [11] Vitales-Noyola M, Serrano-Somavilla A, Martínez-Hernández R, et al. Patients with Autoimmune thyroiditis show diminished levels and defective suppressive function of Tr1 regulatory lymphocytes. *J Clin Endocrinol Metab* 2018;103:3359–67. <https://doi.org/10.1210/jc.2018-00498>
- [12] Verginis P, Li HS, Carayanniotis G. Tolerogenic semimature dendritic cells suppress experimental autoimmune thyroiditis by activation of thyroglobulin-specific CD4+CD25+ T cells. *J Immunol* 2005;174:7433–9.
- [13] Mao C, Wang S, Xiao Y, et al. Impairment of regulatory capacity of CD4+CD25+ regulatory T cells mediated by dendritic cell polarization and hyperthyroidism in Graves' disease. *J Immunol* 2011;186:4734–43. <https://doi.org/jimmunol.0904135> [pii] 10.4049/jimmunol.0904135.
- [14] Pan D, Shin Y-H, Gopalakrishnan G, et al. Regulatory T cells in Graves' disease. *Clin Endocrinol* 2009;71:587–93. <https://doi.org/10.1111/j.1365-2265.2009.03544.x>
- [15] Glick AB, Wodzinski A, Fu P. Impairment of regulatory T-cell function in autoimmune thyroid disease. *Thyroid Off J Am Thyroid Assoc* 2013;23:871–8. <https://doi.org/10.1089/thy.2012.0514>
- *[16] Weetman A.P.W., DeGroot L.J. Autoimmunity to the thyroid gland. In: *Thyroid disease manager*. n.d.
- [17] Figueroa-Vega N, Alfonso-Pérez M, Benedicto I. Increased circulating pro-inflammatory cytokines and Th17 lymphocytes in Hashimoto's thyroiditis. *J Clin Endocrinol Metab* 2010;95:953–62. <https://doi.org/jc.2009-1719> [pii] 10.1210/jc.2009-1719.
- [18] Vitales-Noyola M, Ramos-Leví AM, Martínez-Hernández R, et al. Pathogenic Th17 and Th22 cells are increased in patients with autoimmune thyroid disorders. *Endocrine* 2017;57:409–17. <https://doi.org/10.1007/s12020-017-1361-y>
- [19] Fang S, Huang Y, Wang N, et al. Insights into local orbital immunity: evidence for the involvement of the Th17 cell pathway in thyroid-associated ophthalmopathy. *J Clin Endocrinol* 2018;23.
- [20] Zúñiga LA, Jain R, Haines C, Cua DJ. Th17 cell development: from the cradle to the grave. *Immunol Rev* 2013;252:78–88. <https://doi.org/10.1111/jimr.12036>
- [21] Grant CR, Liberal R, Vergani D, et al. Regulatory T-cells in autoimmune diseases: challenges, controversies and yet-unanswered questions. *Autoimmun Rev* 2015;14:105–16. <https://doi.org/10.1016/j.autrev.2014.10.012>
- *[22] Baulina NM, Kulakova OG, Favorova OO. MicroRNAs: the role in autoimmune inflammation. *Acta Naturae* 2016;8:21–33.
- [23] Rebane A, Akdis CA. MicroRNAs: essential players in the regulation of inflammation. *J Allergy Clin Immunol* 2013;132:15–26. <https://doi.org/10.1016/j.jaci.2013.04.011>
- [24] Reinhart BJ, Slack FJ, Basson M, et al. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* 2000;403:901–6. <https://doi.org/10.1038/35002607>
- [25] Kamanu TKK, Radovanovic A, Archer JAC. Exploration of miRNA families for hypotheses generation. *Sci Rep* 2013;3:2940. <https://doi.org/10.1038/srep02940>
- *[26] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;116:281–97.
