• Review Article •

Epigenetics effect on pathogenesis of thyroid-associated ophthalmopathy

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Abstract

● Thyroid-associated ophthalmopathy (TAO) is an autoimmune disease. Recent studies have found the aberrant epigenetics in TAO, including DNA methylation, non-coding RNAs, and histone modification. Many genes have an aberrant level of methylation in TAO. For example, higher levels are found in CD14, MBP, ANGLE1, LYAR and lower levels in DRD4 and BOLL. Non-coding RNAs are involved in the immune response (miR-146a, miR-155, miR-96, miR-183), fibrosis regulation (miR-146a, miR-21, miR-29), adipogenesis (miR-27) and are thought to play roles in TAO. MicroRNA is also related to the clinical activity score (miR-Let7d-5p) and may be a predictor of glucocorticoid therapy (miR-224-5p). The quantities of H4 in TAO are increased compared with euthyroid control subjects, and the role of histone modifications in Graves' disease may lead to better understanding of its role in TAO. More studies are needed to explain the role of epigenetics in TAO and provide potential therapeutic strategies.

● **KEYWORDS:** thyroid-associated ophthalmopathy; epigenetics; DNA methylation; non-coding RNA; histone modification

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INTRODUCTION

Thyroid-associated ophthalmopathy (TAO), also known as Graves' ophthalmopathy, Graves' orbitopathy, or thyroid eye disease, is an organ-specific autoimmune disease that was first recognized in the 19th Century[1]. TAO is the most frequent extrathyroidal manifestation of complicating Graves' disease (GD) and occurs in around 25% of patients with GD[2]. Symptoms and signs vary and may include eyelid retraction, proptosis, double vision, periorbital edema, exposure keratitis, and optic neuropathy[3]. Limited knowledge of pathogenesis means that therapeutic strategies also vary and may include glucocorticoid, surgery and immunosuppressive therapy[3]. Currently, it is well accepted that TAO has a complex mechanism including pathological processes such as adipogenesis, edema, and fibrosis[4]. Many factors play important roles in the development of TAO including environmental, immune, and genetic aspects[5] allowing pathogenesis and treatment to be studied from these different perspectives. Increasingly, research has focused on the role of epigenetics in TAO.

Epigenetics is used to explain the divergence between the genotype and phenotype and is defined as the structural adaption of chromosomal regions to register, signal, or perpetuate altered activity states according to usages and requirements of heritability[6]. Previous studies have revealed that epigenetics mainly plays a role in the expression of DNA rather than a change of DNA sequence, and it may influence offspring survival through alteration of chromatin structure[7-8]. Epigenetics is essential not only to normal function and development of cells but also to the pathogenesis of many diseases[9] and may be considered as a bridge connecting genetics with environmental factors. When internal and external environment factors change, gene expression may alter. Epigenetics modification mainly includes DNA methylation, noncoding RNAs and histone modification[10]. As a regulator of gene expression, epigenetics may also play a role in the pathophysiology of TAO, as suggested by studies showing an association between epigenetics and TAO[11].

This review summarizes recent research on epigenetics in TAO, to aid understanding of the pathogenesis of this disease and to highlight factors with potential for therapeutic strategies.

Epigenetic Mechanisms of Thyroid-associated Ophthalmopathy

DNA methylation DNA methylation is a process by which DNA is modified and gene expression is regulated. During this process, the family of DNA methyltransferases transfer a methyl group from S-adenyl methionine (SAM) to cytosine at the position of the fifth carbon to form 5mC[11]. DNA methylation usually occurs at the cytosine-phosphate-guanine (CpG) dinucleotide sequences of DNA, also named CpG island, but may also be observed rarely in non-CpG sequences[12-13]. Interestingly, the DNA methylation in non-CpG is observed mainly in embryonic stem cells and is lost in mature tissues[14]. According to one review, DNA methylation is widespread in around 70% of CpG dinucleotides which are enriched in heterochromatin and numerous gene promoters[13]. Three enzymes (DNMT1, DNMT3A, and DNMT3B) are known to play an important role in DNA methylation and maintenance of genomic methylation patterns. Among them, DNMT1 affects the replication and copy of the methylation pattern from the parental DNA to the new DNA; DNMT3A and DNMT3B affect the unmodified DNA, establishing new methylation patterns[11]. DNMT3B can also prevent aberrant transcription initiation events, and is responsible for the fidelity of mRNA transcription initiation[15]. DNA methylation silences gene transcription in 5' promoters and regulates cell context-specific alternative promoters in gene bodies[16]. Recent studies have focused on DNA methylation in TAO, but this process remains poorly understood.

