Non-Coding RNAs in Autoimmune Disorders: Therapeutic Potentials and Integrative Network-Based Computational Insights

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I, Aashi Barwal, bearing Roll Number 23/MSCBIO/01, hereby certify that the work which is presented in the project entitled "Non-coding RNAs in autoimmune disorders: therapeutic potentials and integrative network-based computational insights" in partial fulfilment of the requirement for the award of the degree of Master of Science (M.Sc.) in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, New Delhi, is an authentic record of my own carried out during a period from January 2025 to May 2025, under the supervision of Dr Asmita Das.

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Non-Coding RNAs in Autoimmune Disorders: Therapeutic Potentials and Integrative Network-Based Computational Insights

ABSTRACT

The recent breakthrough in the discovery of therapeutic applications of RNA have established oligonucleotide-based drugs as a promising therapeutic modality for targeting diverse diseases including the autoimmune ones- a group of complex immunological disorders resulting from a breach of tolerance. The current treatment approaches for rely on symptom management with the use of non-specific broad immunosuppressants which have their own associated adverse effects. This calls for the need of precise and target specific therapeutics. With recent progress in deciphering their molecular pathogenesis, have allowed the application of RNA based therapeutic strategies for their treatment. This thesis explores the different types of RNA therapeutics- antisense oligonucleotide (ASO), splice switching oligonucleotide (SSO), RNA interference based and aptamers, describing their mechanism of action and their applicability as therapeutic modalities, further investigating their potential for treatment of autoimmune disorders. A curated set of dysregulated microRNAs from five exemplar autoimmune diseases—rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), Type 1 diabetes mellitus(T1DM), and autoimmune thyroid disease (AITD)—were taken for construction of miRNA–gene interaction networks in miRNet. Network-topology metrics pinpointed a small set of high-degree "master-regulator" miRNAs that control disproportionately large gene sets across multiple diseases. Downstream KEGG pathway enrichment of the shared targets revealed a conserved signalling core—PI3K–Akt, MAPK, JAK–STAT, and the canonical Pathways in cancer module—highlighting a molecular intersection between autoimmunity and oncogenic signalling. These computational insights guide target identification which can be acted upon by the RNA therapeutics. Finally, it addresses the hurdles that limit clinical applicability and propose strategies to accelerate bench to bedside journey of RNA therapeutics in autoimmune disorders.

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LIST OF ABBREVIATIONS

S.No.	Abbreviation	Explanation
1.	ASO	Antisense oligonucleotide
2.	SSO	Splice switching oligonucleotide
3.	DMD	Duchene muscular dystrophy
4.	SMA	Spinal muscular atrophy
5.	ncRNA	Non coding RNAs
6.	miRNA	Micro RNA
7.	SELEX	Systemic evolution of ligands by exponential enrichment
8.	FDA	Food and drug administration
9.	AMD	Age related macular degeneration
10.	VEGF	Vascular endothelial growth factor
11.	RA	Rheumatoid arthritis
12.	SLE	Systemic lupus erythematosus
13.	SS	Sjogren syndrome
14.	T1DM	Type 1 diabetes mellitus
15.	AITD	Autoimmune thyroid disease
16.	RISC	RNA induced silencing complex
17.	AGO	Argonaute endonuclease
18.	NSAID	Non-steroidal anti-inflammatory drugs
19.	DMARD	Disease modifying anti rheumatic drugs.
20.	CIA	Collagen induced arthritis
21.	TNF	Tumor necrosis factor
22.	BTK	Bruton’s tyrosine kinase
23.	PBMCs	Peripheral blood mononuclear cells.
24.	KEGG	Kyoto encyclopedia of genes and genomes
25.	FDR	False discovery rate
26.	HT	Hashimoto’s thyroiditis
27.	GD	Grave’s disease
28.	CRP	C-reactive protein
29.	ESR	Erythrocyte sedimentation rate
30.	ESE	Exonic splicing enhancer
31.	ISE	Intronic splicing enhancer
32.	ISS	Intronic splicing silencer

CHAPTER 1

INTRODUCTION

Autoimmune disorders- are a class of immunological diseases characterized by breach of immune tolerance. They encompass a broad spectrum of conditions resulting from aberrant activation of autoreactive B and T cells, which target the body's own tissues through various effector mechanisms, leading to inflammation, tissue destruction, and organ dysfunction. To date, over 150 autoimmune disorders have been identified—some organ-specific, others affecting multiple organ systems—highlighting the complexity and diversity of immune dysregulation. Once considered rare, epidemiological data now indicate a significant global burden, making autoimmune diseases a growing concern in both public health and pharmaceutical research sectors [1]. Owing to a rather limited understanding of the underlying complex mechanisms involved in autoimmune disorders, there is no definite cure available till date. Currently available therapeutic strategies typically focus on managing the symptoms, using non-specific, broad range immunosuppressive agents, which suppress the immune system, as a whole, to modulate uncontrolled inflammation. They are not very successful in heterogeneous patient populations and are associated with systemic side effects, including infection, allergy, malignant disease, and incomplete disease remission. This underlines the urgent need for advanced therapeutic options with fewer undesirable effects and improved efficacy.[2] With the increasing understanding of the pathogenesis, numerous biological drugs are being developed to treat autoimmune diseases. Recent advances in molecular medicine have established RNA therapeutics as a novel class of treatment. Given the number of roles RNA plays in biological processes, RNA therapeutics leverage the ability of RNA molecules to regulate gene expression. Functional studies reveal the implication of dysregulation of a spectrum of ncRNAs in the pathogenesis of multiple autoimmune diseases, which exert their effects by modulating the key pathological mechanisms underlying autoimmunity. The identification of ncRNAs as pivotal regulators of gene activity, along with their abnormal expression patterns in various diseases, has sparked interest in utilizing them as therapeutic targets. Oligonucleotide-based inhibitors and mimics have shown promise in modulating activity of non-coding RNAs in vitro. These therapeutic agents have also exhibited partial efficacy in preclinical models, thereby, advancing development of innovative treatments hoping for their successful clinical trials in autoimmune diseases.[3] They can be designed to target even the genes considered to be undruggable by conventional approaches. In addition, they offer several advantages over conventional drugs. Computational approaches involving

miRNA networks and KEGG enrichment analysis help identify such hub miRNAs and targets that control convergent inflammatory circuits, whose simultaneously targeting by RNA therapeutics can theoretically revert dysregulated edges across the disorders. This is currently an emerging area. In the wake of their success in clinical trials in various other diseases like DMD, SMA, RNA therapeutics are being put forward as a potential therapeutic for autoimmune diseases as well.

CHAPTER: 2

LITERATURE REVIEW

2.1 Types of RNA Therapeutics

2.1.1 Antisense oligonucleotides (ASO)

ASOs are short, synthetic strands of nucleic acid that bind specifically to target RNA sequences, thereby modulating protein expression.[1] This can be achieved via distinct mechanisms. Once delivered into the body, these ASO bind to the target mRNA, forming an RNA-DNA hybrid. One of the mechanisms involves binding of ASO at translation initiation site acting as a stearic block for the assembly of translation machinery and hence leading to translational arrest.[1] Another mechanism involves RNase H which acts to cause degradation of mRNA in RNA-DNA hybrid as depicted in figure 2.1.1[2–4]. ASO based therapies target the source of pathogenesis of the disease, thus are relatively more successful over other therapies targeting downstream pathways.[5] Fomivirsen (1998) was the first FDA approved ASO drug designed for-HCV-induced retinitis in HIV patients.[6, 7] Mipomersen was the second one to be approved by FDA. Its subcutaneous administration targets apolipoprotein B-100 mRNA to treat familial hypercholesterolemia.[8] A wide range of ASOs of different generations are under clinical trials aimed at treatment of different diseases.

