Review

MicroRNA-Mediated Regulatory Flux: Navigating the Nexus Between Thermal Stress and Host Immunity

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Posted Date: 5 May 2026

doi: 10.20944/preprints202605.0252.v1

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Review

MicroRNA-Mediated Regulatory Flux: Navigating the Nexus Between Thermal Stress and Host Immunity

Running Title: miRNA Nexus in Heat and Immune Stress

Olanrewaju B. Morenikeji [1,*], Marvellous Agbanusi [2], Oluwatobi Fijabi [3], Muyiwa S. Adegbaju [4], Kyra A Baker-Waters [1], Maneiqua C. Marshall [1], Prakash K Singh [1], Xiomara Lane [1], Adeola O Ayoola [5], Adedeji O Adetunji [6], Jason W. Younkin [1], Godstime Taiwo [3], Mabel O Akinyemi [7], Ibukun M. Ogunade [8], Bolaji N. Thomas [4], Sunday O. Peters [9] and Kathryn H. Shows [1,*]

[1] Department of Biology, Virginia State University, Petersburg, VA, 23806, United States
[2] Division of Biological and Health Sciences, University of Pittsburgh at Bradford, Bradford PA 16701, United States
[3] Department of Animal Science and Range Sciences, New Mexico State University, Las Cruces, NM 88003, United States
[4] Department of Biomedical Sciences, College of Health Science and Technology, Rochester Institute of Technology, Rochester, United States
[5] Department of Human Genetics David Geffen School of Medicine, University of California, Los Angeles, United States
[6] Department of Agriculture, University of Arkansas at Pine Bluff, Pine Bluff, AR 71601, USA
[7] Department of Biology, Montclair State University, Montclair NJ United States
[8] Division of Animal and Nutritional Science, West Virginia University, Morgantown, WV, 26505, United States
[9] Department of Animal and Dairy Science, University of Georgia, Athens, GA 30602, United States
* Correspondence: omorenikeji@vsu.edu (O.B.M.); kshows@vsu.edu (K.H.S.)

Abstract

MicroRNAs (miRNAs) are evolutionarily conserved, ~22-nucleotide non-coding RNAs that orchestrate post-transcriptional gene silencing across virtually all metazoan lineages. Since the landmark discovery of *lin-4* in *Caenorhabditis elegans* over three decades ago, the miRNA field has expanded to encompass more than 2,600 annotated human miRNAs collectively targeting over 60% of protein-coding genes, establishing miRNAs as master regulators of cellular homeostasis, differentiation, and stress adaptation. This review articulates a conceptual framework positioning miRNAs as integrative molecular nodes at the intersection of two fundamental biological processes: the heat shock response (HSR) and host immunity. We synthesize evidence demonstrating that thermal stress, whether arising from environmental hyperthermia, febrile immune responses, or climate-driven ecological perturbation, profoundly reshapes miRNA biogenesis, stability, and target engagement, with cascading consequences for innate and adaptive immune signaling. Mechanistically, we trace how heat shock factor 1 (HSF1) undergoes temperature-dependent liquid–liquid phase separation to activate transcription of heat shock proteins (HSPs) and specific miRNA loci, generating feedback circuits that calibrate inflammatory tone through NF-κB, TLR, and inflammasome pathways. Multi-omics integration including transcriptomics, proteomics, metabolomics, and small RNA sequencing has revealed a systems-level "regulatory flux" in which miRNAs dynamically buffer cellular responses to concurrent thermal and immunological challenges. We further examine how pathogens exploit and subvert host miRNA circuits during infection, and how extracellular vesicle-mediated miRNA transfer enables intercellular and inter-organ stress communication. Finally, we survey the translational horizon, including miRNA mimics, antagomiRs, engineered delivery systems, and synthetic miRNA circuits, highlighting both therapeutic promise and outstanding challenges. By reframing miRNAs as rheostatic integrators bridging stress physiology and immunology, this review establishes a foundation for interdisciplinary research at the thermal stress–immunity nexus.

Keywords: microRNA; heat shock response; immunity; thermal stress; extracellular vesicles; multi-omics; miRNA therapeutics; host–pathogen interaction

1. Introduction

The discovery of microRNAs (miRNAs) stands as one of the most transformative advances in molecular biology since the elucidation of the genetic code. In 1993, Victor Ambros and colleagues identified *lin-4*, a small non-coding RNA in *Caenorhabditis elegans* that regulated developmental timing through antisense complementarity to the 3′ untranslated region (UTR) of *lin-14* mRNA [1]. Concurrently, Ruvkun et al. demonstrated that this regulatory interaction mediated post-transcriptional repression of LIN-14 protein without substantially altering mRNA abundance [2]. These findings, initially regarded as a nematode-specific curiosity, were dramatically recontextualized seven years later when *let-7* was identified as a second heterochronic small RNA in *C. elegans* with deeply conserved homologs across bilaterian phyla, including humans [3,4]. The floodgates subsequently opened: systematic cloning efforts, computational prediction algorithms, and deep sequencing technologies collectively expanded the known miRNA repertoire to more than 2,600 annotated human miRNAs catalogd in miRBase v22.1 [5,6], with recent evidence suggesting that a substantial fraction of additional functional small RNAs remain to be characterized [7].

The regulatory reach of miRNAs is staggering. Computational analyses estimate that more than 60% of human protein-coding genes harbor conserved miRNA binding sites within their 3′ UTRs [8], and individual miRNAs can simultaneously modulate hundreds of transcripts, thereby functioning as master regulators of gene expression networks [9]. The mechanistic basis for this regulation involves loading of the mature ~22-nucleotide (nt) miRNA guide strand onto Argonaute (AGO) family proteins to form the RNA-induced silencing complex (RISC), which recognizes target mRNAs through seed-region complementarity (typically nucleotides 2–8 of the guide strand) and induces translational repression, mRNA deadenylation, and/or endonucleolytic cleavage [9,10]. A landmark comprehensive review by Kim et al. in 2025 synthesized decades of biogenesis research, revealing that the apparent simplicity of miRNA maturation belies an extraordinarily complex regulatory architecture involving post-transcriptional modifications, phase separation of processing complexes, target-directed miRNA decay (TDMD), and nuclear functions of miRNA-AGO complexes [7].

Beyond their housekeeping roles in development and tissue homeostasis, miRNAs have emerged as critical mediators of cellular stress responses. The heat shock response (HSR), a phylogenetically ancient cytoprotective program triggered by proteotoxic stress, represents one of the most intensively studied stress pathways in biology [11,12]. Activation of heat shock factor 1 (HSF1) drives rapid transcription of heat shock proteins (HSPs), which function as molecular chaperones to maintain proteostasis under thermal duress [13,14]. Recent groundbreaking work has revealed that HSF1 itself undergoes temperature-dependent liquid–liquid phase separation (LLPS) with lower critical solution temperature (LCST) behavior, establishing a direct biophysical link between temperature sensing and transcriptional activation [15]. Simultaneously, the mammalian immune system has evolved to deploy fever as a frontline defense mechanism against pathogenic challenge [16,17]. This evolutionary convergence of thermal stress physiology and host immunity suggests the existence of shared molecular circuitry, yet the regulatory logic connecting these two fundamental processes remains incompletely understood.

MicroRNAs are uniquely positioned to serve as integrative nodes bridging the HSR and immune signaling. Several lines of evidence support this assertion. First, thermal stress profoundly alters miRNA biogenesis at multiple levels, from transcription of primary miRNA (pri-miRNA) transcripts through nuclear processing, cytoplasmic maturation, and RISC assembly [18,19]. Second, key immune-regulatory miRNAs including miR-146a, miR-155, miR-21, and miR-223 are themselves thermally responsive, with expression levels that shift dynamically during febrile episodes [20,21]. Third, HSPs released into the extracellular milieu during thermal stress function as damage-associated molecular patterns (DAMPs) that activate innate immune receptors, particularly Toll-like receptor 2 (TLR2) and TLR4, initiating signaling cascades that are themselves subject to miRNA-mediated fine-tuning [22,23]. Fourth, the advent of multi-omics technologies integrating transcriptomics, proteomics, metabolomics, and epigenomics has enabled systems-level interrogation of miRNA regulatory networks under combined thermal and immunological stress, revealing emergent properties that cannot be captured by single-pathway analyses [24,25].

The urgency of understanding miRNA-mediated regulatory flux at the thermal stress–immunity nexus is amplified by contemporary global challenges. Anthropogenic climate change is driving unprecedented increases in ambient temperature extremes, with profound consequences for both human health and agricultural productivity [26]. Heat waves are associated with increased susceptibility to infectious diseases, impaired vaccine efficacy, and dysregulated inflammatory responses [27]. In livestock species, chronic heat stress compromises immune competence and elevates disease burden, with substantial economic implications for food security [28,29]. Moreover, the emergence and re-emergence of zoonotic pathogens exemplified by SARS-CoV-2, influenza H5N1, and dengue virus occur against a backdrop of shifting thermal ecologies that modulate both vector biology and host immunity [30,31]. Understanding how miRNAs integrate thermal and immunological signals is therefore not merely an academic exercise but a prerequisite for developing rational therapeutic and agricultural interventions.

This review provides a comprehensive, mechanistically detailed synthesis of the current understanding of miRNA-mediated regulatory flux at the interface of thermal stress and host immunity. We begin by surveying the molecular machinery of miRNA biogenesis and its multi-layered regulation (Section 2), followed by an in-depth examination of the heat shock response and its integration with miRNA networks (Section 3). We then dissect the roles of miRNAs in innate and adaptive immune signaling, with particular attention to the convergence with thermal stress pathways (Section 4), and explore miRNA dynamics at the host–pathogen interface (Section 5). Subsequent sections address extracellular vesicle-mediated miRNA transfer (Section 6), therapeutic and engineering applications (Section 7), and emerging frontiers (Section 8). Throughout, we emphasize multi-omics perspectives, evolutionary conservation, and translational relevance, aiming to catalyse interdisciplinary research at this critical biological nexus.

2. miRNA Biogenesis: Molecular Machinery and Regulatory Layers

2.1. Canonical Biogenesis Pathway

As shown in Figure 1, the canonical miRNA biogenesis pathway begins with transcription of miRNA genes by RNA polymerase II (Pol II), yielding primary miRNA transcripts (pri-miRNAs) that possess 5′ 7-methylguanosine caps and 3′ poly(A) tails, features shared with protein-coding mRNAs [32,33]. Pri-miRNAs contain one or more hairpin structures, each consisting of an imperfectly base-paired stem of approximately 33–35 base pairs (bp), a terminal loop, and flanking single-stranded RNA segments [7,34]. The nuclear processing of pri-miRNAs is executed by the Microprocessor complex, a heterotrimeric assembly comprising one molecule of the RNase III enzyme Drosha and two molecules of the double-stranded RNA-binding protein DGCR8 (DiGeorge syndrome critical region 8, also known as Pasha in invertebrates) [35,36]. Structural and biochemical studies have established that DGCR8 recognizes the pri-miRNA hairpin through a combination of *cis*-acting sequence and structural motifs: the basal UG motif at the single-stranded RNA–stem junction, the apical UGU or UGUG motif near the terminal loop, a mismatched GHG motif within the lower stem, and the overall stem length of ~35 bp [7,37,38]. DGCR8 anchors the apical junction while Drosha measures approximately 11 bp from the basal junction to introduce staggered cuts, generating a ~60–70 nt precursor miRNA (pre-miRNA) hairpin with a characteristic 2-nt 3′ overhang [39,40].

doi:10.20944/preprints202605.0252.v1

Recent work has added nuance to this canonical framework. Yang et al. (2025) demonstrated that intrinsically disordered regions (IDRs) within Drosha selectively promote the biogenesis of a specific subset of miRNAs, suggesting that the Microprocessor is not a monolithic processing machine but rather a tunable complex whose substrate selectivity is modulated by the conformational dynamics of its own subunits [41]. Furthermore, evidence has emerged that the Microprocessor can undergo liquid–liquid phase separation (LLPS) in certain cellular contexts, potentially concentrating processing substrates and enhancing catalytic efficiency in a manner analogous to other nuclear condensates [42,43]. These findings underscore the principle that miRNA biogenesis is not a constitutive, invariant process but rather a dynamically regulated program subject to modulation by cellular state, including thermal stress.

Following nuclear processing, pre-miRNAs are exported to the cytoplasm through the nuclear pore complex by Exportin-5 (XPO5) in a Ran-GTP–dependent manner [44,45]. XPO5 recognizes the ~2-nt 3′ overhang and the double-stranded stem of the pre-miRNA, protecting it from nuclear degradation during transit [46]. In the cytoplasm, the pre-miRNA is cleaved by a second RNase III enzyme, Dicer, which operates in concert with the trans-activation response RNA-binding protein (TRBP, also known as TARBP2) [47,48]. Dicer employs two distinct ruler mechanisms for measuring the cleavage site: a 5′-counting rule, in which the PAZ domain anchors the 5′ phosphate of the pre-miRNA and measures ~22 nt to the cleavage site, and a loop-counting rule, in which the distance from the terminal loop determines the cut position [49,50]. The two RNase III domains of Dicer introduce staggered cuts to liberate an ~22-nt miRNA duplex with 2-nt 3′ overhangs at both ends [51].

The final step of canonical biogenesis involves loading the miRNA duplex onto an AGO protein to form the RISC. In humans, four AGO paralogs (AGO1–4) can load miRNAs, but only AGO2 retains endonucleolytic (“slicer”) activity [52,53]. Guide strand selection is governed by the thermodynamic asymmetry rule: the strand whose 5′ end is less stably base-paired is preferentially retained as the guide strand, while the opposing “passenger” strand (miRNA*) is ejected and degraded [54,55]. Recent cryo-electron microscopy (cryo-EM) structures of human AGO2 in complex with fully paired guide-target RNA, reported by Mohamed et al. (2025), have revealed the structural rearrangements that enable the slicing-competent conformation [56]. Critically, the N domain of AGO2 rotates to permit unimpeded passage of the RNA duplex through the central channel, while a conserved loop in the PIWI domain secures the RNA near the catalytic site, enhancing both slicing rate and specificity [56]. These structural insights have profound implications for the rational design of siRNA-based therapeutics and illuminate how target-binding geometry modulates AGO2 activity under varying cellular conditions, including thermal stress.