- [27] Guo H, Ingolia NT, Weissman JS. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature* 2010;466:835–40. <https://doi.org/10.1038/nature09267>
- *[28] Kozomara A, Birgaoanu M, Griffiths-Jones S. miRBase: from microRNA sequences to function. *Nucleic Acids Res* 2019;47:D155–62. <https://doi.org/10.1093/nar/gky1141>

- [29] Tomankova T, Petrek M, Gallo J. MicroRNAs: emerging regulators of immune-mediated diseases. *Scand J Immunol* 2012;75:129–41. <https://doi.org/10.1111/j.1365-3083.2011.02650.x>
- [30] Sonkoly E, Pivarcsi A. Advances in microRNAs: implications for immunity and inflammatory diseases. *J Cell Mol Med* 2009;13:24–38. <https://doi.org/10.1111/j.1582-4934.2008.00534.x>
- [31] Jiang Q, Wang Y, Teng M, et al. miR2Disease: a manually curated database for microRNA deregulation in human disease. *Nucleic Acids Res* 2009;37:D98–104. <https://doi.org/10.1093/nar/gkn714>
- [32] Long H, Wang X, Chen Y, et al. Dysregulation of microRNAs in autoimmune diseases: pathogenesis, biomarkers and potential therapeutic targets. *Cancer Lett* 2018;428:90–103. <https://doi.org/10.1016/j.canlet.2018.04.016>
- [33] Podshivalova K, Salomon DR. MicroRNA regulation of T-lymphocyte immunity: modulation of molecular networks responsible for T-cell activation, differentiation, and development. *Crit Rev Immunol* 2013;33:435–76. <https://doi.org/10.1615/critrevimmunol.2013006858>
- [34] Gantier MP. New perspectives in MicroRNA regulation of innate immunity. *J Interferon Cytokine Res Off J Int Soc Interferon Cytokine Res* 2010;30:283–9. <https://doi.org/10.1089/jir.2010.0037>
- [35] O'Neill LA, Sheedy FJ, McCoy CE. MicroRNAs: the fine-tuners of Toll-like receptor signalling. *Nat Rev Immunol* 2011;11:163–75. <https://doi.org/10.1038/nri2957>
- [36] Lopez-Pedreira C, Barbarroja N, Luque-Tévar M, et al. Role of microRNAs in the development of cardiovascular disease in systemic autoimmune disorders. *Int J Mol Sci* 2020;21:E2012. <https://doi.org/10.3390/ijms21062012>
- [37] Zhou X, Jeker LT, Fife BT, et al. Selective miRNA disruption in T reg cells leads to uncontrolled autoimmunity. *J Exp Med* 2008;205:1983–91. <https://doi.org/jem.20080707> [pii] 10.1084/jem.20080707.
- [38] Xu S, Guo K, Zeng Q, et al. The RNase III enzyme Dicer is essential for germinal center B-cell formation. *Blood* 2012;119:767–76. <https://doi.org/10.1182/blood-2011-05-355412>
- [39] Nimoto T, Nakasa T, Ishikawa M, et al. MicroRNA-146a expresses in interleukin-17 producing T cells in rheumatoid arthritis patients. *BMC Musculoskelet Disord* 2010;11:209. <https://doi.org/10.1186/1471-2474-11-209>
- [40] Kmiołek T, Paradowska-Gorycka A. miRNAs as biomarkers and possible therapeutic strategies in rheumatoid arthritis. *Cells* 2022;11:452. <https://doi.org/10.3390/cells11030452>
- [41] Lu L-F, Liston A. MicroRNA in the immune system, microRNA as an immune system. *Immunology* 2009;127:291–8. <https://doi.org/10.1111/j.1365-2567.2009.03092.x>
- [42] Keller A, Leidinger P, Bauer A, et al. Toward the blood-borne miRNome of human diseases. *Nat Methods* 2011;8:841–3. <https://doi.org/10.1038/nmeth.1682>
- *[43] Igaz P, editor. *Circulating microRNAs in Disease Diagnostics and their Potential Biological Relevance*, 106. Basel: Springer Basel; 2015. <https://doi.org/10.1007/978-3-0348-0955-9>
- [44] Martínez-Hernández R, Sampedro-Núñez M, Serrano-Somavilla A, et al. A MicroRNA signature for evaluation of risk and severity of autoimmune thyroid diseases. *J Clin Endocrinol Metab* 2018;103:1139–50. <https://doi.org/10.1210/jc.2017-02318>