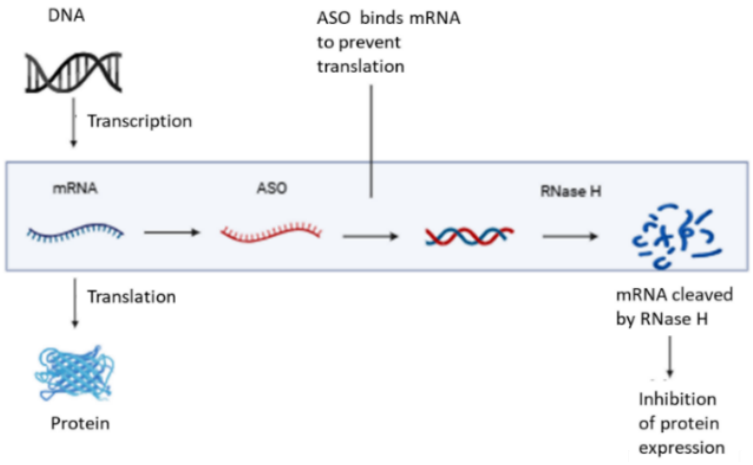

Figure 2.1.1: Mechanism of action of ASO.

Under normal conditions DNA is transcribed into single stranded mRNA which is then translated into protein product. In the presence of ASO which is complementary to the target mRNA, a double stranded DNA-RNA hybrid is formed, which is acted upon by RNase H to cleave RNA component of the hybrid, thereby inhibiting translation.

2.1.2 Splice switching oligonucleotides

Pre-mRNA transcripts from most protein-coding genes can undergo alternative splicing, resulting in multiple isoforms from a single gene. Splicing is regulated by a combination of cis- & trans-acting elements that influence splice site selection. These regulatory factors can either enhance or suppress splicing at specific sites. Splicing enhancers are sequence elements that facilitate the inclusion of nearby exons when bound by their corresponding proteins. In contrast, splicing silencers inhibit splicing at particular sites. These enhancers and silencers may be present within exons or introns.[9] Several diseases are attributed to aberrant RNA-splicing and inclusion of pseudo exons (eukaryotes) leading to non-functional proteins.[10] Provided the crucial role splicing plays in regulating gene expression and its widespread dysregulation in several diseases, there is rising interest in development of drugs that can precisely modulate splicing in ways that can help alleviate disease symptoms.[9] This concept paved the way for the development of a distinct class of RNA-based therapeutics known as splice-switching oligonucleotides (SSOs). They act to restore the production of functional proteins by modulating the splicing patterns of pre-mRNA from defective genes by interfering with the interactions between protein and RNA that direct splicing. [11] These molecules are engineered to bind their target RNA sequences with high affinity, effectively obstructing and redirecting spliceosome activity through steric hindrance. This can occur via exon skipping,

exon inclusion and, exon shuffling, intron retention, alternative splice site selection, shifting of polyadenylation and promoter sites.[11] The mechanism of action of exon skipping and exon inclusion is depicted in figure 2.1.2. The SSOs are chemically modified to prevent recruitment and action of RNase H on pre-mRNA-SSO complex.[9] SSOs have been successfully designed and used as therapeutic agents for DMD and SMA.

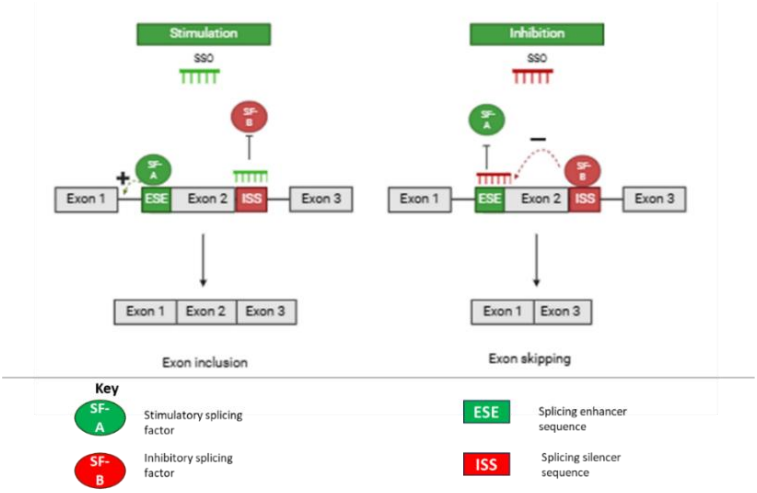

Figure 2.1.2: Mechanism of action of splice switching oligonucleotides.

Normally, splicing is regulated by splicing factor A (stimulatory, green) binding to the ESE to promote exon inclusion, while splicing factor B (inhibitory, red) binds to the ISS to inhibit it. SSOs can modify these interactions to control splicing. In stimulation, the SSO binds to the ISS, blocking factor B and allowing factor A to bind the ESE, leading to exon inclusion. In inhibition, the SSO binds to the ESE, preventing factor A from binding and enabling factor B to bind the ISS, resulting in exon skipping.

2.1.3 Aptamers

Aptamers are single stranded 20-100 nucleotides long oligomers that adapt complex well defined 3-D structures that enabling their highly specific interaction with protein targets, typically achieving nM- to pM binding affinities as depicted in figure 2.1.3.[12] First described in 1990, they are analogous to antibodies in a sense that both of them function as affinity reagents and bind specifically to their target via induced fit mechanism.[13] However aptamers offer an edge over antibodies as they have low manufacturing costs, short generation time, low or no batch to batch variability, better thermal stability, higher modifiability and target potential.. Thereby providing a highly consistent, cost-effective, and easily modifiable alternative to antibodies.[14] They can be used for therapeutic purposes in a manner similar to mAbs. Aptamer-based therapeutics are based on one of the following strategies:

- Antagonist aptamers interfere with the interaction between disease targets (protein-protein/ receptor-ligand interactions).[14].
- Agonist aptamers serve to stimulate or positively influence the function of target receptors.[14]
- Cell type-specific aptamers functions to deliver other therapeutic--agents to the targeted cells or tissues. [15]

The standard methodology for generating aptamers, virtually against any protein is SELEX (systematic evolution of ligands by exponential enrichment). It is a iterative process which consists of repeated binding, partitioning, recovery, and re-amplification steps.[9] Macugen (pegaptanib), was the first aptamer approved by US-FDA for AMD. It is a 27nt chemically stabilized RNA oligomer which blocks VEGF-receptor-induced neovascularization.[16, 17]

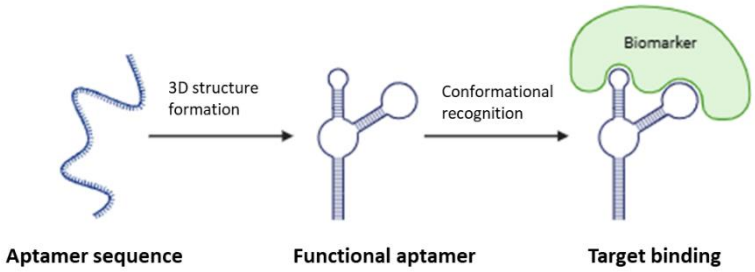

Figure 1.1.3: Mechanism of action of aptamers

Long oligonucleotide sequence, adapt 3-D configuration to form functional aptamers which specifically recognizes and binds to the target molecules, thus inhibiting or enhancing signal transduction.