2.2. Non-Canonical Biogenesis Pathways

While the canonical Drosha–Dicer–AGO pathway accounts for the maturation of the majority of miRNAs, several non-canonical routes have been delineated, each with potential significance for stress-responsive small RNA generation [7,57]. Mirtrons constitute a class of Drosha-independent miRNAs in which the pre-miRNA hairpin corresponds precisely to a short intron, such that splicing and lariat debranching yield a pre-miRNA substrate directly accessible to Dicer without Microprocessor cleavage [58,59]. First identified in *Drosophila melanogaster* and *C. elegans*, mirtrons have subsequently been catalogd in mammals and may expand the repertoire of miRNAs available under conditions such as thermal stress where Drosha activity is limiting [60].

The most extensively characterized Dicer-independent miRNA is miR-451, which is processed by AGO2 itself [61,62]. Following Drosha cleavage, the pre-miR-451 hairpin is too short (~18 bp stem) for Dicer recognition and is instead loaded directly onto AGO2, which cleaves the 3′ arm of the hairpin through its slicer activity (Figure 1). The resulting intermediate is subsequently trimmed by the poly(A)-specific ribonuclease PARN to generate the mature miR-451 [63]. This pathway is of particular immunological significance because miR-451 plays a critical role in erythropoiesis and has been implicated in sickle cell trait-mediated resistance to *Plasmodium falciparum* malaria [64,65]. Additional non-canonical pathways include small nucleolar RNA (snoRNA)-derived miRNAs, which are generated by Dicer processing of snoRNA precursors [66], and transfer RNA-derived fragments (tRFs), which can associate with AGO proteins and exert miRNA-like post-transcriptional regulatory functions [67,68]. The relevance of these non-canonical small RNAs to the thermal stress–immunity interface is an emerging area of investigation, as several tRFs are stress-responsive and accumulate under conditions of heat shock, oxidative stress, and nutrient deprivation [69,70].

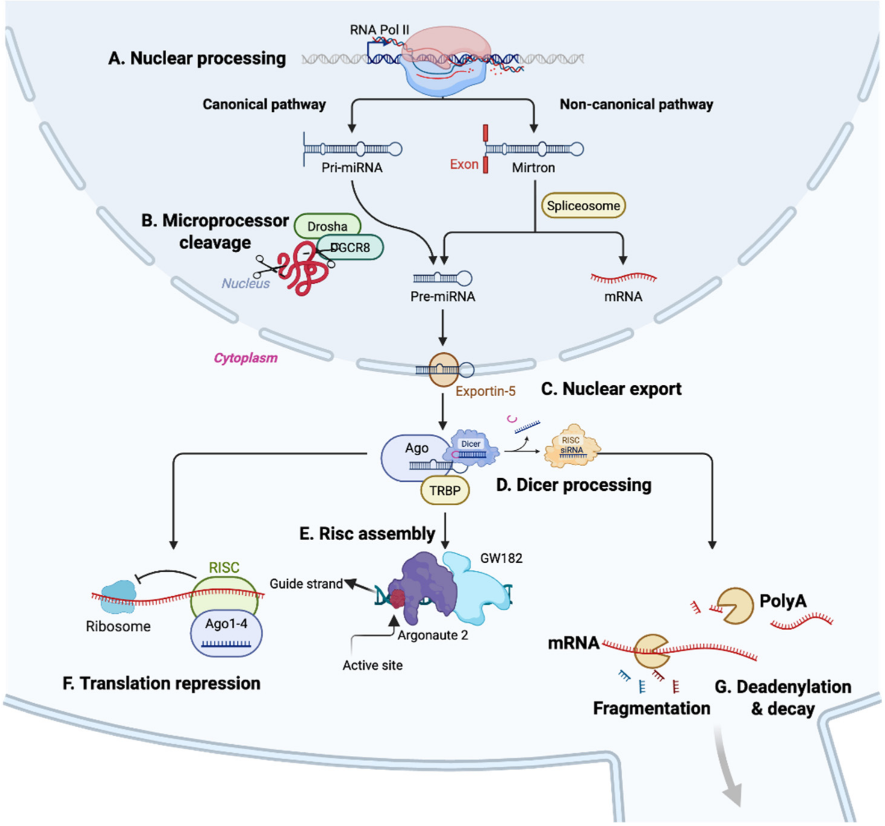

Figure 1. Canonical and non-canonical miRNA biogenesis pathways and regulatory checkpoints. This schematic delineates the spatiotemporal lifecycle of miRNA maturation from transcription to target silencing. (A) Canonical pathway: RNA Pol II transcribes pri-miRNA, which is processed by the Drosha–DGCR8 Microprocessor complex in the nucleus, recognizing the basal UG motif, apical UGU motif, GHG mismatch, and ~35-bp stem length. The resulting pre-miRNA is exported via Exportin-5/RanGTP and cleaved by the Dicer–TRBP complex in the cytoplasm using 5′-counting and loop-counting ruler mechanisms. The miRNA duplex is loaded onto AGO2, with guide strand selection determined by thermodynamic asymmetry, forming the mature RISC. (B) Non-canonical pathways: mirtrons bypass Drosha via splicing; miR-451 bypasses Dicer via AGO2-mediated slicing and PARN trimming; snoRNA-derived and tRNA-derived fragments (tRFs) represent additional stress-responsive small RNA sources. (C) Regulatory checkpoints (indicated by numbered callout boxes): (1) RNA editing (ADAR-mediated A-to-I) at the pri-miRNA level; (2) m6A modification enhancing Microprocessor recognition; (3) 3′ tailing uridylation (TUT4/TUT7) promoting degradation via DIS3L2, adenylation (TENT2) promoting stabilization; (4) Target-directed miRNA decay (TDMD) mediated by ZSWIM8 ubiquitin ligase; (5) ceRNA sponging by lncRNAs and circRNAs; (6) Nuclear AGO2 functions in transcriptional regulation. Stress-responsive modulation points are highlighted with thermal stress indicators. Arrows in red denote pathways enhanced under thermal stress; dashed arrows in blue denote pathways suppressed during heat shock. Created in BioRender https://BioRender.com/.

2.3. Post-Transcriptional Regulation of miRNA Biogenesis

The abundance and activity of mature miRNAs are modulated by a sophisticated array of post-transcriptional regulatory mechanisms that operate at every step of the biogenesis pathway, from pri-miRNA processing to mature miRNA turnover [7,71]. RNA editing by adenosine deaminases acting on RNA (ADARs) can convert adenosine to inosine (A-to-I editing) within pri-miRNA or pre-miRNA hairpins, altering processing efficiency, strand selection, and target specificity [72,73]. For example, A-to-I editing of pri-miR-142 by ADAR1 impairs Drosha cleavage and redirects the edited substrate to degradation by Tudor staphylococcal nuclease (TSN), demonstrating that RNA editing serves as a quality-control checkpoint in miRNA biogenesis [74].

The 3′ terminal modification of pre-miRNAs and mature miRNAs by nucleotidyl transferases constitutes another critical regulatory layer. Uridylation of pre-miRNAs by terminal uridylyltransferases TUT4 (ZCCHC11) and TUT7 (ZCCHC6) promotes degradation by the exonuclease DIS3L2, particularly for pre-miRNAs with non-canonical 1-nt 3′ overhangs [75,76]. Conversely, adenylation of the 3′ end of mature miRNAs by TENT2 (GLD-2) can stabilize certain miRNAs, extending their half-lives and augmenting their regulatory impact [77]. This tailing-dependent regulation of miRNA stability has implications for thermal stress responses, as the activities of TUT4/TUT7 and TENT2 are themselves subject to post-translational regulation by stress-responsive kinases [78].

Target-directed miRNA decay (TDMD) has emerged as a particularly important mechanism for selective miRNA turnover [7,79]. In TDMD, a highly complementary target RNA typically possessing extensive 3′ supplementary pairing beyond the seed region, induces conformational changes in AGO that expose the miRNA to degradation [80,81]. The ZSWIM8 ubiquitin ligase has been identified as a central effector of TDMD, mediating the polyubiquitination and proteasomal degradation of AGO proteins engaged in TDMD-competent interactions [82,83]. Well-characterized TDMD substrates include miR-7 (regulated by the circular RNA ciRS-7/CDR1as) and miR-29b (regulated by the long non-coding RNA NREP) [84,85]. TDMD provides a mechanism for rapid, target-specific elimination of individual miRNAs, enabling cells to dynamically reconfigure their miRNA landscape in response to environmental cues, including thermal perturbation.

RNA modifications on pri-miRNAs further modulate Microprocessor recognition and processing efficiency. N6-methyladenosine (m6A), installed by the METTL3–METTL14 methyltransferase complex and recognized by the reader protein HNRNPA2B1, marks a subset of pri-miRNAs for enhanced Drosha processing [86,87]. 5-Methylcytosine (m5C) modifications have also been detected on pri-miRNAs, although their functional consequences for biogenesis are less well characterized [88]. These epitranscriptomic marks create an additional layer of regulation through which signaling pathways potentially including thermal stress signaling can modulate the miRNA repertoire.

Finally, the competing endogenous RNA (ceRNA) hypothesis posits that long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) bearing multiple miRNA response elements (MREs) can function as miRNA “sponges,” titrating miRNAs away from their cognate mRNA targets and thereby derepressing gene expression [89,90]. While the physiological relevance of ceRNA interactions remains debated, particularly regarding whether endogenous ceRNAs achieve sufficient stoichiometric abundance to appreciably sequester miRNAs under normal conditions, evidence supports ceRNA functionality in specific contexts, including cancer and stress responses where individual ceRNA and miRNA expression levels are substantially perturbed [91,92].

2.4. Nuclear Functions of miRNAs

Although miRNAs have been predominantly studied in the context of cytoplasmic post-transcriptional gene silencing, a growing body of evidence indicates that AGO-miRNA complexes also reside in the nucleus, where they engage chromatin-associated RNAs and participate in transcriptional gene regulation [93,94]. Nuclear AGO2-miRNA complexes have been implicated in both transcriptional gene silencing (TGS), through recruitment of chromatin-modifying complexes to

doi:10.20944/preprints202605.0252.v1

target gene promoters, and transcriptional gene activation (TGA), through interactions with enhancer-associated RNAs [95,96]. Recent chimeric eCLIP (enhanced crosslinking and immunoprecipitation) data generated by Hofman et al. demonstrated that the canonical let-7/HMGA2 regulatory interaction occurs in both the nucleus and the cytoplasm, questioning the prevailing view that miRNA-mediated regulation is exclusively a cytoplasmic phenomenon [97]. These nuclear functions expand the potential regulatory repertoire of miRNAs under stress conditions, as thermal stress induces substantial reorganization of nuclear architecture, chromatin accessibility, and transcription factor occupancy [98,99]. Whether stress-responsive nuclear miRNA complexes contribute to the epigenetic memory of thermal stress in plants and animals represents a compelling question for future investigation.

3. The Heat Shock Response: Molecular Architecture and miRNA Integration

3.1. Heat Shock Factors and Transcriptional Activation

The heat shock response (HSR) is orchestrated primarily by heat shock factor 1 (HSF1), a transcription factor that serves as the master regulator of proteostasis under conditions of thermal and proteotoxic stress [11,100]. The domain architecture of HSF1 comprises a winged helix-turn-helix DNA-binding domain (DBD) that recognizes heat shock elements (HSEs) inverted repeats of the pentameric sequence nGAAn in the promoters of target genes; a hydrophobic heptad repeat domain (HR-A/B) that mediates ligand-independent trimerization; a regulatory domain (RD) that integrates post-translational modification signals; and a C-terminal transactivation domain (TAD) that recruits transcriptional co-activators and the Mediator complex [101,102]. Under non-stress conditions, HSF1 exists predominantly as an inactive monomer, sequestered in a repressive complex with HSP90 and co-chaperones [103]. Upon thermal stress, the accumulation of misfolded proteins titrates HSP90 away from HSF1, releasing the monomer for trimerization, nuclear accumulation, and DNA binding [104,105]. This binding initiates the rapid transcriptional upregulation of a suite of cytoprotective Heat Shock Proteins (HSPs). As shown in the final stages of the cascade in Figure 2, these chaperones function as ATP-dependent foldases, facilitating the re-folding of nascent polypeptides and preventing the formation of toxic insoluble aggregates, thereby preserving cellular proteostatic stoichiometry.

A paradigm-shifting advance in understanding HSF1 activation was reported by Ren et al. (2025), who elucidated the molecular mechanism of temperature-dependent phase separation of HSF1 [15]. Using a combination of in vitro reconstitution, all-atom molecular dynamics simulations, and live-cell imaging, these authors demonstrated that the regulatory domain (RD) of HSF1 undergoes LLPS with lower critical solution temperature (LCST) behavior that is, phase separation is promoted by increasing temperature, in direct contrast to the more commonly observed upper critical solution temperature (UCST) behavior of most intrinsically disordered protein regions [15]. This LCST behavior provides an elegant “chemical code” through which HSF1 directly senses physiological temperature: at normal body temperature (~37 °C), the RD remains soluble, but at febrile or heat-shock temperatures (>39 °C), it phase-separates into condensates that concentrate HSF1 at HSE-containing promoters, dramatically amplifying transcriptional output [15]. Post-translational modifications, particularly phosphorylation of key serine residues (S326, S303, S307), modulate the LCST threshold, providing a mechanism through which signaling pathways can tune the temperature set point for HSF1 activation [15,106]. The conceptual significance of this discovery extends well beyond the HSR: it establishes that biomolecular phase separation can serve as a bona fide thermosensor, with direct implications for understanding febrile immune responses and their miRNA-mediated regulation.

The post-translational modification (PTM) landscape of HSF1 is extraordinarily complex and constitutes a critical regulatory layer [107,108]. Phosphorylation at S326, mediated by mTOR, MEK1, and other kinases, is essential for full transactivation competence, while phosphorylation at S303 and S307, mediated by GSK3β and ERK1/2, promotes transcriptional attenuation and nuclear export [109,110]. Acetylation of HSF1 at K80 by p300/CBP impairs DNA-binding activity and promotes HSF1 release from HSEs, contributing to the attenuation phase of the HSR [111]. Sumoylation at K298 further modulates HSF1 transcriptional activity in a phosphorylation-dependent manner [112]. These PTMs do not operate in isolation but interact synergistically and antagonistically to fine-tune HSF1 activity across different temporal phases of the stress response and in different tissue contexts.