- [45] Nemtsova MV, Zaletaev DV, Bure IV, et al. Epigenetic changes in the pathogenesis of rheumatoid arthritis. *Front Genet* 2019;10:570. <https://doi.org/10.3389/fgene.2019.00570>
- [46] Liu Q, Wu D-H, Han L, et al. Roles of microRNAs in psoriasis: immunological functions and potential biomarkers. *Exp Dermatol* 2017;26:359–67. <https://doi.org/10.1111/exd.13249>
- [47] Liang H, Gong F, Zhang S, et al. The origin, function, and diagnostic potential of extracellular microRNAs in human body fluids: extracellular microRNAs in human body fluids. *Wiley Interdiscip Rev RNA* 2014;5:285–300. <https://doi.org/10.1002/wrna.1208>
- [48] Heegaard NHH, Carlsen AL, Skovgaard K, et al. Circulating extracellular microRNA in systemic autoimmunity. *Exp Suppl* 2012 2015;106:171–95. https://doi.org/10.1007/978-3-0348-0955-9_8
- [49] Salvi V, Gianello V, Tiberio L, et al. Cytokine targeting by miRNAs in autoimmune diseases. *Front Immunol* 2019;10. <https://doi.org/10.3389/fimmu.2019.00015>
- [50] Keller S, Ridinger J, Rupp A-K, et al. Body fluid derived exosomes as a novel template for clinical diagnostics. *J Transl Med* 2011;9:86. <https://doi.org/10.1186/1479-5876-9-86>
- [51] Lässer C. Identification and Analysis of Circulating Exosomal microRNA in Human Body Fluids. In: Kosaka N, editor. *Circ. MicroRNAs*, 1024. Totowa, NJ: Humana Press; 2013. p. 109–28. https://doi.org/10.1007/978-1-62703-453-1_9
- [52] Camussi G, Deregibus MC, et al. Bruno S. Exosomes/microvesicles as a mechanism of cell-to-cell communication. *Kidney Int* 2010;78:838–48. <https://doi.org/10.1038/ki.2010.278>
- [53] Wahlgren J, Karlson TDL, et al. Plasma exosomes can deliver exogenous short interfering RNA to monocytes and lymphocytes. *Nucleic Acids Res* 2012;40:e130. <https://doi.org/10.1093/nar/gks463>
- [54] Montecalvo A, Larregina AT, Shufesky WJ, et al. Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. *Blood* 2012;119:756–66. <https://doi.org/10.1182/blood-2011-02-338004>
- [55] Liu R, Ma X, Xu L, et al. Differential microRNA expression in peripheral blood mononuclear cells from Graves' disease patients. *J Clin Endocrinol Metab* 2012;97:E968–72. <https://doi.org/jc.2011-2982> [pii] 10.1210/jc.2011-2982.
- [56] Bernecker C, Halim F, Lenz L, et al. microRNA expressions in CD4+ and CD8+ T-cell subsets in autoimmune thyroid diseases. *Exp Clin Endocrinol Diabetes* 2014;122:107–12.
- [57] Abou-Zeid A, Saad M, Soliman E. MicroRNA 146a expression in rheumatoid arthritis: association with tumor necrosis factor- α and disease activity. *Genet Test Mol Biomark* 2011;15:807–12.
- [58] Lu L-F, Boldin MP, Chaudhry A, et al. Function of miR-146a in controlling treg cell-mediated regulation of Th1 responses. *Cell* 2010;142:914–29. <https://doi.org/10.1016/j.cell.2010.08.012>
- [59] Taganov KD, Boldin MP, Chang KJ, et al. NF- κ B-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci USA* 2006;103:12481–6.
- [60] Kohlhaas S, Garden OA, Scudamore C, et al. Cutting edge: the Foxp3 target miR-155 contributes to the development of regulatory T cells. *J Immunol* 2009;182:2578–82.
- [61] Yao R, Ma Y-L, Liang W, et al. MicroRNA-155 modulates treg and Th17 cells differentiation and Th17 cell function by targeting SOCS1. *PLoS ONE* 2012;7:e46082. <https://doi.org/10.1371/journal.pone.0046082>
- [62] Bernecker C, Lenz L, Ostapczuk MS, et al. MicroRNAs miR-146a1, miR-155, 2, and miR-200a1 are regulated in autoimmune thyroid diseases. *Thyroid* 2012;22:1294–5. <https://doi.org/10.1089/thy.2012.0277>

- [63] Wang Z, Fan X, Zhang R, et al. Integrative analysis of mRNA and miRNA array data reveals the suppression of retinoic acid pathway in regulatory T cells of Graves' disease. *J Clin Endocrinol Metab* 2014;99:E2620–7.