2.1.4 Therapeutic RNA Interference

Small non-coding RNAs have revealed RNAi as a key regulatory mechanism for post transcriptional gene silencing, which has significant therapeutic implications. [18–20] It interferes with gene expression in multiple ways, including, endonucleolytic cleavage of target mRNA and/or by recruitment of de-adenylation / de-capping enzymes.[21] Non-coding RNAs including miRNA, circRNA, lncRNA and siRNA are the mediators of RNAi, which can theoretically silence any gene (disease associated) in a sequence-specific manner making them a promising therapeutic modality.[22] Owing to the slight differences, they have different roles in pharmaceutical practice, but converge into the RISC (RNA induced silencing complex),[23] which binds to complementary sequences in target mRNAs, thus causing mRNA destabilization, degradation and eventually translational inhibition.[24] siRNA is typically

more efficient at triggering more specific gene silencing, as compared to miRNA, which can target several genes simultaneously, owing to complete and partial complementarity of siRNA and miRNA respectively.[22] The detailed mechanism of siRNA and miRNA action is described in figure 2.1.4. Two common strategies are used by these non-coding RNA targeted therapeutics based on whether the target ncRNA expression needs to be downregulated or re-introduced to restore the normal function. These two strategies are inhibition and replacement.[25] Inhibition involves suppression of the action of endogenous ncRNAs involved in disease pathogenesis and replacement involves restoration of the function of the endogenous ncRNA by introduction of synthetic ncRNAs (mimics) to activate the target gene expression. The replacement approach is especially beneficial when an autoimmune disorder arises due to the insufficient expression of a specific ncRNA.[25, 26] 'Patisiran', the first FDA approved siRNA drug to be commercialized.[27] Next was, 'Givosiran', approved for acute hepatic porphyria. Then came other candidates, Nedosiran, Vutrisiran, Inclisiran, Teprasiran, Fitusiran, Cosdosiran, and Tivanisiran, which are in stage 3 clinical trials.[28] miRNA mimics are being currently explored in preclinical development as putative therapeutic agents.[29] To mention a few, drug named MRG-110, which inhibits Mir-92a, (implied in development of cardiovascular diseases and delayed wound healing), has shown safety and efficacy in phase 1 clinical trials in humans.[30] Another drug, RGLS8429, inhibitor of miR-17 is a promising candidate for polycystic kidney disease. [31] Another miRNA drug, MRG-201 (miR-29 mimic) is being explored for the treatment of keloid and fibrous scar formation.[32]

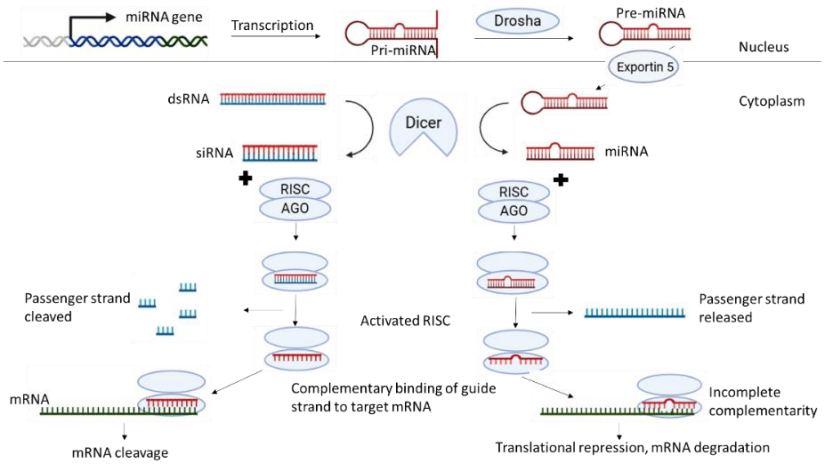

Figure 2.1.4: Mechanism of action of RNA interference

miRNA gene is transcribed into Pri-miRNA, which undergoes processing by enzyme 'Drosha,' to form Pre-miRNA, which is translocated to the cytoplasm by exportin 5 transporter. In the cytoplasm, dsRNA and pre-miRNA are acted upon by 'Dicer' to form siRNA and miRNA respectively. These ds-siRNA/miRNA associate with 'argonaute-2' endonuclease to form RNA induced silencing complex. The passenger strand is then released to form activated RISC. The RISC with guide strand, goes and binds to the target complementary RNA. siRNA being fully complementary to target mRNA, promotes cleavage by AGO-2, whereas miRNA containing loops and bulges prohibit cleavage by AGO-2 but alter stability and causes translational repression.

2.2 Non coding RNAs in autoimmune disorders

2.2.1 Rheumatoid arthritis (RA)

RA is a persistent autoimmune condition primarily affecting synovial joints. It is characterized by chronic inflammation, synovial hyperplasia, and the generation of autoantibodies such as rheumatoid factor and anti-citrullinated protein antibodies. The disease progression involves immune-mediated bone and cartilage, often resulting in significant pain, disability, & systemic complications if left untreated.[33]

Current therapies for rheumatoid arthritis (RA) aim at early detection and inflammation control to prevent joint damage. Common treatments include NSAIDs, corticosteroids, and DMARDs, which target inflammatory pathways but often cause adverse effects and show limited efficacy in many patients. Biologics like monoclonal antibodies (adalimumab, infliximab) and JAK inhibitors (tofacitinib, baricitinib) offer targeted relief but are costly and require frequent dosing. Despite these advances, RA remains incurable, highlighting the need for improved therapies. Emerging targets such as interleukins, ROS, and notably non-coding RNAs (ncRNAs), offer promise for novel, RNA-based therapeutics that could enable precise gene silencing with fewer side effects. [34]

Emerging research has revealed that ncRNAs, especially microRNAs (miRNAs), are intricately involved in regulating immune responses in RA. For instance, miR-146a is often found upregulated in T lymphocytes of RA patients but fails to sufficiently regulate its downstream targets TRAF6 and IRAK1, leading to persistent TNF-α signalling and exacerbated inflammation.[35, 36] Pauley et al. through his in vitro experiments, demonstrated that normalizing miR-146a levels can suppress TNF-α production, indicating a regulatory role in inflammation control.[36, 37] Other dysregulated miRNAs include miR-223, overexpressed in CD4+ naive T-cells [36, 38] and miR-346, which indirectly enhances IL-8 production in synovial fibroblasts [36, 39]. Among the most extensively studied is miR-155, which is also elevated in peripheral monocytes, synovial fluid, & tissue. [36, 40] Pauley et al., first reported the link between miR-155 expression and autoimmune diseases, showing that PBMCs of RA patients had significantly elevated levels of miR-155.[26, 37] It inhibits Jarid2(DNA binding protein), which is involved in repression of pro-inflammatory genes and thus leading to defective Th17 cell function.[26, 41] In addition, reduced miR-155 leads to an increased PU.1 expression and suppressed BCR induced antibody production, highlighting its role in regulating activation of B-cell in RA.[26, 42] Mice deficient in miR-155 also exhibited lesser

local bone destruction in Collagen induced arthritis (CIA), emphasizing its role in RA pathology.[26, 43] Level of miR-221/222, miRNA-23b show positive correlation with clinical indicators such as CRP, ESR, disease activity score, anti-citrullinated protein antibodies, and rheumatoid factor making them potential biomarkers for RA.[44, 45] Additionally the expression of miR-223 & miR-16 vary in RA patients from healthy individuals.[34, 46]

Various RNAi-based strategies have been explored in RA models. In 2005, Schiffelers et al. showed that intra-articular delivery of siRNA targeted to TNF-α reduced joint inflammation in CIA models.[47, 48] Khoury et al. further reported that siRNA-mediated TNF-α silencing led to over 70% reduction in TNF-α levels and substantial remission of arthritic symptoms with weekly dosing. In a separate study.[49] In yet another study Chen et al. used short hairpin RNA (shRNA) targeting TLR7, resulting in downregulation of inflammatory mediators and amelioration of disease symptoms in treated mice.[48, 50] TNF-α silencing, thus presents a prospective approach for treating arthritis and TNF-α-mediated chronic inflammation. Another important molecular target is BTK (Bruton's tyrosine kinase), which is expressed in macrophages and B cells. BTK-specific siRNA therapy in CIA mice significantly reduced joint inflammation.[51] Instead of monotherapy with miRNA or siRNA, their combination therapy with specific drug treatments was proposed to be a better approach with greater efficacy.[51] A study investigating the co-delivery of methotrexate and indomethacin along with MMP-9-targeting siRNA in a macrophage cell line demonstrated effective downregulation of inflammatory cytokines, as well as MMP-9.[51] On administration in arthritic mice, the synergistic action of siRNA and chemical drugs in the formulation significantly alleviated joint swelling and lowered TNF-α, MMP-9, and IL-6 in the knee joint and ankle fluid, restoring near-normal ankle joint morphology, enhanced anti-inflammatory effects and improved cartilage integrity.[51, 52] Macrophages produce a wide range of pro-inflammatory mediators involved in RA, which can be efficiently targeted by RNA therapeutics thus representing a powerful anti-rheumatic strategy.[53] Injection of anti-IL-6, -IL-18 or -IL-1β siRNA lipoplexes were found to abrogate joint swelling alongside cartilage and bone destruction, with IL-6 targeting proving to be most effective. However, a combination of all three siRNAs (IL-1, IL-6, and IL-18) gave most significant therapeutic benefits by comprehensively reducing pathological features of CIA, and improving overall parameters. [53] Intra-articular administration of shRNA against B-cell activating factor (BAFF) achieved local gene silencing, alleviating inflammation & joint damage in CIA mouse by particularly targeting DCs from ankle joint and impairing Th17 cell expansion and B cell function [34, 54].