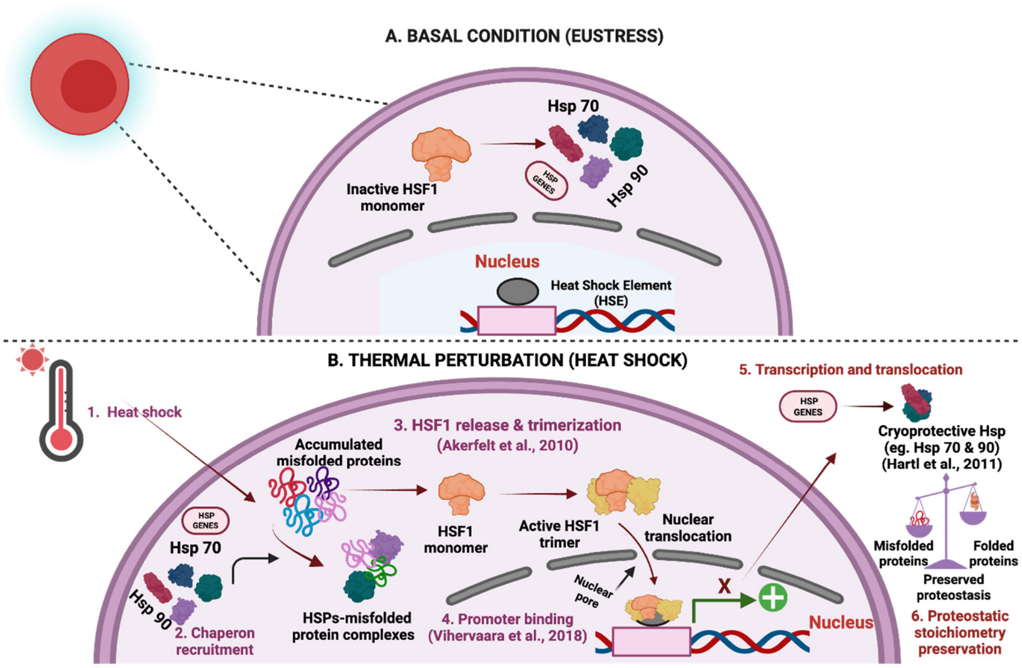

Figure 2. The canonical HSF1-HSP activation cascade. Schematic representation of the cellular Heat Shock Response (HSR) mechanism. (A) Basal conditions (Eustress): In the absence of thermal stress, Heat Shock Factor 1 (HSF1) is sequestered in the cytoplasm as an inactive monomer, bound by molecular chaperones HSP70 and HSP90 (represented by molecular brackets). (B) Thermal Perturbation (Heat Shock): Hyperthermia induces the accumulation of denatured, misfolded proteins. The requirement for immediate refolding creates a competitive demand (indicated by dynamic arrows), recruiting the HSP70/90 chaperones away from HSF1. This titration facilitates the release, homotrimerization, and nuclear translocation of activated HSF1 (represented by the orange molecular trimer). The trimer binds with high affinity to Heat Shock Elements (HSEs) within the promoter regions of target genes, initiating the rapid transcription of cytoprotective Heat Shock Proteins (HSPs). These newly synthesized foldases restore proteostatic stoichiometry and prevent the formation of toxic aggregates. Created in BioRender https://BioRender.com/.

Beyond its canonical role in the HSR, HSF1 has been increasingly recognized as a multifaceted transcription factor with constitutive functions in cancer biology, neurodegeneration, and metabolic regulation [113,114]. Xu et al. (2023) demonstrated that HSF1 forms a specific transcription factor complex with c-MYC, potentiating c-MYC-mediated transcription of oncogenic target genes independently of thermal stress [115]. This HSF1–c-MYC axis provides a mechanistic basis for the well-documented oncogenic role of HSF1 in diverse malignancies and raises the question of whether miRNAs that regulate HSF1 expression may also modulate oncogenic transcriptional programs. Among the other heat shock factor family members, HSF2 plays a role in development and spermatogenesis and can hetero-oligomerize with HSF1 to modulate the target gene repertoire of the HSR [116]. HSF4 is predominantly expressed in the lens and has been implicated in cataractogenesis [117].

3.2. Heat Shock Proteins as Molecular Chaperones

The transcriptional targets of HSF1 include the major families of heat shock proteins, which function as molecular chaperones to maintain proteostasis under thermal and proteotoxic stress [118,119]. HSP90 is among the most abundant and functionally complex chaperones in eukaryotic cells, constituting 1–2% of total cellular protein under non-stress conditions, with expression further induced during heat shock [120]. A comprehensive review by Chiosis et al. (2023) elucidated the structural and functional complexity of HSP90, including its domain architecture (N-terminal ATPase domain, middle domain for client and co-chaperone binding, and C-terminal dimerization domain), its extensive co-chaperone network (CDC37, AHA1, HOP/STIP1, p23/PTGES3), and the concept of "epichaperomes", pathological higher-order assemblies of HSP90 with co-chaperones and client proteins that form in disease states [14]. HSP90 clients include numerous protein kinases, steroid hormone receptors, and transcription factors, many of which are central to immune signaling, including JAK kinases, IκB kinase (IKK), and components of the TLR signaling apparatus [121,122].

The HSP70 family (including the stress-inducible HSPA1A/HSPA1B and the constitutive HSC70/HSPA8) operate through an ATP-dependent substrate binding cycle in which the nucleotide-binding domain (NBD) undergoes conformational changes that allosterically regulate the substrate-binding domain (SBD) [123,124]. J-domain proteins (JDPs, also known as HSP40/DNAJ co-chaperones) stimulate the ATPase activity of HSP70 and deliver substrate proteins, while nucleotide exchange factors (NEFs), including the HSP110 family (HSPH1–3) and BAG domain proteins, promote ADP-to-ATP exchange and substrate release [125,126]. The HSP70 system acts as a central hub for protein quality control, triaging substrates for productive folding, refolding, or proteasomal/autophagic degradation depending on the severity and duration of proteotoxic stress [127].

Small heat shock proteins (sHSPs), exemplified by HSP27 (HSPB1), function as ATP-independent "holdase" chaperones that bind unfolded client proteins and prevent their irreversible aggregation, maintaining substrates in a folding-competent state for subsequent processing by the HSP70/HSP90 machinery [128,129]. sHSPs exhibit remarkable oligomeric plasticity, assembling into dynamic higher-order complexes of variable size and composition, with oligomeric equilibrium influenced by temperature, phosphorylation, and redox state [130]. HSP27 phosphorylation by MAPKAPK2 downstream of p38 MAPK promotes dissociation of large oligomers into smaller, more active chaperone species, establishing a direct link between stress-activated kinase cascades and sHSP chaperone function [131]. Additionally, HSP60 (HSPD1) functions primarily within the mitochondrial matrix as a chaperonin that facilitates the folding of imported mitochondrial proteins through an encapsulation mechanism involving its co-chaperonin HSP10 (HSPE1) [132]. Extracellular release of HSP60, whether through active secretion or passive release from necrotic cells, triggers innate immune activation through TLR2 and TLR4, positioning HSP60 at the interface of mitochondrial proteostasis and immune regulation [133,134].

3.3. miRNA-Mediated Regulation of the Heat Shock Response

The intersection of miRNA regulatory networks with the HSR operates bidirectionally: specific miRNAs target components of the HSR machinery, while HSF1-driven transcription activates the expression of specific miRNA loci, generating complex feedback circuits that calibrate the magnitude and duration of the stress response [135,136]. Several miRNAs have been identified as direct regulators of HSF1 expression. miR-644a directly targets the 3′ UTR of HSF1 mRNA, suppressing HSF1 protein levels and attenuating the transcriptional amplitude of the HSR in cancer cells [137]. Similarly, miR-216a-5p has been reported to repress HSF1 expression in the context of cardiac ischemia–reperfusion injury, where HSF1 downregulation exacerbates mitochondrial dysfunction and apoptosis [138]. These miRNAs may serve as "safety valves" that prevent excessive or prolonged HSR activation, which can be detrimental as exemplified by the oncogenic consequences of constitutive HSF1 activity [114].

The HSP70 family members HSPA1A and HSPA1B are targeted by multiple miRNAs across diverse biological contexts. miR-1, a muscle-enriched miRNA, directly suppresses HSP70 expression in cardiomyocytes, and its downregulation during cardiac ischemia permits HSP70 upregulation as a cytoprotective response [139]. miR-378 targets HSP70 in the context of skeletal muscle hypertrophy, while the miR-181 family, particularly miR-181a regulates HSP70 expression in T cells, with implications for both thermal tolerance and immune cell activation thresholds [140,141]. HSP90 is targeted by miR-628-3p and miR-223, the latter of which is also a critical regulator of inflammasome activation, suggesting a mechanistic link between HSP90 chaperoning of immune signaling clients and miRNA-mediated inflammatory regulation [142,143].

Reciprocally, HSF1-driven transcription activates specific miRNA loci during the HSR. Heat shock induces the expression of miR-214, miR-320a, and several members of the miR-17-92 cluster through direct HSF1 binding to HSE motifs in their genomic regulatory regions [144,145]. These HSF1-induced miRNAs can target pro-apoptotic factors, cell cycle regulators, and immune signaling components, thereby contributing to the cytoprotective and immunomodulatory consequences of HSR activation [146]. The resulting feedforward and feedback loops create a regulatory architecture in which miRNAs both modulate and are modulated by the HSR, enabling precise temporal control over the cellular stress response. A comprehensive summary of experimentally validated miRNA–HSR target pairs, including their mechanisms of action, model organisms, and effects on thermal tolerance, is provided in Table 1.

Thermal stress also exerts profound effects on the miRNA processing machinery itself. Elevated temperatures can alter the enzymatic activities of Drosha and Dicer, the conformational dynamics of RISC assembly, and the subcellular localization of AGO proteins [18,147]. During severe heat shock, AGO2 and associated miRNAs are rapidly sequestered into stress granules (SGs) cytoplasmic ribonucleoprotein condensates that form upon translational arrest, potentially reducing the effective concentration of functional RISC available for target silencing [148,149]. This SG sequestration represents a mechanism through which acute thermal stress can transiently reprogram the miRNA regulatory landscape, with implications for the post-stress recovery of gene expression homeostasis. The bidirectional regulatory architecture linking miRNAs with the HSR encompassing miRNA-mediated suppression of HSF1 and HSP targets, HSF1-driven transcription of miRNA loci, and thermal stress-induced perturbation of miRNA processing machinery is depicted schematically in Figure 3.

Table 1. Key miRNAs modulating heat shock response components.

miRNA	**Target gene(s)**	**Mechanism of action**	**Organism/Model**	**Effect on thermal tolerance**	**Reference(s)**
miR-644a	HSF1	Direct 3′ UTR targeting; translational repression	Human (cancer cell lines)	Reduced HSR amplitude; increased thermosensitivity	[137]
miR-216a-5p	HSF1	Direct 3′ UTR targeting; mRNA destabilization	Mouse (cardiac I/R)	Impaired cardioprotection; increased apoptosis	[138]

miR-1	HSPA1A/HSPA1B (HSP70)	Direct 3′ UTR targeting	Human/mouse (cardiomyocytes)	Attenuated cytoprotection; exacerbated ischemic injury	[139]
miR-378	HSPA1A (HSP70)	Direct 3′ UTR targeting	Mouse (skeletal muscle)	Modulated muscle recovery under heat stress	[140]
miR-181a	HSPA1A (HSP70)	3′ UTR targeting; translational repression	Human (T cells)	Altered T cell activation threshold under febrile conditions	[141]
miR-628-3p	HSP90AA1	Direct 3′ UTR targeting	Human (cancer cells)	Destabilization of HSP90 client proteins; reduced thermotolerance	[142]
miR-223	HSP90B1	Indirect regulation via NLRP3 inflammasome pathway	Human/mouse (myeloid cells)	Modulated inflammatory response to thermal stress	[143]
miR-214	PTEN, BAX	HSF1-induced; targets pro-apoptotic genes	Human (hepatocytes)	Enhanced cell survival under heat stress	[144]
miR-320a	MAPK1, NFKB1	HSF1-induced; attenuates inflammatory signaling	Human (endothelial cells)	Dampened inflammation during hyperthermic stress	[145]
miR-27a	HSPA5 (GRP78/BiP)	3′ UTR targeting; ER stress modulation	Bovine (mammary epithelial cells)	Compromised UPR under combined heat/ER stress	[150]
miR-143	HSPA6	Direct targeting; mRNA degradation	Bovine (PBMCs)	Impaired leukocyte thermotolerance	[151]

let-7a	HMGA2, HSPA8	Translational repression	Human (multiple tissues)	Modulated constitutive chaperoning under mild stress	[97,152]
miR-17-92 cluster	BIM, PTEN	HSF1-coregulated; anti-apoptotic	Mouse (lymphocytes)	Enhanced lymphocyte survival during febrile response	[145,155]
miR-34a	SIRT1, HSF1	Indirect regulation via SIRT1 deacetylation axis	Human (neuronal cells)	Modulated HSF1 acetylation; altered neuronal thermotolerance	[156]
miR-199a	HSF1, HSPA5	Dual targeting; coordinated ER/heat stress attenuation	Human (liver cells)	Reduced combined stress response capacity	[157]

Summary of experimentally validated miRNA–heat shock response (HSR) target pairs. The table catalogs 15 miRNAs that either suppress core HSR effectors (HSF1, HSP90, HSP70 family members, HSP27) or are transcriptionally induced by HSF1 during thermal stress. For each entry, the direct target gene(s), experimentally established mechanism of action (3′ UTR targeting, translational repression, mRNA destabilization, or indirect regulation), organism and cell/tissue model system, functional effect on thermal tolerance, and primary literature reference(s) are provided. miRNAs are grouped by directionality: those that attenuate the HSR by repressing its core components (miR-644a through miR-223) and those that are induced as part of the HSR transcriptional program and shape downstream cytoprotective or immunomodulatory outcomes (miR-214 through miR-17–92 cluster). Abbreviations: HSF1, heat shock factor 1; HSP, heat shock protein; HSPA, heat shock protein family A; HSPB1, heat shock protein family B member 1; I/R, ischemia–reperfusion; ER, endoplasmic reticulum; UPR, unfolded protein response; PBMCs, peripheral blood mononuclear cells.

