REVIEW ARTICLE

Functional Roles of Non-Coding RNAs in Graves' Disease

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Abstract: Graves' disease (GD) is a disorder marked by an enlarged and overactive thyroid gland (Graves' hyperthyroidism), ocular abnormalities (Graves' orbitopathy; GO), and localized dermopathy (pretibial myxoedema; PTM). It is recognized as the most common cause of hyperthyroidism worldwide. Patients with GD most frequently exhibit elevated thyroid hormone secretion from thyroid cells as a result of autoantibodies acting as thyroid-stimulating hormone receptor (TSHR) agonists. Numerous investigations have examined the elements that contribute to the pathogenesis of GD, focusing on different components, such as molecular factors like non-coding RNAs (ncRNAs). NcRNAs represent a type of RNA transcript that, while not encoding proteins, are essential in the regulation of numerous aspects of cellular biology. NcRNAs include major groups, such as circular RNAs (circRNAs), long non-coding RNAs (lncRNAs), and small non-coding RNAs (sncRNAs), all of which are garnering increasing interest in the scientific community. This review will provide a comprehensive analysis of the function of ncRNAs in the development, diagnosis, and treatment of GD, and investigate the latest research in this area.

Keywords: Graves' disease, thyroid gland, hyperthyroidism, TSHR, ncRNAs, diagnosis.

1. INTRODUCTION

Graves' disease (GD), also known as von Basedow disease, is characterized by the distinctive combination of an enlarged and hyperactive thyroid gland (Graves' hyperthyroidism), localized skin conditions like pretibial myxoedema (PTM), and Graves' orbitopathy (GO) [1]. It is recognized as the most common cause of hyperthyroidism worldwide [2]. Originally, it was thought that the pituitary gland's overproduction of thyroid-stimulating hormone (TSH) was the cause of Graves' hyperthyroidism [3]. However, in 1956, identifying TSH receptor (TSHR) autoantibodies proved that GD is an autoimmune disease. Patients with GD most frequently exhibit elevated thyroid hormone secretion from thyroid cells as a result of these autoantibodies acting as TSHR agonists [4]. There are two extremes to GD symptoms: one is a mild, asymptomatic form that is typically diagnosed by low serum TSH levels during standard thyroid function tests, the other, known as accelerated hyperthyroidism, is a severe, potentially fatal "thyroid storm" that is linked to tachycardia, elevated blood pressure, fever, delirium, and a high death rate. The Graves' Triad, or the "complete" disease, accompanied by additional thyroidal problems (GO and PTM), is caused by TSHR autoantibodies and T lymphocytes specific to TSHR acting on TSHR that is expressed in tissues other than the thyroid, especially in adipocytes and fibroblasts. Less than 5% of individuals have clinically obvious GO, although comprehensive imaging indicates that GO is much more prevalent in a moderate form among patients with GD [5]. Retro-orbital inflammation that disrupts the extraocular muscle fibers and accumulates glycosaminoglycan, resulting in edema, is a typical characteristic of GO. PTM is an infiltrating dermopathy caused by a buildup of glycosaminoglycans in the dermis that is characterized by a non-pitting, slowly progressing edema [6]. PTM can progress to significantly disfigure certain GD patients. Several