2.2.2 Systemic lupus erythematosus (SLE)

SLE is a complex, chronic autoimmune disorder that can affect almost every organ system, thus, often presenting with a broad range of clinical manifestations ranging from mild mucocutaneous symptoms to severe multiorgan and central nervous system involvement. These symptoms can range from mild mucocutaneous lesions to severe complications involving the central nervous system, kidneys, and other vital organs, making diagnosis and treatment challenging. The underlying mechanism involves the generation of autoreactive B cells producing autoantibodies owing to defects in both innate and adaptive immunity, leading to. These antibodies bind to self-antigens to form immune complexes that accumulate in tissues, leading to inflammation and tissue injury via complement activation and immune cell recruitment.[55]

Therapeutic management of SLE focuses primarily on preventing organ damage and maintaining remission. Treatment plans are personalized based on the severity and organ involvement. Mild cases are typically managed with NSAIDs, antimalarials like hydroxychloroquine, & immunomodulatory agents. However, more severe manifestations often require potent immunosuppressants such as corticosteroids, cyclophosphamide, or azathioprine. Prolonged use of these agents carries risks of adverse effects, including bone marrow suppression, hepatotoxicity, and increased susceptibility to infections. Although biological therapies like rituximab (targeting CD20) and belimumab (targeting BAFF) have shown modest success, many B cell-directed therapies have failed to yield favourable clinical outcomes due to safety limitations. Consequently, the development of safer, more effective therapies remains a high priority.[56]

Recent advances in transcriptomic studies have uncovered substantial dysregulation of non-coding RNAs, especially microRNAs (miRNAs), in SLE. These miRNAs play pivotal roles in modulating immune responses and have emerged as key players in the disease's pathophysiology. In one study, Dai et al. profiled miRNA expression and identified seven downregulated miRNAs (miR-196a, miR-383, miR-17-5p, miR-184, miR-409-3p, miR-141, and miR-112) and nine upregulated miRNAs (miR-21, miR-198, miR-61, miR-299-3p, miR-142-3p, miR-78, miR-189, miR-342, and miR-289) in SLE patients compared to healthy individuals.[57, 58] miR-146a has been shown to be downregulated in SLE and is closely linked to the activation of the type I interferon (IFN) pathway—a hallmark of SLE pathology.

Its diminished expression fails to control signalling through TRAF6 and IRAK1, thereby amplifying inflammatory responses. [59] Similarly, miR-21 is markedly upregulated in multiple autoimmune disorders, including SLE. It exerts its pathogenic effects by targeting and downregulating several genes such as PDCD4, SMAD7, and BCL2, collectively contributing to an exaggerated immune response. Experimental studies in mouse models have demonstrated that inhibiting miR-21 ameliorates disease symptoms, suggesting its therapeutic relevance.[26]

In this context, Regulus Therapeutics has developed RG-012, a single-stranded antisense oligonucleotide that targets miR-21. This compound has progressed into Phase II clinical trials, reinforcing the potential of miRNA-targeted therapies for SLE. Additional preclinical investigations involving miRNA knockdown and gene knockout strategies have shown promising improvements in disease markers in SLE mouse models.[60–62] Beyond miRNA-based strategies, aptamer-based therapies are also being explored for SLE. One potential target is the G6-9 anti-DNA autoantibody, which binds to nuclear antigens. An RNA aptamer with high affinity for this autoantibody has been engineered, providing opportunities for diagnostic and therapeutic applications.[63, 64] Moreover, chemokines such as CCL2 and its receptor CCR2 are involved in the recruitment of inflammatory cells in lupus nephritis. Aptamers targeting this axis could potentially disrupt immune cell infiltration in the kidneys. One such compound, mNOX-E36–Spiegelmer, is a CCL2-specific RNA aptamer that has demonstrated efficacy in mice model for lupus nephritis. Treatment with this aptamer reduced monocyte migration from bone marrow and decreased renal macrophage and T-cell infiltration. It has also successfully completed Phase I clinical trials without inducing immunostimulatory side effects.[64–66]. Overall, both miRNA- and aptamer-based therapeutic strategies offer considerable promise for the treatment of SLE. Their ability to precisely modulate pathogenic molecular pathways, combined with their favourable safety profiles, makes them attractive candidates for future clinical application in autoimmune diseases like lupus.[64]

2.2.3 Sjögren's syndrome

Sjögren's syndrome (SS) is a systemic autoimmune disease primarily characterized by exocrine gland dysfunction, which results in symptoms such as dry eyes (xerophthalmia) and dry mouth (xerostomia). The condition arises due to the infiltration of autoreactive lymphocytes into salivary and lacrimal glands, leading to apoptosis of the secretory epithelial cells and consequent glandular hypofunction.[67] SS is clinically classified into two types: primary SS

(pSS), which occurs as an isolated autoimmune disorder[68], and secondary SS (sSS), which coexists with other autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus. [69]

The management of primary Sjögren's syndrome (pSS) focuses on symptom relief and management of systemic complications, as no single treatment effectively addresses both aspects. Current options include secretagogues like pilocarpine and symptom-based treatments such as saliva and tear substitutes. For severe systemic cases, corticosteroids and immunosuppressants are used, though their efficacy remains limited and unproven. csDMARDs like methotrexate are used empirically but lack formal approval or strong evidence of benefit. Hydroxychloroquine is commonly prescribed, yet its effectiveness is modest. The absence of targeted therapies and the limited success of conventional immunosuppressive agents underscore the pressing need for more effective treatment options.[70]

Recent studies have highlighted the involvement of non-coding RNAs—particularly microRNAs (miRNAs)—in the pathogenesis of SS. These molecules regulate gene expression post-transcriptionally and have been implicated in key pathological mechanisms of the disease. Wang et al. conducted microarray profiling of labial salivary gland biopsies and reported significant downregulation of miR-181a and miR-16 in SS patients compared to healthy controls. These miRNAs are believed to influence immune responses through their interactions with intracellular ribonucleoproteins La/SSB and Ro/SSA, which are major autoantigens in SS. Although a direct mechanistic link has not been fully established, these associations suggest a regulatory role in disease progression. [71, 72] Further research by Carvajal et al. investigated the expression of hsa-miR-513c-3p and hsa-miR-424-5p in labial salivary gland tissue from pSS patients.[73] Their findings revealed an upregulation of hsa-miR-513c-3p and a downregulation of hsa-miR-424-5p. Notably, the target genes of hsa-miR-513c-3p—XBP-1s and GRP78—were found to be downregulated in patient samples, while the targets of hsa-miR-424-5p—activating transcription factor 6α (ATF6α) and protein SEL1 homolog 1—were upregulated. These findings suggest that altered miRNA expression may disrupt the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress pathways, both of which are implicated in autoimmune disease pathology.[72, 73] Gallo et al. in his microRNA expression studies in patients with varying salivary flow rates, revealed significant dysregulation of 126 miRNAs, among which, four (miR-106a, miR-20a, miR-18b, and miR-146b) were upregulated, while two (miR-635, miR-372) were downregulated.[72] Overexpression of certain other miRNAs like miR-17/92, miR-200, miR-30, and miR-let-7 families was also