3.4. Multi-Omics Perspectives on Thermal Stress

The advent of multi-omics technologies has enabled comprehensive, systems-level characterization of the molecular responses to thermal stress, revealing previously unappreciated complexity in the regulatory networks that link the HSR to broader cellular physiology. A landmark study by Wen et al. (2024) employed longitudinal multi-omics profiling of acute heat exposure in human subjects, quantifying 478 differentially expressed proteins and 1,995 altered metabolites [24]. This molecular maneuvering encompassed coordinated changes across immune response pathways, coagulation cascades, oxidative stress markers, and energy metabolism networks, demonstrating that the HSR in humans extends far beyond the canonical HSF1–HSP axis to engage virtually every major physiological system [24]. Critically, pathway analysis revealed that innate immune signaling including complement activation, cytokine production, and acute-phase protein induction was among the most significantly perturbed functional categories, underscoring the intimate connection between thermal stress and immune regulation at the systems level.

Transcriptomic approaches have yielded important insights into the specificity of thermal stress responses across different pathophysiological contexts. Duan et al. (2025) used comparative transcriptomic profiling to distinguish heatstroke from fever at the molecular level, identifying heatstroke-specific marker genes including *Hspa1a*, *Hspa1b*, *Cyp7a1*, *Arrdc3*, and *G6pc*, that are mechanistically distinct from the immune/inflammatory gene signatures characteristic of febrile responses [25]. This molecular dissection has practical implications for forensic medicine, clinical diagnosis, and the understanding of how different thermal stress intensities differentially engage miRNA regulatory networks.

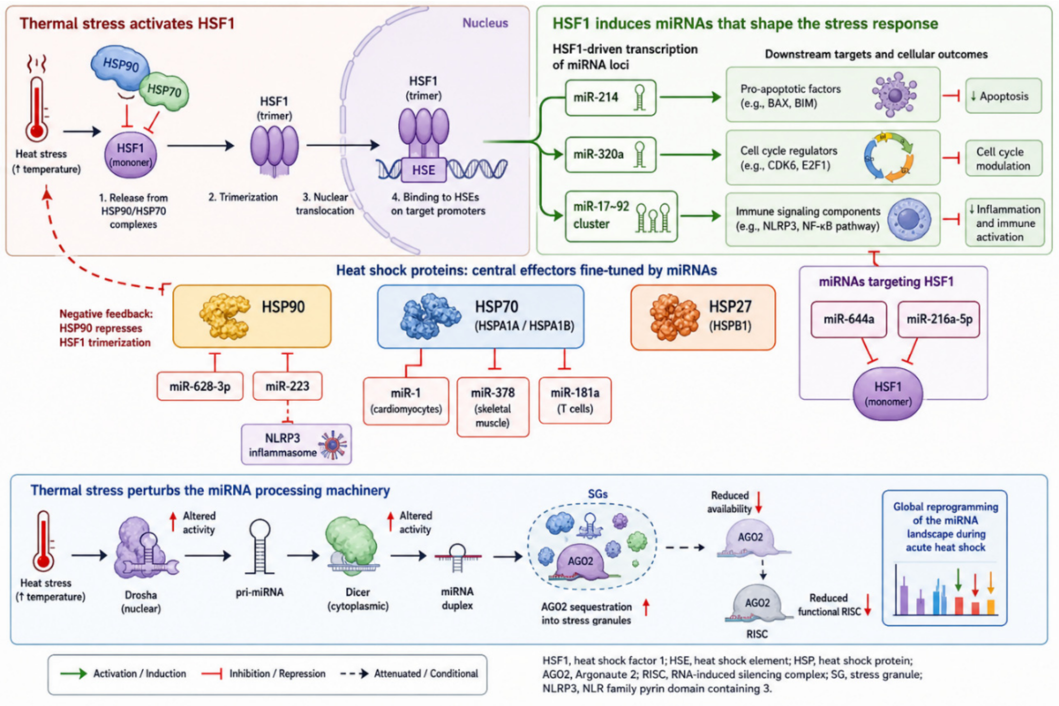

Figure 3. Bidirectional miRNA regulatory architecture in the heat shock response. This schematic illustrates the multilayered regulatory circuitry through which miRNAs both modulate and are modulated by the heat shock response (HSR). (Top left) Thermal stress triggers the release of HSF1 monomers from inhibitory HSP90/HSP70 complexes, enabling trimerization, nuclear translocation, and binding to heat shock elements (HSEs) on target gene promoters. (Top right) HSF1-driven transcription induces specific miRNA loci including miR-214, miR-320a, and the miR-17–92 cluster which in turn target pro-apoptotic factors, cell cycle regulators, and immune signaling components, thereby shaping the downstream cellular stress response. (Center) The major heat shock protein families (HSP90, HSP70/HSPA1A/HSPA1B, HSP27/HSPB1) are depicted as central effector nodes, each subject to post-transcriptional fine-tuning by specific miRNAs: miR-644a and miR-216a-5p suppress HSF1 directly; miR-1, miR-378, and miR-181a target HSP70 family members in tissue-specific contexts (cardiomyocytes, skeletal muscle, and T cells, respectively); miR-628-3p and miR-223 repress HSP90, with miR-223 providing a dual link to NLRP3 inflammasome regulation. A negative feedback loop is shown in which HSP90 represses HSF1 trimerization, closing the transcriptional circuit. (Bottom) Thermal stress-induced perturbation of the miRNA processing machinery is depicted: elevated temperatures alter Drosha and Dicer enzymatic activities, promote AGO2 sequestration into cytoplasmic stress granules (SGs), and reduce functional RISC availability collectively resulting in a transient, global reprogramming of the miRNA landscape during acute heat shock. Red flat-headed arrows denote inhibitory/repressive interactions; green pointed arrows denote activating/inductive interactions; dashed arrows indicate attenuated or conditionally active pathways. Abbreviations: HSF1, heat shock factor 1; HSE, heat shock element; HSP, heat shock protein; AGO2, Argonaute 2; RISC, RNA-induced silencing complex; SG, stress granule; NLRP3, NLR family pyrin domain containing 3. Created in BioRender https://BioRender.com/.

In livestock species, where chronic heat stress poses a significant threat to animal welfare and agricultural productivity, integrated miRNAome–transcriptome–proteome analyses have identified hub miRNAs and their regulatory circuits in cattle, poultry, swine, and aquaculture species [28,150,151]. These studies have revealed that thermally responsive miRNAs in livestock including members of the miR-27, miR-143, miR-181, and let-7 families, converge on pathways regulating apoptosis, oxidative stress, immune function, and energy metabolism, paralleling the multi-omics findings in human thermal stress [29,152]. Systems biology approaches, including weighted gene co-expression network analysis (WGCNA), Bayesian network inference, and causal mediation analysis, have been employed to infer regulatory hierarchies and identify master regulatory miRNAs "hub miRNAs" that disproportionately influence the thermal stress response [153,154]. These computational frameworks provide a foundation for prioritizing candidate miRNAs for functional validation and therapeutic or agricultural intervention.

4. miRNAs in Innate and Adaptive Immune Signaling

4.1. miRNAs in Pattern Recognition and TLR Signaling

The innate immune system relies on pattern recognition receptors (PRRs) to detect conserved molecular structures associated with invading pathogens pathogen-associated molecular patterns (PAMPs) and endogenous danger signals released from damaged tissues, damage-associated molecular patterns (DAMPs) [158]. Toll-like receptors (TLRs) constitute the best-characterized family of PRRs, comprising 10 functional members in humans and 13 in mice [159]. A comprehensive review by Kawai et al. (2024) provided an updated framework for understanding TLR biology, distinguishing cell surface TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, TLR10) that primarily detect extracellular PAMPs, including lipopolysaccharide (LPS), lipoproteins, and flagellin, from endosomal TLRs (TLR3, TLR7, TLR8, TLR9) that sense nucleic acids derived from phagocytosed pathogens [160]. TLR engagement initiates signaling cascades through adaptor proteins (MyD88, TRIF, MAL, TRAM) that converge on the activation of NF-κB, MAP kinases, and interferon regulatory factors (IRFs), driving transcription of pro-inflammatory cytokines, chemokines, and type I interferons [161,162].

MicroRNAs serve as critical rheostats of TLR signaling, fine-tuning both the amplitude and duration of innate immune responses [163]. miR-146a and miR-146b are among the best-characterized negative regulators of the NF-κB pathway (Figure 4). The kinetic stoichiometry of this axis is defined by the antagonistic roles of miR-146a and miR-155, a cross-talk mechanism illustrated in Figure 4. Induced by NF-κB itself, miR-146a directly targets TRAF6 (TNF receptor-associated factor 6) and IRAK1 (interleukin-1 receptor-associated kinase 1) two essential signal transduction adaptor proteins downstream of TLR and IL-1R signaling thereby creating a negative feedback loop that restrains inflammatory signaling and prevents excessive tissue damage [164,165]. The functional importance of this feedback loop is demonstrated by miR-146a-deficient mice, which develop spontaneous autoimmunity, myeloproliferative disease, and chronic inflammation [166]. miR-146a also contributes to the phenomenon of endotoxin tolerance, the hyporesponsiveness of macrophages to repeated LPS stimulation through sustained suppression of TRAF6 and IRAK1 [167].

In counterpoint to the anti-inflammatory role of miR-146a, miR-155 functions as a potent pro-inflammatory miRNA that amplifies innate immune signaling [168,169]. Induced by TLR ligands, TNF-α, and interferons, miR-155 targets negative regulators of inflammation, including SOCS1 (suppressor of cytokine signaling 1), SHIP1 (SH2-containing inositol-5′-phosphatase 1), and TAB2 (TGF-beta activated kinase 1 binding protein 2), thereby enhancing NF-κB and MAP kinase activation [170,171]. miR-155 occupies a dual role in immunity and oncogenesis: while essential for productive antiviral and antibacterial responses, sustained miR-155 overexpression is associated with B cell lymphoma, highlighting the fine line between protective immunity and pathological inflammation [172,173]. As shown in Figure 4, miR-146a functions as a negative feedback rheostat by targeting the signal transducers IRAK1 and TRAF6. This interaction effectively decouples the TLR from the NF-κB machinery, facilitating a controlled resolution of the inflammatory phase. The temporal balance between these two regulatory nodes determines the magnitude and duration of the inflammatory

doi:10.20944/preprints202605.0252.v1

response, ensuring that the NF-κB nexus remains responsive to pathogenic threats while remaining homeostatically constrained to prevent metabolic exhaustion.

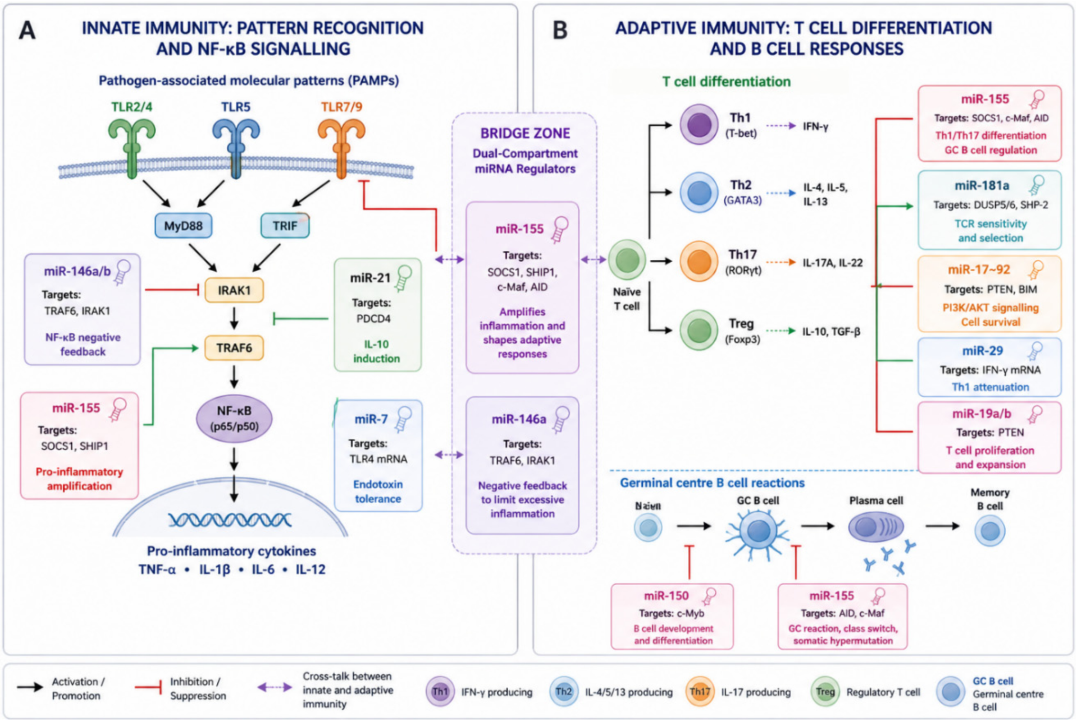

Figure 4. miRNA-mediated regulation of innate pattern recognition and adaptive immune signaling. (**A**) Toll-like receptors (TLR2/4, TLR5, TLR7/9) signal via MyD88/TRIF to activate TRAF6/IRAK1 and NF-κB, inducing pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-12). Four miRNA modules regulate this cascade: miR-146a/b targets TRAF6/IRAK1 (NF-κB negative feedback); miR-155 targets SOCS1/SHIP1 (pro-inflammatory amplification); miR-21 targets PDCD4 (IL-10 induction); let-7 targets TLR4 mRNA (endotoxin tolerance). (B) Naïve T cell differentiation into Th1, Th2, Th17, and Treg subsets alongside germinal center B cell reactions is governed by six miRNA modules: miR-155 (targets SOCS1, c-Maf, AID; Th1/Th17 and GC B cell regulation), miR-181a (targets DUSP5/6, SHP-2; TCR sensitivity), miR-17~92 (targets PTEN, BIM; PI3K/AKT survival), miR-150 (targets c-Myb; B cell development), miR-29 (targets IFN-γ mRNA; Th1 attenuation), and miR-19a/b (targets PTEN; T cell proliferation). Purple bridge zone highlights miR-155 and miR-146a as dual-compartment regulators linking innate and adaptive immunity. Created in BioRender https://BioRender.com/.