- [64] Peng H, Liu Y, Tian J, et al. Decreased expression of microRNA-125a-3p upregulates interleukin-23 receptor in patients with Hashimoto's thyroiditis. *Immunol Res* 2015;62:129–36.
- [65] Kagawa T, Watanabe M, Inoue N, et al. Increases of microRNA let-7e in peripheral blood mononuclear cells in Hashimoto's disease. *Endocr J* 2016.
- [66] Tokić S, Štefanić M, Glavaš-Obrovac L, et al. miR-29a-3p/T-bet regulatory circuit is altered in T cells of patients with Hashimoto's thyroiditis. *Front Endocrinol* 2018;9:264. <https://doi.org/10.3389/fendo.2018.00264>
- [67] Chen J, Tian J, Tang X, et al. MiR-346 regulates CD4+CXCR5+ T cells in the pathogenesis of Graves' disease. *Endocrine* 2015;49:752–60. <https://doi.org/10.1007/s12020-015-0546-5>
- [68] Qi Y, Zhou Y, Chen X, Ye L, et al. MicroRNA-4443 causes CD4+ T cells dysfunction by targeting TNFR-associated factor 4 in Graves' disease. *Front Immunol* 2017;8:1440. <https://doi.org/10.3389/fimmu.2017.01440>
- [69] Zhang D, Qiu X, Li J, et al. MiR-23a-3p-regulated abnormal acetylation of FOXp3 induces regulatory T cell function defect in Graves' disease. *Biol Chem* 2019;400:639–50. <https://doi.org/10.1515/hsz-2018-0343>
- [70] Yin X, Ge J, Ge X, Gao J, Su X, et al. MiR-363-5p modulates regulatory T cells through STAT4–HSPB1–Notch1 axis and is associated with the immunological abnormality in Graves' disease. *J Cell Mol Med* 2021;25:9364–77. <https://doi.org/10.1111/jcmm.16876>
- [71] Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci* 2008;105:10513–8. <https://doi.org/10.1073/pnas.0804549105>
- [72] Yamada H, Itoh M, Hiratsuka I. Circulating microRNAs in autoimmune thyroid diseases. *Clin Endocrinol Oxf* 2014;81:276–81.
- [73] Hiratsuka I, Yamada H, Munetsuna E. Circulating MicroRNAs in Graves' disease in relation to clinical activity. *Thyroid* 2016;26:1431–40. <https://doi.org/10.1089/thy.2016.0062>
- [74] Zheng L, Zhuang C, Wang X, Ming L. Serum miR-146a, miR-155, and miR-210 as potential markers of Graves' disease. *J Clin Lab Anal* 2018;32:e22266. <https://doi.org/10.1002/jcla.22266>
- [75] Martínez-Hernández R, Fuente H de la, Lamana A, et al. Utility of circulating serum miRNA profiles to evaluate the potential risk and severity of immune-mediated inflammatory disorders. *J Autoimmun* 2020;111:102472. <https://doi.org/10.1016/j.jaut.2020.102472>
- [76] Chen X, Huang F, Qi X, et al. Serum and thyroid tissue level of let-7b and their correlation with TRAb in Graves' disease. *J Transl Med* 2018;16:188. <https://doi.org/10.1186/s12967-018-1565-9>
- [77] Trummer O, Foessler I, Schweighofer N, et al. Expression profiles of miR-22-5p and miR-142-3p indicate Hashimoto's disease and are related to thyroid antibodies. *Genes* 2022;13:171. <https://doi.org/10.3390/genes13020171>
- [78] Rodríguez-Munoz A, Martínez-Hernández R, Ramos-Leví AM, et al. Circulating microvesicles regulate treg and Th17 differentiation in human autoimmune thyroid disorders. *J Clin Endocrinol Metab* 2015;100:E1531–9.