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clinical manifestations of GD indicate that each patient's etiology may be influenced by different variables. Although the course of treatment for GD has remained largely unchanged in recent years, there have been advancements in the use of traditional remedies. Even though the majority of patients attempt to avoid surgery, the unpleasant cases of recurrence following surgery have been averted by substituting a whole thyroidectomy for a partial thyroidectomy. Due to its inherent characteristics and the possibility of exacerbating GO following treatment, radioiodine therapy has also lost some of its appeal. Nonetheless, methimazole, also known as carbimazole, has become much more popular as an antithyroid medication, particularly in light of the problems with liver toxicity that come with propylthiouracil [7]. Methimazole can, on the other hand, very rarely result in agranulocytosis and congenital abnormalities. As a result, there is still room for progress in the medical care of GD. Numerous investigations have examined the elements that contribute to the pathogenesis of GD, focusing on different components, such as molecular factors like ncRNAs. Recent research has sought to deepen its knowledge of GD's pathophysiology by examining the case and control groups' RNA and protein expression. Of particular focus are non-coding RNAs (ncRNAs), RNA transcription products that don't encode proteins [8]. ncRNAs have the capacity to regulate whole organisms and cells through a variety of mechanisms, including molecular functions and their influence on biological mechanisms [9]. ncRNAs comprise circular RNAs (circRNAs), long ncRNAs (lncRNAs), and small ncRNAs (sncRNAs) [10]. Endogenous sncRNAs referred to as microRNAs (miRNAs) have become a major area of study in the bioinformatics profession in recent years. miRNAs, *20–25* nucleotides in *length*, play a role in key biological processes like cell polarity, growth, senescence, and death. They can also target mRNAs for posttranscriptional control [11]. Furthermore, current studies suggest that miRNAs are essential in the pathophysiology of GD. For instance, in OFs of GD patients, inflammatory stress upregulates miR-146a, which then inhibits the NF-κB pathway to reduce or stop the immunoreaction by downregulating associated target genes, including IRAK1, an IL-1 receptor-related kinase, and TRAF6 [12]. The previously mentioned Treg cells and Th17 cells are essential to the pathophysiology of GD, and miR-155 influences the formation of Th17 cells in the autoimmune process. Furthermore, miR-155 is essential for the development of fibrous tissue and facilitates the TGF-β signaling pathway to promote collagen synthesis [13]. Moreover, studies investigating The functions of circRNAs and lncRNAs in the pathophysiology of Thyroid-Associated Ophthalmopathy (TAO) have revealed that ncRNAs have a significant role in the pathophysiology of GD [14]. lncRNAs are RNAs longer than 200 nucleotides. Despite not encoding proteins, lncRNAs share structural similarities with mRNAs and have distinct biological roles in addition to interacting with DNA, RNA, and protein [15]. Reverse splicing results in the covalent attachment of the 3′ and 5′ ends, which forms the closed loop structure of circRNAs. Hence, circRNAs have organization, timing, and structural specialization and are unaffected by RNA exonucleases. Similar to miRNAs, circRNAs can attach to RNA-binding proteins and take part in ordinary biological processes in addition to being involved in gene transcription and posttranscriptional control [16]. Generally, these activities can play a substantial function in the pathogenesis and development of GD. This review aims to perform a comprehensive analysis of the role of ncRNAs in the development, diagnosis, and treatment of GD, while also investigating the most recent studies in this area.

2. FUNCTIONAL ROLES OF NON-CODING RNAS IN GRAVES' DISEASE

One of the most common autoimmune disorders is autoimmune thyroid disease (AITD). The pathogenesis of AITD is unknown, but it is caused by genetic and environmental factors. Two types of AITDs are most common: GD and Hashimoto's disease (HT). A growing body of evidence indicates that abnormal expression of ncRNAs is closely associated with the etiopathogenesis of AITD [17]. Crucial regulators of gene transcription and protein translation, ncRNA have been found to impact both normal physiology and major chronic diseases like atherosclerosis, cancer, and type 2 diabetes [18-20]. Nevertheless, little is known about how ncRNA species contribute to the AITD onset and progression. Through a review of recently published literature, a list of some functional ncRNAs was compiled, potential targets, and mechanisms (Table **1**). The main focus was on the significant roles that miRNA, lncRNA, and circRNA play in the pathogenesis of GD, which is the most common cause of hyperthyroidism.

2.1. The Role of miRNAs in GD

Circulating miRNAs have recently been shown to be biomarkers of numerous autoimmune diseases and to play a significant role in disease pathogenesis. Yao *et al*. have reported that in GD plasma, miR-762 was markedly upregulated and miR-144-3p was significantly reduced. Furthermore, a positive correlation between miR-762 expression and both free triiodothyronine (FT3) and thyrotropin receptor antibody (TRAb) levels was found. Both miR-144-3p and miR-762 demonstrated good sensitivity and specificity in separating GD patients from the other subjects [21]. Circulating miR-23b-5p and miR-92a-39 were higher in GD patients in remission than in patients with intractable GD. On the other hand, GD patients in remission had lower levels of let-7g-3p and miR-339-5p than patients with intractable GD. Compared to GD patients in remission or healthy controls, intractable GD patients' exosomes stimulated mRNA expression for TNF-α and IL-1β. Therefore, intractable GD is associated with different levels of circulating miRNAs. In addition, intractable GD patients' serum exosomes

Table 1. Non-coding RNAs as key players in Graves' disease pathogenesis.