In one study on the correlation between DNA methylation and TAO[17], six Chinese TAO and six age-matched healthy people with normal thyroid function and without manifestations of eye disease were enrolled. DNA methylation was significantly different between the groups (n=1583, P<0.05) and 841 DNA methylation sites were extracted after removing missing samples[17]. Hyper-methylated genes (>2-fold) such as *CD14*, *MBP*, *ANGLE1*, *LYAR*, and hypo-methylated genes (<0.5-fold) such as *DRD4*, *BOLL* total 148[18]. Hyper-methylation is defined as a differentially methylated region (DMR) with greater than 95% modification and hypo-methylated as a DMR with less than 5% modification. A total of 42 hyper-methylated (>2.193-fold change) and 42 hypo-methylated (<0.393-fold change) genes have been identified[18]. In TAO, a high level of methylation of CpG has been found in immunoregulatory-related genes such as *CD14*, *IL17RE*, *BECN1*, *CDK5*, and low level in thyroid carcinoma genes such as *LDOC1*, *FHIT*, *RUNX3*, thyroid function genes such as *DRD4*, *DOT1L*, *LHX2*, and oxidative stress genes such as *ABCD1*, *HOXB13*[17].

The level of methylation is correlated with clinical phenotype. According to previous research, the methylation of IL17RE (r=0.967) and myelin basic protein (r=0.971) are related to clinical activity score (CAS); the methylation of cyclin dependent kinase 5 (r=0.84), angel homolog 1 (r=-0.868), Ly1 antibody reactive (r=-0.868) and boule homolog (r=0.816) are correlated with the level of thyrotropin receptor antibody; the methylation levels of cyclin dependent kinase 5 (r=0.599), ZCCHC6 (r=0.796), and GLI family zinc finger 3 (r=0.657) are related to age; the methylation levels of Beclin 1 (r=-0.655), EFBAC1 (r=0.882), and frizzled class receptor 7 (r=0.947) are related to DBP; and the methylation levels of ZNE843 (r=0.886), CD14 (r=-0.879), and CZNE354A (r=-0.851) are related to body-mass index[18]. The level of methylation is also related to the risk of TAO. For example, low levels of methylation of ZCCHC6 and GLI3 are associated with lower risk of TAO (OR=0.15, P=0.039 and OR=0.65, P=0.042, respectively)[17]. The methylation levels of other genes such as *JRK*, *ST14*, *WDR60*, *ABHD14A*, *RTN4RL2*, *FUT3*, *EEFEA2*, *SLC12A7*, *CSNK1G2*, and *CDH22* are also related to the incidence of TAO[17].

Another study classified the 841 DMR on the genes noted above into three clusters based on median absolute deviation and compared the counts of genes in each cluster between TAO and healthy groups[19]. Four significant pathways were extracted: toxoplasmosis, axon guidance, focal adhesion, and proteoglycans in cancer, helping to advance understanding of DNA methylation in the pathogenesis of TAO[19].