- [79] Zhang L, Masetti G, Colucci G, et al. Combining micro-RNA and protein sequencing to detect robust biomarkers for Graves' disease and orbitopathy. *Sci Rep* 2018;8:8386. <https://doi.org/10.1038/s41598-018-26700-1>
- [80] Zhao L, Zhou X, Shan X, et al. Differential expression levels of plasma microRNA in Hashimoto's disease. *Gene* 2018;642:152–8. <https://doi.org/10.1016/j.gene.2017.10.053>
- [81] Yao Q, Wang X, He W, et al. Circulating microRNA-144-3p and miR-762 are novel biomarkers of Graves' disease. *Endocrine* 2019;65:102–9. <https://doi.org/10.1007/s12020-019-01884-2>
- [82] Dorris ER, Smyth P, O'Leary JJ, et al. MIR141 expression differentiates hashimoto thyroiditis from PTC and benign thyrocytes in Irish archival thyroid tissues. *Front Endocrinol Lausanne* 2012;3:102.
- [83] Zhu J, Zhang Y, Zhang W, et al. MicroRNA-142-5p contributes to Hashimoto's thyroiditis by targeting CLDN1. *J Transl Med* 2016;14:166. <https://doi.org/10.1186/s12967-016-0917-6>
- [84] Pohl M, Grabelius F, Worm K, et al. Intermediate microRNA expression profile in Graves' disease falls between that of normal thyroid tissue and papillary thyroid carcinoma. *J Clin Pathol* 2017;70:33–9. <https://doi.org/10.1136/jclinpath-2016-203739>
- [85] Qin Q, Wang X, Yan N, et al. Aberrant expression of miRNA and mRNAs in lesioned tissues of Graves' disease. *Cell Physiol Biochem* 2015;35:1934–42. <https://doi.org/10.1159/000374002>
- *[86] Martínez-Hernández R, Serrano-Somavilla A, Ramos-Leví A, et al. Integrated miRNA and mRNA expression profiling identifies novel targets and pathological mechanisms in autoimmune thyroid diseases. *EBioMedicine* 2019;50:329–42. <https://doi.org/10.1016/j.ebiom.2019.10.061>
- [87] Yang W-J, Ma P-F, Li S-P, et al. MicroRNA-146a contributes to CD4+ T lymphocyte differentiation in patients with thyroid ophthalmopathy. *Am J Transl Res* 2017;9:1801–9.
- [88] Hu Z-J, He J-F, Li K-J, et al. Decreased microRNA-146a in CD4+T cells promote ocular inflammation in thyroid-associated ophthalmopathy by targeting NUBB. *Eur Rev Med Pharmacol Sci* 2017;21:1803–9.
- [89] Thiel J, Alter C, Luppus S, et al. MicroRNA-183 and microRNA-96 are associated with autoimmune responses by regulating T cell activation. *J Autoimmun* 2019;96:94–103. <https://doi.org/10.1016/j.jaut.2018.08.010>
- [90] Shen L, Huang F, Ye L, et al. Circulating microRNA predicts insensitivity to glucocorticoid therapy in Graves' ophthalmopathy. *Endocrine* 2015;49:445–56.
- [91] Lamana A, Castro-Vázquez D, de la Fuente H, et al. VIP/VPAC axis expression in immune-mediated inflammatory disorders: associated miRNA signatures. *Int J Mol Sci* 2022;23:8578. <https://doi.org/10.3390/ijms23158578>
- [92] Wei H, Guan M, Qin Y, et al. Circulating levels of miR-146a and IL-17 are significantly correlated with the clinical activity of Graves' ophthalmopathy. *Endocr J* 2014;61:1087–92.
- [93] Sun J, Wei J, Zhang Y, et al. Plasma exosomes transfer miR-885-3p targeting the AKT/NFκB signaling pathway to improve the sensitivity of intravenous glucocorticoid therapy against graves ophthalmopathy. *Front Immunol* 2022;13:819680. <https://doi.org/10.3389/fimmu.2022.819680>
- [94] Koumas L, Smith TJ, Phipps RP. Fibroblast subsets in the human orbit: Thy-1+ and Thy-1- subpopulations exhibit distinct phenotypes. *Eur J Immunol* 2002;32:477–85. [https://doi.org/10.1002/1521-4141\(200202\)32:2<477::AID-IMMU477>3.0.CO;2-U](https://doi.org/10.1002/1521-4141(200202)32:2<477::AID-IMMU477>3.0.CO;2-U)
- [95] Tong BD, Xiao MY, Zeng JX, et al. MiRNA-21 promotes fibrosis in orbital fibroblasts from thyroid-associated ophthalmopathy. *Mol Vis* 2015;21:324–34.