NcRNAs	Expression		Functions	Refs.
miRNAs	miR-762 miR-144-3p	Upregulated Downregulated	Positive correlation between miR-762 expression and both FT3 and TRAb levels	[21]
	miR-23b-5p and miR-92a-39 let-7g-3p and miR-339-5p	Upregulated in GD patients in remission Downregulated in GD patients in remission	Different levels of circulating miRNAs are associated with intractable GD	[22]
	miR-23a-3p	Downregulated	Regulates Treg dysfunction	[23]
	miR-363	Upregulated	Suppress Treg cell proliferation, differentiation, and function	[24]
	miR-346	Downregulated	Correlated with increased CD4+CXCR5+ T cells proportion as well as disease severity	[25]
	miR-29a-3p	Downregulated	Higher levels of circulating Tfh memory cells, IL-21 transcript levels, IL-21 plasma concentrations, increased expression of ICOS, and a negative correlation between serum levels of TRAb and miR-29a-3p expression	[26]
	miR-142-3p and let-7b	Upregulated in untreated GD	let-7b was strongly correlated with disease severity, and it may participate in the production of TRAb via targeting PLZF	[27]
	miRNA 146a_1 miRNA 155_2 miRNA 200a_1 and miRNA 200a2*	Upregulated Downregulated Downregulated	Posttranscriptional gene regulation in the causative cells	[28]
	miR-15a-5p miR-142-5p miR-126-3p	Downregulated Upregulated Downregulated	Develop focal lesions in the thyroid gland and carefully follow up for the development of focal lesions in the thyroid gland and evaluation for potential malignancy.	[29]
	miR-4443	Upregulated	Induced CD4+ T cells dysfunction by targeting TRAF4, which may cause GD	[30]
	miR-210 miR-155 and miR-146a	Upregulated Downregulated	Serum miR-210 and miR-155 were associated with the extent of goiter, and there was a positive correlation between serum miR-210 and the levels of TRAb and FT4.	[31]
	miR-154*, miR-376b, and miR-431*	Downregulated	Differentially expressed miRNAs were associated with GD and T3 exposure, which may be new biomarkers of GD and potential treatment targets.	[32]
	miR-22 and miR-183 miR-101, miR-197, and miR- 660	Upregulated Downregulated	Potential role of miRNA-target gene networks in the pathogenesis of GD	[33]
	miR-125a	Downregulated	Development and prognosis of AITD were associated with the rs12976445 C/T polymorphism and miR-125a expression in PBMCs	[34]
LncRNAs	HMlincRNA1474 and TCONS_00012608 AK021954 and AB075506	Downregulated Upregulated	These lncRNAs might be involved in the pathophysiology of GD	[35]
	Heg	-	Heg was negatively related to TRAb in untreated patients with GD and to CD14 mRNA in treated patients and controls. Heg RNA cannot account for the decrease in TRAb seen during antithyroid treatment; instead, it might be the result of a reduction in Cdk1 mRNA	[36]
	NONHSAT093153.2, NONHSAT118924.2, and NONHSAT209004.1	Downregulated	lncRNAs are closely related to the GD recurrence	[37]

(Table 1) Contd….

NcRNAs	Expression		Functions	Refs.
	ENST00000604491	Downregulated	Contributes to the GD pathogenesis by regulating FOXP1 and represents a potential GD biomarker	[38]
	RUNX1-IT1	Upregulated	Regulates the expressions of T-bet, CXCL10, and IFN-γ by regulating NrCAM transcription	[39]
CircRNA	hsa_circRNA_000102	Upregulated	hsa_circRNA_000102-associated genes were expected to be involved in immune system activation pathways, such as viral infection and interferon-beta signaling	[40]
	hsa_circ_0090364	Upregulated	Regulates the JAK-STAT pathway through the hsa-miR-378a-3p/IL-6ST/IL21R axis to increase cell growth	[41]
	circZNF644	Upregulated	Positively correlated with the serum levels of TRAb, circZNF644 served as a ceRNA for miR-29a-3p to enhance ICOS expression, which in turn led to a rise in cTfh cells, and GD patients showed a strong relationship between circZNF644 and IL-21	[42]
	hsa_circ_0090364	Upregulated	Regulates the expression of IL6ST, which, in turn, influences the expression and secretion of IL-17A through interacting with miR-378a-3p	[43]

may activate immune cells, which may play a key role in the pathogenesis of GD [22].