High levels of TPOAb are found in the toxoplasmosis pathway, with more toxoplasma-positive women having a high level of TPOAb than in toxoplasma-negative women [25/281 (8.9%) versus 43/967 (4.4%), P=0.004][20]. In addition, latent toxoplasmosis is related to decreased thyroid stimulating hormone (TSH) (P=0.049) in pregnancy[20], and an association has been found between latent toxoplasmosis and autoimmune thyroid disease, but is not clinically significant[20]. As an axon guidance glycoprotein, slit2 is expressed by $CD34^-$ orbital fibroblasts, the level of which is elevated by TSH. According to a recent study[21], slit2 inhibits the differentiation of fibrocytes and markedly increases the generation of inflammatory cytokines from fibrocytes. This effect of modulating inflammation phenotype of $CD34^+$ orbital fibroblasts enables slit2 to play a role in regulating the progress and manifestation of TAO[21]. Focal adhesion participates in cell migration, and research has shown that the activation of focal adhesion kinase is essential for lung fibroblast migration induced by platelet-derived growth factor-BB[22]. The migration of fibroblasts contributes to fibrosis in the lung and thus is a potential target in the search for therapeutics against fibrosis[22]. The migration of fibrocytes and fibrosis also exists in TAO with 63% of genes having a lower level of methylation in the focal adhesion pathway[19]. Proteoglycans participate in the inflammation process as autoantigens by activating $CD4^+$ T cells[19]. Overall,

the latent correlation between these four pathways and TAO has been suggested, not only by different levels of gene methylation in the pathways in TAO and healthy groups, but also the mechanism of these pathways may also exist in TAO. However, there is still a lack of good evidence demonstrating a direct correlation between these pathways and TAO. Data from small samples using blood rather than orbital tissue indicate that existing studies have demonstrated a correlation between methylation and GD[17]. More evidence is needed including large samples to demonstrate that methylation participates in the pathogenesis of TAO.

Non-coding RNAs A recent study has demonstrated that most of the human genome can be transcribed, but that only a small component (less than 2%) of RNAs function as coding RNAs, which can be translated into proteins[23]. The other transcribed RNAs, which have the relatively minor function of coding protein messages, are classified as non-coding RNAs (ncRNAs). As an important component of epigenetics, ncRNAs have been studied extensively in recent years, increasing understanding of their effect on cell survival and pathogenesis of cancer[24]. These ncRNAs comprise tRNAs, rRNAs, and other diverse RNAs. Both tRNAs and rRNAs are associated with protein translation. Other diverse RNAs are divided into two types according to their length. These RNAs range from small non-coding RNAs to long non-coding RNAs (lncRNAs), the latter of length exceeding 200 nucleotides[23,25]. lncRNAs can be classified into different categories based on the position relationships with protein-coding genes[26]. Small non-coding RNA includes siRNA, sRNA, snRNA, snoRNA, piRNA and miRNA classified according to their positions and functions in the cell. Recently, numerous studies have demonstrated a correlation between miRNAs and TAO.

The miRNAs, with length from about 19 to 25 nucleotides, participate in the regulation of post-transcription in target genes by reducing the quantities of mRNA transcription and controlling translation[27-28]. To date, the mechanism of this process has been widely studied. One review concludes that miRNAs take effect by binding to 3' UTR of complementary seed sequences that are in the untranslated region, 5' UTR or translated regions in message RNAs, leading to the degeneration and inhibition of message RNA translation[29]. In contrast, some miRNAs such as miRNA-24-1 have been found to enhance rather than inhibit gene expression according to Xiao *et al*[30]. miRNAs are associated not only with normal biology processes, but also pathogenesis in some conditions such as metabolic disorders, cancer progression, and fibrosis[31]. In TAO, microRNAs such as miR-146a, miR-155, miR-21, miR-27, miR-29, miR-96, miR-183 participate in immune regulation, adipogenesis, fibrosis, and other pathogenesis processes, and additional microRNAs such as miR-Let7d-5p, miR-224-5p are related to CAS and other therapy outcomes.