observed in pSS patients. Pathway analysis revealed their role in mucin-type O-glycan biosynthesis. miR-let-7 overexpression notably led to the downregulation of GALNT1(N-Acetylglucosaminyltransferase), which encodes MUC7(mucin 7), a key salivary protein responsible for oral lubrication.[72, 74] Since glycosylated mucins are crucial for saliva composition, disruptions in this biosynthetic pathway can lead to the symptoms observed in SS patients.[75] miR-181d-5p regulate downstream genes involved in the mucin-type O-glycan biosynthesis pathway. It additionally regulates other pro- and anti-inflammatory pathways along with the ones involved in ER stress and TNF-α. [72, 76] 'miR-181d-5p' was reported to be inversely related to TNF-α expression by Castro et al. The dysregulation of glandular function and morphological alterations observed in pSS patients were attributed to the increase in TNF-α that resulted from downregulation of miR-181d-5p.[72, 77] This association establishes hsa-miR-181d-5p as a novel therapeutic target in Sjögren's syndrome.[76]

Pauley et al. explored a novel strategy to preserve secretory function in SS by developing a conjugate of a siRNA targeting caspase-3 and carbachol, a muscarinic receptor agonist. Carbachol being a secretagogue, facilitates secretion by binding to muscarinic receptors and undergoes receptor-mediated endocytosis, enabling targeted delivery of the siRNA specifically to muscarinic receptor-expressing cells such as salivary acinar cells. The conjugate was reported to successfully reduce caspase-3 gene/protein expression, indicating effective cellular uptake. Additionally, it inhibited cytokine-induced apoptosis, critical for maintaining fluid secretion, in epithelial cells.[51, 78] These findings suggest that this siRNA-based silencing approach may help protect secretory acinar cells from apoptosis, maintaining their secretory function and improving the quality of life in SS patients.

2.2.4 Type 1 Diabetes Mellitus

Type 1 Diabetes Mellitus (T1DM) is a chronic autoimmune condition characterized by the selective destruction of insulin-producing β-cells in the pancreatic islets of Langerhans. This destruction results in a lifelong dependency on exogenous insulin and a progressive decline in endogenous insulin secretion.[79] Irrespective of age of occurrence, insulin deficiency manifests itself as low/undetectable levels of plasma C peptide.[80] The disease is associated with several immune markers particularly autoantibodies targeting pancreatic β-cell.

Current treatment regimens for T1DM are centered on glycemic control through insulin therapy, relying on lifelong administration of exogenous insulin combined with strict dietary

management. While modern humanized and genetically modified insulin analogues offer improved glycemic control, they still fall short of mimicking natural insulin function and carry risks such as hypoglycemia, insulin resistance, and dependency. Islet transplantation offers a promising alternative, but its use is limited by donor shortages and challenges in xenotransplantation. These limitations highlight the pressing need for innovative therapies that can restore natural glycemic control and reduce reliance on insulin.[81]

MicroRNAs (miRNAs) play a crucial role in pancreatic homeostasis, regulating β-cell function, survival, and insulin secretion. Given their significant involvement in pancreatic biology, they are potential therapeutic targets for type 1 diabetes mellitus. RNA interference-based strategies aimed at modulating specific ncRNAs could thus provide novel approaches to, preventing autoimmunity, and improving glycemic control in T1DM patient. Emerging research has shed light on the pivotal role of non-coding RNAs (ncRNAs), particularly microRNAs (miRNAs), in regulating immune responses associated with T1DM.[82] Upregulation of miR-21 is associated with disruption of β-cell development in T1DM animal models.[26, 83] Its overexpression causes an increase in caspase-3 level, eventually leading to increased apoptosis of β-cell, by specific targeting of bcl-2 gene expression.[84, 85] miR-29 is another miRNA implicated in β-cell dysfunction. Its overexpression in pancreatic islets was found to impair glucose-induced insulin secretion.[26] It was also found to promote β-cell apoptosis thereby leading to pancreatic cells dysfunction in the early stages of disease development. This occurs by targeting of the antiapoptotic protein myeloid cell leukemia-1 (Mcl1), which is essential for β-cell survival. The downregulation of Mcl1 leads to increased β-cell death, exacerbating insulin deficiency.[26, 86] The upregulation of miR-181 in T1DM patients show a negative correlation with levels of SMAD7 and C-peptide, suggesting its significant role in pancreatic β-cell dysfunction.[87, 88] The overexpression of miR-7 and miR-124 negatively regulates differentiation of pancreatic α- and β-cell via different mechanisms, thus negatively impacting B-cell differentiation.[89, 90] In diabetic mice, elevated levels of miR-34a were linked to reduced B lymphocyte production by inhibiting Foxp1 gene expression, a key regulator of B lymphopoiesis. This suppression weakens pancreatic islet defence, increasing susceptibility to damage.[91, 92] The increased expression of miR-23, miR-590, and miR-98 has been associated with enhanced generation of autoreactive CD8+ T cells that specifically attack pancreatic islet antigens. This effect is mediated through the downregulation of critical apoptotic regulators such as RAIL (TNF-related apoptosis-inducing ligand), FAS, and FAS ligand, suggesting a gene-silencing

mechanism that contributes to the initiation of autoimmune responses in T1DM.[93, 94] In addition to these, miR-375 and miR-143 are recognized for their involvement in the regulation of insulin secretion, lipid metabolism, and adipocyte differentiation, pointing to their role in maintaining metabolic homeostasis. miR-29b, which shows elevated expression across multiple autoimmune disorders, has a prominent role in modulating inflammatory cytokine production. It achieves this either by directly targeting Eomes and T-bet, or via the Sp1–NFκB–HDAC–miR-29b regulatory axis, highlighting its contribution to immune response regulation and inflammation.

Beyond diagnostic potential, therapeutic strategies targeting miRNAs in T1DM are currently under preclinical development. RNA-based therapeutics—such as antagomirs (miRNA inhibitors) and miRNA mimics—offer the possibility of restoring immune balance and protecting β-cells by modulating miRNA activity. For example, Therapeutic application of miR-29b has demonstrated promise in mitigating diabetic nephropathy by dampening the Th1-mediated immune response and attenuating both renal inflammation and fibrosis. A synthetic miR-29b mimic, known as 'MRG-201', has been developed by 'miRagen Therapeutics' and is currently undergoing clinical evaluation.[26] Since excessive glucagon secretion is a contributing factor to hyperglycemia in individuals with T1DM, glucagon receptor antagonists offer a potential therapeutic approach to help manage blood glucose levels.[95, 96] Building up on this strategy, Vater et al. engineered a 39-nucleotide anti-glucagon DNA/RNA Spiegelmer, termed NOX-G15. This molecule features 2'-O-modifications on the sugar backbone and 5'-end PEGylation to enhance stability against nucleases. NOX-G15 was shown to bind glucagon with high affinity and inhibit glucagon-induced cAMP production in CHO-K1 cells expressing the human glucagon receptor. [64, 97] If successful in diabetic patients, this Spiegelmer could potentially eliminate the need for frequent glucose monitoring and insulin injections. [64, 98]

2.2.5 Autoimmune thyroid disease

Autoimmune thyroid disorders or AITD are a group of, organ specific disorders, affecting thyroid gland, arising due to breakdown of immunological tolerance to self-antigens such as thyroglobulin, thyroperoxidase and the thyrotropin receptor, triggering an infiltration of B and T cells, autoantibody production and, eventual onset of clinical symptoms.[99] Autoimmune

thyroid diseases primarily include Hashimoto's thyroiditis (HT) and Graves' disease (GD), both of which are more prevalent in women. [99]