Additional miRNAs participate in TLR signaling modulation at multiple levels. miR-21, one of the most abundant miRNAs in immune cells, targets the pro-inflammatory tumor suppressor PDCD4 (programd cell death protein 4) downstream of TLR4/NF-κB signaling, simultaneously promoting IL-10 production and restraining IL-12 secretion, thus shifting the inflammatory balance toward resolution [174,175]. The let-7 family, among the most evolutionarily conserved miRNAs, regulates TLR4 expression itself and modulates endotoxin responsiveness in macrophages and epithelial cells [176]. A comprehensive review of the NF-κB signaling network by Guo et al. (2024) emphasized the extraordinary complexity of NF-κB regulation, with dozens of miRNAs targeting various nodes of the pathway, creating a complex web of positive and negative regulatory interactions that collectively determine the cellular inflammatory output [177].

4.2. *miRNAs in Inflammasome Activation*

The NLRP3 inflammasome is a cytoplasmic multiprotein complex that, upon activation by diverse danger signals, catalyzes the processing and secretion of the pro-inflammatory cytokines IL-1β and IL-18 via caspase-1–mediated proteolytic cleavage [178,179]. Activation of the NLRP3 inflammasome requires two signals: a priming signal (typically NF-κB–dependent transcriptional upregulation of NLRP3 and pro-IL-1β) and an activation signal (e.g., K^+ efflux, mitochondrial ROS, lysosomal disruption, or extracellular ATP) [180]. Given the potentially destructive consequences of unrestrained inflammasome activation including pyroptotic cell death, tissue damage, and autoinflammatory disease the NLRP3 inflammasome is subject to stringent regulation at transcriptional, post-transcriptional, and post-translational levels [181].

miR-223 serves as a direct negative regulator of the NLRP3 inflammasome, targeting the 3′ UTR of NLRP3 mRNA in macrophages and neutrophils [182,183]. Downregulation or genetic deletion of miR-223 results in elevated NLRP3 protein levels and enhanced inflammasome activation, contributing to exacerbated inflammatory pathology in murine models of experimental colitis, atherosclerosis, and metabolic syndrome [184]. Additional miRNAs targeting inflammasome components include miR-22, which suppresses NLRP3 expression in the context of renal inflammation [185], and miR-30e, which targets the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD), reducing inflammasome assembly [186]. miR-223 also regulates pyroptosis by modulating GSDMD expression, adding another layer to its anti-inflammatory function [187].

The intersection of thermal stress with inflammasome biology creates a particularly complex regulatory landscape. Moderate febrile temperatures (~39–40 °C) have been reported to enhance NLRP3 inflammasome activation in macrophages, potentially through increased mitochondrial ROS production and altered membrane fluidity, while severe heat shock (>42 °C) can paradoxically suppress inflammasome assembly through HSP90/HSP70-mediated stabilization of inhibitory complexes [188,189]. miRNA-mediated fine-tuning of these opposing thermal effects on inflammasome function likely plays a critical role in determining the inflammatory outcome of febrile episodes, although the precise miRNA circuits involved remain to be fully elucidated.

4.3. miRNAs in Adaptive Immunity

The adaptive immune system generates antigen-specific responses through the activation, proliferation, and differentiation of T and B lymphocytes, processes that are exquisitely regulated by miRNAs at virtually every developmental and functional checkpoint [190,191]. The miR-17-92 cluster (comprising miR-17, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a) is a polycistronic miRNA unit that is essential for lymphocyte development and function [192,193]. Deletion of the miR-17-92 cluster in mice causes severely impaired B cell development and T cell-dependent antibody responses, while T cell-specific overexpression promotes lymphoproliferative disease and autoimmunity [194,195]. Within the cluster, miR-19a and miR-19b play particularly important roles by targeting PTEN and BIM, thereby promoting PI3K/AKT signaling and inhibiting apoptosis in activated T cells [196].

miR-155 is indispensable for both T cell and B cell immunity. In T cells, miR-155 promotes Th1 and Th17 differentiation while restraining Treg function, at least in part through targeting of SOCS1 and the transcription factor c-Maf [197,198]. In B cells, miR-155 is essential for germinal center reactions, class-switch recombination, and somatic hypermutation, where it targets activation-induced cytidine deaminase (AID/AICDA) to calibrate the rate of antibody diversification [199,200]. miR-150 serves as a critical regulator of B cell development, targeting the transcription factor c-Myb to control the transition from pro-B to pre-B cells [201]. miR-181a has a unique role in T cell receptor (TCR) signaling: by targeting multiple phosphatases, including DUSP5, DUSP6, SHP-2, and PTPN22, miR-181a augments TCR sensitivity in developing thymocytes, effectively lowering the threshold for both positive and negative selection [202,203]. The developmental downregulation of miR-181a in mature peripheral T cells desensitizes TCR signaling, contributing to the age-related decline in T cell responsiveness (immunosenescence) [204].

The miR-29 family (miR-29a, miR-29b, miR-29c) regulates interferon-γ (IFN-γ) production in T cells and natural killer (NK) cells, directly targeting the IFN-γ mRNA 3′ UTR and modulating Th1 immune responses [205,206]. Cytokine signaling cascades are further fine-tuned by miRNAs that

doi:10.20944/preprints202605.0252.v1

target components of the JAK-STAT pathway: miR-155 and miR-19a target SOCS1, a negative regulator of JAK-STAT signaling, while miR-146a targets STAT1 in specific contexts [207,208]. These miRNA–cytokine interactions create interconnected regulatory loops that precisely calibrate the magnitude and polarization of adaptive immune responses. The integrated regulatory architecture through which miRNAs coordinate innate pattern recognition via TLR/NF-κB signaling with adaptive lymphocyte differentiation highlighting the bridging roles of miR-155 and miR-146a across both immune arms is depicted in Figure 4.

4.4. miRNAs in Macrophage Polarization

Macrophages exhibit remarkable functional plasticity, with the capacity to adopt distinct activation states along a spectrum conventionally described as M1 (classically activated, pro-inflammatory) and M2 (alternatively activated, anti-inflammatory/pro-resolution) [209,210]. Each polarization state is associated with a characteristic miRNA signature that both reflects and reinforces the underlying transcriptional program [211]. M1 polarization, driven by IFN-γ and TLR ligands, is promoted by miR-155, which targets IL-13Rα1, C/EBPβ, and SOCS1 to sustain NF-κB–dependent pro-inflammatory gene expression [212,213]. Conversely, M2 polarization, induced by IL-4, IL-13, and IL-10, is associated with upregulation of miR-124, which suppresses C/EBPα and the NF-κB p65 subunit, and miR-223, which dampens NLRP3-dependent inflammatory signaling [214,215]. miR-let-7c targets the M1-associated transcription factor C/EBPδ, promoting M2 polarization in the context of tissue repair [216].

The miRNA cargo of exosomes released by polarized macrophages plays a critical role in intercellular communication and can reprogram the polarization state of recipient macrophages and other immune cells [217,218]. Tumor-associated macrophages (TAMs), which predominantly exhibit an M2-like phenotype that promotes immune evasion and angiogenesis, release exosomes enriched in miR-21, miR-29a, and miR-223 that suppress anti-tumor immunity in the tumor microenvironment [219,220]. Conversely, pro-inflammatory exosomal miRNAs from M1 macrophages can promote anti-tumor responses, suggesting therapeutic potential for engineered exosomal miRNA delivery in cancer immunotherapy [221].

4.5. Convergence of Thermal Stress and Immune miRNA Networks

The convergence of thermal stress and immune signaling through shared miRNA regulatory networks represents a central theme of this review. Febrile temperatures (38–40 °C) alter the miRNA landscape of immune cells through several non-mutually exclusive mechanisms [222]. First, elevated temperature directly modulates the expression of thermally responsive miRNAs in immune cells, including upregulation of miR-155 and downregulation of miR-146a in LPS-stimulated macrophages incubated at 39.5 °C versus 37 °C, effectively shifting the inflammatory balance toward enhanced pro-inflammatory signaling [223]. Second, extracellular HSPs released during thermal stress, particularly HSP60, HSP70, and HSP90 function as DAMPs that activate TLR2/TLR4-dependent signaling, initiating miRNA-regulated inflammatory cascades [133,224]. Third, the temperature-dependent phase separation of HSF1 can directly activate transcription of miRNA loci containing HSE motifs, as discussed in Section 3.3, thereby coordinating HSR activation with miRNA-mediated immunomodulation [15,145].

HSP-mediated antigen presentation provides another mechanistic link between thermal stress and adaptive immunity that is subject to miRNA regulation. HSP70 and HSP90 facilitate peptide loading onto MHC class I molecules and enhance cross-presentation of exogenous antigens by dendritic cells, processes that can be modulated by miRNAs targeting components of the antigen processing and presentation machinery [225,226]. The temperature dependence of miRNA stability and RISC efficiency in activated immune cells further contributes to the thermal modulation of immune responses: miRNA half-lives and AGO2-binding affinities are temperature-sensitive in vitro, and elevated temperatures may alter the kinetic parameters of miRNA-mediated silencing in vivo [227,228].

Collectively, these observations support a conceptual model in which thermal stress functions as an "immunological rheostat" that is calibrated, at least in part, through miRNA-mediated regulatory networks. The breadth of immunomodulatory miRNAs operating across innate and adaptive immune compartments, together with their direct targets, pathway affiliations, and known thermal stress contexts, is systematically catalogd in Table 2. The three convergence mechanisms linking thermal stress to immune miRNA networks direct febrile modulation, extracellular HSP-mediated DAMP signaling, and HSF1 phase separation-driven miRNA transcription, together with the resulting immunological rheostat balancing pro-inflammatory and anti-inflammatory outcomes, are depicted in Figure 5.

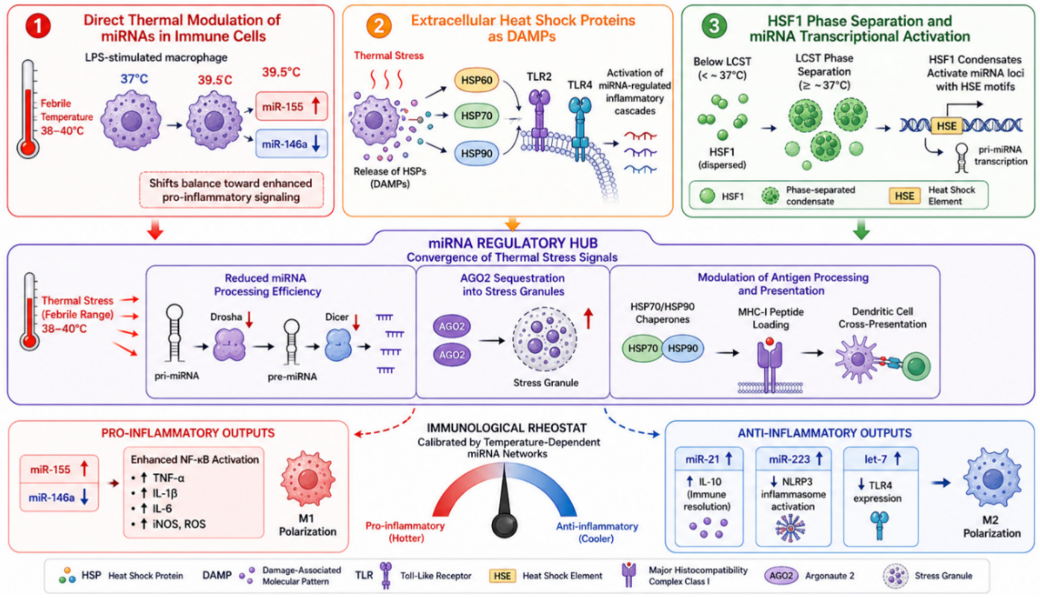

Figure 5. Convergence of thermal stress and immune miRNA networks: The immunological rheostat model. Three non-mutually exclusive mechanisms link thermal stress to miRNA-mediated immune regulation. (1) Febrile temperatures (38–40 °C) directly modulate thermally responsive miRNAs in immune cells, miR-155 is upregulated and miR-146a downregulated in LPS-stimulated macrophages at 39.5 °C versus 37 °C, shifting the inflammatory balance toward enhanced pro-inflammatory signaling. (2) Extracellular HSP60, HSP70, and HSP90, released during thermal stress, function as DAMPs that activate TLR2/TLR4, initiating miRNA-regulated inflammatory cascades. (3) Temperature-dependent LCST phase separation of HSF1 activates transcription of miRNA loci containing HSE motifs, coordinating the heat shock response with immunomodulation. These inputs converge on a miRNA regulatory hub where thermal stress reduces Drosha/Dicer processing efficiency, promotes AGO2 sequestration into stress granules, and modulates HSP70/HSP90-dependent MHC-I peptide loading and dendritic cell cross-presentation. The resulting balance between pro-inflammatory (miR-155↑, miR-146a↓ → enhanced NF-κB, TNF-α, IL-1β, IL-6; M1 polarization) and anti-inflammatory (miR-21↑ → IL-10; miR-223↑ → NLRP3 suppression; let-7↑ → TLR4 downregulation; M2 polarization) outputs is conceptualized as an immunological rheostat calibrated by temperature-dependent miRNA networks. Created in BioRender https://BioRender.com/.