The miR-146a family includes miR-146a and miR-146b, which are on chromosomes 5 and 10, respectively[32]. To our knowledge, the level of miR-146a varies between different types of tissue. In TAO, the expression of miR-146a is increased in fat tissues or connective tissues compared with a control group[33-35]. One study including 27 TAO tissues and 15 healthy tissues revealed that the expression of miR-146a is up-regulated in TAO compared with normal tissue (P<0.05), and that the level of miR-146a is positively correlated with CAS, with higher levels in active than inactive TAO (P<0.05)[33]. Jang *et al*[35] used microarray analysis to show that miR-146a is overexpressed by a factor of 3.15 in TAO compared with a control group and found increased qPCR of miR-146a in TAO (P=0.032). However, in study subjects with $CD4^+$ T cells measured from venous peripheral blood, the expression of miR-146a seems to be decreased in TAO, with three measures of qRT-PCR showing that miR-146a is expressed at a lower level compared with adjacent normal control tissue (P<0.05)[36]. Some studies have also revealed significantly lower circulating levels of miR-146a in TAO (both active and inactive) than in control groups (P=0.002, 0.009, respectively)[37-38]. The active TAO group has a lower circulating level of miR-146a than that in the inactive group (P=0.039)[37]. The finding that the level of miR-146a in TAO compared with controls varies between different tissues raises questions on the way in which miR-146a participates in TAO. There are at least two possible mechanisms that may address this question.

The miR-146a inhibits the process of fibrosis. In the pathogenesis of TAO, fibrocytes and fibroblasts play an essential role in fibrosis. When exposed to TGF-β *in vitro*, the phosphorylation of ERK, p38, JNK, NF-κB, PI3K, Smad2, mTOR, and AKT are increased in a time-dependent manner[34]. In addition, Thy-1^+ fibroblasts and fibrocytes differentiate into myofibroblasts and express α-smooth muscle-specific actin (α-SMA) under the influence of TGF-β, which promotes fibrosis[39]. According to one study[34], miR-146a acts as a negative regulator in TGF-β signaling and inhibits the function of orbital fibroblasts thus alleviating fibrosis. It also reduces the proteins which are related to fibrosis including collagen Iα, TGF-β-induced fibronectin and α-SMA protein[34]. Smad4 and TRAF6 are also down-regulated by miR-146a mimics, which leads to the attenuation of fibrosis[34]. Furthermore, miR-146a targets IGF1R, TRAF6, SMAD4, FAS and promotes fibroblast mitosis and proliferation while suppressing apoptosis, thus facilitating the progress of TAO[33,38].

The miR-146a regulates the innate immune response and participates in autoimmune disease such as systemic lupus erythematosus and rheumatoid arthritis[32]. Its effect

in the immune system is thought to parallel its role in the pathogenesis of TAO. Wang *et al*[33] identified that after stimulating orbital fibroblasts with miR-146a mimics, the level of Notch2 is down-regulated while the level of IL-6 was up-regulated compared with a control group (P<0.01), which participate in the process of differentiation from T cell to TH2. miR-146a inhibits the differentiation of Th1 and proliferation of T cells in peripheral blood by targeting NUMB which is a membrane protein related to the balance of differentiation/proliferation[36,40]. Jang *et al*[35] hold that miR-146a may act as an anti-inflammation factor, participating in the pathogenesis of TAO, and is up-regulated by IL-1β. This process could be inhibited through NF-κB, JNK-1/2, and PI3K inhibitors[35]. The miR-146a mimics significantly downregulated the level of IL-6 induced by IL-1β compared with control mimics and the effect varies according to the dosage of miR-146a (480.59±17.80 pg/mL at 30 nmol/L and 418.15±15.83 pg/mL at 100 nmol/L; P=0.017 and 0.001, respectively)[35]. These conflicting results of relationships between miR-146a and IL-6 need to be further clarified (Figure 1).

Contrary to the effect of miR-146a involved in inflammation, miR-155, which is increased in peripheral venous blood in TAO patients, may promote inflammation by suppression of cytokine signaling 1 (SOCS1) and SH2 domain-containing inositol-5-phosphatase 1 (SHIP1) in the TLR4/NF-κB pathway[38]. miR-155 also inhibits the differentiation of orbital fibroblasts through C/EBP-β, which restricts the generation of adipocytes and myofibroblasts[38]. miR-155 may also affect the cytokines associated with $CD4^+$ T cells[38].