Recent research highlights the involvement of non-coding RNAs, particularly microRNAs (miRNAs), in the immunopathogenesis of AITDs. Several studies have reported the differential expression of multiple miRNAs in PBMCs, serum, plasma, and T cells of AITD patients. In a study conducted by Liu et al., a total of 16 miRNAs were found to be differentially expressed in PBMCs of individuals with Graves' disease (GD) compared to healthy controls. Among them, miR-154, miR-376b, and miR-431 were notably downregulated.[99].In related work, Bernecker et al. reported upregulation of miR-146a in PBMCs of GD patients. In contrast, they observed a reduction in miR-155 levels in CD8+ T cells from both GD and Hashimoto's thyroiditis (HT) patients. Since miR-155 plays a crucial role in the development of regulatory T cells (Tregs) and the differentiation of Th17 cells, its downregulation may contribute to immune imbalance. The same study also identified reduced expression of miR-200a-1 and miR-200a-2 in both CD4+ and CD8+ T cells from GD and HT patients, which are linked to enhanced production of pro-inflammatory Th1 cytokines. [99, 100] Additional insights from microarray analyses of plasma samples from GD patients revealed five significantly altered miRNAs. Four—miR-16-1-3p, miR-221-3p, miR-122-5p, and miR-762—were elevated, whereas miR-144-3p showed decreased expression. In the case of HT, elevated levels of six distinct miRNAs were identified in peripheral plasma, including miR-375, miR-451, and miR-500a, which showed a strong correlation with thyroid-stimulating hormone (TSH) concentrations. Additionally, miR-20a-3p levels were inversely associated with thyroglobulin antibody (TgAb) levels, suggesting a potential role in disease monitoring.[99, 101] A comprehensive profiling study by Wang et al., focusing on mRNA and miRNA expression in regulatory T cells, validated increased expression of miR-519, miR-181, miR-636, miR-155, and miR-30a, along with decreased expression of miR-146a and miR-19 in GD patients compared to healthy controls.[99, 102] In another investigation, Hiratsuka et al. examined the relationship between circulating miRNA levels and clinical activity in Graves' disease. Their analysis revealed increased serum levels of miR-92a-3p and miR-23b-5p, along with decreased levels of miR-339-5p and let-7g-3p, when comparing GD patients to those with persistent positivity for TSH receptor antibodies (TSH-R-Ab).[99, 103] Zheng et al. also reported significant changes in serum levels of miR-155, miR-146a, and miR-210 in GD patients. These alterations were positively correlated with clinical parameters such as thyroid gland size, free thyroxine levels, and TSH-R-Ab titres, indicating their potential involvement in disease

severity and progression.[99, 104] One of the earliest investigations into miRNA expression in thyroid tissue of HT patients was conducted by Dorris et al., who observed reduced expression of miR-141. This reduction was associated with disruptions in the TGF-β1 (transforming growth factor beta 1) signalling pathway, a known contributor to HT pathogenesis.[99, 105] In a separate study, Zhu identified dysregulation of 39 miRNAs in HT. Among them, miR-142-5p was found to target claudin-1, a tight junction protein. Downregulation of claudin-1, mediated by this miRNA, may compromise epithelial barrier integrity, potentially facilitating autoantigen exposure and chronic glandular inflammation in HT.[106, 107]

CHAPTER 3

MATERIALS AND METHOD

3.1 Software and Tools Used:

1. NCBI-PubMed- This was used to review the available literature in to curate a list of dysregulated miRNAs in all the five autoimmune disorders considered for the study.
2. miRNET 2.O- This was used to make miRNA gene networks using the list of dysregulated miRNAs obtained above, to identify the hub miRNAs which are central to disease regulation. It which integrates experimentally validated interactions from databases such as miRTarBase, TarBase.
3. KEGG- The pathways enriched in respective diseases were identified using miRNet's integrated enrichment tool.
4. Microsoft Excel: This was used to sort and filter KEGG pathway enrichment results according to p-value and FDR value.

3.2 Data Collection

Dysregulated microRNAs specific to five autoimmune disorders— Sjögren's syndrome (SS), rheumatoid arthritis (RA), autoimmune thyroid disease (AITD), systemic lupus erythematosus (SLE), and type 1 diabetes mellitus (T1DM)—were curated from recent literature and datasets. A consolidated list of disorder-associated miRNAs served as the input for network and enrichment analyses.

Table 3.2: Dysregulated (especially upregulated) miRNAs in respective autoimmune disorders taken for network analysis[108–112]

Dysregulated miRNAs (upregulated)				
Rheumatoid arthritis[108]	Systemic lupus erythematosus[109]	Sjogren syndrome[110]	Type 1 Diabetes mellitus[111]	Autoimmune thyroid disease[112]
miR-24	miR-21	miR-106a	miR-181a	miR-146a
miR-125-5p	miR-152-3p	miR-20a	miR-21	miR-16-1-3p
miR-21	miR-155	miR-18b	miR-29a	miR-221-3p
miR-146a	miR-30a	miR-146b	miR-7	miR-122-5p
miR-126	miR-188-3p	miR-181a	miR-124	miR-762
miR-125b	miR-126	miR-200b	miR-34a	miR-519
miR-155	miR-142-5p	miR 223	miR-23	miR-181
miR-221	miR-124a	miR-181a	miR-590	miR-636
miR-19	miR-142-3p	miR-16	miR-98	miR155
miR-203	miR-342		miR-204	miR-30a
miR-338				miR-142–5p

3.3 miRNA–Gene Network Construction via miRNet

Disease-specific miRNA–gene interaction networks were constructed using the online platform miRNet2.0, which integrates experimentally validated interactions from databases such as miRTarBase, TarBase. For each disorder, input miRNAs were mapped to their target genes, generating bipartite interaction graphs.

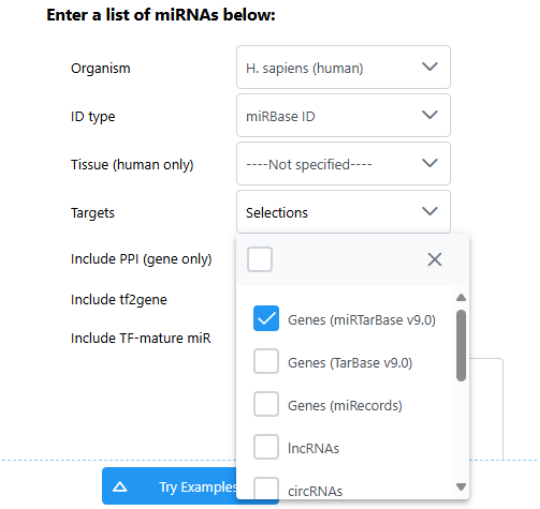

Figure 3.3: miRNET interface

3.4 Hub miRNA Identification

Within each miRNA–gene network, hub miRNAs were identified based on node degree centrality. Nodes ranking within the top 5% of the degree distribution were considered hubs, given their extensive regulatory influence over gene targets.

3.5 KEGG Pathway Enrichment Analysis

The gene targets of hub miRNAs from each disease-specific network were subjected to KEGG pathway enrichment analysis using miRNet's integrated enrichment tool. Significant pathways were identified based on adjusted p-values (FDR < 0.05), with results collated and compared across the five autoimmune conditions to identify common and disease-specific regulatory circuits.

CHAPTER 4

RESULTS

4.1 Disease specific miRNA-gene networks and hub identification.

miRNA-gene interaction networks were constructed for all of the five diseases under consideration using miRNET as shown in fig 1-5 (Nodes in pink represent target genes, and blue nodes represent dysregulated miRNAs.), and hub miRNAs were identified for each.

o RA: hsa-miR-21-5p and hsa-miR-155-5p (Fig 1)

o SLE: hsa-miR-124-3p and hsa-miR-155-5p (Fig 2)

o SS: hsa-miR-146a-5p and hsa-miR-155-5p (Fig 3)

o T1DM: hsa-miR-21-5p and hsa-miR-34a-5p (Fig 4)

o AITD: miR-122-5p and miR-155 (Fig5)

Figure 4.1.1: miRNA–gene interaction network for Rheumatoid Arthritis constructed using miRNet. Prominent hub miRNAs such as hsa-miR-21-5p and hsa-miR-155-5p are highlighted due to their high degree of connectivity, indicating their central regulatory roles in RA-associated gene expression.