- [96] Lee J-Y, Yun M, Paik J-S, et al. PDGF-BB enhances the proliferation of cells in human orbital fibroblasts by suppressing PDCD4 expression Via Up-regulation of microRNA-21. *Investig Ophthalmol Vis Sci* 2016;57:908. <https://doi.org/10.1167/iov.15-18157>
- [97] Jang SY, Chae MK, Lee JH, et al. Role of miR-146a in the regulation of inflammation in an in vitro model of Graves' orbitopathy. *Investig Ophthalmol Vis Sci* 2016;57:4027. <https://doi.org/10.1167/iov.16-19213>
- [98] Choi YJ, Kim C, Choi EW, et al. MicroRNA-155 acts as an anti-inflammatory factor in orbital fibroblasts from Graves' orbitopathy by repressing interleukin-2-inducible T-cell kinase. *PLOS ONE* 2022;17:e0270416. <https://doi.org/10.1371/journal.pone.0270416>
- [99] Jang SY, Park SJ, Chae MK, et al. Role of microRNA-146a in regulation of fibrosis in orbital fibroblasts from patients with Graves' orbitopathy. *Br J Ophthalmol* 2018;102:407–14. <https://doi.org/10.1136/bjophthalmol-2017-310723>
- [100] Woeller CF, Roztocil E, Hammond C, et al. TSHR signaling stimulates proliferation through PI3K/Akt and induction of miR-146a and miR-155 in thyroid eye disease orbital fibroblasts. *Investig Ophthalmol Vis Sci* 2019;60:4336. <https://doi.org/10.1167/iov.19-27865>
- [101] Jang SY, Chae MK, et al. MicroRNA-27 inhibits adipogenic differentiation in orbital fibroblasts from patients with Graves' orbitopathy. *PLOS ONE* 2019;14:e0221077. <https://doi.org/10.1371/journal.pone.0221077>
- [102] Hammond CL, Roztocil E, Gonzalez MO, et al. MicroRNA-130a is elevated in thyroid eye disease and increases lipid accumulation in fibroblasts through the suppression of AMPK. *Investig Ophthalmol Vis Sci* 2021;62:29. <https://doi.org/10.1167/iov.62.1.29>
- [103] Hou T-Y, Wu S-B, Kau H-C, Tsai C-C. The role of oxidative stress and therapeutic potential of antioxidants in Graves' ophthalmopathy. *Biomedicine* 2021;9:1871. <https://doi.org/10.3390/biomedicine9121871>
- [104] Craps J, Joris V, Baldeschi L, et al. miR-199a downregulation as a driver of the NOX4/HIF-1 α /VEGF-A pathway in thyroid and orbital adipose tissues from Graves' patients. *Int J Mol Sci* 2021;23:153. <https://doi.org/10.3390/ijms23010153>
- *[105] Garo LP, Murugaiyan G. Contribution of MicroRNAs to autoimmune diseases. *Cell Mol Life Sci* 2016;73:2041–51. <https://doi.org/10.1007/s00018-016-2167-4>
- [106] Mortazavi-Jahromi SS, Aslani M, Mirshafiey A. A comprehensive review on miR-146a molecular mechanisms in a wide spectrum of immune and non-immune inflammatory diseases. *Immunol Lett* 2020;227:8–27. <https://doi.org/10.1016/j.imlet.2020.07.008>
- [107] Boldin MP, Taganov KD, Rao DS, et al. miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. *J Exp Med* 2011;208:1189–201. <https://doi.org/10.1084/jem.20101823>
- [108] Cho S, Lee H-M, Yu I-S, et al. Differential cell-intrinsic regulations of germinal center B and T cells by miR-146a and miR-146b. *Nat Commun* 2018;9:2757. <https://doi.org/10.1038/s41467-018-05196-3>
- [109] Xu W-D, Feng S-Y, Huang A-F. Role of miR-155 in inflammatory autoimmune diseases: a comprehensive review. *Inflamm Res Off J Eur Histamine Res Soc* 2022;71:1501–17. <https://doi.org/10.1007/s00011-022-01643-6>
- [110] Dawson O, Piccinini AM. miR-155-3p: processing by-product or rising star in immunity and cancer? *Open Biol* 2022;12:220070. <https://doi.org/10.1098/rsob.220070>