Prior research has indicated that patients with GD have elevated Th17 cells and a functional deficiency of regulatory T cells (Tregs). The mechanism by which miR-23a-3p regulates Treg dysfunction in GD was examined in a study by Zhang *et al*. In GD patients, Sirtuin 1 (SIRT1) and RORγt were up-regulated, while significant down-regulation of FOXP3 and miR-23a-3p was observed. The GD group's level of FOXP3 acetylation was lower than that of the control groups. They showed that GD patients have a defect in Treg function as a result of abnormal acetylation of FOXP3, which is regulated by miR-23a-3p via targeting SIRT1 [23]. It has been demonstrated that miR-363 may suppress Treg cell proliferation, differentiation, and function by regulating the STAT4-heat-shock protein family B (small) member 1 (HSPB1)-Notch1 axis via the target gene STAT4. miR-363-5p may significantly influence Treg cell dysfunction and immune tolerance abnormalities in GD patients [24].

Follicular helper T (Tfh) cells have increasingly been recognized as participating in autoimmune diseases, such as GD. It has been shown that miR-346 could regulate CD4+CXCR5+T cells by targeting Bcl-6. In GD patients, miR-346 expression was down-regulated, which was correlated with an increase in the proportion of CD4+CXCR5+ T cells as well as disease severity. MiR-346 may be a novel therapeutic target for treating GD based on its findings [25]. Patients with GD had higher peripheral blood levels of circulating Tfh memory cells, IL-21 transcript levels, and IL-21 plasma concentrations. Peripheral blood mononuclear cells (PBMCs) from GD patients showed markedly increased expression of inducible co-stimulator (ICOS), a crucial molecule expressed on Tfh cells. This expression showed a positive correlation with the percentage of circulating Tfh memory cells and the transcript levels of IL-21 in GD. Expression of miR-29a-3p was downregulated and inversely correlated with expression of ICOS and the frequency of circulating Tfh memory cells in GD patients as determined by miRNA sequencing. The direct target of miR-29a-3p was ICOS, and miR-29a-3p had the ability to inhibit ICOS both transcriptionally and translationally. The percentage of circulating Tfh memory cells decreased when miR-29a-3p was overexpressed. Furthermore, in GD patients, a negative correlation between serum levels of TRAb and miR-29a-3p expression was observed [26].

It has been suggested that the mechanism underlying GD involves aberrant miRNAs. In GD, miR-590-5p, let-7b, miR-142-3p, miR-154-3p, and miR-431-3p were present in serum. Patients with untreated GD had significantly higher serum levels of let-7b and miR-142-3p, while in patients with GD in remission, these gradually decreased to normal levels. The let-7b and TRAb levels had a strong correlation. let-7b directly suppressed the expression of promyelocytic leukemia zinc finger (PLZF) and enhanced the expression of TSHR in thyroid cells *in vitro*. Additionally, it was discovered that there was an inverse correlation between let-7b levels in GD thyroid tissue with PLZF levels. In thyroid tissue from GD patients, there was a decrease in PLZF and an increase in TSHR. In patients with GD, let-7b may be utilized as a therapeutic target and disease biomarker [27]. Another study demonstrated that in comparison to controls, the miRNA 146a_1 was significantly elevated in the PBMC of GD patients. The sequence of miRNA155_2 was also significantly decreased in GD patients' CD8+ T-cells vs. controls. They found a reduction of miRNA 200a_1 and miRNA 200a2* in CD4+ and CD8+ T-cells in peripheral blood but not in PBMCs of GD patients. It may be possible to use these data to better comprehend the gene regulation in the cells causing