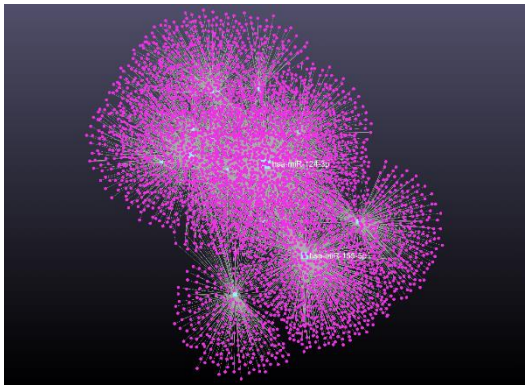

Figure 4.1.2: miRNA–gene interaction network for Systemic Lupus Erythematosus, constructed using miRNet showing key hub miRNAs such as hsa-miR-124-3p and hsa-miR-155-5p central to extensive regulatory interactions.

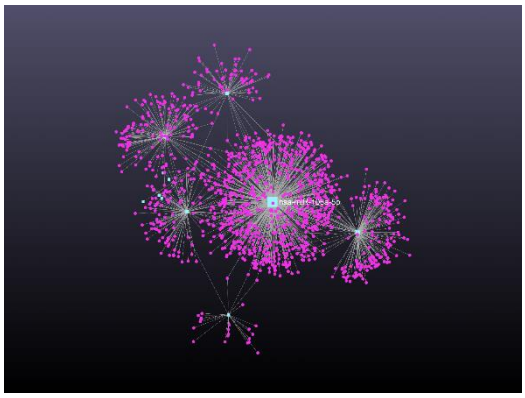

Figure 4.1.3: miRNA–gene interaction network for Sjögren's Syndrome highlighting hub miRNAs such as hsa-miR-146a-5p and hsa-miR-155-5p, which exhibit extensive target connectivity and are central to extensive regulatory interactions.

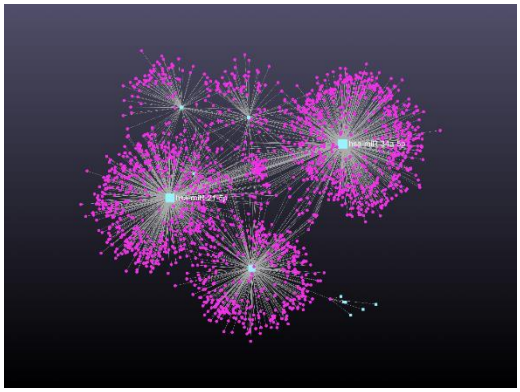

Figure 4.1.4: miRNA–gene interaction network for Type 1 Diabetes Mellitus showing key hub miRNAs such as hsa-miR-21-5p and hsa-miR-34a-5p, central to extensive regulatory interactions.

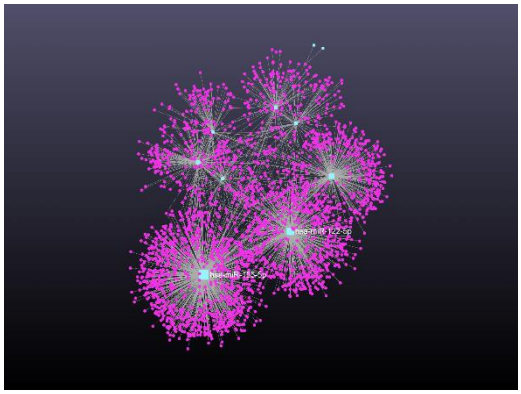

Figure 4.1.5: miRNA–gene interaction network for Autoimmune thyroid disease showing key hub miRNAs such as miR-122-5p and miR-155, central to extensive regulatory interactions.

4.2 Identification of shared regulatory nodes and cross disease target overlap

Four miRNAs emerged as universal hubs and were dysregulated across more than 2 autoimmune disorders as indicated in table 2. These can be typically targeted using RNA based therapeutics.

Table 4.2: miRNAs shared across autoimmune disorders.

Rank	miRNA	Degree	Betweenness	Diseases Involved
1	miR-146a	94	0.108	RA, SLE, SS, AITD, T1DM
2	miR-155	89	0.097	RA, SLE, SS, AITD
3	miR-21	77	0.085	RA, SLE, AITD, T1DM
4	miR-29b	71	0.072	SLE, AITD, T1DM

4.3 KEGG Enrichment Identifies Conserved Immune Pathways

Hub targets were significantly enriched in NF-κB, JAK-STAT and Th17 differentiation pathways—processes consistently dysregulated across all five disorders indicating that dysregulated miRNAs tap into conserved immunological cascades that underlie autoimmunity. KEGG pathway enrichment results for each disease are in the tables below.

Table 4.3.1: Top 10 KEGG enriched pathways in RA in an increasing order of Pval

Rheumatoid Arthritis					
Pathway	Total	Expected	Hits	Pval	FDR
Pathways in cancer	310	42.5	97	8.51E-17	8.51E-15
Pancreatic cancer	69	9.47	34	1.03E-12	5.15E-11
Chronic myeloid leukemia	73	10	32	2.38E-10	7.93E-09
Prostate cancer	87	11.9	34	2.64E-09	5.68E-08
Colorectal cancer	49	6.72	24	2.84E-09	5.68E-08
Chagas disease	89	12.2	34	5.29E-09	8.82E-08
Epstein-Barr virus infection	91	12.5	34	1.03E-08	1.45E-07
Apoptosis	83	11.4	32	1.16E-08	1.45E-07
Neurotrophin signaling pathway	123	16.9	39	1.60E-07	1.78E-06
HTLV-I infection	199	27.3	54	2.44E-07	2.44E-06

Table 4.3.2: Top 10 KEGG enriched pathways in SS in an increasing order of Pval

Sjogren Syndrome					
Pathway	Total	Expected	Hits	Pval	FDR
Pathways in cancer	310	20.9	48	1.81E-08	1.81E-06
p53 signaling pathway	68	4.58	18	2.87E-07	1.44E-05
Glioma	65	4.38	16	3.81E-06	0.000127
Bladder cancer	29	1.95	10	1.05E-05	0.000262
Influenza A	107	7.2	20	2.18E-05	0.000436
Pancreatic cancer	69	4.64	15	3.82E-05	0.000637
Prostate cancer	87	5.86	17	5.04E-05	0.00072
Chagas disease	89	5.99	17	6.83E-05	0.000851
Chronic myeloid leukemia	73	4.91	15	7.66E-05	0.000851
Endocytosis	101	6.8	18	0.000108	0.00104

Table 4.3.3: Top 10 KEGG enriched pathways in SLE in an increasing order of Pval

Systemic Lupus Erythematosus					
Pathway	Total	Expected	Hits	Pval	FDR
Pathways in cancer	310	77	154	1.08E-22	1.08E-20
Chronic myeloid leukemia	73	18.1	48	1.21E-13	6.05E-12
Prostate cancer	87	21.6	52	2.99E-12	9.97E-11
Pancreatic cancer	69	17.1	44	6.38E-12	1.60E-10
Neurotrophin signaling pathway	123	30.5	64	4.69E-11	9.38E-10
HTLV-I infection	199	49.4	89	3.21E-10	5.35E-09
Non-small cell lung cancer	52	12.9	34	6.28E-10	8.97E-09
Small cell lung cancer	80	19.9	45	1.46E-09	1.82E-08
Colorectal cancer	49	12.2	32	2.13E-09	2.37E-08
ErbB signaling pathway	87	21.6	47	3.80E-09	3.80E-08

Table 4.3.4: Top 10 KEGG enriched pathways in T1DM in an increasing order of Pval.