The miR-96 and miR-183 are thought to be related to the function of T cells and tumor cells[41-42], and a recent study revealed that they are also up-regulated in $CD4^+$ cell expression in TAO and activated T cells in human and mouse models (P<0.001)[43]. After stimulation of $CD4^+$ and $CD8^+$ T cells with anti-CD3/anti-CD28, the levels of miR-96 and miR-183 are both elevated compared with unstimulated cells (P<0.001)[43]. The overexpression of miR-96 and miR-183 in murine $CD4^+$ T cells contributes to the elevation of Akt phosphorylation, down-regulating growth response protein 1 (EGR-1) and phosphatase and tensin homolog (PTEN)[43]. miR-96 and miR-183 both target EGR-1 and then directly activate PTEN thus participating in the Akt signaling pathway[43]. By regulating the activation of T cells, miR-96 and miR-183 may affect the immune function and thus participate in the regulation of severity and development of TAO[43].

The miR-21 is a type of ncRNA related to fibrosis. The level of miR-21 has been found to be elevated in patients with TAO compared with normal controls (P<0.05)[44]. Its expression is upregulated by TGF-β1, which in turn activates TGF-β/Smad signaling by phosphorylating Smad3 and elevating

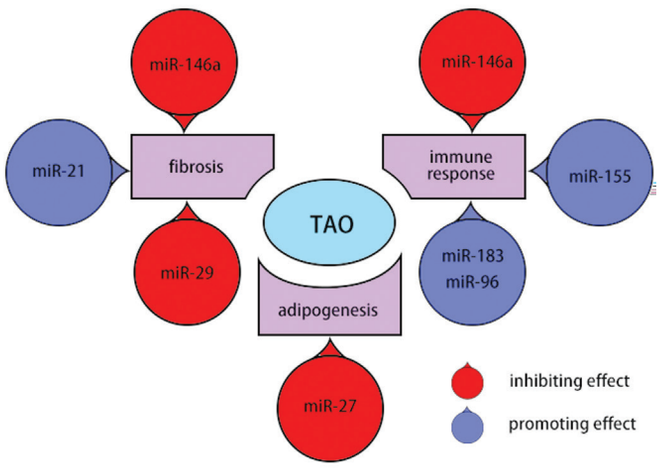

Figure 1 MicroRNAs involved in the pathogenesis of TAO miR146-a: 1) inhibits TGF-β signaling; 2) decreases collagen Iα, fibronectin and α-SMA; 3) decreases Smad4 and TRAF6; 4) promotes fibroblast mitosis and proliferation while suppressing apoptosis; 5) increases or decreases IL-6; 6) inhibits the differentiation of Th1 and proliferation of T cells. miR-21: 1) activates TGF-β/Smad signaling; 2) increases α-SMA; 3) promotes proliferation and development of orbital fibroblasts; 4) decreases apoptosis. miR-29: 1) inhibits wnt3α/β-catenin signaling pathway; 2) decreases collagen Iα; 3) inhibits proliferation. miR-155: 1) inhibits TLR4/NF-κB pathway. miR-96 and miR-183: 1) increases Akt phosphorylation; 2) decreases EGR-1 and phosphatase and tensin homolog (PTEN). miR-27: 1) decreases PPARγ, C/EBPα, and C/EBPβ; 2) decreases lipid droplets.

collagen production[44]. In addition to TGF-β, platelet-derived growth factor (PDGF-BB) enhances the expression of miR-21 in orbital fibroblasts to inhibit PDCD4 expression, which results in the proliferation of orbital fibroblasts[45]. MiR-21 is known to promote the proliferation and development of orbital fibroblasts[44]. Both the cell proliferation and the level of α-SMA mRNA are inhibited by anti- miR-21 and upregulated by miR-21 mimics (all P<0.05)[44]. The latter also decrease the proportion of apoptotic cells compared with controls (P<0.05), which provides evidence of the inhibitory effect of miR-21 in orbital fibroblast apoptosis[44].

The miR-29 seems to prevent fibrosis and have different effects on orbital fibroblasts compared with miR-21. According to Tan *et al*[46], miR-29 is decreased under the effect of TGF-β1 in orbital fibroblasts. After treatment with TGF-β1 for 48h, the miR-29 family including miR-29a, miR-29b, and miR-29c are down-regulated in a time-dependent manner compared with untreated orbital fibroblasts (all P<0.05)[46]. miR-29 decreases the proliferation and clone-forming efficiency of orbital fibroblasts induced by TGF-β1, which may alleviate the progression of TAO[46]. miR-29 also acts as a negative factor in regulating the expression of collagen, type I, alpha 1 (COL1A1) by suppressing the wnt3α/β-catenin signaling pathway[46].