- [111] Fenoglio C, Cantoni C, De Riz M, et al. Expression and genetic analysis of miRNAs involved in CD4+ cell activation in patients with multiple sclerosis. *Neurosci Lett* 2011;504:9–12. <https://doi.org/10.1016/j.neulet.2011.08.021>
- [112] Garchow BG, Bartulos Encinas O, Leung YT, et al. Silencing of microRNA-21 in vivo ameliorates autoimmune splenomegaly in lupus mice. *EMBO Mol Med* 2011;3:605–15. <https://doi.org/10.1002/emmm.201100171>
- [113] Ruan Q, Wang T, Kameswaran V, et al. The microRNA-21-PDCD4 axis prevents type 1 diabetes by blocking pancreatic beta cell death. *Proc Natl Acad Sci USA* 2011;108:12030–5. <https://doi.org/10.1073/pnas.1101450108>
- [114] Punga AR, Andersson M, Alimohammadi M, et al. Disease specific signature of circulating miR-150-5p and miR-21-5p in myasthenia gravis patients. *J Neurol Sci* 2015;356:90–6. <https://doi.org/10.1016/j.jns.2015.06.019>
- [115] Wang S, Wan X, Ruan Q. The MicroRNA-21 in autoimmune diseases. *Int J Mol Sci* 2016;17:864. <https://doi.org/10.3390/ijms17060864>
- [116] Murugaiyan G, da Cunha AP, Ajay AK, et al. MicroRNA-21 promotes Th17 differentiation and mediates experimental autoimmune encephalomyelitis. *J Clin Invest* 2015;125:1069–80. <https://doi.org/10.1172/JCI74347>
- [117] Chakraborty C, Sharma AR, Sharma G, et al. Therapeutic miRNA and siRNA: moving from bench to clinic as next generation medicine. *Mol Ther Nucleic Acids* 2017;8:132–43. <https://doi.org/10.1016/j.omtn.2017.06.005>
- [118] Christopher AF, Kaur RP, Kaur G. MicroRNA therapeutics: discovering novel targets and developing specific therapy. *Perspect Clin Res* 2016;7:68–74. <https://doi.org/10.4103/2229-3485.179431>
- [119] Bader AG, Brown D, Winkler M. The promise of microRNA replacement therapy. *Cancer Res* 2010;70:7027–30. <https://doi.org/10.1158/0008-5472.CAN-10-2010>
- [120] Kota J, Chivukula RR, O'Donnell KA, et al. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* 2009;137:1005–17. <https://doi.org/10.1016/j.cell.2009.04.021>
- [121] Esau C, Davis S, Murray SF, et al. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab* 2006;3:87–98. <https://doi.org/10.1016/j.cmet.2006.01.005>
- [122] Murugaiyan G, Beynon V, Mittal A, et al. Silencing microRNA-155 ameliorates experimental autoimmune encephalomyelitis. *J Immunol Baltim Md* 2011;187:2213–21. <https://doi.org/10.4049/jimmunol.1003952>
- [123] Zhang J, Cheng Y, Cui W, et al. MicroRNA-155 modulates Th1 and Th17 cell differentiation and is associated with multiple sclerosis and experimental autoimmune encephalomyelitis. *J Neuroimmunol* 2014;266:56–63. <https://doi.org/10.1016/j.jneuroim.2013.09.019>
- [124] Mattes J, Collison A, Phipps S, et al. Antagonism of microRNA-126 suppresses the effector function of TH2 cells and the development of allergic airways disease. *Proc Natl Acad Sci U S A* 2009;106:18704–9. <https://doi.org/10.1073/pnas.0905063106>
- [125] Kent OA, Chivukula RR, Mullendore M, et al. Repression of the miR-143/145 cluster by oncogenic Ras initiates a tumor-promoting feed-forward pathway. *Genes Dev* 2010;24:2754–9. <https://doi.org/10.1101/gad.1950610>
- [126] McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet Lond Engl* 2017;389:2328–37. [https://doi.org/10.1016/S0140-6736\(17\)31472-1](https://doi.org/10.1016/S0140-6736(17)31472-1)
- *[127] Hanna J, Hossain GS, Kocerha J. The potential for microRNA therapeutics and clinical research. *Front Genet* 2019;10:478. <https://doi.org/10.3389/fgene.2019.00478>