Table 2. Immunomodulatory miRNAs: targets, pathways, and immune outcomes.

miRNA	Immune pathway	Direct target(s)	Immune cell type	Functional outcome	Thermal stress context	Ref.
miR-146a	TLR/NF-κB	TRAF6, IRAK1	Macrophages, DCs	Negative feedback;	Downregulated at febrile temperatures	[164,165]

				endotoxin tolerance		
miR-155	TLR/NF-κB, JAK-STAT	SOCS1, SHIP1, TAB2	Macrophages, T cells, B cells	Pro-inflammatory amplification; Th1/Th17 promotion	Upregulated at febrile temperatures	[168–171]
miR-21	TLR4/NF-κB	PDCD4	Macrophages	IL-10 induction; anti-inflammatory resolution	Context-dependent thermal regulation	[174,175]
miR-223	NLRP3 inflammasome	NLRP3	Macrophages, neutrophils	Inflammasome suppression; M2 polarization	Modulated during febrile inflammasome activation	[182–184]
let-7	TLR4	TLR4, HMGA2	Macrophages, epithelial cells	Reduced endotoxin responsiveness	Expression altered by HSP-DAMP signaling	[176]
miR-181a	TCR signaling	DUSP5, DUSP6, SHP-2, PTPN22	Thymocytes, T cells	TCR sensitivity enhancement; selection threshold	Temperature-sensitive phosphatase targeting	[202,203]
miR-17-92 cluster	PI3K/AKT, apoptosis	PTEN, BIM	T cells, B cells	Lymphocyte survival and proliferation	HSF1 co-regulation during febrile response	[192–196]
miR-150	B cell development	c-Myb	B cell progenitors	Pro-B to pre-B transition control	Indirect (HSP-mediated B cell stress)	[201]
miR-29a/b/c	IFN-γ signaling	IFN-γ, T-bet	T cells, NK cells	Th1 response modulation	TDMD-regulated under stress	[205,206]
miR-124	NF-κB	C/EBPα, p65	Macrophages, microglia	M2 polarization; anti-inflammatory	Modulated during CNS thermal stress	[214]

miR-22	NLRP3 inflammasome	NLRP3	Macrophages, renal cells	Inflammasome suppression	Responsive to heat-induced kidney injury	[185]
miR-30e	NLRP3/ASC	ASC (PYCARD)	Macrophages	Reduced inflammasome assembly	Context-dependent regulation	[186]
miR-34a	NF-κB, p53	SIRT1, Notch1	T cells, macrophages	Senescence-associated inflammation	Links HSF1 acetylation to immune aging	[156]
miR-10a	Th17/Treg balance	BCL6, NCOR2	T cells, intestinal DCs	Treg stabilization; mucosal homeostasis	Altered during gut thermal/infectious stress	[229]
miR-126	PI3K/AKT	IRS1, PIK3R2	Endothelial cells, T cells	Modulation of vascular inflammation	Heat-responsive vascular miRNA	[230]
miR-9	NF-κB	NFKB1 (p50)	Monocytes, neutrophils	NF-κB negative feedback	Upregulated by LPS and febrile temperatures	[231]
miR-125b	TNF signaling	TNF-α	Macrophages	Dampened TNF production; tolerance induction	Thermal regulation of TNF circuit	[232]
miR-132	MAPK/ERK	IRAK4, FOXO3	Macrophages, DCs	Anti-inflammatory; M2 skewing	Context-dependent stress modulation	[233]
let-7c	M1/M2 polarization	C/EBPδ	Macrophages	M2 promotion; tissue repair	Post-heat stress resolution phase	[216]
miR-301a	NF-κB/Th17	PIAS3	T cells, macrophages	Enhanced NF-κB and Th17 responses	Thermal stress-responsive in autoimmune models	[234]

Comprehensive catalog of 20 miRNAs operating across innate and adaptive immune compartments with documented thermal stress context. For each miRNA, the table specifies the primary immune signaling pathway (TLR/NF-κB, JAK-STAT, PI3K/AKT, NLRP3 inflammasome, TCR signaling, or cytokine-specific circuits), validated direct target gene(s), immune cell type(s) in which regulation has been demonstrated, functional outcome on immune polarization or effector function, thermal stress context linking the miRNA to temperature-dependent regulation, and supporting reference(s). Entries span pattern recognition receptors (miR-146a, miR-155, let-7), inflammasome regulators (miR-223, miR-22, miR-30e), adaptive immunity modulators (miR-181a, miR-17–92, miR-150, miR-29a/b/c, miR-10a), macrophage polarization determinants (miR-124, let-7c, miR-132), and NF-κB/cytokine circuit fine-tuners (miR-9, miR-125b, miR-301a). Abbreviations: TLR, Toll-like receptor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; SOCS1, suppressor of cytokine signaling 1;

doi:10.20944/preprints202605.0252.v1

SHIP1, SH2-containing inositol-5′-phosphatase 1; NLRP3, NLR family pyrin domain containing 3; TCR, T cell receptor; DC, dendritic cell; NK, natural killer; TDMD, target-directed miRNA degradation; CNS, central nervous system; PKD, polycystic kidney disease.

5. miRNAs at the Host–Pathogen Interface

5.1. Viral Infections

The interaction between host miRNAs and viral pathogens represents one of the most intensively studied areas at the intersection of RNA biology and immunology. Viruses both trigger and subvert host miRNA networks to modulate antiviral defense, immune evasion, and viral replication [235,236]. The COVID-19 pandemic catalysed an unprecedented effort to understand miRNA-mediated immunoregulation in the context of SARS-CoV-2 infection. A comprehensive analysis by Arziman and colleagues (2026) revealed that SARS-CoV-2 infection reprograms the host miRNA landscape, with miR-21, miR-146a, and miR-155 serving as central nodes modulating NF-κB, MAPK, and JAK-STAT signaling pathways in infected epithelial and immune cells [237]. Competing endogenous RNA (ceRNA) networks were identified in which viral RNA sequences and host lncRNAs compete for binding to shared miRNAs, altering the post-transcriptional regulatory equilibrium and contributing to the cytokine storm pathology characteristic of severe COVID-19 [237,238].

In HIV-1 infection, miR-29a directly targets the 3′ UTR of the HIV-1 genome, suppressing viral replication in CD4+ T cells and macrophages [239]. The antiviral activity of miR-29a is counteracted by the viral Tat protein, which suppresses Dicer activity and impairs miRNA maturation [240]. Additionally, miR-150 and miR-223, which are normally expressed at high levels in CD4+ T cells, contribute to the restriction of HIV-1 replication in resting T cells but are downregulated upon T cell activation, potentially facilitating viral propagation [241,242]. In hepatitis B virus (HBV) infection, the liver-enriched miR-122 plays a unique dual role: while miR-122 is essential for hepatitis C virus (HCV) replication through direct binding to the HCV 5′ UTR, it exerts antiviral activity against HBV by targeting viral mRNAs [243,244]. HBV counteracts host miRNA defense by suppressing global miRNA processing through viral protein HBx-mediated downregulation of Drosha [245].

Herpes simplex virus (HSV) employs multiple strategies to modulate the host miRNA landscape. The HSV-1 immediate-early protein ICP4 modulates the expression of host miRNAs, while the virus itself encodes miRNAs from its latency-associated transcript (LAT) that target viral and host genes to maintain latency [246,247]. Influenza A virus suppresses host miRNA biogenesis through its NS1 protein, which binds double-stranded RNA and interferes with Drosha and Dicer processing, representing a global strategy for miRNA evasion [248,249]. Host miRNAs miR-323, miR-491, and miR-654 directly target the influenza PB1 gene, restricting viral replication in an miRNA-dependent manner [250]. In dengue and Zika virus infections, the febrile response associated with flaviviral disease may modulate miRNA-mediated antiviral defense, as fever-range temperatures alter the expression and stability of key antiviral miRNAs in infected cells [251,252].

5.2. Bacterial Infections

The role of miRNAs in host defense against bacterial pathogens has been extensively characterized, particularly in the context of intracellular pathogens that subvert macrophage killing mechanisms [253,254]. *Mycobacterium tuberculosis* modulates host miRNA expression to promote its intracellular survival, and a comprehensive analysis by Aguilar, Mano, and Eulalio (2019) provided a systematic framework for understanding the multifaceted roles of miRNAs in host–mycobacterial interactions [255]. miR-155 is upregulated upon *M. tuberculosis* infection and promotes macrophage activation through SOCS1 suppression, enhancing pro-inflammatory cytokine production and mycobacterial killing [256]. However, miR-21 is also induced during infection and may counteract antimicrobial responses by promoting IL-10 production and suppressing Th1 immunity [257]. miR-144 regulates autophagy, a critical antimicrobial mechanism, by targeting autophagy-related genes, and its upregulation by *M. tuberculosis* impairs autophagic clearance of intracellular bacilli, contributing to pathogen persistence [258].

Salmonella infection triggers suppression of the let-7 family in macrophages, resulting in upregulation of let-7 target genes involved in cytokine signaling and cytoskeletal reorganization, facilitating bacterial invasion [259]. miR-128 targets macrophage colony-stimulating factor (M-CSF/CSF1), modulating macrophage differentiation and antimicrobial function during *Salmonella* infection [260]. *Listeria monocytogenes* infection induces miR-146a in intestinal epithelial cells, where it regulates the balance between bacterial clearance and inflammatory tissue damage [261]. *Helicobacter pylori* infection of gastric epithelial cells upregulates miR-155 and miR-21, contributing to chronic gastric inflammation and, ultimately, gastric carcinogenesis through sustained NF-κB activation and epithelial-to-mesenchymal transition [262,263]. In *Staphylococcus aureus* infections, both host and bacterial regulatory small RNAs influence virulence gene expression, biofilm formation, and immune evasion, although the precise mechanisms of host miRNA–bacterial interaction remain an active area of investigation [264,265].

5.3. Parasitic Infections

Parasitic infections present unique challenges for the host immune system, requiring a coordinated Th2-skewed response for helminth clearance while maintaining Th1 competence against intracellular protozoan parasites [266]. miR-451 has been implicated in the protective mechanism of sickle cell trait against *Plasmodium falciparum* malaria: aberrant miR-451 expression in sickle cell erythrocytes is translocated into the parasite via erythrocyte-derived vesicles, where it impairs parasite gene expression and development [65,267]. miR-223 regulates erythrocytic-stage *Plasmodium* infection by modulating the inflammatory response in the liver and spleen, influencing disease severity [268]. In *Leishmania* infections, miR-21 and miR-155 play opposing roles in macrophage activation and deactivation during leishmaniasis: miR-155 promotes classically activated macrophages (M1) that kill intracellular parasites, while miR-21 promotes alternatively activated macrophages (M2) that permit parasite survival [269,270]. Helminth infections, including *Schistosoma* and *Heligmosomoides polygyrus*, modulate host miRNA profiles to promote Th2 skewing: miR-10a and let-7 family members contribute to the expansion of Treg and Th2 populations during chronic helminth infection [271,272].

5.4. Virus-Encoded miRNAs and Pathogen Counter-Strategies

Several virus families, particularly the herpesviruses, encode their own miRNAs that target both viral and host transcripts to modulate the intracellular environment in favor of viral persistence [273,274]. Epstein-Barr virus (EBV) encodes 25 pre-miRNAs yielding at least 44 mature miRNAs, many of which target host immune genes, including MICB (a ligand for the NK cell activating receptor NKG2D), CXCL11, and components of the interferon signaling pathway [275,276]. Kaposi's sarcoma-associated herpesvirus (KSHV) encodes 12 pre-miRNAs that target host anti-apoptotic and immune-regulatory genes, including TWEAKR and BACH1, promoting viral latency and immune evasion [277]. Human cytomegalovirus (CMV) encodes miRNAs that target the host miRNA processing machinery itself, including Dicer, creating a feed-forward loop that progressively disarms the host miRNA-mediated antiviral defense [278].

Pathogen counter-strategies against host miRNA defense extend beyond viral miRNA expression. As noted above, influenza NS1 protein sequesters dsRNA intermediates from Drosha and Dicer, HIV Tat protein suppresses Dicer expression, and HBV HBx protein downregulates Drosha, collectively illustrating a convergent evolutionary strategy in which diverse pathogens disrupt host miRNA biogenesis to evade immune detection [240,245,248]. These host–pathogen miRNA dynamics represent an ongoing evolutionary arms race, with therapeutic implications for developing miRNA-based antiviral strategies that might be resistant to pathogen counter-measures.

5.5. Thermal Stress as a Modifier of Host–Pathogen miRNA Dynamics

Fever is among the most phylogenetically conserved defense mechanisms in the animal kingdom, observed in endotherms and ectotherms alike, suggesting a fundamental role in host resistance to infection [16,279]. Febrile temperatures alter miRNA expression in infected cells through at least three mechanisms: direct temperature-dependent regulation of miRNA gene transcription, HSF1-mediated activation of miRNA loci, and altered processing efficiency of thermally sensitive pri-miRNA and pre-miRNA substrates [223,280]. In ectotherms and livestock species, environmental heat stress can compromise miRNA-mediated antiviral and antibacterial defense by globally suppressing miRNA biogenesis, as the enzymatic activities of Drosha and Dicer exhibit temperature optima that may be exceeded during severe hyperthermia [28,281].

Multi-omics studies in thermally stressed livestock have revealed coordinated shifts in miRNA, mRNA, and protein expression that impair innate immune function, including reduced expression of miR-155, miR-146a, and miR-223 in heat-stressed poultry, with concomitant dysregulation of TLR signaling and inflammasome pathways [151,282]. The implications of climate change for host–pathogen miRNA dynamics are profound: as ambient temperatures rise, the chronic thermal stress experienced by both wildlife and livestock may progressively erode miRNA-mediated immune competence, increasing susceptibility to emerging and re-emerging pathogens [27,283]. Understanding how temperature modulates the host miRNA arsenal against specific pathogens represents an urgent research priority with direct relevance to global health security and food system resilience. A detailed compilation of pathogen-responsive miRNAs across viral, bacterial, and parasitic infections including their host cell targets, functional effects, and mechanistic classifications is presented in Table 3.

Table 3. Pathogen-responsive miRNAs and their functional roles.