Table 1 MicroRNAs in the mechanism of TAO

MicroRNAs	Tissue	Expression level	Function	References
miR-146a	Orbital tissue	Increased	Inhibit fibrosis	[33-35]
miR-146a	Peripheral blood	Decreased	Regulate immune response	[38,40]
miR-155	Peripheral blood	Increased	Promote immune response	[38]
miR-96	Peripheral blood	Increased	Promote immune response	[43]
miR-183	Peripheral blood	Increased	Promote immune response	[43]
miR-21	Orbital tissue	Increased	Promote fibrosis	[44]
miR-29	Orbital tissue	Decreased	Inhibit fibrosis	[46]
miR-27	Orbital tissue	Decreased	Inhibit adipogenesis	[47]
miR-Let7d-5p	Peripheral blood	Decreased	Negatively relate to CAS	[48]
miR-224-5p	Peripheral blood	-	Predict GC therapy outcome	[49]

CAS: Clinical activity score; GC: Glucocorticoid.

Another type of ncRNA, miR-27, has been found to decrease adipogenic differentiation. One study found that qRT-PCR measurements of the levels of miR-27a and miR-27b from orbital fat tissue were significantly down-regulated in TAO patients compared with non-TAO patients (P=0.038, 0.047, respectively) and were gradually decreased during differentiation from orbital fibrosis to adipocytes (all P<0.01)[47]. After treating orbital fibroblasts with miR-27a and miR-27b mimics, the mRNA level of PPARγ and C/EBPα, which are essential to adipogenic differentiation, are significantly decreased (all P<0.05)[47]. Adipogenic proteins such as PPARγ, C/EBPα, and C/EBPβ are also decreased under the effect of miR-27a and miR-27b mimics. miR-27a and miR-27b mimics also reduce lipid droplets (P<0.01), which are indicative of the inhibition of adipogenesis, suggesting that the mimics limit this inhibitory process[47].

The miR-Let7d-5p is related to the clinical features of TAO and is negatively correlated with CAS. According to one study[48], miR-Let7d-5p in serum samples from patients with TAO is lower than serum samples from patients with GD (without TAO; P=0.016) and is negatively associated with TPO-ab (r=-0.38)[48].

Apart from its involvement in the pathogenesis and clinical features of TAO, microRNA also plays a role in the prediction of the outcome of therapy. Shen *et al*[49] found that patients who were insensitive to glucocorticoid had lower expression of miR-224-5p and higher TRAb in serum. The combination of miR-224-5p at baseline and TRAb acts as an independent factor related to the outcome of glucocorticoid therapy, with a positive predictive value of 91.37% and negative predictive value 69.56%[49] (Table 1).

Histone modification Histones are proteins that exist in eukaryotes, including H1, H2A, H2B, H3, and H4[50]. These proteins together with about 200 DNA base pairs comprise a nucleosome, repeating units of which form chromatin[51]. Some post-translation modifications are based on histones, which may affect gene expression[52]. Histone modifications include methylation, phosphorylation, acetalation, sumoylation, and other changes on the N-terminal tails of histone, which may activate or suppress gene expression[53-54]. Among these modifications, methylation and acetylation are the most-characterized representations which regulate the chromatin structure[55]. Enzymes which mediate histone modification include lysine acetyltransferases, lysine deacetylases, histone methyltransferases and lysine demethylases[52]. Histone modifications are also connected with CpG methylation through CpG-binding proteins, which may reveal the association between histone modifications and DNA methylation in a specific region of the genome[56-57]. Recent research on histone modifications in TAO is not extensive but has shed some light onto histone modifications in GD and may provide some clues to understand the effect of modifications on the pathogenesis of TAO.

According to Matheis *et al*[58], the quantities of H4 are significantly higher (by a factor of 2.13) in orbital adipose tissue in TAO compared with euthyroid control subjects, and H4 in TAO is down-regulated in orbital adipose compared with peripheral subcutaneous fat (ratio, 0.25)[58].