this autoimmune process [28]. Children with GD exhibited a statistically significant reduction in miR-15a-5p expression. Patients who have lower blood levels of miR-15a-5p are more likely to develop focal lesions in the thyroid gland. Thyroid disease patients had higher levels of the miR-142-5p molecule. In addition, the GD group had lower levels of miR-126-3p than the control group. They came to the conclusion that children with GD who have lower expression of the miR-126-3p molecule in their blood need close monitoring for the development of focal lesions in their thyroid gland and assessment for possible malignancy [29]. It was discovered that CD4+ T cells from untreated GD (uGD) patients had elevated levels of miR-4443, which was highly associated with GD clinical parameters. Additionally, they discovered that ectopic expression of miR-4443 targeted TNFR-associated factor (TRAF4) to cause aberrant cytokine secretion and proliferation of CD4+ T cells via the NF-κB pathway. These results demonstrated that increased levels of miR-4443 contribute to the immune pathogenesis of GD [30]. One study examined the expression of serum Treg-associated miRNAs and determined whether serum miRNAs could serve as GD biomarkers. GD patients had significantly higher serum levels of miR-210 than healthy controls, while miR-155 and miR-146a were lower. Furthermore, in GD, serum levels of miR-210 and miR-155 were associated with the extent of goiter. The levels of three miRNAs were different by gender. Additionally, there was a positive correlation between serum miR-210 and the levels of TRAb and free thyroxine (FT4) [31]. In PBMC from initial GD patients, the expression of miR-431*, miR-154*, and miR-376b was suppressed. Additionally, in GD patients in remission, their expression levels were recovered. Meanwhile, in cultured PBMC from healthy subjects, T3 treatment was able to directly inhibit the expression of these miRNAs. Therefore, differentially expressed miRNAs were associated with GD and T3 exposure, which could serve as novel biomarkers for GD and possible therapeutic targets [32]. In GD patients, miR-22 and miR-183 were shown to be increased while their potential target mRNAs were decreased. In addition, miR-101, miR-197, and miR-660 were reduced while their potential target mRNAs were increased. This study highlights the potential role of miRNA-target gene networks in the pathogenesis of GD, providing new insights into understanding the pathophysiological mechanisms underlying GD [33]. A separate study found that patients with intractable GD showed a significantly higher prevalence of the CC genotype and C allele in the rs12976445 C/T polymorphism of the MIR125A gene compared to those with GD in remission. Compared to controls, GD patients exhibited down-regulated miR-125a expression, which was negatively correlated with age. In conclusion, both the rs12976445 C/T polymorphism and miR-125a expression in PBMCs were associated with the development and clinical progression of AITD [34].

2.2. The Role of lncRNAs in GD

The study by Yin *et al.* provided comprehensive lncRNA and mRNA expression profiles in GD CD4+ T cells. GD CD4+ T cells exhibited differential expression of HMlincRNA1474, TCONS_00012608, AK021954, and AB075506. Specifically, HMlincRNA1474 and TCONS_00012608 were downregulated, whereas AK021954 and AB075506 were upregulated in newly diagnosed GD patients. These findings suggest that the identified lncRNAs may contribute to the pathogenesis of GD. The differentially expressed lncRNAs discovered in this study represent potential novel biomarkers for GD and may offer new therapeutic targets for disease intervention [35]. According to another study, TRAb concentrations may be regulated by two distinct factors. A noncoding RNA transcript, Heg, was negatively related to TRAb in untreated patients with GD and to CD14 mRNA in treated patients and controls. Heg RNA cannot account for the decrease in TRAb seen during antithyroid treatment; instead, it might be the result of a reduction in Cdk1 mRNA, which is crucial for regulating cell cycle activity. During treatment, Cdk1 gene expression falls to levels lower than those seen in normal subjects, which may be a pharmacological effect of antithyroid drugs [36]. The expression profile of lncRNAs and mRNAs in CD4+ T cells in patients with recurrent GD was constructed in one study. They discovered that the expression of NONHSAT093153.2, NONHSAT118924.2, NONHSAT209004.1, RPL8, OAS2, NFAT5, and DROSHA was significantly decreased in the GD group. Therefore, lncRNAs are closely related to the GD recurrence [37]. In patients with GD, the relative expression of ENST00000604491 was significantly reduced and negatively correlated with the serum levels of thyroid-stimulating hormone receptor antibodies (TRAb). Subsequent research verified that in GD patients, reduced FOXP1 expression was inversely correlated with serum levels of TRAb. Furthermore, there was a notably positive correlation between the expression of ENST00000604491 and the FOXP1 transcript levels in GD. Thus, this lncRNA represents a potential biomarker for GD [38]. Another study demonstrated that the expressions of lncRNA RUNX1-IT1 and Neural cell adhesion molecule (NrCAM) were most significantly increased in CD4+ T cells of GD patients. Furthermore, p53 was an NrCAM transcription factor, which could interact with NrCAM. Down-regulation of NrCAM and RUNX1-IT1 could reduce the mRNA and protein levels of transcriptional regulator T-bet and CXC chemokine ligand 10 (CXCL10) in CD4+ T cells. lncRNA RUNX1-IT1 contributed to the occurrence and development of GD by regulating the transcription of NrCAM and the expression of the crucial Th1 factors T-bet, CXCL10, and interferon γ (IFN-γ) [39].