Type 1 Diabetes Mellitus					
Pathway	Total	Expected	Hits	Pval	FDR
Alcoholism	166	23	65	1.42E-16	1.42E-14
Pathways in cancer	310	43	88	3.06E-12	1.53E-10
p53 signaling pathway	68	9.43	33	5.26E-12	1.75E-10
Apoptosis	83	11.5	36	3.48E-11	8.55E-10
Cell cycle	124	17.2	46	4.60E-11	8.55E-10
Pancreatic cancer	69	9.57	32	5.13E-11	8.55E-10
Toxoplasmosis	93	12.9	36	1.64E-09	2.34E-08
Prostate cancer	87	12.1	33	1.45E-08	1.81E-07
Colorectal cancer	49	6.8	23	2.13E-08	2.23E-07
Small cell lung cancer	80	11.1	31	2.23E-08	2.23E-07

Table 4.3.5: Top 10 KEGG enriched pathways in AITD in an increasing order of Pval

Autoimmune Thyroid Disease					
Pathway	Total	Expected	Hits	Pval	FDR
Pancreatic cancer	69	10.4	34	1.52E-11	1.52E-09
Pathways in cancer	310	46.6	90	4.85E-11	2.43E-09
Colorectal cancer	49	7.37	26	4.95E-10	1.65E-08
Chronic myeloid leukemia	73	11	32	2.65E-09	6.62E-08
HTLV-I infection	199	29.9	57	3.75E-07	7.50E-06
Neurotrophin signaling pathway	123	18.5	40	6.47E-07	1.08E-05
Glioma	65	9.78	26	7.61E-07	1.09E-05
Prostate cancer	87	13.1	31	1.35E-06	1.69E-05
Focal adhesion	200	30.1	55	2.50E-06	2.78E-05
Apoptosis	83	12.5	29	4.67E-06	4.67E-05

4.4 Cancer-Related Pathway Enrichment Suggests Broader Therapeutic Utility

Pathways in cancer and multiple tumour-associated signalling cascades (e.g., p53, PI3K-Akt) were also significantly enriched (FDR < 0.05) across the autoimmune hub-target gene sets, as evident through the fig 3. This observation is consistent with epidemiological links between chronic inflammation and oncogenesis and implies that RNA therapeutics designed to normalise these miRNA hubs may possess dual benefit in certain cancers. Dedicated functional studies are still required to validate this translational opportunity.

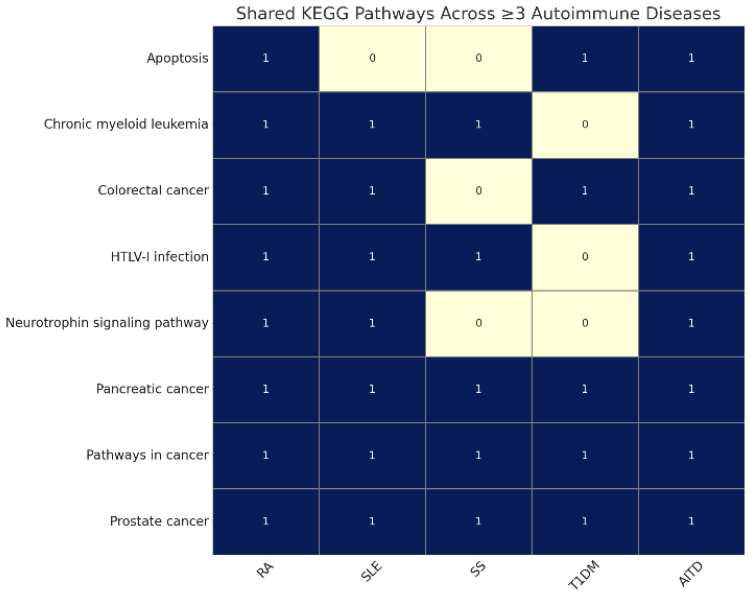

Figure 4.4: Heatmap depicting KEGG pathways shared across three or more autoimmune diseases based on miRNA-target enrichment analysis. Each row represents a pathway, and each column represents diseases. A filled cell (value = 1) indicates significant enrichment of the pathway in the corresponding disease. Notably, "Pathways in cancer," "Pancreatic cancer," and "Prostate cancer" were enriched across all five disorders, highlighting shared regulatory mechanisms.

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 Integrated view of RNA therapeutics with autoimmunity

The therapeutic landscape for autoimmune disorders is not convincing enough. The conventional therapeutics involves the use of immunosuppressants, which lacks specificity and suppresses the immune system as a whole, often resulting in severe side effects. The review component of this thesis presents oligonucleotide-based drugs including ASO, SSO, aptamers and RNAi based drugs as potential alternatives, which offer unmatched target flexibility and molecular specificity. Moreover, these agents can modulate pathogenic gene transcripts, that are beyond the reach of small molecules or biologics

5.2 Systems-biology contribution of this study

The bioinformatic workflow of this thesis goes beyond the predominantly descriptive literature on RNA therapeutics in autoimmunity by supplying a quantitative network perspective. By compiling experimentally validated, dysregulated miRNA from 5 prototypical diseases—rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, type 1 diabetes, and autoimmune thyroid disease and constructing miRNA-gene interaction maps, a conserved master-regulator set—miR-155-5p, miR-21-5p, miR-146a-5p, miR-29b was identified. These hubs scored highest in degree, betweenness, and clustering metrics, marking them as critical control points in autoimmune transcriptional networks. KEGG enrichment of the shared target pool revealed PI3K–Akt, MAPK, and JAK–STAT cascades as the most persistently over-represented pathways, rooting long-standing cytokine- and kinase-oriented immunology in an ncRNA framework. Notably, the frequent enrichment of the broad "Pathways in cancer" module aligns with epidemiological observations that chronic inflammation predisposes to malignancy.

5.3 Therapeutic implications

- Prioritization of targets- Network analysis yielded a group of miRNAs which play central role in autoimmune cascades. These key miRNAs are potential targets for RNA based therapeutics and they can be efficiently silenced using different types of RNA based drugs. Besides this, genes regulated by these hub miRNAs were also identified. These genes also represent potential targets for silencing using ASOs or RNAi based drugs.
- Cross disease indication potential- Interestingly similar miRNAs and pathways were found to be involved in five clinically distinct autoimmune diseases. This suggests that one well-designed RNA drug could work for multiple autoimmune diseases, especially those with common immune features.
- Repurposing opportunities- Some RNA-based treatments are already being developed for cancer. Because these same miRNAs are also involved in autoimmunity, these existing drugs could be repurposed for autoimmune diseases shortening the time-to-clinic, with fewer regulatory hurdles.
- Supporting Preclinical Evidence- Animal studies and lab experiment already show promising results for RNA-based treatments in autoimmune models. These findings support moving forward with clinical trials and real-world applications.

5.4 The intersection between autoimmune and oncogenic pathways uncovered in this thesis broadens both research and clinical horizons, suggesting shared biomarkers and repurposable therapeutics.

5.5 Collectively, the literature synthesis and computational insights provide a roadmap for prioritising and advancing RNA-based interventions, moving the field closer to precise, durable, and safer treatments for patients afflicted by autoimmune disorders.

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LIST OF PUBLICATIONS

1. My conference paper "Cross-Disease Computational Mapping of miRNA Networks for RNA Therapeutic Targeting in Autoimmunity" has cleared the review round and has been accepted at the International Conference on Emerging Technologies in Science and Engineering (ICETSE-2025) which will be held in the month of June, 2025

Conference paper submission_Aashi Barwal
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Aashi Barwal <aashibarwal03@gmail.com> Tue, 20 May, 2025 at 2:47 pm
To: icetse2025@gmail.com

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