Pathogen	miRNA(s)	Target/Pathway	Host cell type	Functional effect	Ref.
SARS-CoV-2	miR-21, miR-146a, miR-155	NF-κB, MAPK, JAK-STAT	Epithelial cells, macrophages	Cytokine storm modulation; ceRNA network perturbation	[237,238]
HIV-1	miR-29a	HIV-1 3′ UTR (direct)	CD4+ T cells, macrophages	Viral replication suppression	[239]
HIV-1	miR-150, miR-223	Viral receptors; replication	CD4+ T cells	Restriction in resting T cells	[241,242]
HBV	miR-122	HBV mRNAs (direct targeting)	Hepatocytes	Antiviral suppression of HBV replication	[243,244]
HCV	miR-122	HCV 5′ UTR (stabilization)	Hepatocytes	Pro-viral: essential for HCV replication	[243]
HSV-1	LAT-encoded miRNAs	ICP0, ICP4 (viral), TGF-β (host)	Neurons, epithelial cells	Latency maintenance; immune evasion	[246,247]
Influenza A	miR-323, miR-491, miR-654	PB1 (viral polymerase)	Epithelial cells	Viral replication restriction	[250]

Dengue virus	miR-252, miR-146a	DENV NS proteins; NF-κB	Monocytes, DCs	Antiviral defense modulation during fever	[251]
EBV	BART miRNAs (viral)	MICB, CXCL11, IFN pathway	B cells, epithelial cells	NK cell evasion; latency promotion	[275,276]
KSHV	miR-K12 cluster (viral)	TWEAKR, BACH1	Endothelial cells	Anti-apoptotic; latency maintenance	[277]
M. tuberculosis	miR-155	SOCS1; SHIP1	Macrophages	Enhanced M1 activation; mycobacterial killing	[255,256]
M. tuberculosis	miR-144	ATG genes (autophagy)	Macrophages	Autophagy suppression; pathogen persistence	[258]
Salmonella	let-7 family	Cytokine signaling; cytoskeleton	Macrophages	Facilitated bacterial invasion	[259]
L. monocytogenes	miR-146a	TRAF6, IRAK1	Intestinal epithelial cells	Balanced clearance vs. tissue damage	[261]
H. pylori	miR-155, miR-21	NF-κB, EMT regulators	Gastric epithelial cells	Chronic inflammation; carcinogenesis	[262,263]
S. aureus	miR-24, miR-31	IL-10, biofilm regulators	Keratinocytes, macrophages	Modulated biofilm and immune evasion	[264,265]
P. falciparum	miR-451	Parasite gene expression (trans-species)	Erythrocytes	Sickle trait protection; impaired parasite development	[65,267]
Leishmania	miR-155, miR-21	M1/M2 polarization	Macrophages	Opposing roles in parasite clearance vs. persistence	[269,270]
Schistosoma	miR-10a, let-7	Th2/Treg pathways	T cells, DCs	Th2 skewing; chronic infection tolerance	[271,272]
CMV	cmv-miR-UL112 (viral)	MICB, Dicer (host)	Fibroblasts, monocytes	NK cell evasion; miRNA processing disruption	[278]

doi:10.20944/preprints202605.0252.v1

Compilation of 20 host and pathogen-encoded miRNAs involved in host–pathogen interactions across viral, bacterial, and parasitic infections. For each entry, the table provides the causative pathogen, responsive miRNA(s), targeted pathway or gene, host cell type in which the interaction has been characterized, and functional effect on infection outcome. Viral pathogens include SARS-CoV-2, HIV-1, HBV, HCV, HSV-1, influenza A, dengue virus, EBV, KSHV, and CMV encompassing both host miRNAs that restrict or facilitate viral replication and virus-encoded miRNAs (LAT miRNAs, BART miRNAs, miR-K12 cluster, cmv-miR-UL112) that promote immune evasion and latency maintenance. Bacterial pathogens include Mycobacterium tuberculosis, Salmonella, Listeria monocytogenes, Helicobacter pylori, and Staphylococcus aureus. Parasitic pathogens include Plasmodium falciparum (with the sickle trait–miR-451 trans-species regulatory mechanism), Leishmania, and Schistosoma. Entries are organized by pathogen class (viral → bacterial → parasitic). Abbreviations: ceRNA, competing endogenous RNA; DENV, dengue virus; EBV, Epstein–Barr virus; KSHV, Kaposi sarcoma-associated herpesvirus; CMV, cytomegalovirus; EMT, epithelial–mesenchymal transition; MICB, MHC class I polypeptide-related sequence B.

6. Extracellular Vesicle-Mediated miRNA Transfer in Stress and Immunity

6.1. Biogenesis and Cargo Loading of Exosomal miRNAs

Extracellular vesicles (EVs), including exosomes (30–150 nm diameter), microvesicles (100–1000 nm), and apoptotic bodies, serve as vehicles for intercellular transfer of proteins, lipids, and nucleic acids, including miRNAs [284,285]. Exosomes originate from the endosomal pathway through inward budding of multivesicular body (MVB) membranes, generating intraluminal vesicles (ILVs) that are released upon MVB fusion with the plasma membrane [286]. Biogenesis proceeds through ESCRT (endosomal sorting complexes required for transport)-dependent and ESCRT-independent (ceramide/sphingomyelinase-dependent) pathways, with pathway selection influencing EV size, composition, and functional properties [287,288].

The loading of specific miRNAs into exosomes is a selective, non-random process governed by multiple sorting mechanisms. Sumoylated heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) recognizes specific sequence motifs termed “EXOmotifs” (e.g., GGAG, CCCU) in mature miRNAs and directs their sorting into exosomes [289]. Neutral sphingomyelinase 2 (nSMASE2/SMPD3) controls ceramide-dependent exosome biogenesis and influences miRNA cargo composition [290]. Additional sorting determinants include miRNA 3′ terminal modifications, AGO2 association status, and the abundance of intracellular target RNAs, which can compete with exosomal sorting [291]. Thermal stress profoundly alters EV miRNA cargo: heat-shocked cells release exosomes enriched in stress-responsive miRNAs, including miR-214, miR-320a, and members of the HSP-targeting miRNA families, potentially serving as systemic “stress signals” that precondition recipient cells against subsequent thermal challenge [292,293].

6.2. Intercellular and Inter-Organ Communication

The recognition that EV-mediated miRNA transfer enables cell-to-cell and organ-to-organ communication has prompted the development of computational frameworks for systematically mapping these interactions. Shao et al. (2025) introduced miRTalk, a computational framework for inferring EV-derived miRNA-mediated cell–cell communication from single-cell RNA sequencing (scRNA-seq) data [294]. By integrating miRNA expression profiles, target prediction, and cell type-specific pathway enrichment, miRTalk enables reconstruction of intercellular miRNA communication networks at unprecedented resolution, revealing that immune cells, fibroblasts, and epithelial cells engage in extensive EV miRNA cross-talk that is dynamically remodelled during inflammation and stress [294].

Dendritic cell-to-T cell communication via exosomal miRNAs has emerged as a mechanism for antigen-independent immune modulation, in which DC-derived exosomes carrying miR-155 and miR-146a regulate the activation threshold of recipient T cells [295,296]. Macrophage-to-epithelial cell transfer of miR-223 via exosomes regulates intestinal barrier function and inflammasome activation in epithelial cells, contributing to mucosal immune homeostasis [297]. In the context of exercise physiology and thermal stress, muscle-derived EVs containing miRNAs (myomiRs, including miR-1, miR-133a, and miR-206) circulate systemically and reach distant organs, including the liver, brain, and adipose tissue, where they exert metabolic and immunomodulatory effects analogous to myokines [298,299].

A technically significant advance was reported by Messios et al. (2025), who demonstrated that intramuscular delivery of antagomiRs achieves widespread tissue distribution beyond the injection site, including detectable knockdown in the liver, kidney, and spleen [300]. This finding has important implications for therapeutic miRNA delivery, suggesting that local administration routes may achieve systemic effects through a combination of direct tissue absorption and EV-mediated secondary distribution.

6.3. EV miRNAs in Disease and Therapeutic Potential

Exosomal miRNAs have been implicated in the pathogenesis and progression of diverse diseases, including heart failure (miR-21, miR-29b in cardiac fibrosis and remodeling), neurodegenerative disorders (miR-137, miR-124 in Alzheimer's and Parkinson's disease), and cancer (miR-21, miR-10b, miR-200 family in tumor metastasis and immune evasion) [301–303]. The stability of miRNAs within exosomal membranes protected from RNase degradation and their detectability in accessible biofluids (blood, urine, saliva, cerebrospinal fluid) have fueled intense interest in circulating EV miRNAs as minimally invasive diagnostic biomarkers [304,305]. Panels of exosomal miRNAs have been proposed for early detection of cancer, cardiovascular disease, and transplant rejection, although clinical validation remains ongoing [306]. Engineered EVs, equipped with targeting ligands (e.g., RVG peptide for brain targeting, GE11 peptide for EGFR-positive tumors) and loaded with therapeutic miRNAs or antagomiRs, represent a promising delivery platform that combines the natural biocompatibility and immune-evasive properties of endogenous EVs with the precision of synthetic targeting [307,308].

7. Therapeutic and Engineering Applications

7.1. miRNA-Based Therapeutics

The translation of miRNA biology into clinical therapeutics has progressed substantially over the past decade, yielding several candidates in clinical development and establishing design principles for miRNA-based drugs [309,310]. miRNA therapeutics fall into two broad categories: miRNA mimics, which are synthetic double-stranded RNA molecules designed to restore the function of endogenously deficient miRNAs, and anti-miRs (antagomiRs), which are chemically modified antisense oligonucleotides that sequester and inactivate overexpressed miRNAs [311]. A comprehensive review by Wang et al. (2025) provided an updated assessment of miRNA-based drug development, highlighting both the therapeutic promise and the formidable challenges associated with this modality [312].

Chemical modifications are essential for conferring metabolic stability, nuclease resistance, and favorable pharmacokinetic properties to synthetic miRNAs and antagomiRs [313,314]. The 2′-O-methyl (2′-OMe) modification, in which the 2′ hydroxyl group of the ribose sugar is replaced with a methyl group, enhances nuclease resistance while maintaining Watson-Crick base-pairing capacity [315]. Locked nucleic acid (LNA) modifications, which introduce a methylene bridge between the 2′ oxygen and 4′ carbon of the ribose, dramatically increase binding affinity for complementary RNA and enhance both stability and potency [316]. Phosphorothioate (PS) backbone modifications, in which one non-bridging oxygen of the phosphodiester bond is replaced with sulfur, improve nuclease resistance and promote protein binding, enhancing cellular uptake and tissue distribution [317]. These modifications can be combined in various configurations to optimize the pharmacological properties of miRNA therapeutics for specific clinical applications.

The clinical development of miRNA-based therapeutics has yielded both successes and setbacks that have informed the field's maturation. Miravirsen (SPC3649), an LNA-modified anti-miR-122 oligonucleotide developed by Santaris Pharma (now Roche), was the first miRNA therapeutic to enter clinical trials, demonstrating dose-dependent and durable suppression of HCV RNA in chronically infected patients without evidence of viral resistance [318,319]. MRX34, a liposomal miR-34a mimic developed by Mirna Therapeutics for advanced solid tumors, showed promising anti-tumor activity in a Phase I trial but was discontinued due to immune-related serious adverse events, highlighting the challenge of managing the immunostimulatory properties of double-stranded RNA therapeutics [320,321]. Cobomarsen (MRG-106), an LNA-modified anti-miR-155 oligonucleotide developed for cutaneous T cell lymphoma (CTCL), demonstrated clinical activity in Phase I/II trials but was ultimately discontinued for strategic reasons [322]. These clinical experiences have refined the field's understanding of the safety, efficacy, and design requirements for miRNA therapeutics. The current landscape of miRNA-based therapeutics in clinical development, encompassing drug names, miRNA targets, therapeutic modalities, disease indications, delivery systems, and key clinical findings, is summarized in Table 4.

Table 4. miRNA-based therapeutics in clinical development.

Drug name	**miRNA target**	**Modality**	**Disease indication**	**Clinical stage**	**Delivery system**	**Key findings**	**Ref.**
Miravirsen (SPC3649)	anti-miR-122	LNA anti-miR	Hepatitis C virus	Phase II (completed)	Naked (subcutaneous)	Dose-dependent HCV RNA reduction; durable; no resistance	[318,319]
RG-101	anti-miR-122	GalNAc-conjugated anti-miR	Hepatitis C virus	Phase II (discontinued)	GalNAc conjugate (subcutaneous)	Potent viral suppression; jaundice adverse events	[359]
MRX34	miR-34a mimic	miRNA mimic	Advanced solid tumors	Phase I (discontinued)	Liposomal (IV)	Anti-tumor activity observed; immune-related SAEs	[320,321]

Cobomarsen (MRG-106)	anti-miR-155	LNA anti-miR	CTCL, DLBCL	Phase II (discontinued)	Naked (subcutaneous/IV)	Clinical activity in CTCL; manageable safety profile	[322]
TargomiRs (MesomiR-1)	miR-16 mimic	miRNA mimic	Malignant pleural mesothelioma	Phase I (completed)	Bacterial minicells (IV)	Objective responses in some patients; tolerable	[360]
MRG-110	anti-miR-92a	LNA anti-miR	Heart failure; wound healing	Phase I (completed)	Naked (intradermal)	Enhanced angiogenesis in wound healing model	[361]
RGLS4326	anti-miR-17	Short anti-miR	Autosomal dominant PKD	Phase I (completed)	Naked (subcutaneous)	Reduced kidney cyst growth in preclinical models	[362]
Remlarsen (MRG-201)	miR-29 mimic	miRNA mimic	Keloid/pathological fibrosis	Phase II	Naked (intradermal)	Reduced collagen deposition; anti-fibrotic	[363]
INT-1B3	miR-193a-3p mimic	miRNA mimic	Advanced solid tumors	Phase I	LNP (IV)	Multi-pathway anti-tumor activity in preclinical models	[364]
TTX-MC138	anti-miR-10b	Nanoparticle anti-miR	Metastatic breast cancer	Phase I (ongoing)	Dextran-coated iron oxide NP	Metastasis suppression in	[365]

						preclinical; MRI-detectable	
ABX464	miR-124 (indirect upregulation)	Small molecule	Ulcerative colitis, HIV	Phase II/III	Oral	Anti-inflammatory via miR-124 induction; well-tolerated	[366]

Overview of 11 miRNA-based therapeutic agents that have entered clinical trials, representing the current translational landscape of miRNA medicine. For each agent, the table specifies the drug name, miRNA target, therapeutic modality (LNA anti-miR, miRNA mimic, GalNAc conjugate, nanoparticle, small molecule, or bacterial minicell), disease indication, clinical development stage (Phase I through Phase III), delivery system, key clinical findings to date, and primary reference(s). Modalities span three mechanistic categories: anti-miR oligonucleotides that sequester endogenous oncogenic or pro-viral miRNAs (miravirsen, RG-101, cobomarsen, MRG-110, RGLS4326, TTX-MC138), miRNA mimics that restore tumor-suppressive or anti-fibrotic miRNA activity (MRX34, TargomiRs, INT-1B3, remlarsen), and a small-molecule approach that indirectly upregulates a therapeutic miRNA (ABX464 → miR-124). Notable clinical milestones include the first-in-human miRNA therapeutic (miravirsen), the first liposomal miRNA mimic (MRX34), and the first MRI-detectable nanoparticle anti-miR (TTX-MC138). Abbreviations: LNA, locked nucleic acid; GalNAc, N-acetylgalactosamine; LNP, lipid nanoparticle; IV, intravenous; SAE, serious adverse event; CTCL, cutaneous T cell lymphoma; DLBCL, diffuse large B cell lymphoma; PKD, polycystic kidney disease; HCV, hepatitis C virus.