2.3. The Role of circRNAs in GD

In primary GD cases, 15 differentially expressed circRNAs (DEcRs) were found. In plasma exosomes from GD patients, hsa_circRNA_000102 was confirmed

to be an increased component. The circRNA/miRNA/mRNA interaction network revealed the most potential targeting miRNAs of hsa_circRNA_000102 and its related genes. According to the functional analyses, hsa_circRNA_000102-associated genes were expected to be involved in immune system activation pathways, such as viral infection and interferon-beta signaling [40]. When comparing the GD group to healthy controls, 366 significantly differentially expressed circular RNAs were found. Patients with GD had higher levels of hsa_circ_0090364, which was positively correlated with thyroid-stimulating hormone receptor antibodies. hsa_ circ_0090364 may regulate the JAK-STAT pathway through the hsa-miR-378a-3p/IL-6ST/IL21R axis to enhance cell growth. These findings provide new clues into the pathophysiological mechanisms of GD and possible therapeutic targets [41]. circZNF644 was highly expressed in GD patients' PBMCs and showed a positive correlation with the serum levels of TSH receptor autoantibodies (TRAb). The proportion of circulating follicular helper T (cTfh) cells *in vitro* decreased as a result of circZNF644 knockdown. circZNF644 served as a ceRNA for miR-29a-3p to enhance the expression of ICOS, which subsequently caused an increase in cTfh cells. A positive correlation was observed between circZNF644 expression and ICOS expression and the proportion of cTfh cells in PBMCs of GD patients. However, there was a negative correlation between circZNF644 expression and miR-29a-3p expression. Furthermore, GD patients showed a strong relationship between circZNF644 and IL-21, and circZNF644 silencing inhibited the expression of IL-21. Therefore, in GD, circZNF644 expression is a key feature, contributing to cTfh cells' pathogenic function [42]. It was discovered that circPHF16 (hsa_circ_0090364) was highly expressed in GD patients' serum and PBMCs. IL-17A expression and secretion were suppressed by circPHF16 silencing, whereas the opposite result was obtained by circPHF16 overexpression. Additionally, bioinformatics analysis showed the circPHF16/miR-378a-3p/IL6ST pathway, in which circPHF16 regulates the expression of IL6ST, which, in turn, influences the expression and secretion of IL-17A through interacting with miR-378a-3p. Together, this study identifies circPHF16 as a possible target in the development of new strategies for the diagnosis and treatment of GD [43].

2.4. Gene-lncRNA Network Analyses in GD

Recent studies have applied gene co-expression network analyses to GD, revealing significant insights into its pathogenesis. For instance, a study utilizing weighted gene co-expression network analysis (WGCNA) identified 16 gene modules associated with GD, highlighting immune regulation and response pathways [44]. Another investigation constructed lncRNA-mRNA co-expression networks in relapsed GD patients, identifying specific lncRNAs linked to disease recurrence [37]. These findings underscore the potential of gene–lncRNA network analyses in elucidating GD mechanisms. Recent pan-cancer studies have underscored the significance of large-scale bioinformatics approaches in elucidating cancer-specific regulatory modules, particularly those involving non-coding RNAs. For instance, a comprehensive analysis of disulfidptosis-related gene sets across various cancers revealed associations with patient survival and immune cell infiltration, suggesting potential roles in tumor progression and therapy response [45]. Similarly, investigations into cuproptosis and copper metabolism-related networks highlighted the impact of copper-induced cell death pathways on tumor biology, emphasizing their relevance in cancer prognosis and treatment strategies [46]. Furthermore, profiling of mitotic DNA integrity checkpoint protein kinases across multiple cancer types identified their critical functions in maintaining genomic stability, with implications for cancer development and potential therapeutic targeting [47]. These studies collectively demonstrate the power of multi-omics data integration in uncovering novel regulatory mechanisms in cancer, aligning with the objectives of this research.