Some histone modifications occur in GD. One study revealed down-regulated levels of histone 3 lysine 4 trimethylation (H3K4me3) and histone 3 lysine 27 acetylation (H3K27ac) modifications in LCK, CD247 and ZAP70 of T cells, demonstrating the impairment of epigenetic regulations in GD[57]. Another study based on human thyroid cells found that when exposed to interferon α (IFNα), levels of H3K4me3 and histone 3 lysine 4-monomethylated (H3K4me1) were increased preferentially in non-coding gene regions[59]. Global histone 3 at lysine 9 (H3K9) methylation and histone 4 acetylation in peripheral blood mononuclear cells are also both downregulated in GD[60-61]. These histone modifications are all identified in autoimmune thyroid disease. Whether these changes also appear in TAO and whether there are other histone modifications remain to be investigated by further research in the future.

Epigenetics in Other Inflammatory Disease Compared with malignant disease, research on epigenetics in inflammatory disease is less prolific. Genetic factors have been found to affect the process of inflammatory disease. For example, 14 lncRNAs were thought to regulate centrally the inflammation response of idiopathic pulmonary lung fibroblasts induced by IL-6, however, research increasingly suggests that epigenetics may also play a role in pathogenesis of orbital inflammatory disease[62]. Orbital inflammatory diseases other than TAO include granulomatosis with polyangiitis, sarcoidosis, and nonspecific orbital inflammation[63]. Since they are all inflammatory, these diseases may share some similar pathways in pathogenesis and have some common regulatory sites. According to Laban *et al*[64], miR-365 and miR-148a were increased slightly in idiopathic orbital inflammation compared with orbital non-Hodgkin lymphoma since these two microRNAs are associated with B cell mechanisms which regulate IL-6 signaling and B cell tolerance, respectively. In addition, miR-29a and miR-223-3p were linked with lymphoid and myeloid, respectively. Epigenetics also affects the process of granulomatosis with polyangiitis. Dekkema *et al*[65] found 19 miRNAs expressed differently between patients and controls, extracted using miRNA microarray of T cells, the top five miRNA being hsa-let-7g-5p, hsa-miR-20a-5p, hsa-miR-26a-5p, hsa-miR-142-3p, and hsa-miR-146b-5p. Among these, miR-142-3p was significantly overexpressed in Treg and could suppress the capacity of Tregs, while miR-142-3p also decreased the expression of cAMP and adenylate cyclase 9. Research on epigenetics of sarcoidosis such as miR-34a and miR-let7 is linked with pulmonary, which regulates the process of inflammation and fibrosis[66]. While this research has had little focus on orbital tissue, it provides a perspective from which to plan future research on sarcoidosis of orbital tissue.

CONCLUSIONS AND PROSPECTS

As an autoimmune disease, TAO has not been well understood; in particular, its pathogenesis remains unclear. Epigenetics is thought to play a role in the incidence, process and outcomes of TAO. Some genes are aberrantly methylated in TAO, for example, CD14, MBP, ANGLE1, LYAR are hyper-methylated and DRD4, BOLL is hypo-methylated. Better understanding is needed about the effect these genes have on the pathogenesis of TAO, and whether these gene loci could be used as therapeutic sites. microRNAs participate in TAO in multiple ways, which include not only pathogenesis and clinical features, but also outcomes of therapy. For example, miR-146a, miR-155, miR-96, miR-183 regulate the immune response of TAO; miR-21 and miR-29 regulate fibrosis; miR-27 inhibits adipogenesis; miR-Let7d-5p are negatively correlated with CAS; and miR-224-5p predict GC therapy outcomes. Furthermore, there seem to be more complex interactions between microRNA and TAO, and we anticipate that evidence of microRNA related to specific cell processes in TAO will be found in future. Despite few studies on histone modifications in TAO, we infer that histone modifications may also play a role in the pathogenesis of TAO based on its function in regulating gene transcription and previous findings on the histone modifications of GD which is thought to be closely associated with TAO.

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