7.2. Delivery Systems

Effective delivery to target tissues remains the principal challenge for miRNA-based therapeutics, as naked oligonucleotides are rapidly degraded by serum nucleases, cleared by the kidneys, and exhibit poor cellular uptake [312,323]. Viral vectors, particularly adeno-associated viruses (AAVs), offer efficient and durable delivery of miRNA expression cassettes, with tissue tropism determined by capsid serotype [324]. AAV-mediated delivery of miR-26a has shown efficacy in hepatocellular carcinoma models, while AAV-miR-1 delivery has been explored for cardiac disease [325,326]. However, concerns regarding immunogenicity, insertional mutagenesis, and manufacturing scalability limit the broad clinical applicability of viral vectors for miRNA delivery.

Lipid nanoparticles (LNPs), whose clinical potential was validated by the mRNA COVID-19 vaccines, represent the most advanced non-viral delivery platform for RNA therapeutics [327,328]. LNPs encapsulate miRNA mimics or antagomiRs within ionizable lipid bilayers that promote endosomal escape following cellular uptake, achieving efficient cytoplasmic delivery primarily to hepatocytes following systemic administration [329]. Polymer-based nanoparticles including poly lactic-co-glycolic acid (PLGA), polyethylenimine (PEI), and chitosan-based formulations offer tunable degradation kinetics, surface functionalization, and controlled release properties [330,331]. Inorganic nanoparticles, including gold, mesoporous silica, and superparamagnetic iron oxide nanoparticles, provide additional platforms with unique optical, diagnostic, or magnetic targeting capabilities [332,333].

Exosome-based delivery systems leverage the natural biocompatibility, immune-evasive properties, and tissue tropism of endogenous EVs [307,334]. Engineering strategies include surface display of targeting ligands (e.g., Lamp2b fusion proteins for tissue-specific delivery) and electroporation-mediated loading of therapeutic miRNAs into purified exosomes [335]. Stimuli-responsive delivery systems represent an emerging frontier in which miRNA release is triggered by specific environmental cues, including pH (tumor microenvironment), temperature (febrile or hyperthermic conditions), redox potential (oxidative stress), or enzyme activity [312,336]. Thermoresponsive miRNA delivery systems are of particular relevance to this review, as they leverage heat-activated phase transitions in carrier materials (e.g., thermosensitive hydrogels, temperature-responsive polymeric nanoparticles) to achieve precision miRNA release during hyperthermic therapy or febrile immune responses, effectively coupling the pharmacological intervention to the pathophysiological state [337,338].

7.3. Synthetic Biology and miRNA Engineering

Advances in synthetic biology have enabled the construction of artificial miRNA circuits that function as programmable gene regulatory modules [339,340]. Synthetic miRNA-responsive gene switches in which transgene expression is controlled by endogenous miRNA activity, have been applied in cell-based therapies to ensure tissue-specific and state-dependent therapeutic gene expression [341]. For example, miRNA-responsive mRNA constructs that are selectively silenced in off-target tissues (e.g., liver, where miR-122 is abundant) can reduce hepatotoxicity of systemically administered mRNA therapeutics [342]. In the context of chimeric antigen receptor T (CAR-T) cell therapy, synthetic miRNA circuits have been engineered to modulate CAR expression in response to the tumor microenvironment, improving the therapeutic index by coupling cytotoxic activity to specific molecular cues [343].

CRISPR-based approaches for miRNA modulation include CRISPR-Cas13–mediated knockdown of specific miRNAs, CRISPR interference (CRISPRi) silencing of miRNA gene promoters, and CRISPR activation (CRISPRa) of endogenous miRNA loci [344,345]. These tools provide unprecedented precision for dissecting miRNA function in loss-of-function and gain-of-function settings and have been adapted for high-throughput functional screens of miRNA regulatory networks [346]. The integration of thermoresponsive elements such as heat-inducible promoters driving Cas13 expression with miRNA-targeting CRISPR systems could enable temperature-gated miRNA modulation, a strategy with potential applications in both research and thermally triggered gene therapy [347].

7.4. Agricultural and Veterinary Applications

The economic impact of heat stress on livestock production, estimated at billions of dollars annually in losses due to reduced growth, impaired reproduction, compromised immunity, and increased mortality, has motivated research into miRNA-based strategies for improving thermal tolerance [28,348]. Profiling studies have identified candidate miRNAs associated with heat tolerance phenotypes in cattle (e.g., *Bos indicus* breeds versus *Bos taurus*), poultry, and swine, and these miRNAs converge on pathways regulating HSP expression, apoptosis, oxidative stress, and immune function [29,349,350]. Genomic selection strategies incorporating miRNA-related quantitative trait loci (QTLs) may enable breeding for enhanced thermal resilience, while direct miRNA-based interventions such as in ovo delivery of miRNA mimics to improve hatchling thermotolerance in poultry are under investigation [351,352].

Cross-kingdom miRNA transfer, the concept that plant-derived miRNAs consumed in the diet can survive gastrointestinal digestion and modulate gene expression in the consuming animal, has generated both excitement and controversy [353,354]. While initial reports of dietary plant miR-168a regulating mammalian LDLRAP1 expression have been challenged on methodological grounds, more recent studies employing improved controls and delivery systems have provided evidence supporting functional cross-kingdom transfer in specific contexts [355,356]. In aquaculture, miRNA regulation of thermal tolerance in commercially important fish species including salmon, tilapia, and catfish is an active area of research, with species-specific miRNA profiles associated with upper thermal tolerance limits and heat-shock survival [357,358].

8. Challenges, Emerging Frontiers, and Future Directions

8.1. Technical Challenges

Despite the remarkable progress in miRNA biology, several technical challenges impede the translation of basic research findings into robust, reproducible, and clinically actionable knowledge. miRNA target prediction remains an imperfect science: while seed-based complementarity algorithms (TargetScan, miRanda, DIANA-microT) have achieved reasonable sensitivity, they suffer from high false-positive rates, as not all computationally predicted miRNA-target interactions are functional in physiological contexts [367,368]. The requirement for experimental validation through luciferase reporter assays, AGO-CLIP (crosslinking and immunoprecipitation), and functional rescue experiments substantially slows target identification throughput [369]. Standardization of experimental protocols including cell type, miRNA delivery method, timepoint, and readout is essential for improving the reproducibility of miRNA target validation studies [370].

Quantification of miRNA expression presents its own challenges. RT-qPCR, the gold standard for miRNA quantification, requires careful normalisation using stably expressed endogenous controls (e.g., RNU6B, miR-16, miR-191), but the selection of appropriate normalizers is tissue- and context-dependent [371]. Small RNA sequencing offers transcriptome-wide miRNA profiling but introduces biases during library preparation, particularly through adapter ligation bias, PCR amplification bias, and the influence of miRNA terminal modifications on capture efficiency [372,373]. Cross-platform comparability between RT-qPCR, microarray, and sequencing-based miRNA profiling remains limited, complicating meta-analyses and the development of diagnostic biomarker panels [374].

8.2. Emerging Technologies

Single-cell miRNA profiling represents a transformative frontier that will enable cell type-resolved mapping of miRNA regulatory networks in heterogeneous tissues and tumor microenvironments [375]. Integration of single-cell miRNA data with scRNA-seq, CITE-seq (protein-level information), and spatial transcriptomics will provide multi-modal, spatially resolved views of miRNA function in situ [376,377]. Long-read sequencing technologies (Oxford Nanopore, PacBio) offer the possibility of isoform-resolved miRNA analysis, enabling direct detection of isomiRs, miRNA variants differing in length or sequence at their 5′ or 3′ termini, without the fragmentation and amplification biases inherent in short-read approaches [378]. Artificial intelligence and machine learning, including deep learning architectures trained on AGO-CLIP data, are dramatically improving miRNA target prediction accuracy and enabling in silico drug design for miRNA-based therapeutics [379,380]. CRISPR-based functional screens including genome-wide miRNA knockout libraries and combinatorial perturbation experiments are enabling systematic dissection of miRNA function in specific biological contexts, including stress responses and immune activation [346,381].

8.3. Integrative Multi-Omics Frameworks

The integration of miRNA expression data with matched mRNA, protein, and metabolite profiles from the same biological samples represents the most powerful approach for reconstructing miRNA regulatory networks and inferring causal relationships [382,383]. Strategies for miRNA–mRNA–protein integration include correlation-based approaches (identifying anti-correlated miRNA–mRNA pairs), causal inference methods (Mendelian randomization, Bayesian network modelling), and machine learning-based target network reconstruction [384,385]. Metabolomics integration with miRNA networks is particularly informative for thermal stress biology, as acute heat exposure induces dramatic metabolic reprogramming that is at least partially miRNA-dependent [24,386]. Epigenomic profiling of miRNA gene loci including DNA methylation, histone modifications, and chromatin accessibility (ATAC-seq) under thermal stress conditions reveals how epigenetic mechanisms regulate the transcriptional responsiveness of miRNA genes to temperature [387,388]. Network medicine approaches, which model miRNA–disease associations through protein–protein interaction networks and disease module identification, offer a framework for understanding how miRNA dysregulation at the thermal stress–immunity nexus contributes to complex disease phenotypes [389,390].

8.4. Outstanding Questions and Future Perspectives

Several fundamental questions remain unresolved and represent priorities for future investigation. First, how does chronic versus acute thermal stress differentially reprogram miRNA networks? While acute heat shock triggers rapid, transient changes in miRNA expression that are substantially reversed upon recovery, chronic thermal stress such as that experienced by livestock during prolonged heat waves or by human populations in tropical climates, may induce epigenetic reprogramming of miRNA gene loci that produces lasting alterations in the miRNA landscape [391,392]. Second, can miRNA-based interventions modulate febrile immune responses to improve infectious disease outcomes? The dual role of fever, beneficial at moderate temperatures but harmful during hyperpyrexia suggests that precision miRNA therapeutics targeting specific inflammatory nodes could optimize the febrile response without suppressing protective immunity [393]. Third, what is the evolutionary conservation of thermally responsive miRNA circuits across phyla? Comparative genomics and functional studies in ectotherms, endotherms, and invertebrates will reveal the ancient core of thermal miRNA regulation and lineage-specific innovations [394,395]. Fourth, how can multi-omics data be leveraged for personalized miRNA-based diagnostics? The development of patient-specific miRNA profiles integrating circulating EV miRNAs, tissue miRNAs, and multi-omics disease signatures could enable precision medicine approaches to thermal stress-related diseases [396,397].

9. Conclusions

This review has articulated a comprehensive conceptual framework positioning miRNAs as integrative molecular nodes at the nexus of thermal stress and host immunity. The evidence synthesized across eight major sections demonstrates that miRNAs are not mere post-transcriptional fine-tuners but rather dynamic, multi-layered regulatory agents that actively shape the cellular response to concurrent thermal and immunological challenges. From the temperature-dependent phase separation of HSF1 that drives both HSP and miRNA transcription, through the complex feedback circuits connecting miR-146a, miR-155, and miR-223 to NF-κB and inflammasome pathways, to the extracellular vesicle-mediated miRNA transfer that enables intercellular and inter-organ stress communication, a coherent picture emerges of miRNA-mediated regulatory flux as a systems-level phenomenon that cannot be adequately captured by single-pathway analyses.

The multi-omics paradigm shift integrating transcriptomics, proteomics, metabolomics, small RNA sequencing, and epigenomics has been instrumental in revealing the emergent properties of miRNA regulatory networks under thermal stress. Studies employing longitudinal multi-omics profiling of heat-exposed humans and livestock have demonstrated that the molecular choreography of the heat shock response extends far beyond the canonical HSF1–HSP axis to engage virtually every major physiological system, with miRNAs serving as critical integrative mediators. The host–pathogen miRNA interface adds further complexity, as both viral and bacterial pathogens exploit and subvert host miRNA circuits, with thermal stress modifying these dynamics in ways that have profound implications for infectious disease susceptibility in a warming world.

The translational horizon for miRNA-based therapeutics is both promising and challenging. Clinical experience with Miravirsen, MRX34, and Cobomarsen has established proof-of-concept for miRNA therapeutics while illuminating the obstacles delivery, immunogenicity, off-target effects that must be overcome for broad clinical adoption. Thermoresponsive delivery systems that couple miRNA release to the pathophysiological state of the patient represent a particularly elegant strategy at the intersection of thermal biology and RNA therapeutics. Agricultural applications, including miRNA-based strategies for improving livestock thermal tolerance, address an urgent global challenge that will only intensify as climate change progresses.

We call for interdisciplinary convergence between the traditionally siloed fields of stress biology, immunology, and RNA therapeutics. The thermal stress–immunity nexus is not merely an academic curiosity but a critical biological interface with implications for human health, agricultural productivity, and ecosystem resilience. Future research should prioritize: (1) single-cell and spatial multi-omics dissection of miRNA regulatory networks in thermally stressed immune cells; (2) evolutionary comparative studies of thermal miRNA circuits across diverse taxa; (3) clinical development of thermoresponsive miRNA delivery platforms; and (4) integration of miRNA biomarkers into precision medicine frameworks for heat-related illness and infectious disease. By embracing miRNAs as the rheostatic integrators they are, we can unlock new diagnostic, therapeutic, and agricultural opportunities at this vital biological nexus.

Funding: Not applicable.

Acknowledgments: This review was conducted without specific funding from any public, commercial, or not-for-profit funding agency. OBM, MSA and BNT were supported by the USDA-NIFA research grant 2023-67016-39917. We are grateful to the Department of Biology, Virginia State University for ongoing support.

Authors contributions: OBM conceived the idea; OBM and KHS coordinated the project; OBM, MA, OF and MSA performed the primary literature search, curated data, prepared tables and figures, and drafted the manuscript; OBM, OF, and KHS supervised the conceptual framing of the review, provided critical input throughout, and jointly revised and finalized the manuscript with all authors; KAB MCM, PKS, XL, AOA, AOA, JWY, GT, MOA, IMO, BNT and SOP contributed revisions and expertise in their respective fields. All authors critically reviewed and approved the final manuscript.

Competing interests: Authors declare no competing interests.

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