Research has demonstrated that miR-942-5p downregulates PIEZO1, correlating with poor prognosis in non-small cell lung cancer (NSCLC) via the MAPK pathway [48]. Such bioinformatics analyses exemplify how miRNA-target interactions can inform disease prognosis, a methodology that could be applied to GD research. Recent studies have explored the role of miRNAs in GD, highlighting their potential as prognostic biomarkers [49, 21]. The clinical utility of ncRNAs as biomarkers necessitates validation in large cohorts. While specific large-scale validation studies in GD are limited, the identification of deregulated lncRNAs in GD patients' PBMCs suggests potential biomarkers. Further studies focusing on clinical validation are essential to establish their diagnostic and prognostic relevance [38]. Therapeutic strategies targeting ncRNAs, such as antisense oligonucleotides (ASOs), RNA sponges, and small-molecule modulators, have shown promise in various diseases. For instance, small molecules have been developed to modulate ncRNA function in neurodegenerative diseases, offering insights into potential applications in GD. Exploring these therapeutic avenues could provide novel interventions for GD management [50]. Integrating multi-omics approaches, including transcriptomics, epigenomics, and proteomics, can refine our understanding of ncRNA roles in GD. A systematic review highlighted the application of multi-omics in identifying biomarkers for thyroid eye disease, a manifestation of GD, emphasizing the value of comprehensive data integration. Such approaches can uncover complex regulatory networks and potential therapeutic targets [51]. While The Cancer Genome Atlas (TCGA) provides extensive transcriptomic data, its applicability to non-cancer diseases like GD requires caution. Studies have identified technical and biological biases in bulk transcriptomic data from TCGA, including issues related to tumor heterogeneity and sample purity [52]. Researchers should be aware of these limitations when extrapolating findings to GD.

3. FUTURE PERSPECTIVE

The study on the functional role of non-coding RNAs in Graves' disease is very promising in affording a better insight into its pathogenesis and therapeutic interventions. Fast progress in RNA sequencing technologies and bioinformatics could accelerate the way to discover disease-specific signatures of ncRNAs, which may lead to the development of robust biomarkers for early diagnosis, prognosis, and evaluation of treatment responses. Moreover, unraveling how ncRNAs modulate immune pathways in GD may lead to novel RNA-based therapies.

Targeted modulation of miRNAs, lncRNAs, and circRNAs offers an exciting opportunity for personalized medicine. However, several challenges, such as delivery systems, off-target effects, and immune responses to ncRNA-based therapies, are to be overcome. Future studies should focus on large-scale clinical validation of the clinical relevance of ncRNAs in GD and explore their roles in environmental and genetic contexts that might modulate disease susceptibility. The integration of multi-omics data by efforts in collaboration with research into ncRNAs may well turn out to be the sea change in diagnosis and treatment strategies for GD.

CONCLUSION

Non-coding RNAs are considered important modulators in the complex pathophysiological mechanisms underlying Graves' disease, including immune cell activation, thyroid hormone production, and autoimmunity processes. The prospective suppression of ncRNAs is very likely to change the treatment landscape of Graves' disease while offering innovative diagnostic indicators and therapeutic targets. With increasing knowledge, ncRNAs hold the potential to close the gaps in the existing management of GD and step towards precision medicine. Translation of ncRNA research with cutting-edge technologies and interdisciplinary approaches is the key to unlocking new strategies against this complex autoimmune disorder. This review strongly points out the critical need for further studies on elucidating the interaction between ncRNAs and molecular pathways in GD.

AUTHORS' CONTRIBUTIONS

It is hereby acknowledged that all authors have accepted responsibility for the manuscript's content and consented to its submission. They have meticulously reviewed all results and unanimously approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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